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Acupuncture for pain management

Edited by
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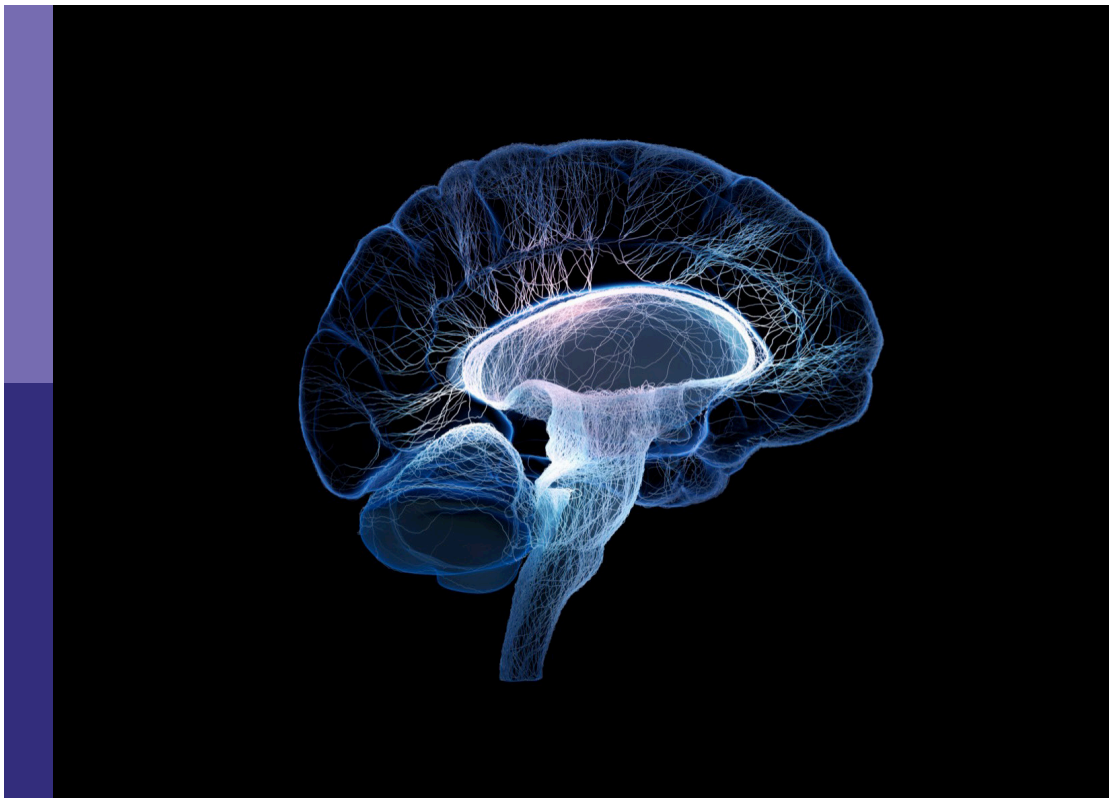

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Acupuncture for pain management

Topic editors

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Editorial: Acupuncture for pain management

Jun Zhou[1,2], Yuke Teng[1,2], Xinyue Zhang[1,2], Shirui Cheng[1,2], Xinling Li[1], Shengjie Hu[1], Weiming Luo[1], Xingyao Chen[1] and Zhengjie Li[1,2]*

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KEYWORDS

pain management, acupuncture, efficacy, mechanisms, evidence-based, clinical applications

Editorial on the Research Topic
Acupuncture for pain management

Pain management poses significant challenges worldwide due to the high prevalence, debilitating effects, and limited effective medication options. In light of the opioid crisis and concerns about drug abuse, regulatory and oversight agencies are advocating for alternative treatment options for pain control. Acupuncture is a potential choice to address these demands. However, despite its growing popularity, there is still limited understanding of the scientific mechanisms underlying acupuncture's effectiveness for pain management, and there is a need for translational research to bridge the gap between mechanisms illustration and clinical applications. To address these gaps, we launched a Research Topic about "*Acupuncture for pain management*" on November 10th, 2021, inviting researchers to contribute their studies and identify potential avenues for future research in this field.

The Research Topic "*Acupuncture for pain management*," published in *Frontiers in Neuroscience*, featured 19 articles involving 161 authors from 5 countries, presenting significant contributions to our understanding of acupuncture's efficacy, safety, and mechanisms for pain management. The articles can be summarized into four categories: efficacy of acupuncture, acupuncture for specific pain conditions, mechanisms illustration of acupuncture analgesia, and technological advances in acupuncture and personalized treatment integration.

(1) Efficacy of Acupuncture: Several studies demonstrated the effectiveness of acupuncture in various pain conditions, including migraines, neck pain, shoulder pain, and osteoarthritis. For instance, Liu C-T. et al. conducted a multicenter randomized controlled trial and found that acupuncture could effectively prevent menstruation-related migraines. Shi et al. conducted a randomized controlled trial evaluating the efficacy and safety of electro-thumbtack needle therapy for chronic neck pain. Heo et al. and Zhan et al. confirmed the benefits of acupuncture in systematic reviews and meta-analyses for shoulder pain in scapulohumeral periarthritis and post-stroke shoulder pain, respectively. Additionally, Hee et al. conducted an overview of systematic reviews and meta-analyses, showing the effectiveness of warm needle acupuncture in treating osteoarthritis. These studies provide evidences for the efficacy of acupuncture in various pain conditions.

(2) Acupuncture for Specific Pain Conditions: Several studies highlighted the efficacy of acupuncture in managing pain associated with traumatic rib fractures, irritable bowel syndrome, and acute renal colic. Liu L-Y. et al. demonstrated that acupuncture was a safe and effective analgesic modality for managing pain in traumatic rib fractures. Yang et al. conducted a systematic review and meta-analysis, finding that acupuncture and moxibustion were effective treatments for irritable bowel syndrome. Chen L. et al. conducted a prospective cohort study and found that acupressure provided significantly faster pain relief (for acute renal colic) compared to parecoxib sodium within 10 min. These studies provide evidences for the effectiveness of acupuncture in specific pain conditions.

(3) Mechanisms illustration of Acupuncture Analgesia: Several studies also provided insights into the mechanisms illustration underlying acupuncture's therapeutic effects. Acupuncture was found to modulate neuronal activity, pain-related ion channels, and the release of inflammatory cytokines and chemokines. It also activated the descending pain control system and demonstrated effects on brain regions involved in musculoskeletal pain and primary dysmenorrhea. Additionally, a proposed mechanism based on tensegrity principles offered potential for treating fibromyalgia. For instance, Ma et al. proposed that the somatosensory system played a potential role in the effectiveness of acupuncture for neuropathic pain. Acupuncture has the ability to inhibit neuronal activity induced by neuropathic pain through the reduction of pain-related ion channel activation and suppression of inflammatory cytokines and chemokines release. Furthermore, it could activate the descending pain control system by increasing the levels of spinal or cerebral neurotransmitters, such as 5-hydroxytryptamine (5HT), norepinephrine (NE), and opioid peptides. In another study, Wang J-y. et al. demonstrated that transcutaneous auricular vagus nerve stimulation (taVNS) increased plasma melatonin concentration, upregulated melatonin receptor (MTR) expression in the amygdala, and relieved peripheral neuropathic pain. Liu, Zhang et al. revealed a correlation between energy metabolism and acupuncture treatment of migraine through proteomic and metabolomic analyses. Moreover, Wang S. et al. discovered that electroacupuncture alleviated hyperalgesia in rats with acute neck pain by regulating neuronal-glial interactions and glutamate transporters. Ha et al. employed the coordinate-based activation likelihood estimation (ALE) method in a meta-analysis on acupuncture for musculoskeletal pain, revealing acupuncture-induced modulation of various brain regions in musculoskeletal pain patients. In the study by Liu, Li et al., acupuncture was found to modulate the functional connectivity density in patients with primary dysmenorrhea. Additionally, Plaut proposed a mechanism illustration for acupuncture as a global percutaneous needle fasciotomy based on tensegrity principles, providing insights into its potential for treating fibromyalgia. These findings shed light on the potential mechanisms illustration underlying acupuncture's efficacy in pain management and offer possible explanations for the observed clinical benefits of this therapeutic approach.

(4) Technological Advances in Acupuncture and Personalized Treatment Integration: Technological advancements in acupuncture and the integration of personalized treatment have expanded the capabilities of traditional acupuncture methods, facilitating customized treatments for individual patients. Fu et al. utilized innovative research strategies, such as machine learning and neuroimaging techniques, to identify potential biomarkers for discriminating patients with migraines and predicting the efficacy of transcutaneous vagus nerve stimulation (tVNS) in reducing migraine attack frequency. In a two-center randomized controlled trial, Chen C. et al. compared the CX-DZ-II smart electronic stimulator to the conventional SDZ-II electronic stimulator for alleviating neck pain caused by cervical spondylosis. The study demonstrated that the smart electronic stimulator was non-inferior to the conventional stimulator. Kim et al. published a protocol for an ongoing multicenter randomized placebo-controlled trial investigating the safety and efficacy of 650 nm invasive laser acupuncture for non-specific chronic low back pain. Furthermore, Li et al. plan to conduct a randomized controlled trial to investigate whether combining transcutaneous electrical acupoint stimulation with electroacupuncture can expedite postoperative recovery after abdominal surgery. These studies aim to facilitate more effective and targeted acupuncture clinical applications in pain management. These technological advancements in acupuncture and personalized treatment integration hold great promise for improving the effectiveness, precision, and accessibility of acupuncture therapy.

Acupuncture's therapeutic potential extends beyond its analgesic properties. Its holistic approach, focusing on the restoration of balance and harmony within the body, offers a unique perspective in the realm of pain management. With the world grappling with the ramifications of the opioid crisis and the limitations of satisfactory pharmacological options, it is imperative to explore alternative treatments that are safe, sustainable, and evidence-based. Acupuncture embodies these qualities and holds immense promise in transforming the landscape of pain management. Acupuncture offers several advantages as a pain management modality, including its non-pharmacological nature, individualized approach, and minimal side effects. However, certain limitations, such as the requirement for experienced acupuncturists, inter-individual variability, and the lack of standardized treatment protocols, should be acknowledged.

Based on the existing evidence, developing personalized treatment plans that incorporate acupuncture for pain management is crucial. Clinicians should consider the specific pain condition, patient characteristics, and clinical guidelines when determining the appropriateness of acupuncture. Additionally, exploring the integration of acupuncture with conventional medicine and other pain management strategies may offer comprehensive solutions for patients.

It is our belief that this Research Topic will serve as a catalyst for innovation and collaboration, bringing together experts from various disciplines to further our understanding of acupuncture's role in pain management. Through rigorous research and a multidimensional approach, we can harness the full potential of acupuncture to alleviate the suffering caused by pain. Together, we can pave the way for a paradigm shift in pain management, transforming it into a more sustainable and patient-centered endeavor.

Author contributions

JZ: conceptualization, writing the original draft, and writing-review, and editing. YT, XZ, SC, XL, SH, WL, and XC: writing-review and editing. ZL: provided oversight and guidance as the corresponding author and contributed significantly to the editing and finalization of the manuscript. All authors approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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STUDY PROTOCOL
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Efficacy and Safety of Electro-Thumbtack Needle Therapy for Patients With Chronic Neck Pain: Protocol for a Randomized, Sham-Controlled Trial

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Background: Chronic neck pain is a prevalent condition adversely impacting patients' wellbeing in both life and work experience. Electro-thumbtack needle (ETN) therapy, combining acupuncture with transcutaneous stimulation, might be one of the effective complementary and alternative medicine (CAM) therapies in treating chronic neck pain, although the evidence is scarce. This study aims to estimate the efficacy and safety of ETN therapy for chronic neck pain.

Methods and Analysis: This is a sham-controlled, randomized clinical trial. A total of 180 subjects will be randomly allocated to either the ETN group or the sham ETN group. Treatment will be administrated three times a week for four consecutive weeks, with a 6-month follow-up. The primary outcome measure will be the Numerical Rating Scale for neck pain (NRS-NP) over a period of the 4 weeks. Secondary outcome measures include the Northwick Park Neck Pain Questionnaire (NPQ), Neck Disability Index (NDI), Patient Global Impression of Change (PGIC), patient expectation, and preference assessment. The chi-square test or Fisher's exact test will be used for proportions of participants having clinically meaningful improvement. Analysis of covariance or repeated-measures analysis of variance will be applied to examine changes in the outcome measures from baseline.

Discussions: This prospective trial will contribute to evaluating the efficacy and safety of ETN in the treatment of chronic neck pain, with an intermediate-term follow-up. This study will provide further evidence for clinical neck pain management.

Ethics and Dissemination: This trial has been approved by the Research Ethical Committee of Guang'anmen Hospital (ethical approval number: 2021-039-KY-01). Recruitment began in March 2022 and will continue until December 2023. Dissemination plans include posters, WeChat, websites, and bulletin boards in hospital and communities.

Clinical Trial Registration: This trial is registered at ClinicalTrials.gov (identifier: NCT04981171).

Keywords: acupuncture, chronic neck pain, RCT, electro-thumbtack needle, efficacy and safety

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INTRODUCTION

Chronic neck pain is a common physical complaint across the world and the fourth leading impairment in terms of years lived with disability (1, 2). Its lifetime prevalence is around 48.5% (3). Neck pain is mainly due to poor posture or anxiety, especially in women and the middle-aged population (4). Previous clinical trials have demonstrated that therapies including exercise, acupuncture, and transcutaneous electrical nerve stimulation (TENS) have moderate effects in pain alleviation (5–8). However, management of chronic neck pain remains complex and challenging.

Acupuncture has been widely used for pain alleviating around the world. In previous studies, acupuncture or electroacupuncture is more effective than sham controls (6, 8–11). Clarified evidence supported that acupuncture could produce opioids and other bioactive antalgic chemicals (12). It is thus recommended for the treatment of chronic neck pain by consensus (6, 8, 13). Nevertheless, the application of acupuncture is limited to a large extent due to the patients' fright of and intolerance to needling pain. TENS, a non-invasive physical therapy, is more popular and comfortable. Evidence from clinical trials supported the effectiveness of TENS for chronic neck pain (14–16). The pain could be alleviated *via* stimulation either on acupoints or trigger points, both immediately after treatment and during follow-up periods (15, 17, 18). However, the difference between TENS and sham control is not significant, especially at the long-term follow-ups (14, 18).

A combination of acupuncture with conservational treatment, such as TENS, is considered to benefit the patients more in comparison with single therapy (8, 13). The emergence of electro-thumbtack needle (ETN) therapy is in response to this recommendation, aiming to achieve better effectiveness while maintaining comfortable. The device of ETN is composed of a thumbtack needle with 2 mm long body and a small rechargeable and portable electric stimulator, which outputs current. The surface of thumbtack needle is modified with conductive material to reinforce electric stimulation on acupoints. It is a convenient therapy that can be easily operated by patients on their own after training. In light of the good effectiveness of acupuncture and TENS published in previous studies, as well as the convenience of ETN, we plan to conduct this study to estimate the efficacy and safety of ETN therapy for chronic neck pain in comparison with sham ETN therapy.

METHODS AND ANALYSIS

Study Design

This study is a single-center, participant-blinded, randomized controlled trial, which will be conducted from March 2022 to December 2023 in Guang'anmen Hospital, China Academy of Chinese Medical Sciences. The study flowchart is shown in **Figure 1**. We design this protocol conforming to the guidelines of the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) (19) (**Table 1**) and the Consolidated Standards of Reporting Trials (CONSORT). This trial has been approved by the Institutional Review Board of Guang'anmen Hospital, China Academy of Chinese Medical Sciences (ethical approval number: 2021-039-KY-01), and it has been registered at ClinicalTrials.gov on July 18, 2021 (Identifier: NCT04981171). Each participant will receive 4-week treatment and will be followed up for 6 months.

Eligibility Criteria

Inclusion Criteria

Participants conforming to the following criteria are included:

(1) Neck pain accompanied with headaches or movement coordination impairments according to the Orthopedic Section of the American Physical Therapy Association (APTA) (7);
(2) Aged 18–65 years;
(3) History of neck pain for at least 3 months;
(4) Scoring at least 4 on Numerical Rating Scale for Neck Pain (NRS-NP) assessing the average neck pain intensity over the last 7 days (12, 20–23).

Exclusion Criteria

Participants who meet any of the following criteria are excluded:

(1) Neck pain with mobility deficits or radiating neck pain according to the Orthopedic Section, APTA (7);
(2) Neck pain secondary to specific diseases, such as tumor, immune disease, endocrine and metabolic disorders, neurological abnormalities, cervical vertebra fracture, and cervical dislocation;
(3) Acute or radiating neck pain or pain accompanied with upper limb symptoms;
(4) Neck pain with sensory or motor disturbance;
(5) Prior cervical spine surgery or congenital abnormalities;
(6) Experiencing medical dispute litigation;
(7) With the experience of acupuncture in the past 30 days;
(8) In the administration of analgesic, muscle relaxant, hormones, or having greater pain in other regions of the body;
(9) Allergic to metal or the adhesive tape, implantation of cardiac pacemaker, or ulceration of skin at selected acupoints;
(10) Disable to communicate or critically ill;
(11) Drug or alcohol dependent;
(12) Currently or planning to be pregnant.

Recruitment

A total of 180 participants with chronic neck pain will be recruited in Guang'anmen Hospital *via* posters, WeChat, websites, and bulletin boards in hospital and communities. Diagnosis will be made by an orthopedist. Eligible participants will be officially enrolled only after signing a written informed consent, and all participants have the right to withdraw their consent later at any time.

Randomization and Allocation

Eligible participants will be randomly assigned in a 1:1 ratio to either the ETN group or the sham ETN group. The

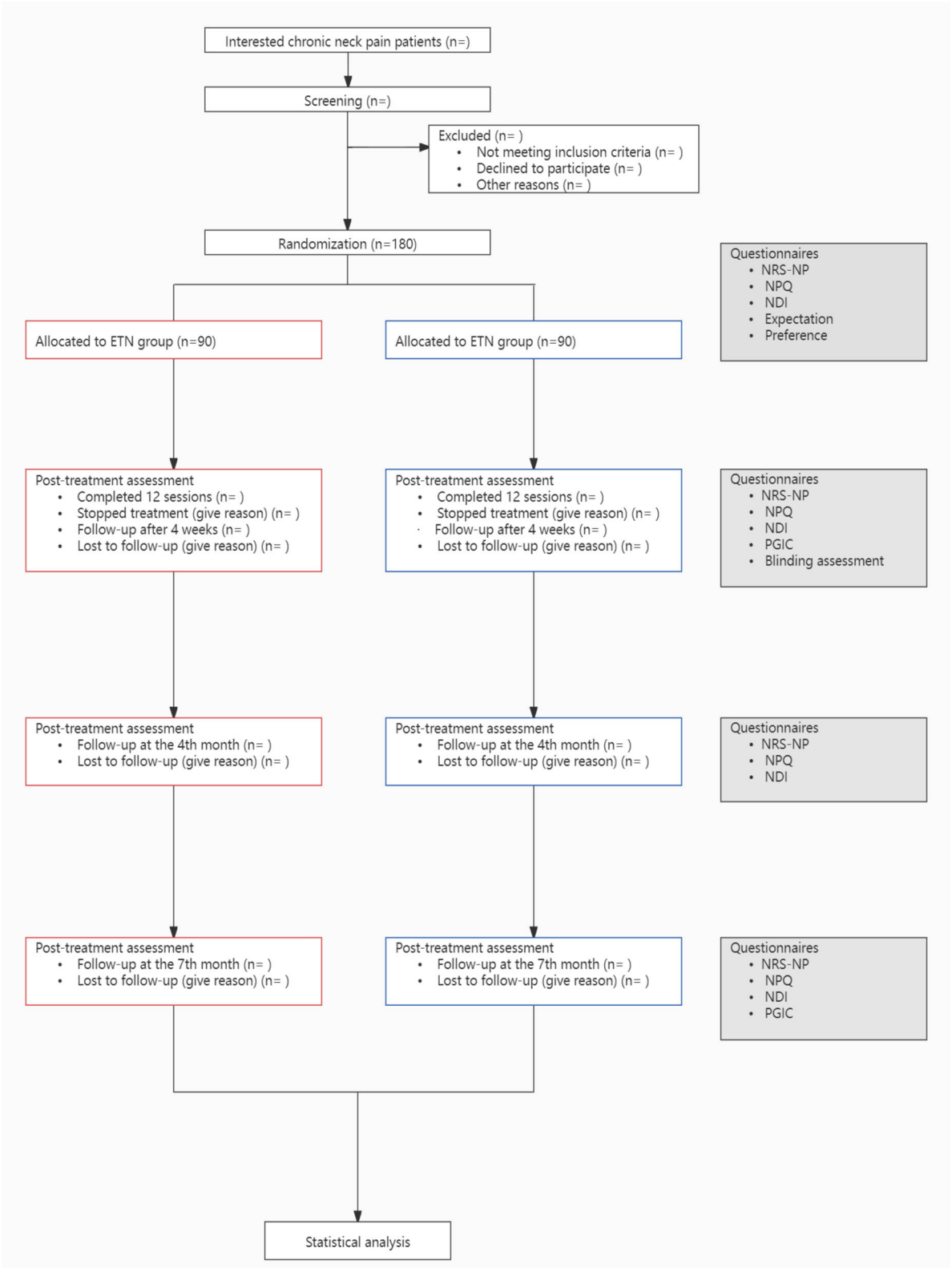

FIGURE 1 | Study flowchart. ETN, electro-thumbtack needle; NRS-NP, numerical rating scale for neck pain; NPQ, the northwick park neck pain questionnaire; NDI, neck disability index; PGIC, patient global impression of change.

TABLE 1 | SPIRIT 2013 checklist: recommended items to address in a clinical trial protocol and related documents*.

Section/item	Item no	Description	Yes/No
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and if applicable, trial acronym	Yes
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	Yes
	2b	All items from the World Health Organization Trial Registration Data Set	Yes
Protocol version	3	Date and version identifier	Yes
Funding	4	Sources and types of financial, material, and other support	Yes
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Yes
	5b	Name and contact information for the trial sponsor	Yes
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	Yes
	5d	Composition, roles, and responsibilities of the coordinating center, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	Yes
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	Yes
	6b	Explanation for choice of comparators	Yes
Objectives	7	Specific objectives or hypotheses	Yes
Trial design	8	Description of trial design including type of trial (e.g., parallel group, crossover, factorial, single group), allocation ratio, and framework (e.g., superiority, equivalence, non-inferiority, exploratory)	Yes
Methods: participants, interventions, and outcomes			
Study setting	9	Description of study settings (e.g., community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	Yes
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centers and individuals who will perform the interventions (e.g., surgeons, psychotherapists)	Yes
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Yes
Interventions: modifications	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (e.g., drug dose change in response to harms, participant request, or improving/worsening disease)	Yes
Interventions: adherence	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (e.g., drug tablet return; laboratory tests)	Yes
Interventions: concomitant care	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Yes
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (e.g., systolic blood pressure), analysis metric (e.g., change from baseline, final value, time to event), method of aggregation (e.g., median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	Yes
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see **Figure 1**)	Yes
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	Yes
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	Yes
Methods: assignment of interventions (for controlled trials)			
Allocation: Sequence generation	16a	Method of generating the allocation sequence (e.g., computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (e.g., blocking) should be provided in a separate document that is unavailable to those who enroll participants or assign interventions	Yes
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (e.g., central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	Yes
Implementation	16c	Who will generate the allocation sequence, who will enroll participants, and who will assign participants to interventions	Yes
Blinding (masking)	17a	Who will be blinded after assignment to interventions (e.g., trial participants, care providers, outcome assessors, data analysts), and how	Yes

(Continued)

TABLE 1 | Continued

Section/item	Item no	Description	Yes/No
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	Yes
Methods: data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (e.g., duplicate measurements, training of assessors) and a description of study instruments (e.g., questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	Yes
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Yes
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (e.g., double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	Yes
Statistical methods	20a	Statistical methods for analyzing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	Yes
	20b	Methods for any additional analyses (e.g., subgroup and adjusted analyses)	No
	20c	Definition of analysis population relating to protocol non-adherence (e.g., as randomized analysis), and any statistical methods to handle missing data (e.g., multiple imputation)	Yes
Methods: monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	Yes
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	Yes
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	Yes
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Yes
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Yes
Protocol amendments	25	Plans for communicating important protocol modifications (e.g., changes to eligibility criteria, outcomes, analyses) to relevant parties (e.g., investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	Yes
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorized surrogates, and how (see Item 32)	Yes
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	No
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Yes
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	Yes
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	Yes
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Yes
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (e.g., *via* publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	Yes
	31b	Authorship eligibility guidelines and any intended use of professional writers	Yes
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	No
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorized surrogates	Yes
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	No

**It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.*

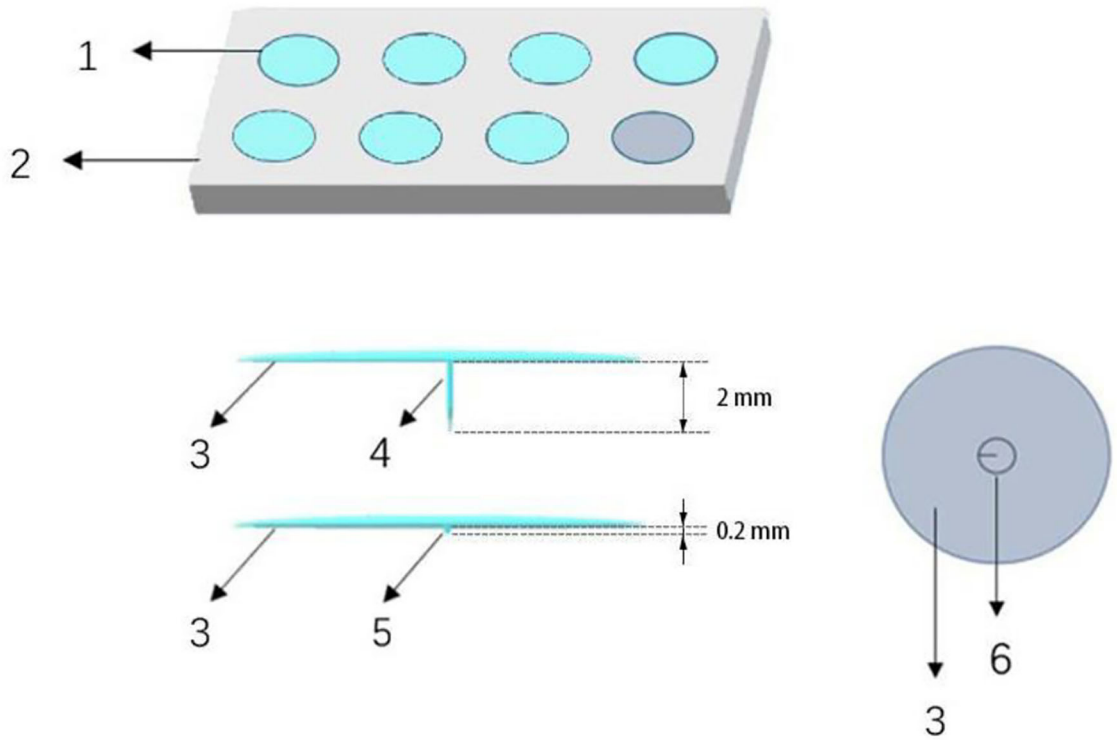

FIGURE 2 | Electro-thumbtack needle and sham thumbtack needle. (1) Thumbtack needle or sham needle, (2) plastic plate, (3) adhesive tape, (4) needle, (5) blunt tip, (6) circular handle.

randomization sequence will be generated using a fixed block size by an independent investigator through the SAS 9.4 (SAS Institute, Cary, NC, USA). The randomization number will be sealed in opaque and sequentially labeled envelopes, with the name of the investigator and the date signed over the seals. The envelop will be opened sequentially after the enrollment of participant, and the group allocation will be only informed to the acupuncturist before treatment.

Blinding

In this trial, the participants, outcome assessors, and the statisticians will all be blinded. The sham ETN was designed to mimic true ETN: the sham thumbtack needle is 0.2 mm long with a blunt tip (instead of a sharp tip), and the electric stimulation is minimal and transient. They will produce a similar sensation of tingling as true ETN, which makes it hard for participants to distinguish. To avoid conversation between groups, participants will be treated separately on alternate days by appointments. After either session in the last week of treatment, blinding assessment will be completed by all participants. They will be told before group allocation: you may be randomly allocated to either the ETN group or the sham ETN group; the electric stimulation is weak in both groups, and you may even be incapable to sense it during the process out of body adaption. Participants will be asked "Which therapy do you think was provided to you?" The available answers include "ETN therapy," "sham ETN therapy," or "unclear."

Interventions

ETN Group

Thumbtack needle is in the shape of a thumbtack. It has a short body and a circular handle buried in a piece of round medical adhesive tape (**Figure 2**), which was produced by conductive material. In this trial, thumbtack needles of 0.25 × 2 mm size (Zhenxing, Hangzhou, China) will be used. Acupoints of Dazhui (GV14), bilateral Wangu (GB1 2), bilateral Jiaji at C4 and C6 levels, two Ashi points, and bilateral Houxi (SI3) will be selected in accordance with the theory of traditional Chinese medicine and the consensus of experts.

After sterilizing the skin around the acupoints, the acupuncturist will hold the handle of the needle between the thumb and forefinger, vertically insert the needle into the skin instantly, and adhere medical adhesive tape to skin. After that, the acupuncturist will setup the gel electrodes and the portable stimulation devices (Zhenxing, Hangzhou, China) (**Figure 3**) to the adhesive tape of all thumbtack needles, switch the mode to low-frequency and discontinuous wave, and adjust the current intensity from level 1 to an appropriate level, which the participant can tolerate. The acupuncturist will gently remove the medical adhesive tape and needles in 30 min.

Participants will receive three 30-min treatment sessions per week (ideally every other day) for 4 consecutive weeks after baseline assessment.

FIGURE 3 | Electric stimulation device and gel electrode.

Sham ETN Group

We will use sham thumbtack needle specially designed to mimic the true needling. The sham thumbtack needle is 0.2 mm in length and has a blunt tip (rather than sharp) at the bottom. Other than these differences, the conductive surface and other structure of sham thumbtack needle are totally the same as true needle (**Figure 2**). In the sham ETN group, the blunt tip of needle will stay on the surface and not penetrate the skin, which avoids treatment effect to the largest extent and confuses the participants meanwhile.

Acupoints used in the sham ETN group are the same as those in the ETN group. Acupuncturist will first sterilize the areas of acupoints and then apply the sham needles on the surface of the skin. After assembling the same gel electrodes and portable stimulation devices as in the ETN group (**Figures 3**, **4**), the acupuncturist will adjust the current intensity to level 1 (i.e., the minimum level). The minimal stimulus will last for only 30 s, after which the device will be turned down to cutoff the electric current. The duration of each session will also be 30 min. The frequency of treatment will be all the same as that in the ETN group.

Neck Exercises

Standardized neck exercise of stretching and strengthening can help participants to relax the neck and acknowledge the appropriate posture. The exercise program consists of head retraction, neck extension, neck flexion, and rotation (**Figure 5**) (24). Each stretch must reach the maximum angle as possible and be hold for 5–10 s with a 10-s interval (15). When a participant is undergoing severe neck pain, he/she could conduct the exercise in clinostatism (24).

All participants will receive standardized neck exercise instruction at their first treatment session. The neck exercise will be performed at home later. A handbook with detailed illustrations to the exercise will be distributed to participants for guidance. Participants will be asked to perform the standardized exercise one time every day (20) (preferably in the morning)

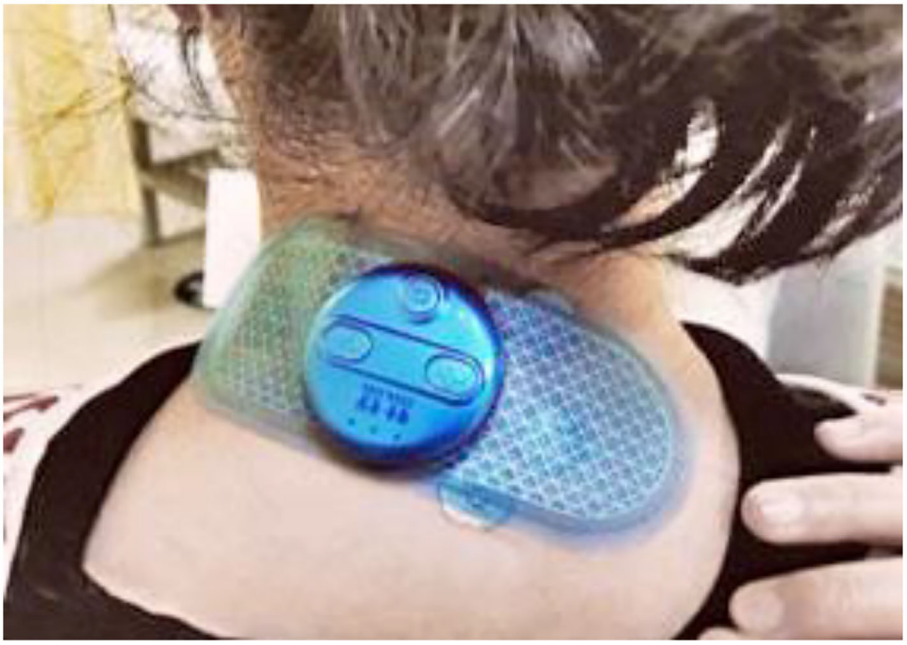

FIGURE 4 | ETN therapy or sham ETN therapy.

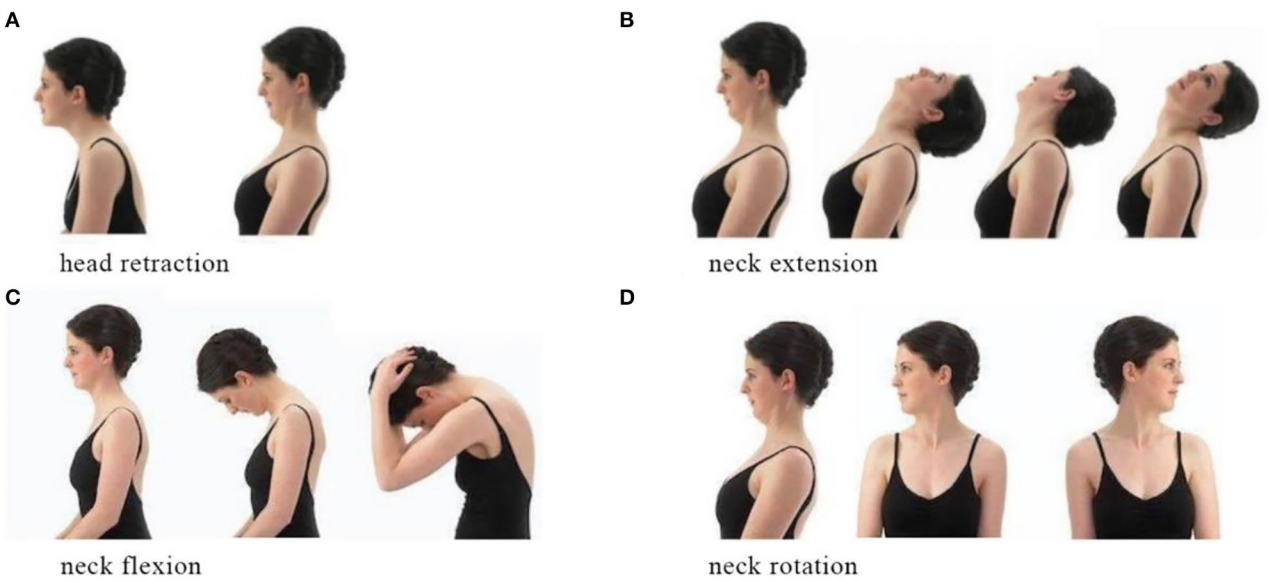

FIGURE 5 | Neck exercise. **(A)** Head retarction. **(B)** Neck extension. **(C)** Neck flexion. **(D)** Neck rotation.

throughout the 4-week treatment. An exercise diary will also be distributed for supervision.

Concomitant Treatment

Any other therapy will be discouraged during this trial. Acetaminophen (sustained release type, 500 mg/T) will be provided as a rescue medication with the largest dose of 4 tablets per day for no more than 3 days in total in the condition of unbearable pain. Rescue medication or any other unexpected intervention will be recorded in detail. Proportion of participants and the total days taking rescue medication will be calculated and properly analyzed.

Outcome Measures

Primary Outcome and Measurement

The primary outcome of this trial will be the proportion of participants with at least 50% decrease from baseline in the score of NRS-NP at the 4th week (25). The NRS-NP is a

valid and reliable 11-point rating scale to assess the average intensity of neck pain during the last 3 days (26, 27). It is sensitive to small changes with a lower failure rate and can be delivered either graphically or verbally (26–28). The score of NRS-NP ranges from 0 (i.e., no pain at all) to 10 (i.e., the worst pain imaginable). Participants will be asked to circle out 1 of the 11 numbers to describe their pain. The scores of NRS-NP can be categorized into four degrees: 0 (i.e., no pain), 1–3 (i.e., mild), 4–6 (i.e., moderate), and 7–10 (i.e., severe). The reduction of 50% from baseline is regarded as golden standard of the NRS-NP to interpret significant clinically improvement (15, 28, 29).

Secondary Outcomes

Secondary outcomes include the proportion of participants with an at least 50% decrease from baseline in NRS-NP score at the 16th and 28th weeks; the changes from baseline in NRS-NP score at the 4th, 16th, and 28th weeks; and the proportion of participants with an at least 30% decrease from baseline in NRS-NP at the 4th, 16th, and 28th weeks. Other secondary outcomes include:

(1) Changes in the Northwick Park Neck Pain Questionnaire (NPQ) percentage score from the baseline at the 4th, 16th, and 28th weeks.
(2) The proportion of participants with an at least 25% decrease in NPQ percentage score together with a "better" or "much better" at the 4th, 16th, and 28th weeks.

The NPQ is a validated questionnaire to assess the overall symptoms of neck pain (30). It contains 9 questions, the score of each ranging from 0 to 4, on the aspects of intensity, duration, numbness, sleep disturbance, and disability in carrying, watching television, working, social life, and driving. The total score of the 9 questions will be converted to NPQ percentage score (divided by 36 for people who drive and by 32 for those who do not), which ranges from 0 to 100%, and with higher scores indicating worse condition. A 25% decrease in NPQ percentage score and a "better" or "much better" for the 5-scale global effectiveness rating are both needed (31) to reach Minimal Clinically Important Difference (MCID).

(3) Changes from the baseline in Neck Disability Index (NDI) score at the 4th, 16th, and 28th weeks.
(4) The proportion of participants with an at least 10-point decrease from the baseline in NDI score at the 4th, 16th, and 28th weeks.

The NDI is a frequently used instrument to evaluate the self-reporting functional status of patients with neck pain. It consists of 10 items concerning pain, headache, concentration, and daily obligatory activities. Each section is scored with an ordinal scale from 0 to 5, with a maximum score of 50 points (32, 33). Higher scores indicate greater functional limitation. The NDI has been translated into the simplified-Chinese version, which has been shown to have reproducibility, reliability, and validity (34). A 10-point decrease is required to achieve clinically important meaning (27, 35–37).

(5) The proportion of participants reported "much improved" or "very much improved" on Patient Global Impression of Change (PGIC) at the 4th week.

PGIC is a seven-point categorical scale to assess the participant's subjective satisfaction of the treatment (38). It is considered the most reliable instrument for trials concerning chronic pain (38–40). Participants are supposed to report their overall conditions in comparison with before the treatment using one of the following options: "very much worse," "much worse," "minimally worse," "no change," "minimally improved," "much improved," and "very much improved." Those reported "much improved" or "very much improved" are considered responders of the intervention. It reflects not only the benefits of treatment but also the importance of the benefits for participants themselves (41).

(6) Expectation and belief.

Two questions will be elicited to assess the expectation of and belief in ETN, respectively. For expectation, participants will be asked "How do you think your neck pain will be in 4 weeks?" with the optional of "worse," "unchanged," "no idea," "better," and "much better." For belief in ETN therapy, they will be asked "Do you think ETN therapy will be effective in treating chronic neck pain?" with the option of "not effective," "little effective," "not sure," "effective," and "very effective." These questions will be elicited at the baseline.

Safety Assessment

Acupuncture-associated side effects such as bruising, dizziness, nausea, hematomas, infection, palpitations, or severe pricking pain [visual analog score (VAS) $\geq$ 4], and all other adverse events (AEs) irrelevant to acupuncture, will be carefully observed and recorded. Serious AEs (SAEs) will be reported to the Medical Ethics Committee of Guang'anmen Hospital within 24 h. After each session, the acupuncturist and outcome assessors will document adverse events and side effects if happens.

Data Management and Quality Control

The outcome assessor will fill the initial data in case report form and then, input the data into an excel template for data collecting. Two inspectors will check and make sure that all initial data have been correctly input. Any deviation from protocol will be reported to ZL, who will make the final decision if we have to change the protocol or terminate the trial.

Both ETN therapy and sham ETN therapy are safe for participants recruited in our trial; therefore, no intervention modification is permitted, and no unblinding during the trial is permissible. Group allocation will be revealed after the completion of statistical analysis. Participants in the sham ETN group will receive 12 supplementary sessions of ETN therapy if they want. The follow-ups can be conveniently finished by filling in a questionnaire in the clinic, over telephone or through WeChat. This approach could probably improve adherence and make it possible for those who have to discontinue the treatment to complete follow-ups.

Sample Size

The sample size calculation was based on the primary outcome of the proportion of participants with an at least 50% decrease from the baseline in average neck pain measured by NRS-NP at the 4th week. According to the previous literature, the response rate of TENS plus neck exercise for 4 weeks was reported to be 50% (15); therefore, we presume a 60% response rate of ETN plus neck exercise. For sham ETN plus neck exercise, we presume the response rate to be 38%. Accordingly, a two-tailed testing with an alpha of 0.05, a desired power set at 0.8, and an allowing dropout rate of 20% resulted in 90 participants needed in each group.

Statistical Analysis

Data calculation will be performed using the SPSS software Ver.20.0 under the ITT principle. Baseline data before intervention will be analyzed to see if the two groups are comparable. The continuous data will be represented as mean ± SD deviation or median [interquartile range (IQR)]. Proportions of participants having clinically meaningful improvement will be analyzed using chi-square test or Fisher's exact test. Changes in the outcome measures from the baseline will be examined by using analysis of covariance for normally distributed data to exclude the possible impact on the effect by covariates of gender and work habits. Repeated-measures analysis of variance will be used if the data are not normally distributed. The Cochran-Mantel-Haenszel test will be used to analyze categorical data, which will be represented by percentages and case. We will use linear regression to discuss the association of participants' expectations and the treatment. Missing research data will be imputed by multiple imputations. For all statistical calculation, the significance level will be two-sided and set at 0.05. A $p < 0.05$ will be considered to indicate statistical significance.

DISCUSSION

The objective of this study is to evaluate the efficacy and safety of ETN therapy for non-specific chronic neck pain. Demographic characteristics will be collected including age, duration, job, psychosocial or physical risk factors, level of exercise, history of injury, and history of autoimmune disease. The outcomes are designed in accordance with the Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials (IMMPACT) recommendations for core outcomes of chronic pain (41). Both the reduction of 30 and 50% on NRS are applied as outcome measures in this trial. Although the reduction of 30% from baseline in NRS is consistently acknowledged as MCID in current studies (15, 23, 25, 27, 28), a 50% reduction is widely used in clinical trials as a rigorous outcome measurement interpreting more significant improvement in symptoms (15, 28, 29). Additionally, multiple outcomes will be evaluated, as various as pain intensity, disability, quality of life, and the degree of subjective satisfaction.

The effectiveness of both manual acupuncture and TENS has been identified by previous studies (15, 42) in reducing chronic neck pain. Since ETN treatment has been an integration of thumbtack needling and electric stimulation, the treatment effect will probably be stronger and comprehensive. Notably, it is easy to administrate thumbtack needles on acupoints, even by patients themselves at home.

In this trial, blunt-tip thumbtack needle with no penetration and transient minimal electric stimulation is designed as sham control. It can produce acupuncture-like sensation of tingling or pain by pressing the blunt tip on acupoints without skin penetrating, similar to the validated placebo needle (43, 44). To maintain the success of blindness, participants from different groups will be avoided to contact with each other. The outcome assessor and the statistician will also be concealed to group allocation. These approaches are constructed to reduce possible biases attached to the results.

Limitations of this study are as follows. Group allocation will be not blinded to the acupuncturist, whose attitude for or against an intervention might be inevitably transferred to participants. Besides, this study does not implement a waitlist control in consideration of ethics principles, in which condition we cannot exclude the influence of spontaneous remission of disease.

This prospective trial will evaluate the efficacy and safety of ETN for chronic neck pain and provide evidence for clinical health recommendations.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Research Ethical Committee of Guang'anmen Hospital, China Academy of Chinese Medical Sciences. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ZL, HS, and XW designed this trial and drafted the manuscript over discussion. The study protocol was revised by YY, LZ, YC, and SG. HS and XW contributed to the ethical application and the implementation of the trial. All authors approved the publication of this final manuscript.

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REFERENCES

1. Blanpied PR, Gross AR, Elliott JM, Devaney LL, Clewley D, Walton DM, et al. Neck pain guidelines: revision 2017: using the evidence to guide physical therapist practice. *J Orthop Sports Phys Ther*. (2017) 47:511–2. doi: 10.2519/jospt.2017.0507
2. Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M, et al. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the global burden of disease study 2010. *Lancet.* (2012) 380:2163–96. doi: 10.1016/S0140-6736(12)61729-2
3. Fejer R, Kyvik KO, Hartvigsen J. The prevalence of neck pain in the world population: a systematic critical review of the literature. *Eur Spine J.* (2006) 15:834–48. doi: 10.1007/s00586-004-0864-4
4. Hoy DG, Protani M, De R, Buchbinder R. The epidemiology of neck pain. *Best Pract Res Clin Rheumatol.* (2010) 24:783–92. doi: 10.1016/j.berh.2011.01.019
5. Cohen SP, Hooten WM. Advances in the diagnosis and management of neck pain. *BMJ.* (2017) 358:j3221. doi: 10.1136/bmj.j3221
6. Côté P, Wong JJ, Sutton D, Shearer HM, Mior S, Randhawa K, et al. Management of neck pain and associated disorders: a clinical practice guideline from the Ontario protocol for traffic injury management (OPTIMa) collaboration. *Eur Spine J.* (2016) 25:2000–22. doi: 10.1007/s00586-016-4467-7
7. Childs JD, Cleland JA, Elliott JM, Teyhen DS, Wainner RS, Whitman JM, et al. Neck pain: Clinical practice guidelines linked to the international classification of functioning, disability, and health from the orthopedic section of the American physical therapy association. *J Orthop Sports Phys Ther.* (2008) 38:A1–A34. doi: 10.2519/jospt.2008.0303
8. Hurwitz EL, Carragee EJ, van der Velde G, Carroll LJ, Nordin M, Guzman J, et al. Treatment of neck pain: noninvasive interventions: results of the bone and joint decade 2000-2010 task force on neck pain and its associated disorders. *Spine.* (2008) 33(4 Suppl):S123–52. doi: 10.1097/BRS.0b013e3181644b1d
9. Yuan Q-L, Wang P, Liu L, Sun F, Cai Y-S, Wu W-T, et al. Acupuncture for musculoskeletal pain: a meta-analysis and meta-regression of sham-controlled randomized clinical trials. *Sci Rep.* (2016) 6:30675. doi: 10.1038/srep30675
10. He D, Bo Veiersted K, Høstmark AT, Ingulf Medbø J. Effect of acupuncture treatment on chronic neck and shoulder pain in sedentary female workers: a 6-month and 3-year follow-up study. *Pain.* (2004) 109:299–307. doi: 10.1016/j.pain.2004.01.018
11. Vas J, Perea-Milla E, Méndez C, Sánchez Navarro C, León Rubio JM, Brioso M, et al. Efficacy and safety of acupuncture for chronic uncomplicated neck pain: a randomised controlled study. *Pain.* (2006) 126:245–55. doi: 10.1016/j.pain.2006.07.002
12. Zhang R, Lao L, Ren K, Berman BM. Mechanisms of acupuncture-electroacupuncture on persistent pain. *Anesthesiology.* (2014) 120:482–503. doi: 10.1097/ALN.0000000000000101
13. Seo SY, Lee K-B, Shin J-S, Lee J, Kim M-R, Ha I-H, et al. Effectiveness of acupuncture and electroacupuncture for chronic neck pain: a systematic review and meta-analysis. *Am J Chin Med.* (2017) 45:1573–95. doi: 10.1142/S0192415X17500859
14. Martimbianco ALC, Porfírio GJ, Pacheco RL, Torloni MR, Riera R. Transcutaneous electrical nerve stimulation (TENS) for chronic neck pain. *Cochrane Database Syst Rev.* (2019) 12:CD011927. doi: 10.1002/14651858.CD011927.pub2
15. Chan DKC, Johnson MI, Sun KO, Doble SJ, Jenkins S. Electrical acustimulation of the wrist for chronic neck pain: a randomized, sham-controlled trial using a wrist-ankle acustimulation device. *Clin J Pain.* (2009) 25:320–6. doi: 10.1097/AJP.0b013e318192ce39
16. Ardiç F, Sarhus M, Topuz O. Comparison of two different techniques of electrotherapy on myofascial pain. *J Back Musculoskelet Rehabil.* (2002) 16:11–6. doi: 10.3233/BMR-2002-16103
17. Maayah M, Al-Jarrah M. Evaluation of transcutaneous electrical nerve stimulation as a treatment of neck pain due to musculoskeletal disorders. *J Clin Med Res.* (2010) 2:127–36. doi: 10.4021/jocmr2010.06.370e
18. Chiu TTW, Hui-Chan CWY, Chein G. A randomized clinical trial of TENS and exercise for patients with chronic neck pain. *Clin Rehabil.* (2005) 19:850–60. doi: 10.1191/0269215505cr920oa
19. Chan A-W, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, KrleŽa-Jerić K, et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. *Ann Intern Med.* (2013) 158:200–7. doi: 10.7326/0003-4819-158-3-201302050-00583
20. Bronfort G, Evans R, Anderson AV, Svendsen KH, Bracha Y, Grimm RH. Spinal manipulation, medication, or home exercise with advice for acute and subacute neck pain: a randomized trial. *Ann Intern Med.* (2012) 156 (1 Pt. 1):1–10. doi: 10.7326/0003-4819-156-1-201201030-00002
21. White P, Lewith G, Prescott P, Conway J. Acupuncture versus placebo for the treatment of chronic mechanical neck pain: a randomized, controlled trial. *Ann Intern Med.* (2004) 141:911–9. doi: 10.7326/0003-4819-141-12-200412210-00007
22. Lee S, Nam D, Leem J, Han G, Lee S, Lee J. Efficacy and safety of myofascial-meridian release acupuncture (MMRA) for chronic neck pain: a study protocol for randomized, patient- and assessor-blinded, sham controlled trial. *BMC Complement Altern Med.* (2016) 16:45. doi: 10.1186/s12906-016-1027-y
23. Farrar JT, Troxel AB, Stott C, Duncombe P, Jensen MP. Validity, reliability, and clinical importance of change in a 0-10 numeric rating scale measure of spasticity: a post hoc analysis of a randomized, double-blind, placebo-controlled trial. *Clin Ther.* (2008) 30:974–85. doi: 10.1016/j.clinthera.2008.05.011
24. McKenzie R. *Treat Your Own Neck.* 3rd ed. Raumati Beach: Spinal Publications New Zealand Limited (2010). p. 50–64.
25. Malfliet A, Kregel J, Coppieters I, Pauw R de, Meeus M, Roussel N, et al. Effect of pain neuroscience education combined with cognition-targeted motor control training on chronic spinal pain: a randomized clinical trial. *JAMA Neurol.* (2018) 75:808–17. doi: 10.1001/jamaneurol.2018.0492
26. Williamson A, Hoggart B. Pain: a review of three commonly used pain rating scales. *J Clin Nurs.* (2005) 14:798–804. doi: 10.1111/j.1365-2702.2005.01121.x
27. Pool JJM, Ostelo RWJG, Hoving JL, Bouter LM, Vet HCW. Minimal clinically important change of the Neck Disability Index and the Numerical Rating Scale for patients with neck pain. *Spine.* (2007) 32:3047–51. doi: 10.1097/BRS.0b013e31815cf75b
28. Farrar JT, Young JP, LaMoreaux L, Werth JL, Poole MR. Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale. *Pain.* (2001) 94:149–58. doi: 10.1016/S0304-3959(01)00349-9
29. Moore A, Moore O, McQuay H, Gavaghan D. Deriving dichotomous outcome measures from continuous data in randomised controlled trials of analgesics: use of pain intensity and visual analogue scales. *Pain.* (1997) 69:311–5. doi: 10.1016/S0304-3959(96)03306-4
30. Leak AM, Cooper J, Dyer S, Williams KA, Turner-Stokes L, Frank AO. The northwick park neck pain questionnaire, devised to measure neck pain and disability. *Br J Rheumatol.* (1994) 33:469–74. doi: 10.1093/rheumatology/33.5.469
31. Sim J, Jordan K, Lewis M, Hill J, Hay EM, Dziedzic K. Sensitivity to change and internal consistency of the northwick park neck pain questionnaire and derivation of a minimal clinically important difference. *Clin J Pain.* (2006) 22:820–6. doi: 10.1097/01.ajp.0000210937.58439.39
32. Vernon H. The neck disability index. *J Musculoskel Pain.* (1996) 4:95–104. doi: 10.1300/J094v04n04_09
33. Vernon H. The neck disability index: state-of-the-art, 1991-2008. *J Manipulative Physiol Ther.* (2008) 31:491–502. doi: 10.1016/j.jmpt.2008.08.006
34. Lim HHR, Tang ZY, Hashim MABM, Yang M, Koh EYL, Koh KH. Cross-cultural adaptation, reliability, validity, and responsiveness of the simplified-chinese version of neck disability index. *Spine.* (2020) 45:541–8. doi: 10.1097/BRS.0000000000003325
35. Cerezo-Téllez E, Torres-Lacomba M, Fuentes-Gallardo I, Perez-Muñoz M, Mayoral-Del-Moral O, Lluch-Girbés E, et al. Effectiveness of dry needling for chronic nonspecific neck pain: a randomized, single-blinded, clinical trial. *Pain.* (2016) 157:1905–17. doi: 10.1097/j.pain.0000000000000591
36. Cleland JA, Fritz JM, Whitman JM, Palmer JA. The reliability and construct validity of the neck disability index and patient specific functional scale in patients with cervical radiculopathy. *Spine.* (2006) 31:598–602. doi: 10.1097/01.brs.0000201241.90914.22

37. Young BA, Walker MJ, Strunce JB, Boyles RE, Whitman JM, Childs JD. Responsiveness of the neck disability index in patients with mechanical neck disorders. *Spine J.* (2009) 9:802–8. doi: 10.1016/j.spinee.2009.06.002
38. Perrot S, Lantéri-Minet M. Patients' global impression of change in the management of peripheral neuropathic pain: clinical relevance and correlations in daily practice. *Eur J Pain.* (2019) 23:1117–28. doi: 10.1002/ejp.1378
39. Salaffi F, Stancati A, Silvestri CA, Ciapetti A, Grassi W. Minimal clinically important changes in chronic musculoskeletal pain intensity measured on a numerical rating scale. *Euro J Pain.* (2004) 8:283–91. doi: 10.1016/j.ejpain.2003.09.004
40. Geisser ME, Clauw DJ, Strand V, Gendreau MR, Palmer R, Williams DA. Contributions of change in clinical status parameters to patient global impression of change (PGIC) scores among persons with fibromyalgia treated with milnacipran. *Pain.* (2010) 149:373–8. doi: 10.1016/j.pain.2010.02.043
41. Turk DC, Dworkin RH, Allen RR, Bellamy N, Brandenburg N, Carr DB, et al. Core outcome domains for chronic pain clinical trials: IMMPACT recommendations. *Pain.* (2003) 106:337–45. doi: 10.1016/j.pain.2003.08.001
42. MacPherson H, Tilbrook H, Richmond S, Woodman J, Ballard K, Atkin K, et al. Alexander technique lessons or acupuncture sessions for persons with chronic neck pain: a randomized trial. *Ann Intern Med.* (2015) 163:653–62. doi: 10.7326/M15-0667
43. Qin Z, Zang Z, Wu J, Zhou J, Liu Z. Efficacy of acupuncture for chronic prostatitis/chronic pelvic pain syndromes: study protocol for a randomized, sham acupuncture-controlled trial. *BMC Complement Altern Med.* (2016) 16:440. doi: 10.1186/s12906-016-1428-y
44. Liu B, Xu H, Ma R, Mo Q, Yan S, Liu Z. Effect of blinding with a new pragmatic placebo needle: a randomized controlled crossover study. *Medicine.* (2014) 93:e200. doi: 10.1097/MD.0000000000000200

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Acupuncture Analgesia in Patients With Traumatic Rib Fractures: A Randomized-Controlled Trial

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Pain management for traumatic rib fracture is important to prevent complications and reduce associated comorbidities. This trial investigated the analgesic efficacy of acupuncture on traumatic rib fracture. Patients with traumatic rib fracture were randomly assigned to traditional acupuncture (TA), laser acupuncture (LA) or sham laser acupuncture (SLA) groups in a 1:1:1 ratio. The intervention was performed on days 1 to 3 after treatment allocation. The acupoints included bilateral LI4 (Hegu), SJ6 (Zhigou), ST36 (Zusanli) and GB34 (Yanglingquan). The primary outcome was Numeric Rating Scale (NRS) scores for pain after the intervention. Secondary outcomes included sustained maximal inspiration (SMI) lung volume, stress responses, the use of analgesics, and associated complications. Data were analyzed via one-way analysis of variance (ANOVA) with Scheffé's *post hoc* testing or chi-squared testing. Of the 120 study participants, 109 completed all interventions and measurements. The primary outcomes, which indicated average pain intensity levels and pain while deep breathing, were both significantly lower in the TA and LA groups than in the SLA group after 2 treatments. No between-group differences were observed in SMI lung volume, stress response, analgesics use or associated complications. These findings suggest that TA and LA are safe and effective analgesic modalities for pain management for traumatic rib fracture.

Clinical Trial Registration: [ClinicalTrials.gov], identifier [NCT03822273].

Keywords: traumatic rib fracture, acupuncture, laser acupuncture, low level laser therapy, acupuncture analgesia

INTRODUCTION

Blunt thoracic trauma can damage vital structures in the chest wall and thereby cause rib fracture, hemothorax, pneumothorax, pulmonary contusion, lung laceration, and blunt cardiac injury (1). Among all of them, rib fracture is the most common injury. In United States hospitals, an estimated 150,000–300,000 patients per year seek treatment for this injury, and mortality rates of up to 10% are reported (2). The chest wall pain induced by thoracic trauma can impair patients' ability

to clear airway secretions by coughing and deep breathing, increasing the risk of pneumonia, and respiratory failure (2). The major goal in the treatment of patients with rib fracture is to reduce pain. Appropriate analgesia and early aggressive care appear to attenuate the development of pulmonary complications. Commonly used oral analgesics such as acetaminophen and non-steroidal anti-inflammatory drugs (NSAIDs) seem to provide limited relief from severe pain (3). For years, narcotic analgesics have remained the main therapy for pain management in patients with traumatic rib fracture (4). However, narcotic regimens are often associated with multiple adverse effects, such as respiratory depression, confusion, dizziness, nausea, vomiting, and constipation. Opioid-induced respiratory depression in patients with traumatic rib fracture is lethal, especially in the presence of pneumothorax and hemothorax. Regional analgesic techniques such as epidural analgesia, thoracic paravertebral block, and intercostal nerve block also have been used for pain management, but there are many limiting factors, such as infection at the site of injection, allergy to local anesthetic drug, coagulopathy, and bleeding disorders (5). In addition, epidural analgesia is contraindicated in patients with hypotension and may increase pulmonary complications (5). Thoracic paravertebral block and intercostal nerve block entail the risks of pneumothorax and vascular puncture (5). Surgery with internal fixation for rib fracture is another effective option for relieving pain, particularly in patients with severe pain and flail chest (6). However, surgical stabilization of rib fracture is costly and may not be cost-effective for patients without flail chest (7). Currently, the optimal analgesic modality remains unknown in adults with blunt thoracic trauma including traumatic rib fracture (3). More cost-effective and harmless treatments for pain management in patients with traumatic rib fracture should be discovered.

Acupuncture is an ancient Chinese medical technique that is commonly used for pain relief. Although the mechanisms of acupuncture are not completely understood, clinical trials of acupuncture treatment increasingly suggest that acupuncture is effective for musculoskeletal pain and even cancer pain (8). The American College of Physicians suggests that acupuncture can be selected as initial non-pharmacologic treatment for the treatment of acute or chronic low back pain (9). Currently, few studies have examined acupuncture treatment for traumatic rib fracture. Of these studies, only one was a randomized controlled study; in that study, 58 patients with rib fracture experienced significant pain relief with acupuncture as compared with controls (10).

The purpose of this study was to investigate the analgesic efficacy of acupuncture for traumatic rib fracture. The hypothesis of the study was that acupuncture could alleviate the pain associated with traumatic rib fracture, improve respiration, and prevent pneumonia.

MATERIALS AND METHODS

Ethics Approval

The protocol was registered with ClinicalTrials.gov (Identifier: NCT03822273). The study was approved by the Human Ethics Committee of Chang Gung Medical Foundation Institutional Review Board, IRB No. 201701455A3. This study was conducted in accordance with the principles of the Declaration of Helsinki. Both verbal and written forms of detailed information about the trial were provided before participation by trauma surgery physicians at Kaohsiung Chang Gung Memorial Hospital (KCGMH). All participants voluntarily signed and provided informed consent that had been approved by the ethics committee prior to enrollment. The participants received no financial benefit from the study and were fully aware of their rights to withdraw at any time. Personal information about potential and enrolled participants was collected, shared, and maintained in an independent and secure storage space to protect the participants' confidentiality before, during, and after the trial.

Study Design

This single-center, prospective, randomized controlled clinical trial (RCT) was conducted from January 2018 to February 2020 at KCGMH, a 2,600-bed tertiary medical center with an average of 9,000 emergency department visits per month. Patients with rib fracture hospitalized in the ward of Trauma Surgery were recruited. Patients with rib fracture who wanted to participate in the study were screened for eligibility through a chart review and interview on the first visit. The participants who met the inclusion criteria were randomly assigned to true acupuncture (TA), laser acupuncture (LA), or sham laser acupuncture (SLA) groups in a 1:1:1 ratio. The study design, developed in accordance with the Consolidated Standards of Reporting Trials (CONSORT) 2010, is depicted in **Figure 1**.

Eligibility Criteria

Participants' eligibility to participate in the study was assessed by trauma surgeons. Written informed consents were obtained for all participating patients before randomization. All the patients with traumatic rib fracture were managed from the time of admission by the trauma surgery team, who had no information about the allocation results.

The study inclusion criteria were as follows: (1) aged 20 years or older; (2) presence of one or more, unilateral or bilateral rib fractures diagnosed with chest X-ray; (3) maximal rib pain score of more than 5 on the numerical rating scale (NRS: 0–10) while doing any one of the following three actions: deep breathing, coughing, or turning over; and (4) ability to describe the sites of pain and evaluate the intensity accurately.

The participants with the following conditions were excluded: (1) inability to be examined for the movements of deep breathing, coughing, or turning over in bed; (2) local skin infection or open wound on the acupoints, or limb amputees; (3) cognitive impairment that precluded the accurate clinical assessment of the NRS score; (4) severe multiple trauma or any poorly controlled diseases such as atelectasis, pneumonia, or other infectious diseases, immune system dysfunction, bleeding tendency, psychiatric disorders, and skin problems; (5) communication difficulties; (6) surgical stabilization of new rib fracture before enrollment; (7) unwillingness to provide informed consent; or (8) determination of unsuitability for this study by medical staff. In addition, participants would be dropped

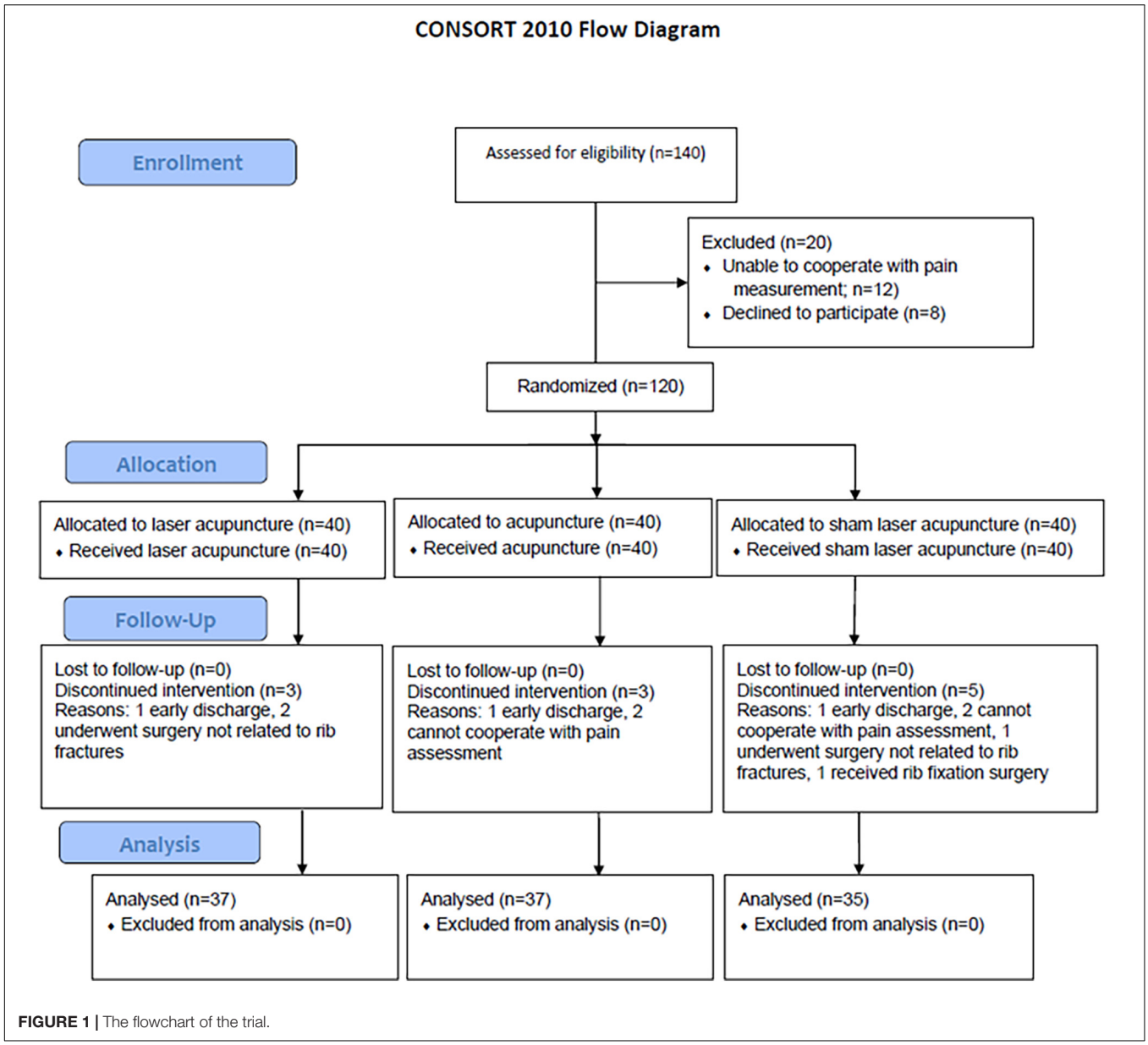

FIGURE 1 | The flowchart of the trial.

from the trial under the following conditions: (1) unstable vital signs and/or need for first aid during study period; (2) surgical stabilization of rib fracture during study period; and (3) voluntary decision by participants to withdraw from the trial at any time.

Sample Size Calculation, Blinding, and Randomization

Based on a previous study (10) on the effectiveness of acupuncture in patients with rib fracture, the mean ± standard deviation of the NRS scores on day 3 after treatment were 1.3 ± 1.4 in the acupuncture group and 2.6 ± 0.5 in the control group. Anticipating a power of 80% ($1 - \beta = 0.8$), statistical significance of 95% ($\alpha = 0.05$), and a dropout rate of 20%, a total of 50 participants were required in each group, according to G*power analysis.

All the participants were told that they would receive one kind of acupuncture treatment in addition to the conventional treatment. The participants were not notified about which group they were allocated to or which kind of acupuncture treatment would be applied as a control before randomization. Enrolled patients were randomly assigned in a ratio of 1:1:1to the TA, LA, and SLA groups for three parallel treatments. Randomization was conducted using block randomization with a block size of 3 for the three groups. Randomization was executed by an independent researcher, who was not involved in the inclusion or exclusion process, treatment, or assessment procedures. Participants randomized to the LA or SLA groups were blinded to laser acupuncture. The outcome assessor was blinded to the group assignments and was well trained in the use of the pain scale and physical tests. To avoid unblinding of the

assessment of outcomes, the participants were instructed not to discuss any aspect of their treatment with the assessor.

Intervention

Regardless of treatment group, the acupuncture regimen included stimulation of the same acupoints by a certified traditional Chinese medicine (TCM) physician, who was also the only person in this trial aware of the group assignment. All participants received conventional pain management offered by trauma surgeons. Drugs used for underlying chronic diseases were not restricted.

Traditional Acupuncture Group

Participants in the TA group were treated with filiform needles once daily for three consecutive days after the day of enrollment. Eight needles were inserted at the following acupoints: the bilateral LI4 (Hegu), SJ6 (Zhigou), ST36 (Zusanli), and GB34 (Yanglingquan) (**Figure 2**), which were located according to the WHO Standardized Acupuncture Point Location guidelines (11). Disposable sterilized stainless-steel needles were inserted to a depth of 15–35 mm. All needles were rotated manually at least once at each session to elicit the needle sensation (*de-qi*). The needle retention time was 15 min.

Laser Acupuncture Group

Participants allocated to the LA group received laser acupuncture treatment at the same acupoints used in the TA group. The laser acupuncture therapy was performed with a gallium aluminum arsenide LaserPen [maximal power, 150 mW; wavelength, 810 nm; area of probe, 0.03 cm^2; power density, 5 W/cm^2; pulsed wave; and Bahr frequencies (B1: 599.5 Hz, B2: 1199 Hz, B3: 2398 Hz, B4: 4776 Hz, B5: 9552 Hz, B6: 19,104 Hz, and B7: 38,208 Hz); RJ-Laser, Reimers & Janssen GmbH, Waldkirch, Germany]. The laser was applied to each point for 5 s and delivered 0.375 J of energy at each of the acupoints.

Sham Laser Acupuncture Group

Participants in the SLA group underwent sham laser acupuncture treatment without any laser output. The acupuncture points, application duration, and total number of treatments were the same as those in the LA group.

Study Outcome

The primary outcome measure was the NRS score for pain on days 1–3 after treatment. The scores of NRS for pain were assessed on average and while deep breathing, coughing, and turning over. Since pain is a subjective experience, the exact quality and quantity of pain are very difficult for people to express, and no diagnostic tests are available for assessing pain. A pain scale such as the NRS is thus a relatively objective tool to measure and describe the pain of an individual. The NRS is a simple tool for verbally describing pain intensity on a scale of 0–10 points, where 0 = no pain and 10 = worst possible pain.

The secondary outcome included sustained maximal inspiration (SMI) lung volume measured by incentive spirometer, stress response, the use of narcotic and non-narcotic medications, and the use of laxatives, antacids, and proton pump inhibitors during the study period. The number of participants who used these drugs was calculated during the intervention on days 1–3 and after the intervention on day 4. The stress responses associated with pain were measured from salivary cortisol level, heart rate variability (HRV), heart rate, mean arterial pressure and sleep quality (0–10; 0 = no problem, 10 = sleep totally interrupted by pain) on days 1–3 after treatment. Finally, the complications of pneumonia and gastrointestinal (GI) bleeding within one month were recorded after enrollment.

Salivary samples were obtained from the participants before the intervention and on days 1–3 after the intervention. After collection, the samples were kept cold in order to prevent bacterial growth in the specimens. The samples were immediately

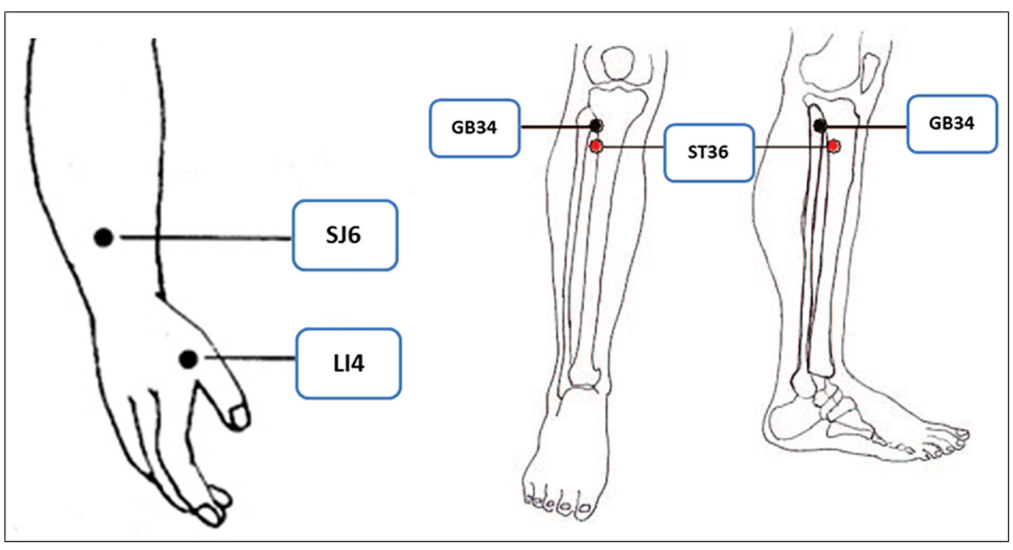

FIGURE 2 | The acupoints selected in the trial.

refrigerated at 4°C and then, within 4 h of collection, stored at −20°C until analysis. Prior to analysis, the samples were thawed completely, vortexed, and centrifuged at 1,500 × *g* for 15 min to remove mucins and other particulate matter which could interfere with the assay and affect the results. Salivary cortisol activity was determined using a Salimetrics® Cortisol Enzyme Immunoassay Kit (Item No. 1-3002, Salimetrics LLC, State College, PA, United States), which is a competitive immunoassay used to quantitatively measure salivary cortisol levels. The cortisol in standards and samples compete with cortisol conjugated to horseradish peroxidase for the antibody binding sites on a microtiter plate. According to the manufacturer's protocol, after incubation, unbound components were washed away, and bound cortisol enzyme conjugate was measured by the reaction of horseradish peroxidase enzyme to the substrate tetramethylbenzidine. This reaction produces a blue color, and a yellow color is formed after the reaction is stopped with an acidic solution. The optical density was read on a standard plate reader at 450 nm.

Statistical Analysis

Both intention-to-treat (ITT) analysis using all available data and per-protocol (PP) analysis using only data from participants who completed all treatment sessions were performed. The clinical characteristics data were analyzed via one-way analysis of variance (ANOVA) with Scheffé's *post hoc* testing or chi-squared testing, as appropriate. Quantitative variables, presented as mean ± SD, were analyzed by ANOVA with Scheffé's *post hoc* testing. Qualitative variables, expressed as a number (percentage), were analyzed using the Chi-Squared test. Lengths of hospital stay in participants with or without comorbid clavicle fracture in each group were analyzed by independent *t*-tests. All analyses were performed in SPSS 22.0 for Windows (Statistics 22.0, SPSS, IBM, New York, NY, United States). Differences were considered statistically significant at $P < 0.05$.

RESULTS

Patient Characteristics

As shown in the flowchart of the trial in **Figure 1**, 140 patients with rib fracture were assessed for eligibility and 120 patients met the criteria. Of the 120 study participants, 109 completed all interventions and measurements.

One patient in each of the TA, LA, and SLA groups was discharged from the hospital early and could not receive complete treatment and assessment. Two patients in each of the TA and SLA groups could not cooperate with pain assessment. Two patients in the LA group and one in the SLA group stopped treatment due to surgery unrelated to rib fracture. Another patient in the SLA group dropped out due to surgical stabilization of the rib fracture. The PP and ITT analyses of the clinical characteristics of the participants showed consistent results. The results of the PP and ITT analyses are listed in **Table 1** and **Supplementary Table 1**, respectively, and statistically significant differences were found. The factors associated with pain intensity, including number of ribs fractured and injury severity scores, were not significantly different between groups.

Primary Outcome

The PP and ITT analyses of the primary outcome also showed consistent results. The NRS scores for pain intensity on days 1 to 3 after treatment using PP and ITT analyses are listed in **Table 2** and **Supplementary Table 2**. The initial average pain intensity levels and pain while coughing, turning over and deep breathing were not significantly different between groups before treatment. Average pain intensity levels and pain while deep breathing were both significantly lower in the TA and LA groups than in the SLA group after two treatments. There were no significant differences in the NRS scores while coughing and turning over after treatment between groups. The primary efficacy variables were the percentage changes in NRS scores between baseline and on day 3 after treatment. The distribution of NRS scores before and after treatment and the statistics for the percentage changes in NRS scores between groups are presented in **Figure 3**. According to the NRS scores on day 3, the percentage improvement was significantly higher in the TA group than in the SLA group in terms of average pain intensity levels, pain while coughing, turning over and deep breathing. The percentage improvements in average pain intensity and pain while turning over were significantly higher in the LA group than in the SLA group.

Secondary Outcome

The data on the secondary outcome were analyzed using PP analysis. No between-group differences were observed in SMI lung volumes, salivary cortisol levels, HRV, heart rate, mean arterial pressure or sleep quality on days 1–3 after treatment (**Table 3**). The use of narcotic and non-narcotic medications for pain between groups was not significantly different during and after intervention (**Table 4**). The use of laxatives, antacids and PPIs for GI disorders are presented in **Table 5**, showing that the use of laxatives between groups was not significantly different during and after intervention. In contrast, the use of antacids and PPIs was significantly lower in the TA group and higher in the SLA group than in the LA group after intervention. The participants in the SLA group tended to use more laxatives, antacids and PPIs during the intervention, but the difference was not statistically significant. One patient in each of the TA and LA groups had pneumonia during hospitalization. One patient in each of the TA and LA groups had GI bleeding within the one-month follow-up period. No between-group differences were observed for the complications of pneumonia and GI bleeding (**Table 5**). The subgroup analysis of the length of hospital stay for participants with or without comorbid clavicle fracture is presented in **Table 6**. Overall, patients with combined rib and clavicle fracture had significantly longer hospital stays than those with rib fracture alone (12.3 ± 5.2 days vs. 9.6 ± 4.4 days; $P = 0.011$). This phenomenon was similar in the SLA group (15.8 ± 3.6 days vs. 9.8 ± 4.4 days; $P = 0.003$), but inconsistent with the TA group (9.9 ± 5.0 days vs. 9.1 ± 2.4 days; $P = 0.645$) and LA group (12.6 ± 5.1 days vs. 9.9 ± 5.7 days; $P = 0.225$). In comparison between groups, however, the patients with

TABLE 1 | Clinical characteristics of participants in the study.

Characteristics	Acupuncture (*N* = 37)	Laser acupuncture (*N* = 37)	Sham Laser acupuncture (*N* = 35)	*P* value
Age, years, mean ± SD[a]	54.49 ± 15.81	54.46 ± 15.87	54.06 ± 13.59	0.991
Male gender, n (%)[b]	22 (59.5%)	27 (73%)	20 (57.1%)	0.317
BMI (kg/m^2)[a]	26.15 ± 5.28	26.38 ± 5.33	24.27 ± 3.46	0.134
Current smoker, n (%)[b]	12 (32.4%)	11 (29.7%)	10 (28.6%)	0.800
Mechanism of injury, n (%)[b]				0.444
Traffic accident	31(83.8%)	27 (73.0%)	25 (71.4%)	
Fall	1 (2.7%)	5 (13.5%)	5 (14.3%)	
Crush	5 (13.5%)	5 (13.5%)	5 (14.3%)	
Number of ribs fractured[a]	4.05 ± 2.26	4.19 ± 2.07	3.89 ± 1.84	0.821
Injury Severity Score[a]	10.95 ± 5.59	9.97 ± 4.32	11.94 ± 6.80	0.337
Complications, n (%)[b]				0.950
Pneumothorax	6 (16.2%)	6 (16.2%)	4 (11.4%)	
Hemothorax	3 (8.1%)	4 (10.8%)	3 (8.6%)	
Hemopneumothorax	1 (2.7%)	3 (8.1%)	2 (5.7%)	
Chest tube/pig tail insertion, n (%)[b]	4 (10.8%)	3 (8.1%)	5 (14.3%)	0.694
Trauma to admission (days)[a]	1.86 ± 1.72	1.54 ± 0.77	1.66 ± 0.97	0.517
Admission to intervention (days)[a]	4.43 ± 2.32	4.05 ± 1.89	4.17 ± 1.86	0.716
Admission to discharge (days)[a]	9.3 ± 3.3	10.6 ± 5.6	10.8 ± 4.8	0.352

[a]*One-way analysis of variance with Scheffé's post hoc testing.* [b]*Chi-squared testing.*
SD, standard deviation; BMI, body mass index.

TABLE 2 | NRS scores for pain intensity before and after treatment on days 1–3.

NRS	Intervention day	Acupuncture	Laser acupuncture	Sham laser acupuncture	*P* value
Average	pre D1	6.19 ± 1.29	6.11 ± 1.27	6.06 ± 1.00	0.894
	post D1	5.22 ± 1.29	5.05 ± 1.20	5.63 ± 1.17	0.128
	post D2	3.73 ± 1.19*	4.00 ± 1.31*	4.91 ± 1.34	<0.001
	post D3	2.73 ± 1.02*	2.73 ± 1.24*	4.00 ± 1.44	<0.001
Deep	pre D1	5.19 ± 2.26	4.62 ± 2.62	5.17 ± 1.86	0.480
breath	post D1	4.27 ± 2.18	3.59 ± 2.18*	5.00 ± 1.88	0.020
	post D2	3.00 ± 1.78*	2.97 ± 1.87*	4.11 ± 1.86	0.013
	post D3	2.19 ± 1.18*	2.11 ± 1.45*	3.26 ± 1.67	0.001
Cough	pre D1	7.97 ± 1.61	7.73 ± 1.95	7.69 ± 1.57	0.745
	post D1	7.57 ± 1.54	6.97 ± 2.24	7.54 ± 1.62	0.293
	post D2	6.24 ± 1.75	6.27 ± 2.19	7.11 ± 2.14	0.127
	post D3	5.46 ± 2.09	5.27 ± 2.47	6.43 ± 2.32	0.078
Turnover	pre D1	8.49 ± 1.69	8.54 ± 1.56	8.14 ± 1.67	0.541
	post D1	7.92 ± 1.82	7.89 ± 1.79	7.86 ± 1.90	0.990
	post D2	6.65 ± 2.12	6.68 ± 2.00	7.20 ± 2.06	0.449
	post D3	5.62 ± 2.13	5.35 ± 2.65	6.54 ± 2.33	0.090

NRS, Numerical Rating Scale; pre D1, NRS score on day 1 before treatment; post D1, D2, D3, NRS score on day 1–3 after treatment.
**Significance when compared with sham laser acupuncture group, *P < 0.05.*

combined rib and clavicle fracture in the SLA group had longer hospital stays, but there was no statistically significant difference from the TA and LA groups.

DISCUSSION

Currently, acupuncture is increasingly used for a variety of diseases worldwide. Although considerably large acupuncture research had demonstrated that acupuncture is safe for many conditions in the past two decades (12, 13), there is still no standard protocol for pain management. The main reason is that there are various types and protocols of acupuncture treatment for one condition or disease. There is currently no standard approach or prescription for acupuncture treatment. Most of the acupoints used in acupuncture treatment are based on the experience of physicians, and these treatments often lack clinical trials to verify their efficacy. Therefore, well-designed randomized controlled trials with a widely accepted protocol of acupuncture treatment are still needed. To the best of our

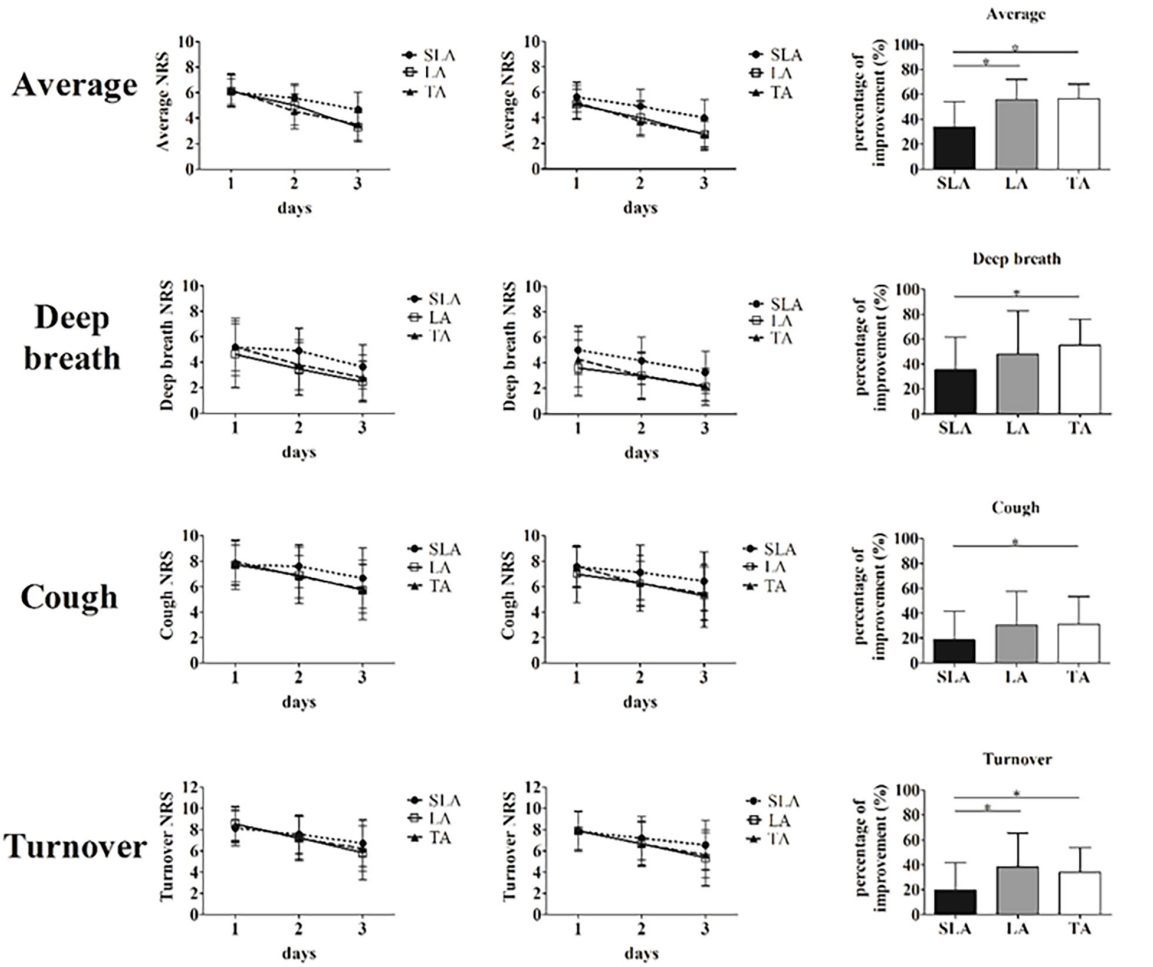

FIGURE 3 | Primary efficacy variables, and distributions of average pain level and pain while deep breathing, coughing and turning over before and after treatment. (Data of (Post3-Pre1)/Pre1 were analyzed via one-way analysis of variance with Scheffé's *post hoc* testing. *Significance when compared with sham laser acupuncture group, $^*p < 0.05$. Pre, before treatment; Post, after treatment; SLA, sham laser acupuncture; LA, laser acupuncture; TA, acupuncture).

knowledge, few publications have described acupuncture for traumatic rib fracture. Only one randomized controlled trial (10) has demonstrated a positive effect on acute pain using a novel acupuncture modality. In that study, the needle insertion sites were on the abdomen or back and not on classical acupoints. Although this novel acupuncture modality is safe, it lacks the theoretical basis of TCM, and the selected needle insertion sites are rarely used in real acupuncture practice.

The present study is the first RCT to investigate acupuncture analgesia using classical acupoints in patients with traumatic rib fracture. The main results of our study showed that the average pain intensity levels of pain while coughing, turning over and deep breathing were all significantly lower in the TA group than in the SLA group, and the average pain intensity and pain while turning over were both significantly lower in the LA group than in the SLA group. The acupuncture modality in our study is commonly implemented and can be widely accepted. The selected acupoints, namely, LI4 (Hegu), SJ6 (Zhigou), ST36 (Zusanli), and GB34 (Yanglingquan), are based on the theory of TCM. All acupoints are located on the extremities, which are completely safe for needle insertion. According to meridian theory, the acupoints LI4 and ST36, belonging to the yang-ming meridian, and SJ6 and GB34, belonging to the shao-yang meridian, can potentially improve *qi* and blood flow and subsequently alleviate pain on the front and lateral areas of the chest, respectively. In acupuncture clinical practice, LI4 is a very common and useful point for any condition related to the face and head, while ST36 is typically used to treat GI disturbance. Combining these two acupoints can produce a good analgesic effect anywhere in the body (14, 15). SJ6 is typically used for constipation and pain in the lateral costal region, ribs and axilla. GB34 is typically used for motor disorders and pain (16, 17), particularly those associated with trauma and in the lateral side of the body.

TABLE 3 | SMI volume, pain-induced stress responses before and after treatment.

	Intervention day	Acupuncture	Laser acupuncture	Sham laser acupuncture	*P* value
SMI	pre D1	627.0 ± 277.3	668.9 ± 219.3	658.0 ± 220.6	0.741
	post D1	773.0 ± 213.6	767.6 ± 208.6	758.6 ± 214.0	0.959
	post D2	914.9 ± 241.8	909.5 ± 211.8	848.6 ± 217.8	0.386
	post D3	944.6 ± 237.6	931.6 ± 229.1	917.1 ± 212.5	0.881
Cortisol	pre D1	0.26 ± 0.14	0.41 ± 0.30	0.38 ± 0.35	0.067
	post D1	0.21 ± 0.12	0.21 ± 0.21	0.20 ± 0.12	0.951
	post D2	0.16 ± 0.09	0.22 ± 0.13	0.17 ± 0.08	0.037
	post D3	0.20 ± 0.18	0.23 ± 0.17	0.23 ± 0.17	0.761
LF	pre D1	998.5 ± 1506.4	416.9 ± 557.7	1162.8 ± 2185.6	0.135
	post D1	1248.5 ± 1718.5	844.0 ± 1512.5	767.3 ± 1314.4	0.387
	post D2	2013.0 ± 4015.1	988.0 ± 1805.6	991.2 ± 1414.2	0.191
	post D3	1170.7 ± 1701.2	1057.3 ± 2474.8	1123.8 ± 1958.5	0.947
HF	pre D1	626.3 ± 691.0	433.78 ± 573.0	560.2 ± 648.6	0.438
	post D1	960.4 ± 1383.0	533.2 ± 674.4	593.6 ± 804.2	0.167
	post D2	766.5 ± 795.3	673.6 ± 879.2	729.5 ± 809.9	0.894
	post D3	773.1 ± 896.2	602.7 ± 779.6	600.1 ± 712.2	0.595
LF/HF	pre D1	1.42 ± 1.20	1.20 ± 0.87	1.57 ± 1.76	0.527
	post D1	1.32 ± 0.90	1.36 ± 1.23	1.32 ± 1.62	0.990
	post D2	1.71 ± 1.83	1.42 ± 1.30	1.55 ± 1.14	0.694
	post D3	1.39 ± 0.96	1.55 ± 1.56	1.84 ± 1.17	0.361
HR	pre D1	77.6 ± 13.3	76.8 ± 11.9	76.5 ± 13.0	0.935
	post D1	75.4 ± 13.0	76.3 ± 12.0	75.1 ± 11.1	0.919
	post D2	79.1 ± 12.0	76.7 ± 10.7	77.7 ± 12.5	0.696
	post D3	78.2 ± 11.7	76.8 ± 10.0	76.9 ± 11.2	0.842
MAP	pre D1	91.4 ± 14.7	100.8 ± 15.0	97.6 ± 18.1	0.124
	post D1	92.2 ± 14.0	100 ± 17.7	93.3 ± 17.6	0.227
	post D2	97.4 ± 15.4	95.8 ± 28.4	95.9 ± 17.5	0.959
	post D3	95.3 ± 12.8	95.4 ± 24.0	95.9 ± 12.3	0.991
Sleep	pre D1	6.1 ± 1.8*	5.6 ± 1.8	4.9 ± 2.3	0.048
	post D1	6.0 ± 2.1	5.4 ± 2.6	5.7 ± 2.7	0.586
	post D2	5.4 ± 2.9	5.5 ± 2.8	5.8 ± 2.3	0.824
	post D3	5.1 ± 2.8	6.5 ± 2.1	6.7 ± 2.0	0.052

pre D1, NRS score on day 1 before treatment; post D1, D2, D3, NRS score on day 1 to 3 after treatment; NRS, Numerical Rating Scale; SMI, sustained maximal inspiration; LF, low frequency; HF, high frequency; LF/HF, LF/HF ratio; HR, heart rate; MAP, mean arterial pressure.
**Significance when compared with sham laser acupuncture group, *P < 0.05.*

TABLE 4 | The use of narcotic and non-narcotic medications during intervention (days 1–3) and after intervention (day 4).

	Intervention day	Acupuncture (37), *n* (%)	Laser acupuncture (37), *n* (%)	Sham laser acupuncture (35), *n* (%)	*P* value
Opioids	During	30 (81.1%)	29 (78.4%)	26 (74.3%)	0.783
	After	16 (43.2%)	14 (37.8%)	17 (48.6%)	0.655
NSAIDs	During	22 (59.5%)	24 (64.9%)	21 (60.0%)	0.871
	After	12 (32.4%)	10 (27.0%)	8 (22.9%)	0.659
Acetaminophen	During	9 (24.3%)	11 (29.7%)	6 (17.1%)	0.455
	After	5 (13.5%)	4 (10.8%)	4 (11.4%)	1.0

NSAIDs, non-steroidal anti-inflammatory drugs.

Regarding clinical trials of acupuncture treatment, an appropriate design for control group and double-blinding procedure remains a methodological challenge. The commonly used controls in acupuncture trials include non-intervention control, non-insertion sham control, and needle insertion at sham or real acupoints (18). Although non-insertion sham control such as non-penetrating devices resembles the real acupuncture needling procedure, patients are very likely to distinguish between real and fake acupuncture because of the lack of needling sensations, such as numbness, soreness, distention, or dull pain, in the sham controls. Another sham needle insertion control using wrong points or superficial penetration may produce non-specific effects of blood circulation and neural stimulation, such as non-specific endorphin release (18, 19). Accordingly, no reliable blinding methodology for needle acupuncture has been achieved so far. Recently, SLA has been used as a control for needle acupuncture trials. It is reported that SLA can serve as a valid placebo control in LA studies due

TABLE 5 | The use of laxatives and antacid/PPI during intervention (days 1–3) and after intervention (day 4), and complications during hospitalization and follow-up for one month.

	Intervention day	Acupuncture (37), n (%)	Laser acupuncture (37), n (%)	Sham laser acupuncture (35), n (%)	*P* value
Laxatives	During	13 (35.1%)	13 (35.1%)	20 (57.1%)	0.095
	After	3 (8.1%)	6 (16.2%)	9 (25.7%)	0.132
Antacid/PPI	During	19 (51.4%)	20 (54.1%)	27 (77.1%)	0.050
	After	5* (13.5%)	16* (43.2%)	10 (28.6%)	0.018
Pneumonia	During hospitalization	1 (2.7%)	1 (2.7%)	0 (0%)	1.000
	f/u 1 month	–	–	–	–
GI bleeding	During hospitalization	–	–	–	–
	f/u 1 month	1 (2.7%)	1 (2.7%)	0 (0%)	1.000

PPI, proton pump inhibitor; GI, gastrointestinal.

TABLE 6 | Length of hospital stay in participants with or without combined clavicle fracture.

	Acupuncture, day (*n*)	Laser acupuncture, day (*n*)	Sham laser acupuncture, day (*n*)	$P^{\#}$ value	All patients; day (*n*)
Rib fractures	9.1 ± 2.4 (27)	9.9 ± 5.7 (28)	9.8 ± 4.4 (29)	0.769	9.6 ± 4.4 (84)
Combined clavicle fracture	9.9 ± 5.0 (10)	12.6 ± 5.1 (9)	15.8 ± 3.6 (6)	0.076	12.3 ± 5.2 (25)
*P** value	0.645	0.225	0.003		0.011

#One-way analysis of variance with Scheffé's post hoc testing.
**Independent t-tests.*

to their similar credibility and the lack of sensory input on the peripheral nervous system, and it can serve as a sham control for acupuncture trials when there is a need to evaluate the effects of needling *per se* (20). A 3-arm parallel randomized trial with TA, LA and SLA was conducted in our study. SLA treatment affords all requirements to produce the same non-specific effects as LA treatment, which can verify the efficacy of the selected acupoints in the study. SLA can serve as a sham procedure, and LA can serve as an active comparator for evaluating the needling effect of real acupuncture. We suggest that it is currently the best of all designs that can both confirm the specific effect of acupuncture and verify the specificity of selected acupoints.

All the participants we enrolled needed to be hospitalized. Twice as many participants as in the previous study were enrolled in our study. The intervention timing in this study was when the patient could not obtain adequate pain control after conventional analgesics. This means that the participants in our study had moderate injuries and uncontrollable pain. During this period, surgeons often need to face some questions, including whether to increase the dose of analgesics, increase other alternative methods of pain relief, or recommend surgical fixation. The present study aimed to investigate the immediate analgesic effect of adjuvant acupuncture in the treatment of traumatic rib fracture. Both PP and ITT analyses were performed on the clinical characteristics and primary outcome. The PP and ITT analyses of the data were consistent in this study. Additionally, none of the participants dropped out of the trial due to rib pain worsening. We considered that it was more important to ensure that the participants received complete treatments than to investigate the effect of acupuncture as an add-on therapy. Therefore, information with PP analysis should be sufficient from the trial.

According to the primary outcome in the study, the analgesic effect appeared to be significant after 2 treatments in the TA and LA groups. This finding suggests that acupuncture can provide good pain relief in about two days during hospitalization. In addition, according to the percentage improvement of the NRS scores in average and pain-inducing movements, the analgesic effect appeared to be greater with TA than with LA. A possible explanation is that TA induces *de-qi* sensation, a distinct skin insertion associated with gentle twisting or up-and-down movement, which can induce more nociceptive stimulus to modulate pain signals than can LA. LA does not produce sensation; however, it has photochemical reactions in cells and provokes nerve fiber activation, resulting in physiologic changes similar to TA (20). Our research results demonstrated that acupuncture is an effective non-pharmacologic treatment for pain management in this period. We think that if the pain control is still insufficient after a patient receives acupuncture treatment for 3 days, surgical fixation may be a good choice.

In this study, we hypothesized that acupuncture could reduce pain associated with rib fracture and subsequently improve respiration and prevent the complication of pneumonia. The results showed that acupuncture had a good analgesic effect for rib fracture, but it did not significantly increase the maximal inspiration lung volume or decrease pneumonia compared to the controls. One possible reason is that the participants enrolled in the study were those with stable vital signs without dyspnea during the study period. This factor might decrease the beneficial effects of acupuncture for pulmonary complications associated with rib fracture.

Other secondary outcomes assessing acute stress responses, including salivary cortisol levels, heart rate, HRV, mean blood pressure and sleep disorder, showed no significant differences between groups. There are several possible reasons for these results. First, the average pain levels and pain while deep breathing were reduced from moderate to mild, and the pain

while coughing and that while turning over were reduced from severe to moderate. Both results show that the pain was not completely improved after full treatment and might still have caused stress responses. Another might be that the psychological impact of the injuries had been sustained for a long time (21), so these stress responses were unable to improve quickly even if the pain was under control.

According to the general perception of pain management, the use of analgesic medicines will be decreased as the pain improves. In contrast to one previous systemic review and meta-analysis (22), which showed that acupuncture could improve acute postoperative pain and reduce opioid use, our results showed that the use of narcotic and non-narcotic medications did not differ significantly between groups during and after intervention. We believe that the reason is mainly due to the short duration of our intervention and different mechanisms of the injuries. In the literature, the duration of pain after rib fracture is considered to be at least 8 weeks (23). Physicians would not stop the analgesics so quickly, even if the patient's pain had been improved during a short-term three-day treatment in our study.

Notably, we found an interesting phenomenon, namely, that participants in the TA and LA groups had lower tendencies to use laxatives and antacids/PPI during the intervention as compared to the SLA group, but without statistical significance. Significantly fewer participants in TA group and more participants in LA group used antacid/PPI after intervention as compared with the SLA group. We think that the benefits on GI disorders mainly come from acupuncture at the ST36 point (24), and the effect of TA lasts longer than that of LA because real needling effect.

GI bleeding is another complication associated with acute stress and the use of NSAIDs. The events of GI bleeding during hospitalization and follow-up for one month showed no significant differences between groups in our study. Finally, we also analyzed the lengths of hospital stay between subgroups based on whether participants had clavicle fracture in addition to rib fracture. Of all the participants, patients having traumatic rib fracture combined with clavicle fracture had significantly longer hospital stays than those of patients with traumatic rib fracture alone. This trend was observed in the SLA group but not in the TA and LA groups. This difference suggests that acupuncture may shorten hospital stays for patients with combined clavicle and rib fracture; however, this benefit is not very significant.

There are several limitations to our study. First, the single-center scope of the study and the relatively small sample size may have led to overestimation of the effects of acupuncture. Secondly, several factors may affect the measurement of HRV and sleep quality, including patients' condition (e.g., pain), environmental factors (e.g., light and sound disturbances), or psychological factors (e.g., anxiety and stress) (25). Finally, we were unable to choose and unify the treatment timing because the participants were recruited at different times post injury. However, the results show that early acupuncture intervention within a few days of the trauma can reduce the rib pain. Further research is needed to understand the potential long-term analgesic effect in patients with traumatic rib fracture.

CONCLUSION

The findings of this study suggest that TA and LA are safe and effective analgesic modalities for pain management in traumatic rib fracture. As a non-pharmacologic adjuvant treatment, acupuncture has considerable potential for aiding in pain reduction. Acute pain management following traumatic rib fracture is a common challenge, and these results can aid physicians seeking feasible and effective methods to manage pain and improve the patient experience.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Human Ethics Committee of Chang Gung Medical Foundation Institutional Review Board, IRB No. 201701455A3. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

C-TL, T-MH, and W-LH: conceptualization. B-YW: data curation. B-YW and Y-CH: formal analysis. C-TL: funding acquisition, project administration, and writing—original draft. C-TL, T-MH, and C-HS: investigation. C-TL and T-MH: methodology. M-YT and Y-HC: supervision and writing—review and editing. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Simon BJ, Cushman J, Barraco R, Lane V, Luchette FA, Miglietta M, et al. Pain management guidelines for blunt thoracic trauma. *J Trauma.* (2005) 59:1256–67. doi: 10.1097/01.ta.0000178063.77946.f5
2. Jensen CD, Stark JT, Jacobson LL, Powers JM, Joseph MF, Kinsella-Shaw JM, et al. Improved outcomes associated with the liberal use of thoracic epidural analgesia in patients with rib fractures. *Pain Med.* (2017) 18:1787–94. doi: 10.1093/pm/pnw199
3. Galvagno SM Jr, Smith CE, Varon AJ, Hasenboehler EA, Sultan S, Shaefer G, et al. Pain management for blunt thoracic trauma: a joint practice management guideline from the eastern association for the surgery of trauma and trauma anesthesiology society. *J Trauma Acute Care Surg.* (2016) 81:936–51. doi: 10.1097/TA.0000000000001209
4. Bayouth L, Safcsak K, Cheatham ML, Smith CP, Birrer KL, Promes JT. Early intravenous ibuprofen decreases narcotic requirement and length of stay after traumatic rib fracture. *Am Surg.* (2013) 79:1207–12.
5. Kim M, Moore JE. Chest trauma: current recommendations for rib fractures, pneumothorax, and other injuries. *Curr Anesthesiol Rep.* (2020) 10:61–8. doi: 10.1007/s40140-020-00374-w
6. He Z, Zhang D, Xiao H, Zhu Q, Xuan Y, Su K, et al. The ideal methods for the management of rib fractures. *J Thorac Dis.* (2019) 11:S1078–89. doi: 10.21037/jtd.2019.04.109
7. Choi J, Mulaney B, Laohavinij W, Trimble R, Tennakoon L, Spain DA, et al. Nationwide cost-effectiveness analysis of surgical stabilization of rib fractures by flail chest status and age groups. *J Trauma Acute Care Surg.* (2021) 90:451–8. doi: 10.1097/TA.0000000000003021
8. Lu W, Rosenthal DS. Acupuncture for cancer pain and related symptoms. *Curr Pain Headache Rep.* (2013) 17:321. doi: 10.1007/s11916-013-0321-3
9. Qaseem A, Wilt TJ, McLean RM, Forciea MA. Noninvasive treatments for acute, subacute, and chronic low back pain: a clinical practice guideline from the American college of physicians. *Ann Intern Med.* (2017) 166:514–30. doi: 10.7326/M16-2367
10. Ho HY, Chen CW, Li MC, Hsu YP, Kang SC, Liu EH, et al. A novel and effective acupuncture modality as a complementary therapy to acute pain relief in inpatients with rib fractures. *Biomed J.* (2014) 37:147–55. doi: 10.4103/2319-4170.117895
11. WHO. *Standard Acupuncture Point Locations in the Western Pacific Region*. Geneva: World Health Organization (2008).
12. Ma Y, Dong M, Zhou K, Mita C, Liu J, Wayne PM. Publication trends in acupuncture research: a 20-year bibliometric analysis based on Pubmed. *PLoS One.* (2016) 11:e0168123. doi: 10.1371/journal.pone.0168123
13. Birch S, Lee MS, Alraek T, Kim TH. Overview of treatment guidelines and clinical practical guidelines that recommend the use of acupuncture: a bibliometric analysis. *J Altern Complement Med.* (2018) 24:752–69. doi: 10.1089/acm.2018.0092
14. Vieira JS, Toreti JA, de Carvalho RC, de Araújo JE, Silva ML, Silva JRT. Analgesic effects elicited by neuroactive mediators injected into the ST 36 acupuncture point on inflammatory and neuropathic pain in mice. *J Acupunct Meridian Stud.* (2018) 11:280–9. doi: 10.1016/j.jams.2018.05.006
15. Yen CM, Wu TC, Hsieh CL, Huang YW, Lin YW. Distal electroacupuncture at the LI4 acupoint reduces CFA-induced inflammatory pain via the brain TRPV1 signaling pathway. *Int J Mol Sci.* (2019) 20:4471. doi: 10.3390/ijms20184471
16. Na BJ, Jahng GH, Park SU, Jung WS, Moon SK, Park JM, et al. An fMRI study of neuronal specificity of an acupoint: electroacupuncture stimulation of yanglingquan (GB34) and its sham point. *Neurosci Lett.* (2009) 464:1–5. doi: 10.1016/j.neulet.2009.08.009
17. Park JY, Park JJ, Jeon S, Doo AR, Kim SN, Lee H, et al. From peripheral to central: the role of ERK signaling pathway in acupuncture analgesia. *J Pain.* (2014) 15:535–49. doi: 10.1016/j.jpain.2014.01.498
18. Chen H, Yang M, Ning Z, Lam WL, Zhao YK, Yeung WF, et al. A guideline for randomized controlled trials of acupuncture. *Am J Chin Med.* (2019) 47:1–18. doi: 10.1142/S0192415X19500010
19. Meyer-Hamme G, Friedemann T, Greten HJ, Plaetke R, Gerloff C, Schroeder S. ACUDIN – ACUpuncture and laser acupuncture for treatment of DIabetic peripheral neuropathy: a randomized, placebo-controlled, partially double-blinded trial. *BMC Neurol.* (2018) 18:40. doi: 10.1186/s12883-018-1037-0
20. Irnich D, Salih N, Offenbächer M, Fleckenstein J. Is sham laser a valid control for acupuncture trials? *Evid Based Complement Alternat Med.* (2011) 2011:485945. doi: 10.1093/ecam/neq009
21. Craig A, Tran Y, Guest R, Gopinath B, Jagnoor J, Bryant RA, et al. Psychological impact of injuries sustained in motor vehicle crashes: systematic review and meta-analysis. *BMJ open.* (2016) 6:e011993. doi: 10.1136/bmjopen-2016-011993
22. Wu MS, Chen KH, Chen IF, Huang SK, Tzeng PC, Yeh ML, et al. The efficacy of acupuncture in post-operative pain management: a systematic review and meta-analysis. *PLoS One.* (2016) 11:e0150367. doi: 10.1371/journal.pone.0150367
23. Tulay CM, Yaldiz S, Bilge A. Do we really know the duration of pain after rib fracture? *Kardiochir Torakochirurgia Pol.* (2018) 15:147–50. doi: 10.5114/kitp.2018.78437
24. Yin J, Chen JD. Gastrointestinal motility disorders and acupuncture. *Auton Neurosci.* (2010) 157:31–7. doi: 10.1016/j.autneu.2010.03.007
25. Kulpatcharapong S, Chewcharat P, Ruxrungtham K, Gonlachanvit S, Patcharatrakul T, Chaitusaney B, et al. Sleep quality of hospitalized patients, contributing factors, and prevalence of associated disorders. *Sleep Disord.* (2020) 2020:8518396. doi: 10.1155/2020/8518396

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Coordinate-based (ALE) meta-analysis of acupuncture for musculoskeletal pain

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Background: Neuroimaging studies have been widely used to investigate brain regions' alterations in musculoskeletal pain patients. However, inconsistent results have hindered our understanding of the central modulatory effects of acupuncture for musculoskeletal pain. The main objective of our investigation has been to obtain comprehensive evidence of acupuncture for musculoskeletal pain diseases.

Methods: The PubMed, Web of Science, Google Scholar, Embase, China National Knowledge Infrastructure (CNKI), VIP Database, China Biology Medicine disc Database, Clinical Trial Registration Platform, and Wanfang Database were searched for neuroimaging studies on musculoskeletal pain diseases published from inception up to November 2021. Then, the relevant literature was screened to extract the coordinates that meet the criteria. Finally, the coordinate-based meta-analysis was performed using the activation likelihood estimation algorithm.

Results: A total of 15 neuroimaging studies with 183 foci of activation were included in this study. The ALE meta-analysis revealed activated clusters in multiple cortical and sub-cortical brain structures in response to acupuncture across studies, including the thalamus, insula, caudate, claustrum, and lentiform nucleus.

Conclusions: The studies showed that acupuncture could modulate different brain regions, including the thalamus, insula, caudate, claustrum, and lentiform nucleus. The findings offer several insights into the potential mechanisms of acupuncture for musculoskeletal pain and provide a possible explanation for the observed clinical benefit of this therapy.

Systematic review registration: https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=227850, identifier: CRD42021227850.

KEYWORDS

musculoskeletal pain, acupuncture, functional magnetic resonance imaging, activation likelihood estimation, meta-analysis

Introduction

Musculoskeletal pain disorders are a group of diseases characterized by nociception in the musculoskeletal system (muscles, ligaments, joints, and tendons), which include but are not limited to neck pain, low back pain, and fibromyalgia (Skootsky et al., 1989; Wolfe et al., 1995; Lawrence et al., 1998; Melhorn, 2014). Nowadays, musculoskeletal pain is a significant complaint. Adults with musculoskeletal pain account for 40.4–69.3% of the population (Abdulmonem et al., 2014). A potential peak of sufferers is anticipated as the population ages and young people adopt improper postures (Yuan et al., 2016). Musculoskeletal pain substantially impacts patients' quality of life and causes considerable societal and economic burdens since it limits daily activities and reduces productivity. On the other hand, the long-term usage of drugs is not indicated in clinical practice due to adverse effects (Ussai et al., 2015). As a result, alternative therapies should be given more consideration.

As an efficacious treatment for musculoskeletal pain, acupuncture has a long history of relieving the symptoms of such diseases in the East (Lenoir et al., 2020). It has been recommended as a positive therapy in a clinical practice guideline from the American College of Physicians (Qaseem et al., 2017). Acupuncture has been used to treat many types of musculoskeletal pain diseases (Mu et al., 2020; Zhang and Wang, 2020). However, we still have a limited understanding of how acupuncture works.

The current study showed that brain changes in musculoskeletal pain are widespread and involve the pain network as well as sensory, emotional, and cognitive control networks that process information (Mitsi and Zachariou, 2016). Various functional neuroimaging techniques have been used to ascertain which brain areas are metabolically activated or deactivated. That would help understand acupuncture analgesia from a central nervous system view. In previous studies, acupuncture has been found to regulate abnormal neural activities of the "pain matrix," mainly the second-order (including posterior parietal, prefrontal and anterior insular areas) and third-order (including the orbitofrontal and perigenual/limbic networks) matrices responsible for pain memory in musculoskeletal pain sufferers (Apkarian et al., 2005; Borsook et al., 2010a; Garcia-Larrea and Peyron, 2013). After extensive literature research, the pain memory matrices, including the insula cortex (IC), inferior frontal cortex, thalamus, anterior cingulate cortex, medial prefrontal cortex (MPFC), and others, were mainly associated with acupuncture analgesia (Henry et al., 2011; Farmer et al., 2012; Chae et al., 2013; Villarreal Santiago et al., 2016). Although these neuroimaging studies have identified several brain regions activated or deactivated by acupuncture, the results still have to be appropriately interpreted. Simultaneously, we found that previous studies have failed to consider heterogeneity due to different imaging modalities (e.g., fMRI, PET, SPECT), study populations, experimental paradigms, and data analysis pipelines. Therefore, further studies are still necessary.

As mentioned above, activation likelihood estimation (ALE) is an excellent method for dealing with heterogeneity. It has been the primary method for integrating neuroimaging data in meta-analyses. Instead of calculating the presence or absence of brain activity in the region of interest, ALE analysis combines the study coordinates of all individuals to determine the statistical probability of brain regions being activated or deactivated. An ALE analysis is required due to the lack of consistent evidence in the current study. This analysis can help us better summarize acupuncture's significant modulatory effects on musculoskeletal pain.

This study aimed to investigate the brain activities of patients with musculoskeletal pain to obtain sufficient evidence of acupuncture's effectiveness in treating musculoskeletal pain diseases. To this end, this study first searched relevant databases, then evaluated the included literature. Finally, a comprehensive conclusion of how acupuncture treats musculoskeletal pain diseases was drawn by combining the ALE analysis results.

Materials and methods

Literature search and selection

Studies were obtained from the following databases: PubMed, Web of Science, Google Scholar, Embase, China National Knowledge Infrastructure (CNKI), VIP Database, China Biology Medicine disc Database, Clinical Trial Registration Platform, and Wanfang Database, searched from inception to November 2021. The relevant references from the retrieved papers have been added to the database for this study. Only whole-brain studies published in English were eligible for the review. Table 1 shows search strategies to replicate the other databases' selection processes. In addition, references to studies included in the review and clinical trial databases were manually screened to avoid omitted studies. The present meta-analysis was registered in PROSPERO (no. CRD42021227850).

Inclusion criteria

1. The study must be performed for whole-brain analysis by fMRI.
2. The research results had to be presented in Talairach or MNI coordinates.
3. Documents presented by the same research team must use different raw data.
4. Cohort studies and randomized controlled trials were included only if neuroimaging results were available.

TABLE 1 Searching strategy.

1.1 PubMed searching strategy	1.2 CNKI searching strategy
#1 musculoskeletal pain (MeSH Terms)	#1肌肉骨骼痛（主题词）
#2 musculoskeletal pain (All Fields)	#2肌肉骨骼疾病（主题词）
#3 musculoskeletal disease (All Fields)	#3肌肉痛（主题词）
#4musculoskeletal disorders (All Fields)	#4肌肉疼痛（主题词）
#5muscular diseases (All Fields)	#5骨骼痛（主题词）
#6chronic musculoskeletal pain (All Fields)	#6关节炎（主题词）
#7musculoskeletal Conditions (All Fields)	#7关节痛（主题词）
#8muscle pain (All Fields)	#8关节疾病（主题词）
#9Myalgia (All Fields)	#9纤维肌痛（主题词）
#10myofascial Pain (All Fields)	#10肌筋膜疼痛（主题词）
#11Fibromyalgia (MeSH Terms)	#11肩痛（主题词）
#12neck pain (MeSH Terms)	#12腰痛（主题词）
#13Osteoarthritis (MeSH Terms)	#13背痛（主题词）
#14Arthritis (MeSH Terms)	#14颈痛（主题词）
#15Arthrosis (MeSH Terms)	#15颈椎痛（主题词）
#16Arthralgia (MeSH Terms)	#16#1OR#2 OR#3 OR#4 OR#5 OR#6 OR#7 OR#8 OR#9 OR#10 OR#11 OR#12 OR#13 OR#14OR#15
#17Joint Diseases (MeSH Terms)	#17功能性磁共振（主题词）
#18Low Back Pain (MeSH Terms)	#18功能磁共振（主题词）
#19Lumbago (MeSH Terms)	#19 fMRI主题词
#20Back Pain (MeSH Terms)	#20#17OR#18 OR#19
#21Backache (MeSH Terms)	#21针刺（主题词
#22Shoulder Pain (MeSH Terms)	#22穴位（主题词
#23Cervicalgia (MeSH Terms)	#23电针（主题词）
#24#1OR#2 OR #3 OR #4 OR #5 OR #6OR#7OR #8 OR #9 OR #10 OR #11 OR#12 OR#13OR #14 OR#15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23	#23电针（主题词）
#25 acupuncture (MeSH Terms)	#24#21OR#22 OR#23
#26 acupuncture Therapy (MeSH Terms)	#25 Final search terms: #16AND #20 AND #24
#27 acupoint (MeSH Terms)	
#28 acupuncture Point (MeSH Terms)	
#29 electroacupuncture (MeSH Terms)	
#30 electro-acupuncture (MeSH Terms)	
#31#25OR#26 OR#27 OR#28 OR#29 OR#30	
#32Functional magnetic resonance imaging (MeSH Terms)	
#33 Functional MRI (MeSH Terms)	
#34 fMRI (MeSH Terms)	
#35#32 OR#33 OR#34	
#36 Final search terms: #24AND #32 AND #35	

Exclusion criteria

1. Area-of-interest scans, hyper-scanning, and small-volume-correction studies were excluded.
2. Other secondary research such as conference articles, reviews, animal experiment articles, case reports, letters, and other second-hand studies were excluded.

After removing duplicates, the specific process is as follows: Two independent reviewers (YL and JT) checked the titles and abstracts to include and exclude irrelevant studies. The full texts are obtained and rechecked in more detail to finalize their inclusion. Any disagreement is resolved through discussion in which a third reviewer (JC) would participate. The final selection is checked and determined by a third reviewer (JC).

BOX 1
Quality assessment of individual studies.

Category 1: sample characteristics (10)

1. Patients are evaluated with specific standardized diagnostic criteria (1).
2. Important demographic data (age and gender) are reported with mean (or median) and SDs (or range) (2).
3. Healthy control subjects are evaluated to exclude psychiatric and medical illnesses and demographic data are reported (1).
4. Important clinical variables (e.g., medication status, illness duration and severity) are reported with mean (or median) and SDs (or range) (4).
5. Sample size per group >10 (2).

Category 2: methodology and reporting (10)

6. Whole brain analysis is automated with no a priori regional selection (3).
7. Magnet strength at least 1.5T (1).
8. At least 5 min of resting state acquisition (1).
9. Whole brain coverage of resting scans (1).
10. The acquisition and preprocessing techniques are clearly described so that they can be reproduced (1).
11. Coordinates reported in a standard space (1).
12. Significant results are reported after correction for multiple testing using a standard statistical procedure (AlphaSim, FDR (False Discovery Rate), FWE (Family Wise Error) or permutation- based methods) (1).
13. Conclusions are consistent with the results obtained and the limitations are discussed (1).

Data extraction

The following items are extracted from each record: (1) publication details: title, first author, publishing year, unit, country, or region; (2) methodology details include: participants, disease types, diagnostic criteria, demographic characteristics (including age and gender), imaging modalities, data analysis strategies, interventions (including acupuncture and electroacupuncture). (3) Outcomes: significantly altered cerebral regions (defined by MNI/Talairach coordinates, cluster size, and statistical threshold), clinical assessment outcomes, and correlations between imaging and clinical data.

In this meta-analysis, team members extracted the participants whose neuroimaging data were analyzed for activation (which increased after the treatments in patients with musculoskeletal pain; POST > PRE), and deactivation (which decreased after the treatments in patients with musculoskeletal pain; PRE > POST) coordinates.

Quality assessment

So far, there has been no standard checklist for quality assessment of individual functional neuroimaging studies. A checklist (Box 1) published in a previous meta-analysis was adopted (Li et al., 2019; Gong et al., 2020).

Activation likelihood estimation analysis

The researchers employed Ginger ALE version 3.0.2 (brainmap.org/ale) to conduct a neural coordinate-based activation likelihood estimation (ALE) meta-analysis on the neuroimaging data. Using Montreal Neuroimaging Institute (MNI) coordinates or converting them into an MNI-based coordinate system ensured the consistency of the coordinates. We used a cluster-level inference threshold correction algorithm for the ALE calculation, with $p < 0.05$ as the cluster-forming threshold and $p < 0.05$ for cluster-level inference. The number of permutations was 5000 for all calculations of simple ALE maps. We did not perform subgroup analyses because of the small number of included studies, foci, and patients.

Results

Search results

The flow diagram of the process depicting the literature search and study selection is shown in Figure 1. Among the 495 articles found in the literature search, 437 were excluded after reviewing the abstract, and another 43 were rejected after reviewing their full text.

Our research identified a total of 15 articles that assessed the effect of acupuncture for musculoskeletal pain on brain activity. All of these studies used functional magnetic resonance imaging (fMRI) scans. The studies reporting ALFF and ReHo as a measure of fMRI with the voxel-wise method of extracting image data were analyzed using ALE. All of them used FWE for multiple comparisons corrections (Table 2).

ALE results

These studies yielded 183 foci of activation from 15 experiments. The ALE meta-analysis showed activated clusters in multiple cortical and sub-cortical brain structures in

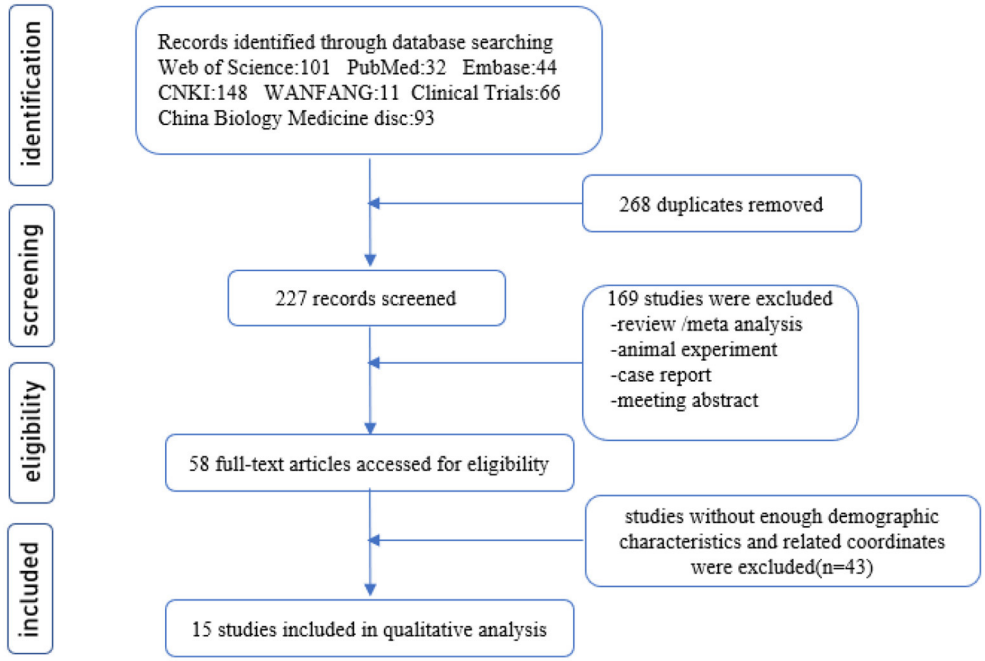

FIGURE 1
Flowchart shows the study selection process.

response to acupuncture across studies, including the thalamus, insula, caudate, claustrum, and lentiform nucleus (Table 3 and Figure 2).

Discussion

This study aims to use voxel-based meta-analysis to identify brain regions commonly activated during acupuncture for musculoskeletal pain in various experimental paradigms. It is interesting to note that the ALE analysis method used in this study could help us to achieve reliable and strong results instead of a gamut of less reproducible findings from the individual studies. The ALE meta-analysis revealed activated clusters in multiple cortical and sub-cortical brain structures in response to acupuncture across studies, including Cerebrum (bilaterally), Left Brainstem (Sub-lobar and Midbrain), and Gyrus (Thalamus, Insula, Caudate, Claustrum, Lentiform Nucleus).

The ALE results suggest a core modulation brain region cluster for musculoskeletal pain treatment by acupuncture, which not only clarifies the pain perception of acupuncture intervention as well as the up and down stable pathways of pain modulation (cerebellum and brainstem) (Moulton et al., 2010; Ruscheweyh et al., 2014; Napadow et al., 2019; Mercer Lindsay et al., 2021) but more importantly, the subcortical pain matrix brain region was found to be involved in the central modulation of acupuncture for musculoskeletal pain, which is an important reference value for others to conduct the research on the central modulation of acupuncture for visceral pain.

Neuroimaging has identified a set of brain regions that respond to noxious stimuli by observing the brain's perception of injurious stimuli and pain modulation. And these regions are often referred to as the "pain matrix," which includes the thalamus, anterior cingulate cortex, posterior cingulate cortex, insula, amygdala, primary and secondary somatosensory cortex (S1 and S2), and periaqueductal gray matter (Melzack, 1999; Apkarian et al., 2005; May, 2007). Among them, the thalamus and insula play an extremely important role in pain perception and pain processing. The activation of the thalamus is mainly connected with the first-order processing of sensory information. It receives signals from its periphery and sends them to the hypothalamus, insula, motor, and somatosensory cortex (Al-Chaer et al., 1996; Aziz et al., 2000; Olesen et al., 2016; Lee et al., 2019). It was found that the post-effects of acupuncture can cause changes in functional connectivity between important brain regions in the pain matrix, exerting the analgesic effects of acupuncture by decreasing the thalamus-anterior cingulate pain upload pathway and strengthening the ventral medial prefrontal-anterior cingulate descending inhibitory pathway (Roy et al., 2012). There is significant evidence that the insula plays a critical role in pain

TABLE 2 Overview of the 15 studies included in the meta-analyses.

Number	Study	Sample size (n)	Gender (M/F)	Age (years ± SD)	Reference space	Foci (n)	Threshold
1	Guo et al. (2015)	MP30	14/16	NA	Talairach	10	$p < 0.05$ cor
2	Chen et al. (2014a)	MP15	7/8	NA	MNI	10	$p < 0.05$ cor
3	Zou et al. (2019)	MP32	15/17	46.371 ± 10.025	MNI	7	$p < 0.05$ cor
		HC25	12/13	40.014 ± 9.765			
4	Qu et al. (2021)	MP80	28/52	52.35 ± 4.62	MNI	5	$p < 0.05$ cor
		HC80	30/50	53.01 ± 4.58			
5	Liu et al. (2013)	MP15	9/6	25.7 ± 2.3	MNI	7	$p < 0.05$ cor
		HC15	9/6	25.7 ± 2.3			
6	Hou et al. (2014)	MP49	19/30	24.73 ± 1.46	MNI	10	$p < 0.05$ cor
		HC19	8/11	25.58 ± 3.32			
7	Chen et al. (2014b)	MP30	17/13	58 ± 8	MNI	4	$p < 0.05$ cor
8	Shi et al. (2015)	MP28	17/11	NA	MNI	69	$p < 0.05$ cor
		HC28	17/11	NA			
9	Zhang et al. (2018)	MP20	10/10	53.33 ± 5.26	MNI	10	$p < 0.05$ cor
10	Jian et al. (2018)	MP46	27/19	61.3 ± 6.9	MNI	7	$p < 0.05$ cor
11	Gollub et al. (2018)	MP43	17/26	57 ± 7	MNI	8	$p < 0.05$ cor
12	Xiang et al. (2019)	MP12	7/5	44.42 ± 6.99	MNI	5	$p < 0.05$ cor
13	Chen et al. (2015)	MP30	17/13	58 ± 8	MNI	22	$p < 0.05$ cor
14	Tu et al. (2019)	MP80	35/45	39.5 ± 13.0	MNI	4	$p < 0.05$ cor
		HC74	39/35	36.9 ± 8.2			
15	Napadow et al. (2012)	MP17	0/17	29.8 ± 4.0	MNI	5	$p < 0.05$ cor

MP, musculoskeletal pain; HC, healthy control; NA, not available; M, male; F, female.

processing, which could cause pain perception after acupuncture intervention and integrate sensory information from visceral and motor activity with limbic system input (Moisset et al., 2010; Olesen et al., 2016; Lee et al., 2019). The activation of the insula indicates that in the acupuncture state, the brain accelerates the processing of pain information, carries out sensory integration more efficiently, provides timely feedback to the various stress systems of the organism, and speeds up the processing of pain stimulation so as to better relieve pain (Zhang et al., 2014). The ALE results also found a significant increase in activity in regions including the caudate, claustrum, and lentiform nucleus on fMRI scans following acupuncture. These regions are the main components of the basal ganglia (Graybiel, 2005; Kreitzer and Malenka, 2008; Yelnik, 2008). The function of the basal ganglia is to control autonomous movement and participate in advanced cognitive functions such as memory, emotion, and reward learning (Herrero et al., 2002; Nagy et al., 2006; Draganski et al., 2008). Previous studies have shown that the basal ganglia may be involved in most aspects of pain processing, including the cognitive dimension of pain and pain modulation (Chudler and Dong, 1995). Also, adequate modulation of the basal ganglia subregions may be related to autonomic dyskinesia caused by musculoskeletal pain (Borsook et al., 2010b). The combination of the above findings provides some support for the evidence of acupuncture's effectiveness in treating musculoskeletal pain diseases.

In general, up till now, a large number of neuroimaging studies have shown that patients with musculoskeletal pain exhibit structural and functional changes in brain regions. The majority of those regions are associated with multiple aspects of pain processing. Specifically, complex neuronal network interactions in the organism are required to form pain perception (Cai et al., 2018). When pain strikes, the brain temporarily and dynamically integrates multiple brain regions to process pain information. The "pain matrix" summarizes these brain regions involved in the process of pain. The brainstem, prefrontal, thalamus, insula, cingulate gyrus, subcortical areas, and somatosensory cortex are all part of this matrix, which is responsible for sensory, emotional, and cognitive functions (Apkarian et al., 2005). In this study, activated signals in some brain regions, such as the caudate, claustrum, and lentiform nucleus, were different from the healthy controls. Previous studies also present the potential for alterations in these brain regions. The cerebral cortex's function as an essential component of the pain modulation system has received widespread attention (Ong et al., 2019). We think that the thalamus, insula, caudate, claustrum, and

TABLE 3 All clusters from the ALE analysis.

Cluster #	x	y	z	ALE	P	Z	Label (nearest gray matter within 5 mm)
1	6	−30	−6	0.018043537	6.81E-06	4.3498716	Right Cerebrum. Sub-lobar. Thalamus. Gray Matter.*.
1	−38	−12	16	0.01651409	2.30E-05	4.074941	Left Cerebrum. Sub-lobar. Insula. Gray Matter. Brodmann area 13
1	−6	−16	−10	0.016219338	2.91E-05	4.0203114	Left Brainstem. Midbrain.*. Gray Matter. Substania Nigra
1	−16	−22	22	0.016133353	3.08E-05	4.006909	Left Cerebrum. Sub-lobar. Caudate. Gray Matter. Caudate Tail
1	−10	−24	22	0.014161315	1.22E-04	3.6681178	Left Cerebrum. Sub-lobar. Thalamus. Gray Matter.*.
1	−32	0	24	0.01126301	6.64E-04	3.209974	Left Cerebrum. Sub-lobar. Insula. Gray Matter. Brodmann area 13
1	−32	−10	20	0.01083934	8.38E-04	3.142502	Left Cerebrum. Sub-lobar. Insula. Gray Matter. Brodmann area 13
1	−40	0	20	0.010651297	9.42E-04	3.1080759	Left Cerebrum. Sub-lobar. Insula. Gray Matter. Brodmann area 13
1	−12	−24	−6	0.010418649	0.001067337	3.0708263	Left Brainstem. Midbrain.*. Gray Matter. Substania Nigra
1	−22	−10	14	0.010235265	0.001180531	3.0406013	Left Cerebrum. Sub-lobar. Thalamus. Gray Matter. Ventral Lateral Nucleus
1	−2	−30	−4	0.010192102	0.001216393	3.0315785	Left Cerebrum. Sub-lobar. Thalamus. Gray Matter. Pulvinar
1	−10	−22	0	0.010163731	0.001239598	3.0258691	Left Cerebrum. Sub-lobar. Thalamus. Gray Matter. Mammillary Body
1	−20	−22	0	0.009802071	0.001657662	2.9368799	Left Cerebrum. Sub-lobar. Thalamus. Gray Matter. Ventral Posterior Lateral Nucleus
1	−36	−12	2	0.009715884	0.001762938	2.9177318	Left Cerebrum. Sub-lobar. Claustrum. Gray Matter.*.
1	−22	−18	10	0.009700557	0.001803456	2.9106383	Left Cerebrum. Sub-lobar. Thalamus. Gray Matter.*.

*Clusters outside the brain atlas.

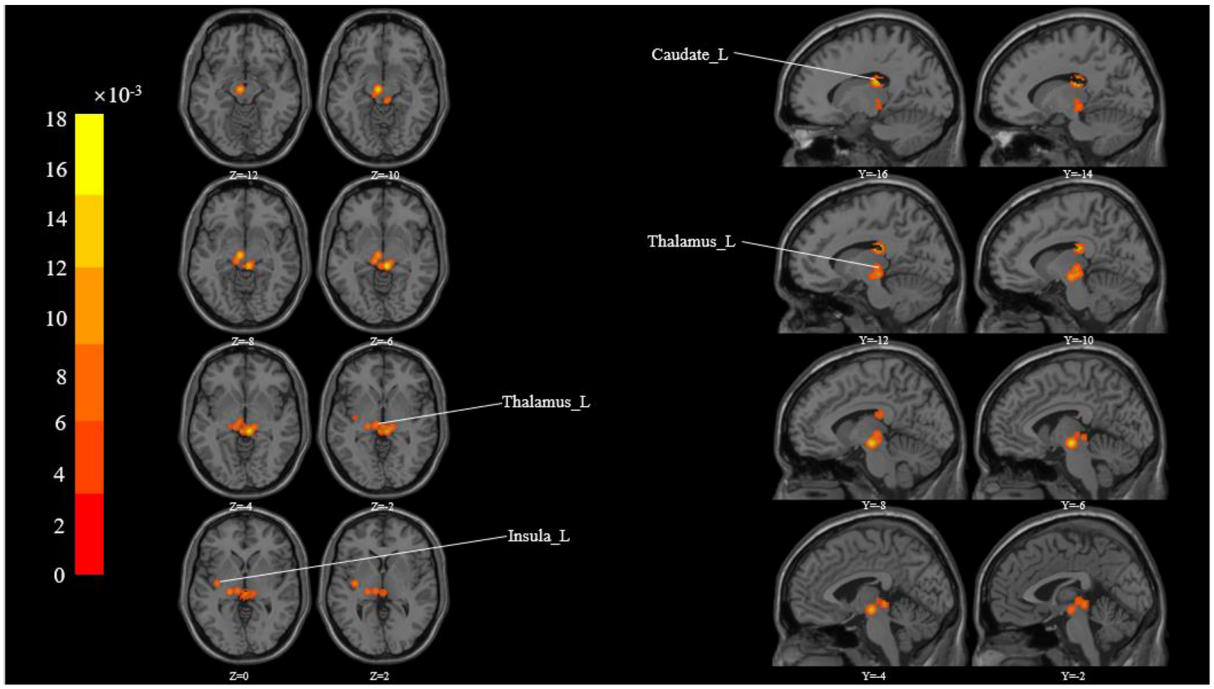

FIGURE 2
All activation likelihood estimate results for studies measuring. Cluster-level Inference P-FWE < 0.05, Permutations = 5000 Cluster-Forming P- Uncorrected < 0.05.

lentiform nucleus are crucial to the acupuncture mechanism previous studies have found that the effect of acupuncture is to elevate mechanical pain thresholds, change signaling levels in several important pain pathway areas, and have positive impacts on a variety of pain syndromes and states (Baeumler et al., 2014). To truly obtain sufficient evidence of acupuncture's effectiveness in treating musculoskeletal pain diseases, a greater homogeneity of the different study populations, experimental paradigms, and data analysis pipelines must be sought. The complexity of musculoskeletal

pain diseases was a prevalent concern in the process of including the literature, and we repeated the ALE analysis after excluding studies with sample sizes <10 to remove the bias introduced by small-study effects. It was also specified that the included studies had to be fMRI whole-brain analyses. Although the paradigms in our study were not identical, the approach to functional neuroimaging techniques was similar enough across studies to warrant comparison. In this study, ALE analysis was used to determine the probability of brain regions being activated or deactivated by integrating the study coordinates of all screened studies. The results provided feedback information for the mechanisms of brain function with acupuncture for musculoskeletal pain. Furthermore, evaluating the study results may indicate the considerable modulatory effects of acupuncture for musculoskeletal pain, which is consistent with the "pain matrix" theory. These findings provide various new insights into the processes of acupuncture for musculoskeletal pain and a possible explanation for the therapy's clinical efficacy.

Limitations

Subjects included in the analysis came from heterogeneous musculoskeletal pain disorders. Consequently, our findings merely give a glimpse into the mechanism of acupuncture's effect on musculoskeletal pain.

Conclusion

The ALE meta-analysis revealed activated clusters in multiple cortical and sub-cortical brain structures, especially basal ganglia, in response to acupuncture across studies.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Author contributions

GH drafted the manuscript and designed the study under the guidance of LL and FZ. GH and ZT performed the study extraction and meta-analysis. JC, SW, AL, and NL helped in literature search and data analyses. YL and JT offered good suggestions. LL and FZ revised the manuscript. All authors contributed toward revising the manuscript and gave the final approval of the version to be published.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2022.906875/full#supplementary-material

References

Abdulmonem, A., Hanan, A., Elaf, A., Haneen, T., and Jenan, A. (2014). The prevalence of musculoskeletal pain & its associated factors among female Saudi school teachers. *Pak. J. Med. Sci.* 30, 1191–1196. doi: 10.12669/pjms.306.5778

Al-Chaer, E. D., Lawand, N. B., Westlund, K. N., and Willis, W. D. (1996). Visceral nociceptive input into the ventral posterolateral nucleus of the thalamus: a new function for the dorsal column pathway. *J. Neurophysiol.* 76, 2661–2674. doi: 10.1152/jn.1996.76.4.2661

Apkarian, A. V., Bushnell, M. C., Treede, R. D., and Zubieta, J. K. (2005). Human brain mechanisms of pain perception and regulation in health and disease. *Eur. J. Pain* 9, 463–484. doi: 10.1016/j.ejpain.2004.11.001

Aziz, Q., Schnitzler, A., and Enck, P. (2000). Functional neuroimaging of visceral sensation. *J. Clin. Neurophysiol.* 17, 604–612. doi: 10.1097/00004691-200011000-00006

Baeumler, P. I., Fleckenstein, J., Takayama, S., Simang, M., Seki, T., and Irnich, D. (2014). Effects of acupuncture on sensory perception: a systematic review and meta-analysis. *PLoS ONE* 9:e113731. doi: 10.1371/journal.pone.0113731

Borsook, D., Sava, S., and Becerra, L. (2010a). The pain imaging revolution: advancing pain into the 21st century. *Neuroscientist* 16, 171–185. doi: 10.1177/1073858409349902

Borsook, D., Upadhyay, J., Chudler, E. H., and Becerra, L. (2010b). A key role of the basal ganglia in pain and analgesia–insights gained through human functional imaging. *Mol. Pain* 6, 27. doi: 10.1186/1744-8069-6-27

Cai, R. L., Shen, G. M., Wang, H., and Guan, Y. Y. (2018). Brain functional connectivity network studies of acupuncture: a systematic review on resting-state fMRI. *J. Integr. Med.* 16, 26–33. doi: 10.1016/j.joim.2017.12.002

Chae, Y., Chang, D. S., Lee, S. H., Jung, W. M., Lee, I. S., Jackson, S., et al. (2013). Inserting needles into the body: a meta-analysis of brain activity associated with acupuncture needle stimulation. *J. Pain* 14, 215–222. doi: 10.1016/j.jpain.2012.11.011

Chen, J., Liu, G., Chen, J., Liu, X., Liu, X., and Ling, Y. (2014a). Resting state fMRI study of different acupuncture analgesia therapies. *Chin. J. Gerontol.* 33, 2977–2979. doi: 10.1155/2021/6616060

Chen, X., Spaeth, R. B., Freeman, S. G., Scarborough, D. M., Hashmi, J. A., Wey, H. Y., et al. (2015). The modulation effect of longitudinal acupuncture on resting state functional connectivity in knee osteoarthritis patients. *Mol Pain* 11, 67. doi: 10.1186/s12990-015-0071-9

Chen, X., Spaeth, R. B., Retzepi, K., Ott, D., and Kong, J. (2014b). Acupuncture modulates cortical thickness and functional connectivity in knee osteoarthritis patients. *Sci. Rep.* 4, 6482. doi: 10.1038/srep06482

Chudler, E. H., and Dong, W. K. (1995). The role of the basal ganglia in nociception and pain. *Pain* 60, 3–38. doi: 10.1016/0304-3959(94)00172-B

Draganski, B., Kherif, F., Klöppel, S., Cook, P. A., Alexander, D. C., Parker, G. J., et al. (2008). Evidence for segregated and integrative connectivity patterns in the human Basal Ganglia. *J. Neurosci.* 28, 7143–7152. doi: 10.1523/JNEUROSCI.1486-08.2008

Farmer, M. A., Baliki, M. N., and Apkarian, A. V. (2012). A dynamic network perspective of chronic pain. *Neurosci. Lett.* 520, 197–203. doi: 10.1016/j.neulet.2012.05.001

Garcia-Larrea, L., and Peyron, R. (2013). Pain matrices and neuropathic pain matrices: a review. *Pain* 154(Suppl 1), S29–s43. doi: 10.1016/j.pain.2013.09.001

Gollub, R. L., Kirsch, I., Maleki, N., Wasan, A. D., Edwards, R. R., Tu, Y., et al. (2018). A functional neuroimaging study of expectancy effects on pain response in patients with knee osteoarthritis. *J. Pain.* 19, 515–527. doi: 10.1016/j.jpain.2017.12.260

Gong, J., Wang, J., Luo, X., Chen, G., Huang, H., Huang, R., et al. (2020). Abnormalities of intrinsic regional brain activity in first-episode and chronic schizophrenia: a meta-analysis of resting-state functional MRI. *J. Psychiatry Neurosci.* 45, 55–68. doi: 10.1503/jpn.180245

Graybiel, A. M. (2005). The basal ganglia: learning new tricks and loving it. *Curr. Opin. Neurobiol.* 15, 638–644. doi: 10.1016/j.conb.2005.10.006

Guo, T., Xiao, S., Chu, W., and Li, M. (2015). Changes of different analgesic acupuncture treatments for regional homogeneity of brain activity in resting state by functional magnetic resonance imaging and the similarities and differences between their mechanisms. *J. Clin. Acupunct. Moxibust.* 31, 25–27.

Henry, D. E., Chiodo, A. E., and Yang, W. (2011). Central nervous system reorganization in a variety of chronic pain states: a review. *PMR* 3, 1116–1125. doi: 10.1016/j.pmrj.2011.05.018

Herrero, M. T., Barcia, C., and Navarro, J. M. (2002). Functional anatomy of thalamus and basal ganglia. *Childs. Nerv. Syst.* 18, 386–404. doi: 10.1007/s00381-002-0604-1

Hou, X., Chen, W., Chen, J., Zhang, D., Liu, X., and Liu, B. (2014). The study of regional homogeneity of DMN in patients with CSNP after acupuncture in group acupoint. *Chin. J. Magn. Resonan. Imag.* 5, 436–440.

Jian, K., Zengjian, W., Jaclyn, L., Domenic, M., Robert, E., Irving, K., et al. (2018). Enhancing treatment of osteoarthritis knee pain by boosting expectancy: A functional neuroimaging study. *NeuroImage Clin.* 18, 325–344. doi: 10.1016/j.nicl.2018.01.021

Kreitzer, A. C., and Malenka, R. C. (2008). Striatal plasticity and basal ganglia circuit function. *Neuron* 60, 543–554. doi: 10.1016/j.neuron.2008.11.005

Lawrence, R. C., Helmick, C. G., Arnett, F. C., Deyo, R. A., Felson, D. T., Giannini, E. H., et al. (1998). Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. *Arthritis Rheum* 41, 778–799. doi: 10.1002/1529-0131(199805)41:5<778::AID-ART4>3.0.CO;2-V

Lee, I. S., Cheon, S., and Park, J. Y. (2019). Central and peripheral mechanism of acupuncture analgesia on visceral pain: a systematic review. *Evid. Based Complement. Alternat. Med.* 2019:1304152. doi: 10.1155/2019/1304152

Lenoir, D., De Pauw, R., Van Oosterwijck, S., Cagnie, B., and Meeus, M. (2020). Acupuncture versus sham acupuncture: a meta-analysis on evidence for longer-term effects of acupuncture in musculoskeletal disorders. *Clin. J. Pain* 36, 533–549. doi: 10.1097/AJP.0000000000000812

Li, S., Hu, N., Zhang, W., Tao, B., Dai, J., Gong, Y., et al. (2019). Dysconnectivity of multiple brain networks in schizophrenia: a meta-analysis of resting-state functional connectivity. *Front. Psychiatry* 10, 482. doi: 10.3389/fpsyt.2019.00482

Liu, Z., Wu, W., Zhang, S., Guo, S., and Yang, J. (2013). Pain matrix response to acupuncture stimuli in individuals with acute low back pain: an fmri study. *Chin. J. Pain Med.* 19, 201–205.

May, A. (2007). Neuroimaging: visualising the brain in pain. *Neurol Sci* 28(Suppl 2), S101–107. doi: 10.1007/s10072-007-0760-x

Melhorn, J. M. (2014). "Epidemiology of musculoskeletal disorders and workplace factors," in: *Handbook of Musculoskeletal Pain and Disability Disorders in the Workplace*, eds R. J. Gatchel and I. Z. Schultz (New York, NY: Springer New York), 175–204. doi: 10.1007/978-1-4939-0612-3_10

Melzack, R. (1999). From the gate to the neuromatrix. *Pain Suppl.* 6, S121–s126. doi: 10.1016/S0304-3959(99)00145-1

Mercer Lindsay, N., Chen, C., Gilam, G., Mackey, S., and Scherrer, G. (2021). Brain circuits for pain and its treatment. *Sci. Transl. Med.* 13, eabj7360. doi: 10.1126/scitranslmed.abj7360

Mitsi, V., and Zachariou, V. (2016). Modulation of pain, nociception, and analgesia by the brain reward center. *Neuroscience* 338, 81–92. doi: 10.1016/j.neuroscience.2016.05.017

Moisset, X., Bouhassira, D., Denis, D., Dominique, G., Benoit, C., and Sabat,é, J. M. (2010). Anatomical connections between brain areas activated during rectal distension in healthy volunteers: a visceral pain network. *Eur. J. Pain* 14, 142–148. doi: 10.1016/j.ejpain.2009.04.011

Moulton, E. A., Schmahmann, J. D., Becerra, L., and Borsook, D. (2010). The cerebellum and pain: passive integrator or active participator? *Brain Res. Rev.* 65, 14–27. doi: 10.1016/j.brainresrev.2010.05.005

Mu, J., Furlan, A. D., Lam, W. Y., Hsu, M. Y., Ning, Z., and Lao, L. (2020). Acupuncture for chronic nonspecific low back pain. *Cochrane Database Syst. Rev.* 12, Cd013814. doi: 10.1002/14651858.CD013814

Nagy, A., Eördegh, G., Paróczy, Z., Márkus, Z., and Benedek, G. (2006). Multisensory integration in the basal ganglia. *Eur. J. Neurosci.* 24, 917–924. doi: 10.1111/j.1460-9568.2006.04942.x

Napadow, V., Kim, J., Clauw, D. J., and Harris, R.E. (2012). Decreased intrinsic brain connectivity is associated with reduced clinical pain in fibromyalgia. *Arthritis Rheum.* 64, 2398–2403. doi: 10.1002/art.34412

Napadow, V., Sclocco, R., and Henderson, L. A. (2019). Brainstem neuroimaging of nociception and pain circuitries. *Pain Rep* 4, e745. doi: 10.1097/PR9.0000000000000745

Olesen, A. E., Farmer, A. D., Olesen, S. S., Aziz, Q., and Drewes, A. M. (2016). Management of chronic visceral pain. *Pain Manag.* 6, 469–486. doi: 10.2217/pmt-2015-0011

Ong, W. Y., Stohler, C. S., and Herr, D. R. (2019). Role of the Prefrontal Cortex in Pain Processing. *Mol. Neurobiol.* 56, 1137–1166. doi: 10.1007/s12035-018-1130-9

Qaseem, A., Wilt, T. J., McLean, R. M., Forciea, M. A., Denberg, T. D., Barry, M. J., et al. (2017). noninvasive treatments for acute, subacute, and chronic low back pain: a clinical practice guideline from the American College of Physicians. *Ann. Intern. Med.* 166, 514–530. doi: 10.732 6/M16-2367

Qu, B., Wang, H., Zhao, C., and Shi, G. (2021). Clinical efficacy evaluation and central mechanism study of acupuncture in treating chronic knee osteoarthritis. *J. Xinjiang Med. Univ.* 44, 600–604.

Roy, M., Shohamy, D., and Wager, T. D. (2012). Ventromedial prefrontal-subcortical systems and the generation of affective meaning. *Trends Cogn. Sci.* 16, 147–156. doi: 10.1016/j.tics.2012.01.005

Ruscheweyh, R., Kühnel, M., Filippopulos, F., Blum, B., Eggert, T., and Straube, A. (2014). Altered experimental pain perception after cerebellar infarction. *Pain* 155, 1303–1312. doi: 10.1016/j.pain.2014.04.006

Shi, Y., Liu, Z., Zhang, S., Li, Q., Guo, S., Yang, J., et al. (2015). Brain network response to acupuncture stimuli in experimental acute low back pain: An fMRI Study. *Evid. Based Complement Alternat. Med.* 2015, 210120. doi: 10.1155/2015/210120

Skootsky, S. A., Jaeger, B., and Oye, R. K. (1989). Prevalence of myofascial pain in general internal medicine practice. *West. J. Med.* 151, 157–160.

Tu, Y., Jung, M., Gollub, R. L., Napadow, V., Gerber, J., Ortiz, A., et al. (2019). Abnormal medial prefrontal cortex functional connectivity and its association with clinical symptoms in chronic low back pain. *Pain* 160, 1308–1318. doi: 10.1097/j.pain.0000000000001507

Ussai, S., Miceli, L., Pisa, F. E., Bednarova, R., Giordano, A., Della Rocca, G., et al. (2015). Impact of potential inappropriate NSAIDs use in chronic pain. *Drug Des. Devel. Ther.* 9, 2073–2077. doi: 10.2147/DDDT.S80686

Villarreal Santiago, M., Tumilty, S., Macznik, A., and Mani, R. (2016). Does acupuncture alter pain-related functional connectivity of the central nervous system? A systematic review. *J. Acupunct. Meridian. Stud.* 9, 167–177. doi: 10.1016/j.jams.2015.11.038

Wolfe, F., Ross, K., Anderson, J., Russell, I. J., and Hebert, L. (1995). The prevalence and characteristics of fibromyalgia in the general population. *Arthritis Rheum.* 38, 19–28. doi: 10.1002/art.1780380104

Xiang, A., Yu, Y., Jia, X., Ma, H., Liu, H., Zhang, Y., et al. (2019). The low-frequency BOLD signal oscillation response in the insular associated to immediate analgesia of ankle acupuncture in patients with chronic low back pain. *J. Pain Res.* 12, 841–850. doi: 10.2147/JPR.S189390

Yelnik, J. (2008). Modeling the organization of the basal ganglia. *Rev. Neurol. (Paris)* 164, 969–976. doi: 10.1016/j.neurol.2008.04.019

Yuan, Q. L., Wang, P., Liu, L., Sun, F., Cai, Y. S., Wu, W. T., et al. (2016). Acupuncture for musculoskeletal pain: A meta-analysis and meta-regression of sham-controlled randomized clinical trials. *Sci. Rep.* 6, 30675. doi: 10.1038/srep30675

Zhang, S., Wang, X., Yan, C. Q., Hu, S. Q., Huo, J. W., Wang, Z. Y., et al. (2018). Different mechanisms of contralateral- or ipsilateral-acupuncture to modulate the brain activity in patients with unilateral chronic shoulder pain: a pilot fMRI study. *J. Pain Res.* 11, 505–514. doi: 10.2147/JPR.S152550

Zhang, S. S., Wu, W., Liu, Z. P., Huang, G. Z., Guo, S. G., and Yang, J. M. (2014). Altered regional homogeneity in experimentally induced low back pain: a resting-state fMRI study. *J. Neuroeng. Rehabil.* 11, 115. doi: 10.1186/1743-0003-11-115

Zhang, Y., and Wang, C. (2020). Acupuncture and Chronic Musculoskeletal Pain. *Curr. Rheumatol. Rep.* 22, 80. doi: 10.1007/s11926-020-00954-z

Zou, Y., Tang, W., Wang, S., Huang, J., and Li, J. (2019). Objective evaluation on brain network imaging of treating same disease with different methods effect of acupuncture for chronic low back pain (cLBP). *Fudan Univ. J. Med. Sci.* 46, 167–173. doi: 10.3969/j.issn.1672-8467.2019.02.004

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The CX-DZ-II intelligent electronic stimulator for neck pain caused by cervical spondylosis: A two-center, randomized, controlled, and non-inferiority trial

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Background: Electroacupuncture (EA) has been commonly used for the management of neck pain caused by cervical spondylosis (NPCS); however, current electrical instruments have limitations on intelligence, digitalization, and visualization. The intelligent electronic stimulator (CX-DZ-II) is a digital device with an evidence-based diagnosis and treatment system. This study aimed to investigate the efficacy and safety of the CX-DZ-II intelligent EA instrument for NPCS.

Materials and Methods: A total of 164 patients with NPCS [mean age (SD), 49.48 (13.47) years] were randomly assigned to receive 8 sessions (over 2 weeks) EA of the intelligent electronic stimulator (CX-DZ-II) or the regular electronic stimulator (SDZ-II). The primary outcome was the change of the visual analog scale (VAS) from baseline to 2 weeks of treatment. Secondary outcomes included mean scores of the VAS after each treatment in 1 week, responder rate, drug-usage rate of non-steroidal antipyretic analgesics (NSAAs), the occurrence rate of adverse events (AEs), proportions of apparatus with defect during treatment, and excellent rate of apparatus.

Results: The intelligent electronic stimulator (CX-DZ-II) was non-inferior to the regular electronic stimulator (SDZ-II) for changes from baseline in the VAS [3.36 vs. 3.23, with a difference of 0.17 (95% CI, −0.36 to 0.69), $P < 0.025$ for non-inferiority]. No between-group differences were found in outcomes of VAS in 1 week, overall responders, and drug-usage rate of NSAAs. The defect rate and excellent rate of the instrument were similar in the CX-DZ-II and SDZ-II groups. Adverse events occurred in 9 (10.84%) patients in the CX-DZ-II group and 4 (5.00%) patients in the SDZ-II group.

Conclusion: The intelligent electronic stimulator (CX-DZ-II) was non-inferior to the regular electronic stimulator (SDZ-II) in relieving neck pain.

The intelligent electronic stimulator (CX-DZ-II) is a promising non-inferior alternative instrument for NPCS.

Clinical Trial Registration: [https://clinicaltrials.gov/], identifier [NCT03005301].

KEYWORDS

electroacupuncture, neck pain, cervical spondylosis, randomized controlled trial, non-inferiority trial

Introduction

Cervical spondylosis (CS) is an age-related degenerative condition presenting structural or functional damage to the cervical spinal cord, nerve roots, and adjacent blood vessels. Worldwide, the prevalence of CS increases by year and affects more younger people. A previous study showed that engaging in mental work, high housework intensity, and sleep duration of less than 7 h/day were the main contributors to the incidence of CS (Lv et al., 2018). Neck pain (NP) is the most common complaint of patients with CS (Vogt et al., 2006). According to the guidelines from America, Netherlands, and Denmark, CS is one of the main causes of NP (Blanpied et al., 2017; Kjaer et al., 2017; Bier et al., 2018). In the 2017 global burden of disease study, the incidence of NP per 100,000 population was 806.6 and the years lived with disability from NP per 100,000 population was 352.0 (Safiri et al., 2020). With the increasing costs and the long-lasting disability associated with CS, conducting research on the effectiveness of interventions designed to prevent and treat CS is crucial (Kuo and Tadi, 2022). Currently, a range of non-surgical treatments for NP caused by CS has been recommended, such as manual therapy, exercise, psychological therapies, and acupuncture (Chinese Medical Association, 2007; Corp et al., 2021).

Acupuncture is a physical intervention used for various pain management. Electroacupuncture (EA) is a prevalent therapy of acupuncture, with the integrated effect of a manual needle and electrical stimulation from instruments. Currently, EA has been widely used for various pain diseases, including chronic non-specific low back pain, post-operative pain, musculoskeletal pain, and pain relief during colonoscopy (Comachio et al., 2020; Huang et al., 2021b; Joan Gan et al., 2021; Mao et al., 2021). Especially, findings of the previous meta-analysis indicated that acupuncture might be effective for NP and EA that may relieve even more pain (Seo et al., 2017; Huang et al., 2021c). EA blocks pain by activating a variety of bioactive chemicals through peripheral, spinal, and supraspinal mechanisms (Zhang et al., 2014).

Electric stimulators are indispensable devices for EA therapy. Since the 1950s, EA instruments with multiple presentation formats had been innovated for clinical treatment, such as wearable devices, single-acupoint electronic apparatuses, and apparatus equipped with manual acupuncture techniques (Hong et al., 2006; Liu et al., 2010; Shen et al., 2016; Feng et al., 2019). Among them, the regular electronic stimulator (SDZ-II) has been widely used in various conditions management and scientific research (Luo, 2014; Su et al., 2016; Huang et al., 2021a), with qualified parameters (Wang, 2019). According to a previous report, the SDZ-II electronic stimulator was also effective for NP relief for patients with CS (Tang et al., 2014). Nonetheless, similar to other devices, the SDZ-II electronic stimulator has deficiencies in parameter adjustment, intelligence, digitalization, and visualization (Yang and Yong, 2009; Liu et al., 2016).

The intelligent electronic stimulator (CX-DZ-II) is a new intelligent device with an evidence-based diagnosis and treatment system (Liang et al., 2017). It is equipped with a display terminal and then accurate settings such as pulse waveform, frequency, intensity, and treatment time that can be provided to users visually. More importantly, the information can also be uploaded instantly, if WiFi is available (Jia et al., 2016). Generally, it owns the advantages of accurate parameter adjustment, visual data storage, and remote data management. Therefore, this study aimed to assess the effect and safety of the intelligent electronic stimulator (CX-DZ-II) in comparison with the regular electronic stimulator (SDZ-II) for NP relief in patients with CS and then provide an alternative for CS management.

Materials and methods

Study design

This randomized, non-inferiority trial (NCT03005301) was performed at the Hospital of Chengdu University of Traditional Chinese Medicine and the West China Hospital, Sichuan University. The study protocol has been approved by the

Sichuan Regional Ethical Review Committee, affiliated with Chengdu University of Traditional Chinese Medicine (Approval No. 2016XL-007).

The total observation period in this study was 2 weeks for each participant. Eligible participants were randomly assigned to receive EA from the CX-DZ-II intelligent electronic stimulator or the SDZ-II regular electronic stimulator. All the outcome measurements were completed at baseline and 2 weeks after randomization. In addition, the visual analog scales (VASs) for each participant were also assessed after each treatment in the first week.

Participants

Patients with CS of neck type or nerve root type were recruited through outpatient clinics from April 2017 to August 2017.

The diagnostic criteria of CS were established according to the Clinical Guidelines for Diagnosis and Treatment: Pain (Chinese Medical Association, 2007): (1) CS of neck type: pain in the neck, the shoulder, and the occipitalia; limited cranial movement with tense muscles and trigger points; X-rays showed that there were changes in cervical curvature, and dynamic radiographs showed instability and loosening of the intervertebral joints. (2) CS of nerve root type: neck, shoulder, and back pain, and even radiation of arm pain to forearm and fingers; sensation of electric touch, numbness, and obvious hypoesthesia in the nerve root innervation area; results of Eaton test were positive; X-rays showed that there were changes in uncinate joint hyperplasia, bone spur formation, narrowing of intervertebral space, and changes and even loss of physiological radians.

Participants who met all the following inclusion criteria were enrolled in this study: (1) male or female between 18 and 75 years of age; (2) a history of recurrent episodes of NP (one or more episodes of NP per month lasting for more than 3 months); (3) the VAS score >3; (4) subject or his supervisor can comprehend the aims and process of this trial; (5) not participating in other trials or receiving other relevant treatments during the trial periods; and (6) willing to sign informed consent. Participants with any of the following conditions were excluded: (1) subject has acute neck trauma; (2) previous medical history of neck trauma treated by surgery, neurological deficit, congenital and developmental spinal disorders, systemic bone diseases, or systemic joint diseases; (3) diagnosis of carotid artery dissection; (4) unable to clearly perceive the pain or express their feelings; (5) infection in the acupoint region; (6) history of acupuncture treatment for NP in the previous week; (7) use of non-steroidal antipyretic analgesics (NSAAs) in the previous 3 days, need to use central analgesics or narcotic analgesics during the period of clinical trial, use of any ointments/medicinal liquors with functions of promoting blood circulation and easing pain, and use of oral and intravenous medicines aiming at opening blood vessels and providing nerve nutrition; (8) combinations of severe diseases, such as myocardial infarction, severe hepatic renal dysfunction, acute infectious diseases, malignant tumors, or severe mental disorders in the previous 12 months; (9) intolerance of acupuncture and EA treatment or allergy to acupuncture; (10) pregnancy or lactation; (11) participation in other clinical trials in the previous 3 months, and (12) unsuitability to this trial as judged by the investigators.

Randomization and masking

Eligible participants were randomized according to a computer-generated randomization list in sealed, opaque envelopes and were divided into two groups: the CX-DZ-II and SDZ-II groups. The randomization was stratified by enrollment site in a block size of 4 with a 1:1 ratio. The randomization list was conserved by the physicians not participating in the study. Participants, acupuncturists, and outcome assessors were not blinded because of the obvious difference in operating interface, shape, and appearance of the two study instruments. Only statisticians were blinded to treatment allocation.

Interventions

Disposable acupuncture needles (0.35 mm $\times$ 25 mm, 0.35 mm $\times$ 40 mm, Huatuo, Suzhou Hualun Medical Appliance), the CX-DZ-II intelligent electronic stimulator (Chengdu Chengxin High-tech Company, Chengdu, China), and the SDZ-II regular electronic stimulator (Suzhou Medical Appliance Factory, registration No. 20133370611) were used.

All the treatments were performed by licensed acupuncturists. Participants in the treatment group received EA at basic acupoints: Dazhui (GV14), bilateral Fengchi (GB20), Jianjin (GB21), and Jiaji (EX-B2). Manual acupuncture was also performed at arbitrary acupoints, which were chosen based on syndrome differentiation: for participants with wind-cold dampness Bi syndrome, bilateral Fengmen (BL12), and Waiguan (TE5) were used; for participants with phlegm stasis in channels syndrome, bilateral Quchi (LI11), Pishu (BL20), Fenglong (ST40), and Geshu (BL17) were used; for participants diagnosed with deficiency of qi and blood syndrome, bilateral Ganshu (BL18), BL20, and Zusanli (ST36) were used; for participants with deficiency of Gan (liver) and Shen (kidney) syndrome, bilateral Yanglao (SI6), BL18, Shenshu (BL23), and Taixi (KI3) were used. The

depth of the inserted needles differed but was approximately 15–40 mm. Following needle insertion, lifting, thrusting, twisting, and rotating, with a frequency of 60–90 times per min were performed on all the needles to achieve the *deqi* sensation. Two paired electrodes from the CX-DZ-II intelligent electronic stimulator were attached transversely to the needle handles at bilateral GB20 and EX-B2. The EA stimulation lasted for 30 min, with a dilatational wave of 2/100 Hz and a current intensity within the patient's tolerance. Participants in the CX-DZ-II group received a total of 8 sessions of treatments: 5 sessions (every day) in the first week and 3 sessions (every other day) in the second week.

Participants in the control group received EA from the SDZ-II regular electronic stimulator. In the SDZ-II group, acupoints selection, depth of needles, needle manipulation for *deqi*, and electrode placements were consistent with the CX-DZ-II group.

Outcomes measurement

The primary outcome was the change in the VAS from baseline to the completion of treatment.

Secondary outcomes included: (1) mean scores of the VAS after each treatment in the first week; (2) responder rate of participants with at least 70% increase from baseline in the treatment score, which was assessed based on the table for lower lumbar vertebral diseases of the Japanese Orthopedic Association; (3) drug-usage rate of NSAAs during treatment; (4) the occurrence rate of adverse events (AEs); (5) proportions of apparatus with defect during treatment; and (6) excellent rate of apparatus, defined as the proportions of instrument assessment with a score <16 points. The scores of the instrument were evaluated after each treatment according to the predesigned operating performance scale (1–5 indicating extremely difficult to easy) (see **Supplementary Table 1**).

Statistical analysis

Based on a pilot study using the SDZ-II regular electronic stimulator for NP coursed by CS, the decline in the VAS score after a course of treatment was 5.18 ± 1.06 (Tang et al., 2014); considering clinical experience, we set -0.53 as the non-inferior margin. Thus, 160 participants were needed to provide 80% power to detect a difference between groups in the VAS score declination after treatment at a one-sided significance level of 0.025, assuming a 20% loss in the dropout rate (Chen et al., 2020).

Outcomes were analyzed according to the intention-to-treat principle, defined as all the randomized participants with baseline data receiving at least one treatment. The primary outcome was also assessed based on the per-protocol (PP) population, defined as all randomized participants without major protocol violations. The primary outcome was assessed using a one-tailed test at a significance level of 0.025, while the secondary outcomes used a two-tailed test at a significance level of 0.05. For the change in mean scores of the VAS after treatment, the *t*-test was used. The mean VAS score after each treatment was analyzed using repeated-measures ANOVA, setting group, time, and the interaction between group and time as fixed effects and center as covariates. For responder rate, the Cochran–Mantel–Haenszel test, stratified by site, was used to test a hierarchical comparison between groups. Drug-usage rate, the occurrence rate of AEs, defect rate, and excellent rate of the instrument between the two groups were compared using the chi-squared test or Fisher's exact test.

Missing data for the primary outcome were imputed from the last observation carried forward. For secondary outcomes, no imputation was used. The results based on PP set were used as sensitivity analysis.

All the analyses were performed by SAS version 9.1 (SAS Institute Incorporation, NC, United States).

Results

Populations and characteristics

Among 185 participants screened, 164 (83 in the CX-DZ-II group, 81 in the SDZ-II group) participants were randomized (**Figure 1**). A total of 6 (3.66%) participants dropped out: 1 withdrew before the first treatment and 5 dropped out during the treatment period. For the primary outcome, data were imputed at 4.82 and 0% of participants in the CX-DZ-II and SDZ-II groups. There were no significant differences between groups in terms of gender, age, height, weight, types of CS, coexisting illness, other treatments, participants using other medicine, and the VAS score (**Table 1**).

Clinical outcomes

The changes from baseline in the VAS throughout 2 weeks were 3.36 in the CX-DZ-II group and 3.23 in the SDZ-II group (difference: 0.17; 95% CI, -0.36 to 0.69, $p < 0.025$ for non-inferiority); similar results were found in the PP set (PPS) (difference: 0.28; 95% CI, -0.38 to 0.93, $p < 0.025$ for non-inferiority). The differences were within the prespecified non-inferiority margin of -0.53, demonstrating that the CX-DZ-II intelligent EA instrument was non-inferiority to the SDZ-II regular electronic stimulator (**Table 2**).

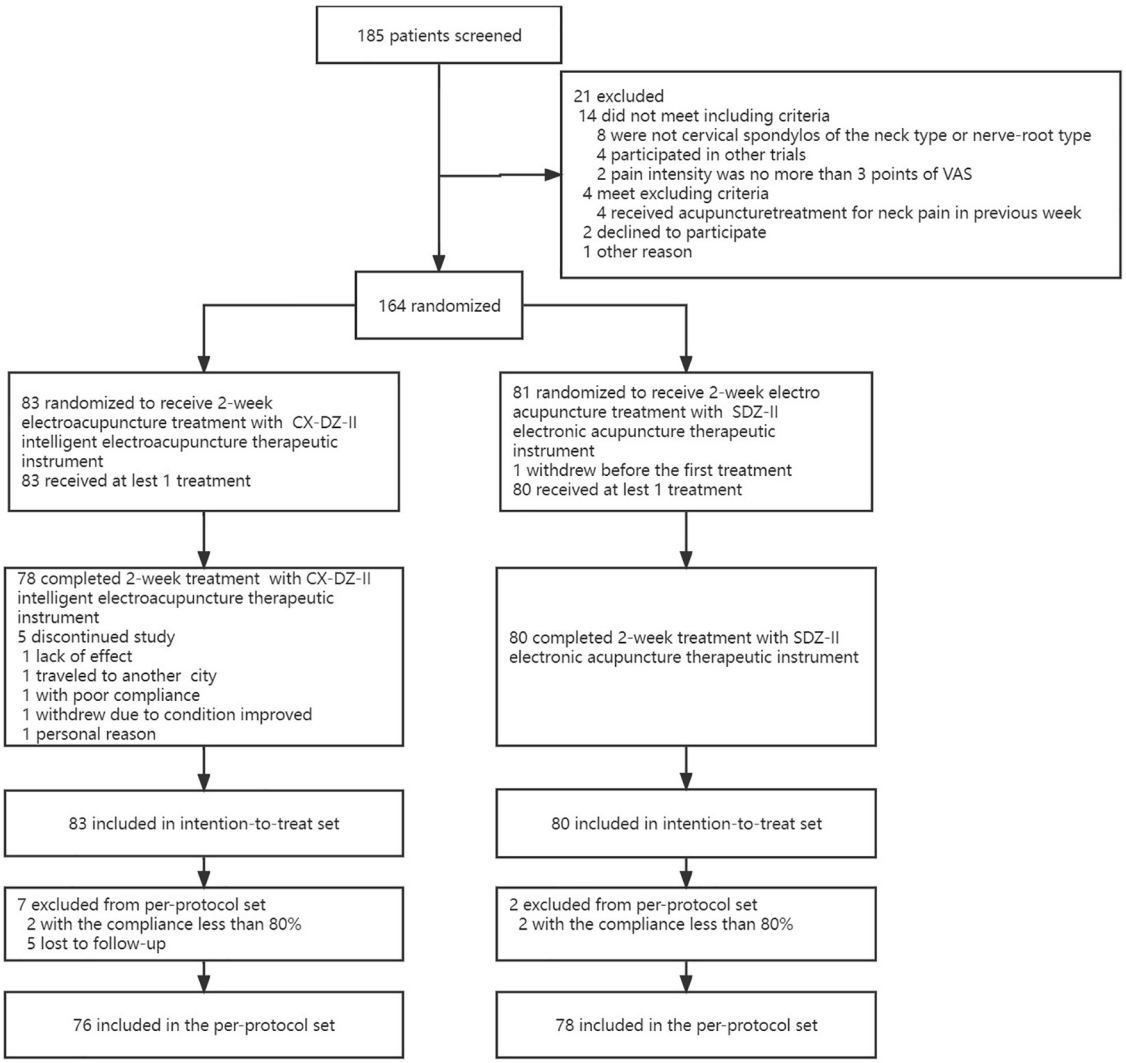

FIGURE 1
Trial flowchart.

No between-group differences were found in the VAS score after treatment at 1 week (all $p > 0.05$, see Supplementary Table 2), overall responders ($p = 0.869$), and drug-usage rate of NSAAs ($p = 1.000$) (Figure 2 and Table 3).

In addition, the defect rate of the instrument and the excellent rate of the instrument in the CX-DZ-II intelligent electronic stimulator were similar to the SDZ-II regular electronic stimulator (Table 3).

Adverse events

No significant difference was observed between the CX-DZ-II group and the SDZ-II group ($p = 0.169$) (Table 4). In terms of non-treatment-related serious AEs, two AEs (got cold) were reported in the CX-DZ-II group, while three AEs (got cold, retinal detachment, and intestinal polypectomy) were reported in the SDZ-II group.

Discussion

In this randomized non-inferiority trial of EA for neck pain caused by cervical spondylosis (NPCS), we compared the efficacy and safety of two different EA devices. Findings from the VAS score indicated that the CX-DZ-II intelligent electronic stimulator was non-inferior to the SDZ-II regular electronic stimulator, with a similar safety profile and practicability.

Currently, EA is widely used based on its combined efficacy of manual needles and electric stimulation, especially for pain management. Studies showed that EA could activate sympathetic nerve fibers to enhance the migration

of opioid-containing cells to the inflammatory sites or trigger the hypothalamus–pituitary–adrenal axis to decrease cyclooxygenase-2 and then lead to an increase in opioids (Zhang et al., 2014). At the spinal level, EA may also induce several neurotransmitters to inhibit pain, including opioids, 5-hydroxytryptamine, norepinephrine, dopamine, and acetylcholine (Munro, 2007; Yang et al., 2011; Zhang et al., 2012). Of note, functional and structural brain changes due to acupuncture for analgesia were also investigated using neuroimaging techniques (Tu et al., 2021). It has been noticed that acupuncture can reduce brain responses to noxious stimuli in typical regions involved in pain processing, such as the thalamus, insula, and prefrontal cortex (Li et al., 2014; Yan et al., 2020).

Previous studies reported that EA with different frequencies has different analgesic effects (Chen and Han, 1992; Lin et al., 2009). Although EA apparatus can adjust stimulus parameters quantitatively, the accuracy of adjustment is limited. Furthermore, few of them are equipped with networking and visualization systems. The CX-DZ-II intelligent electronic stimulator is designed based on microcomputer net technology and engineering technology. It owns the advantage of accurate parameter adjustment, which is critical to clinical efficacy and safety. More importantly, clinicians can get acupuncture prescriptions from the EBAM and upload the therapeutic information to the cloud database through the terminal unit (Jia et al., 2016; Liang et al., 2017). Thus, we conducted this trial to investigate the efficacy and safety of the CX-DZ-II intelligent electronic stimulator for NP relief in patients with CS.

Neck pain is a common symptom for patients with CS, which has a great impact on people's quality of life and health. Pain intensity is thought to be one of the primary factors that determine the impact of NP on a person's overall function and sense of wellbeing. The VAS is a 10-cm scale for pain assessment, which is accessible and easy to administrate. It has been commonly used in pain research and clinical

TABLE 1 Baseline characteristics.

Characteristics	CX-DZ-II (n = 83)	SDZ-II (n = 81)	p-value
Gender, *n* (%)			
Male	18 (21.69)	11 (13.75)	0.185
Female	65 (78.31)	69 (86.25)	
Age, mean (SD), yr	47.75 ± 12.19	48.23 ± 13.57	0.813
Height, mean (SD), cm	159.71 ± 6.19	160.00 ± 6.37	0.538
Weight, mean (SD), Kg	58.83 ± 8.75	56.84 ± 8.40	0.141
Types of CS			
CS of the neck type	52 (62.65)	59 (73.75)	0.129
CS of the nerve-root type	31 (37.35)	21 (26.25)	
Coexisting illness, *n* (%)			
Hypertension	10 (38.46)	1 (5.56)	0.205
Insomnia	4 (15.38)	0 (0.00)	
Others	12 (46.15)	17 (94.44)	
Other treatments			
No	83 (100.0)	80 (100.00)	1.0000
Yes	0 (0.00)	0 (0.00)	
Participants using other medicine, *n* (%)			
No	63 (75.90)	65 (81.25)	0.406
Yes	20 (24.10)	15 (18.75)	
NSAAs	0 (0.00)	0 (0.00)	
others	20 (100.00)	15 (100.00)	
VAS score, mean (SD)	5.55 (1.47)	5.42 (1.75)	0.613

TABLE 2 Primary outcome.

	CX-DZ-II (n = 83)	SDZ-II (n = 80)	Difference (95% CI)	P value
FAS	3.36 (2.92 to 3.79)	3.23 (2.75 to 3.71)	0.17 (-0.36 to 0.69)	0.022
PPS[a]	3.53 (3.08 to 3.97)	3.25 (2.76 to 3.74)	0.28 (-0.38 to 0.93)	0.008

FAS, full analysis set; PPS, per-protocol set; CI, confidence intervals. [a]The number of participants providing data on the VAS score was 76 in the CX-DZ-II group and 78 in the SDZ-II group.

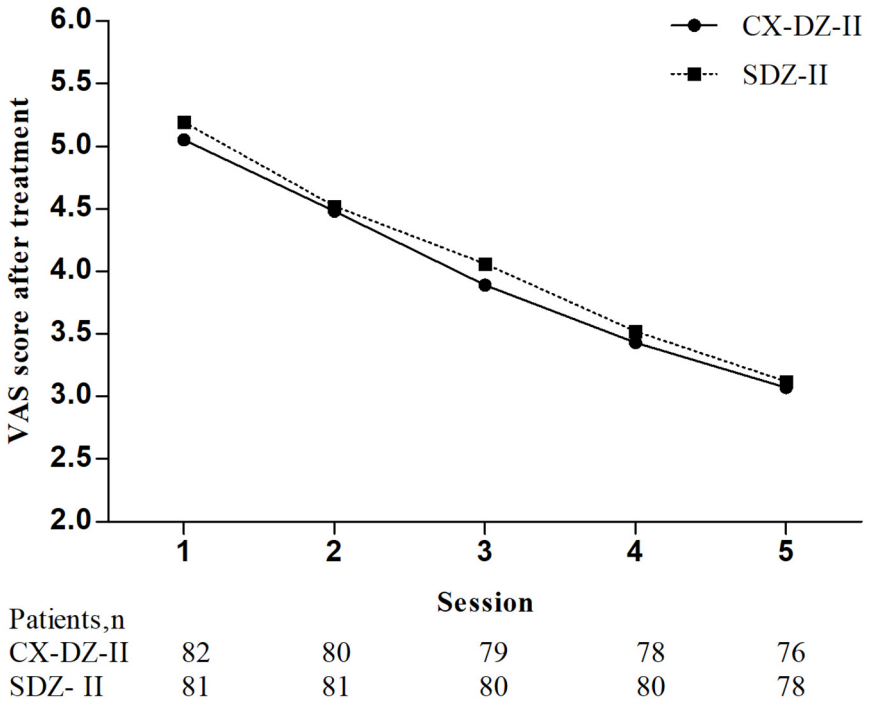

FIGURE 2
The visual analog scale (VAS) score after treatment at 1 week. *The global test was significant ($p < 0.0001$) and repeated-measures ANOVA with Greenhouse–Geisser correction was used. No significant group differences in 1 week were found (Supplementary Table 3).

TABLE 3 Other secondary outcomes.

Outcome measures[a]	CX-DZ-II ($n = 83$)	SDZ-II ($n = 80$)	p value
Overall responders, n (%)	41 (49.40)	41 (51.25)	0.869
Drug-usage rate of NSAAs, n (%)	0 (100)	0 (100)	1.000
Defect rate of the instrument, n (%)	6 (7.23)	3 (3.75)	0.496
Excellent rate of instrument, n (%)			
Score < 16	82 (98.80)	79 (98.75)	1.000
Score > 16	1 (1.20)	1 (1.25)	

[a]Statistical analysis set was based on the intention-to-treat population.

TABLE 4 Adverse events related to treatment.[a]

AEs	Participant, n (%)		p value
	CX-DZ-II ($n = 83$)	SDZ-II ($n = 80$)	
Overall	9 (10.84)	4 (5.00)	0.169
Fainted during acupuncture	3 (3.61)	1 (1.25)	/
Transient sharp pain	4 (4.82)	3 (3.75)	/
Inserting needles again	2 (2.41)	0 (0.00)	/

AEs, adverse events. [a]Adverse events were analyzed based on the full analysis set. AEs with different categories occurring in one participant were defined as different independent AEs. An AE with multiple occurrences in one participant was defined as a different independent AE.

practice, with demonstrated reliability and validity (Li et al., 2007; Moses et al., 2019). Therefore, in this study, a change in the VAS from baseline to the completion of treatment was used as the primary outcome. Moreover, to evaluate the immediate efficacy of the CX-DZ-II intelligent electronic stimulator, the VAS score after each treatment in 1 week has also been reported.

In this study, the changes in the VAS were 3.36 in the CX-DZ-II group vs. 3.23 in the SDZ-II group, within the range of 2.04–4.89 reported in previous trials (Wang, 2012; Wan et al., 2013; Huang, 2015; Garov, 2016). Moreover, findings from the VAS in 1 week indicated that the instant analgesic effect of the CX-DZ-II intelligent electronic stimulator seemed better than the SDZ-II regular electronic stimulator, although no statistical difference was observed. Moreover, similar results of the overall responder and drug-usage rate of NSAAs also verified that the CX-DZ-II intelligent electronic stimulator was non-inferior to the SDZ-II regular electronic stimulator. Although results indicated that the CX-DZ-II intelligent electronic stimulator deserved to be promoted for patients with CS of neck type and nerve root type, the generalizability of the CX-DZ-II regular intelligent electronic stimulator for other CS types still requires further investigation.

The present trial also demonstrated some properties of the CX-DZ-II intelligent electronic stimulator and the SDZ-II regular electronic stimulator. Studies indicate that the sensitivity, manipulation, and therapeutic parameters of devices are critical to the clinical effect and safety of EA. Currently, many EA instruments are equipped with mechanical rotary knobs for parameter adjustment, providing approximate data on electrical frequency and intensity to clinicians (Xu et al., 2016). More importantly, parameter settings of traditional EA instruments need to be reset manually after treatment; otherwise, the excessive current intensity may increase and cause transient discomfort and pain to patients when it is used again. Comparatively, the CX-DZ-II intelligent electronic stimulator is equipped with an ARM-A9 chip, Android 4.0 system, and capacitive touchscreen, which provides conditions for visual and quick parameter adjustment. Moreover, the electrical stimulation parameters can be reset automatically after shutdown, avoiding potential security risks in the next operation. In this study, no between-group differences in the defect rate and the excellent rate were observed, indicating that the CX-DZ-II intelligent electronic stimulator can function, as well as the SDZ-II regular electronic stimulator in terms of performance and operation.

Potential AEs related to treatment should also be noticed. According to previous reports, transient sharp pain is one of the common AEs in trials of EA (Liu et al., 2017, 2021). In this study, similar discomfort appeared in both groups, with no statistical difference. In addition, AEs also involved inserting needles again because of the improper disposal of wire, with no connection with the major structure of the CX-DZ-II intelligent electronic stimulator.

Conclusion

In conclusion, the effect of the CX-DZ-II intelligent electronic stimulator was non-inferior to the SDZ-II regular electronic stimulator in decreasing the VAS of patients with NPCS. Similarly, the performance of safety and manipulation between groups was consistent. Therefore, we believed that the CX-DZ-II intelligent electronic stimulator, a new device characterized by digitization, networking, and visualization, can provide a promising non-inferior alternative in the treatment of EA for NPCS.

Limitation

Some limitations of this trial must be acknowledged. First, only the instant effect and safety of the CX-DZ-II intelligent electronic stimulator were verified in this study, and future trials need to assess its persistent effect in the follow-up periods. Second, the degree of participants' expectations with the results was not evaluated and the pure effect of the CX-DZ-II intelligent electronic stimulator for NPCS needs further assessment. Third, for the CX-DZ-II intelligent electronic stimulator, the specific usage of online functions such as data collection, evidence-based diagnosis, and treatment decision support was not reported because they were not related to the aims of this study.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Sichuan Regional Ethics Review Committee, Affiliated to Chengdu University of Traditional Chinese Medicine. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

YR and FL: conception and design of the study and critical revision of the manuscript for important intellectual content. HL and YZ: data collection. LC and DL: statistical analysis. LC, DL, and JX: drafting of the manuscript. All authors have contributed to the article and approved the submitted version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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References

Bier, J. D., Scholten-Peeters, W. G. M., Staal, J. B., Pool, J., Van Tulder, M. W., Beekman, E., et al. (2018). Clinical practice guideline for physical therapy assessment and treatment in patients with nonspecific neck pain. *Phys. Ther.* 98, 162–171. doi: 10.1093/ptj/pzx118

Blanpied, P. R., Gross, A. R., Elliott, J. M., Devaney, L. L., Clewley, D., Walton, D. M., et al. (2017). Neck pain: revision 2017. *J. Orthop. Sports Phys. Ther.* 47, A1–A83. doi: 10.2519/jospt.2017.0302

Chen, X. H., and Han, J. S. (1992). Analgesia induced by electroacupuncture of different frequencies is mediated by different types of opioid receptors: another cross-tolerance study. *Behav. Brain Res.* 47, 143–149. doi: 10.1016/s0166-4328(05)80120-2

Chen, Z. H., Liang, F. R., Yang, M. X., Li, D. H., Zhang, Y., and Ren, Y. L. (2020). Effect and safety of cx-dz-ii intelligent electroacupuncture therapeutic instrument for neck pain caused by cervical spondylos: study protocol for a randomized controlled trial. *Chin. J. Integr. Med.* 26, 375–381. doi: 10.1007/s11655-019-3038-2

Chinese Medical Association (2007). *Clinical guidelines for diagnosis and treatment: Pain*. Beijing: People's Medical Publishing House.

Comachio, J., Oliveira, C. C., Silva, I. F. R., Magalhães, M. O., and Marques, A. P. (2020). Effectiveness of manual and electrical acupuncture for chronic non-speci?c low back pain: a randomized controlled trial. *J. Acupunct. Meridian Stud.* 13, 87–93. doi: 10.1016/j.jams.2020.03.064

Corp, N., Mansell, G., Stynes, S., Wynne-Jones, G., Morso, L., Hill, J. C., et al. (2021). Evidence-based treatment recommendations for neck and low back pain across europe: a systematic review of guidelines. *Eur. J. Pain* 25, 275–295. doi: 10.1002/ejp.1679

Feng, J., Wen, C. B., Liang, F. R., Luo, Y., Chen, J., Zhao, S. T., et al. (2019). Design of wearable traditional chinese medicine electroacupuncture therapeutic instrument system based on stm32. *J. Tradit. Chin. Med.* 42, 64–68. doi: 10.13593/j.cnki.51-1501/r.2019.02.064

Garov, Y. (2016). *The effectiveness and safety of electro-acupuncture and warm acupuncture for the treatment of cervical spondylosis radiculopathy: A randomized clinical trial*. Jiangsu: Nanjing university of Traditional Chinese Medicine.

Hong, W. X., Chen, L., and Jing, J. (2006). *Design of A Novel Intelligent Electroacupuncture with Reinforcement and Reduction Needle Methods in the Traditional Chinese Medicine*. Beijing: Biomedical Engineering.

Huang, X. Z. (2015). *The clinical study on the treatment of cervical spondylotic radiculopathy with Qi ci cervical Jia ji acupoints methods*. Guangdong: Guangzhou university of traditional Chinese medicine.

Huang, Y., Yu, M., Kuma, A., Klein, J. D., Wang, Y., Hassounah, F., et al. (2021a). Downregulation of let-7 by electrical acupuncture increases protein synthesis in mice. *Front. Physiol.* 12:697139. doi: 10.3389/fphys.2021.697139

Huang, C. Z., Li, Y. L., Lan, X. L., He, B., Yang, J., and Li, J. (2021b). Electroacupuncture combined with acupoint catgut embedding for postoperative pain after fistulotomy. *Zhen Ci Yan Jiu* 46, 421–425. doi: 10.13702/j.1000-0607.200603

Huang, J. F., Zheng, X. Q., Chen, D., Lin, J. L., Zhou, W. X., Wang, H., et al. (2021c). Can acupuncture improve chronic spinal pain? A systematic review and meta-analysis. *Glob. Spine J.* 11, 1248–1265. doi: 10.1177/2192568220962440

Jia, W., Wen, C., Yan, X., Chen, J., and Fu, T. (2016). *Intelligence electric acupuncture apparatus system based on wifi*. China: 206133564U.

Joan Gan, C. Y., Chan, K. K., Tan, J. H., Tan Chor Lip, H., Louis Ling, L. L., and Mohd Azman, Z. A. (2021). Smartphone-controlled patch electro-acupuncture versus conventional pain relief during colonoscopy: a

randomized controlled trial. *ANZ J. Surg.* 91, E375–E381. doi: 10.1111/ans.16870

Kjaer, P., Kongsted, A., Hartvigsen, J., Isenberg-Jørgensen, A., Schiøttz-Christensen, B., Søborg, B., et al. (2017). National clinical guidelines for non-surgical treatment of patients with recent onset neck pain or cervical radiculopathy. *Eur. Spine J.* 26, 2242–2257. doi: 10.1007/s00586-017-5121-8

Kuo, D. T., and Tadi, P. (2022). *Cervical Spondylosis, Statpearls, StatPearls Publishing Copyright 2022,* Treasure Island: StatPearls Publishing LLC

Li, J., Zhang, J. H., Yi, T., Tang, W. J., Wang, S. W., and Dong, J. C. (2014). Acupuncture treatment of chronic low back pain reverses an abnormal brain default mode network in correlation with clinical pain relief. *Acupunct. Med.* 32, 102–108. doi: 10.1136/acupmed-2013-010423

Li, L., Liu, X., and Herr, K. (2007). Postoperative pain intensity assessment: a comparison of four scales in chinese adults. *Pain Med.* 8, 223–234. doi: 10.1111/j.1526-4637.2007.00296.x

Liang, F., Shu, H., Ren, Y., Zeng, F., Li, J., Guo, T., et al. (2017). *Evidence-Based Acupuncture and Moxibustion Meridian-Acupoint Treating and Detecting Instrument Supporting Multiplexed Output and Method of Use Thereof.* Sichuan: Justia

Lin, Y., Peng, Y., Yi, S., Tang, S., and Li, Z. (2009). Effect of different frequency electroacupuncture on the expression of substance p and β-endorphin in the hypothalamus in rats with gastric distension-induced pain. *Zhen Ci Yan Jiu* 34, 252–257.

Liu, B., Wu, J., Yan, S., Zhou, K., He, L., Fang, J., et al. (2021). Electroacupuncture vs prucalopride for severe chronic constipation: a multicenter, randomized, controlled, noninferiority trial. *Am. J. Gastroenterol.* 116, 1024–1035. doi: 10.14309/ajg.0000000000001050

Liu, T., Shen, H., Yang, H., and Gao, M. (2016). Discussion on the industry standard: electroacupuncture therapy device. *Zhongguo Zhen Jiu* 36, 99–101.

Liu, T. Y., Yang, H. Y., Gao, M., Xu, G., and Tang, W. C. (2010). Single-acupoint electroacupuncture based on traditional acupuncture becomes true. *Zhen Ci Yan Jiu* 35, 384–387.

Liu, Z., Liu, Y., Xu, H., He, L., Chen, Y., Fu, L., et al. (2017). Effect of electroacupuncture on urinary leakage among women with stress urinary incontinence: a randomized clinical trial. *JAMA* 317, 2493–2501. doi: 10.1001/jama.2017.7220

Luo, M. (2014). *Clinical Observation on Lumbar Intervertebral Disc Herniation Treated by Electro-Acupunture of Jiaji Points Combined with Mckenzie Therapy*. Guangzhou: University Traditional Chinese Medicine.

Lv, Y., Tian, W., Chen, D., Liu, Y., Wang, L., and Duan, F. (2018). The prevalence and associated factors of symptomatic cervical spondylosis in chinese adults: a community-based cross-sectional study. *BMC Musculoskelet. Disord.* 19:325. doi: 10.1186/s12891-018-2234-0

Mao, J. J., Liou, K. T., Baser, R. E., Bao, T., Panageas, K. S., Romero, S. A. D., et al. (2021). Effectiveness of electroacupuncture or auricular acupuncture vs usual care for chronic musculoskeletal pain among cancer survivors: the peace randomized clinical trial. *JAMA Oncol.* 7, 720–727. doi: 10.1001/jamaoncol.2021.0310

Moses, M. J., Tishelman, J. C., Stekas, N., Jevotovsky, D. S., Vasquez-Montes, D., Karia, R., et al. (2019). Comparison of patient reported outcome measurement information system with neck disability index and visual analog scale in patients with neck pain. *Spine* 44, E162–E167. doi: 10.1097/BRS.0000000000002796

Munro, G. (2007). Dopamine d(1) and d(2) receptor agonism enhances antinociception mediated by the serotonin and noradrenaline reuptake inhibitor duloxetine in the rat formalin test. *Eur. J. Pharmacol.* 575, 66–74. doi: 10.1016/j.ejphar.2007.07.062

Safiri, S., Kolahi, A. A., Hoy, D., Buchbinder, R., Mansournia, M. A., Bettampadi, D., et al. (2020). Global, regional, and national burden of neck pain in the general population, 1990-2017: systematic analysis of the global burden of disease study 2017. *BMJ* 368:m791. doi: 10.1136/bmj.m791

Seo, S. Y., Lee, K. B., Shin, J. S., Lee, J., Kim, M. R., Ha, I. H., et al. (2017). Effectiveness of acupuncture and electroacupuncture for chronic neck pain: a systematic review and meta-analysis. *Am. J. Chin. Med.* 45, 1573–1595. doi: 10.1142/S0192415X17500859

Shen, H., Liu, T. Y., and Yang, H. Y. (2016). Review of application technology of electroacupuncture instrument and new idea of its development. *Shanghai J. Acupunct. Moxibustion* 35, 1016–1020. doi: 10.13460/j.issn.1005-0957.2016.08.1016

Su, Z., Hu, L., Cheng, J., Klein, J. D., Hassounah, F., Cai, H., et al. (2016). Acupuncture plus low-frequency electrical stimulation (acu-lfes) attenuates denervation-induced muscle atrophy. *J. Appl. Physiol.* 120, 426–436. doi: 10.1152/japplphysiol.00175.2015

Tang, S., Luo, X. Y., Shi, Q. D., Lin, Y. P., Lai, L., and He, L. (2014). Observations on the efficacy of electroacupuncture at huatuo jiaji points in treating cervical spondylosis *Shanghai J. Acu-Mox.* 33, 840–842. doi: 10.13460/j.issn.1005-0957.2014.09.0840

Tu, Y., Cao, J., Bi, Y., and Hu, L. (2021). Magnetic resonance imaging for chronic pain: diagnosis, manipulation, and biomarkers. *Sci. Chin. Life Sci.* 64, 879–896. doi: 10.1007/s11427-020-1822-4

Vogt, M. T., Cawthon, P. M., Kang, J. D., Donaldson, W. F., Cauley, J. A., and Nevitt, M. C. (2006). Prevalence of symptoms of cervical and lumbar stenosis among participants in the osteoporotic fractures in men study. *Spine* 31, 1445–1451. doi: 10.1097/01.brs.0000219875.19688.a6

Wan, B. J., Huang, W., Zhang, Y. X., and Zhang, H. S. (2013). Influence of electroacupuncture with penetration needling method on comprehensive pain score in patients with cervical spondylotic radiculopathy. *Zhongguo Zhen Jiu* 33, 407–410.

Wang, W. (2012). *The clinical study by randomized controlled trials on Qi ci cervical Jia ji acupoints in treatment of cervical spondylosis radiculopathy*. Sichuan: Chengdu university of Traditional Chinese Medicine.

Wang, Z. J. (2019). *Research on Traceability Method and Development of the Calibration Device for Electro-Acupuncture Treatment Instrument*. Zhejiang: Association of Pacific Rim Universities.

Xu, F. P., Chen, Z. L., and Guo, Y. (2016). Reports on electroacupuncture device parameters and analysis of its measured output frequencies. *Shanghai J. Acupunct. Moxibustion* 35, 1139–1142.

Yan, C. Q., Huo, J. W., Wang, X., Zhou, P., Zhang, Y. N., Li, J. L., et al. (2020). Different degree centrality changes in the brain after acupuncture on contralateral or ipsilateral acupoint in patients with chronic shoulder pain: a resting-state fmri study. *Neural Plast.* 2020:5701042. doi: 10.1155/2020/5701042

Yang, E. J., Koo, S. T., Kim, Y. S., Lee, J. E., Hwang, H. S., Lee, M. S., et al. (2011). Contralateral electroacupuncture pretreatment suppresses carrageenan-induced inflammatory pain via the opioid-mu receptor. *Rheumatol. Int.* 31, 725–730. doi: 10.1007/s00296-010-1364-y

Yang, Y. X., and Yong, M. C. (2009). Safety problems and countermeasures of electroacupuncture instruments. *Zhongguo Zhen Jiu* 29, 339–341.

Zhang, R., Lao, L., Ren, K., and Berman, B. M. (2014). Mechanisms of acupuncture-electroacupuncture on persistent pain. *Anesthesiology* 120, 482–503. doi: 10.1097/ALN.0000000000000101

Zhang, Y., Zhang, R. X., Zhang, M., Shen, X. Y., Li, A., Xin, J., et al. (2012). Electroacupuncture inhibition of hyperalgesia in an inflammatory pain rat model: involvement of distinct spinal serotonin and norepinephrine receptor subtypes. *Br. J. Anaesth.* 109, 245–252. doi: 10.1093/bja/aes136

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Predicting response to tVNS in patients with migraine using functional MRI: A voxels-based machine learning analysis

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Background: Migraine is a common disorder, affecting many patients. However, for one thing, lacking objective biomarkers, misdiagnosis, and missed diagnosis happen occasionally. For another, though transcutaneous vagus nerve stimulation (tVNS) could alleviate migraine symptoms, the individual difference of tVNS efficacy in migraineurs hamper the clinical application of tVNS. Therefore, it is necessary to identify biomarkers to discriminate migraineurs as well as select patients suitable for tVNS treatment.

Methods: A total of 70 patients diagnosed with migraine without aura (MWoA) and 70 matched healthy controls were recruited to complete fMRI scanning. In study 1, the fractional amplitude of low-frequency fluctuation (fALFF) of each voxel was calculated, and the differences between healthy controls and MWoA were compared. Meaningful voxels were extracted as features for discriminating model construction by a support vector machine. The performance of the discriminating model was assessed by accuracy, sensitivity, and specificity. In addition, a mask of these significant brain regions was generated for further analysis. Then, in study 2, 33 of the 70 patients with MWoA in study 1 receiving real tVNS were included to construct the predicting model in the generated mask. Discriminative features of the discriminating model in study 1 were used to predict the reduction of attack frequency after a 4-week tVNS treatment by support vector regression. A correlation coefficient between predicted value and actual value of the reduction of migraine attack frequency was conducted in 33 patients to assess the performance of predicting model after tVNS treatment. We vislized the distribution of the predictive voxels as well as investigated the association between fALFF change (post-per treatment) of predict weight brain regions and clinical outcomes (frequency of migraine attack) in the real group.

Results: A biomarker containing 3,650 features was identified with an accuracy of 79.3%, sensitivity of 78.6%, and specificity of 80.0% ($p < 0.002$). The discriminative features were found in the trigeminal cervical complex/rostral ventromedial medulla (TCC/RVM), thalamus, medial prefrontal cortex (mPFC), and temporal gyrus. Then, 70 of 3,650 discriminative features were identified to predict the reduction of attack frequency after tVNS treatment with a correlation coefficient of 0.36 ($p = 0.03$). The 70 predictive features were involved in TCC/RVM, mPFC, temporal gyrus, middle cingulate cortex (MCC), and insula. The reduction of migraine attack frequency had a positive

correlation with right TCC/RVM (r = 0.433, $p = 0.021$), left MCC (r = 0.451, $p = 0.016$), and bilateral mPFC (r = 0.416, $p = 0.028$), and negative with left insula (r = −0.473, $p = 0.011$) and right superior temporal gyrus/middle temporal gyrus (r = −0.684, $p < 0.001$), respectively.

Conclusions: By machine learning, the study proposed two potential biomarkers that could discriminate patients with MWoA and predict the efficacy of tVNS in reducing migraine attack frequency. The pivotal features were mainly located in the TCC/RVM, thalamus, mPFC, and temporal gyrus.

KEYWORDS

migraine, transcutaneous vagus nerve stimulation, machine learning, functional magnetic resonance imaging, support vector machine (SVM), vagus nerve

Introduction

Migraine, affecting approximately 1 billion people, is the second most prevalent neurologic disorder, which imposes socioeconomic burdens and absence of work and study, and can be divided into episodic migraine (attacks that occur ≤15 days/month) and chronic migraine (attacks that occur >15 days/month) (Ashina, 2020; Mu et al., 2020). In all subtypes, migraine without aura (MWoA) was the most common one, experienced by the majority of migraineurs (Launer et al., 1999). Currently, the diagnosis of MWoA mainly depends on the International Classification of Headache Disorders (ICHD) (Arnold, 2018), with five criteria including unilateral location with pulsating quality, moderate to severe pain intensity, suffering for 4 to 72 h in one attack, and presence of nausea/vomiting and/or photophobia/phonophobia. Nevertheless, many patients have difficulties in meeting the entire criteria of MWoA in clinical practice (Ozge et al., 2015). Therefore, it is necessary to find an objective as well as an accurate method to diagnose MWoA in the "gray zones."

In addition, as the pathophysiology remains misty, the pharmacotherapy of migraine is far from satisfactory. Accumulating evidence suggests that transcutaneous vagus nerve stimulation (tVNS) at the external ear can induce anti-nociception. By stimulating the auricular and cervical branches of the vagus nerve non-invasively, migraineurs experienced a significant decrease in the attack frequency and intensity (Straube et al., 2015; Diener et al., 2019; Zhang et al., 2020). Consequently, tVNS has got a Class I recommendation for patients with episodic migraine (Tassorelli et al., 2018; Blech et al., 2020). Moreover, another two studies investigated the mechanism of tVNS in the treatment of migraine and found that tVNS could inhibit the transmission of trigeminal nociception and cortical spreading depression (Cornelison et al., 2020; Morais et al., 2020). Taken together, the above evidence suggested that tVNS should be considered in the clinical practice of migraine. On the other way, despite the effectiveness of tVNS for MWoA, the efficacy varies considerably across different subjects. Therefore, identifying a valid and objective biomarker for treatment response will be of great importance. Intriguingly, emerging functional magnetic resonance imaging (fMRI) has provided an innovative perspective for migraine, greatly contributing to the understanding of its pathophysiology and therapeutics (Ashina et al., 2021). For example, migraineurs exhibited aberrant patterns in the trigeminal cervical complex (TCC), thalamus, medial prefrontal cortex (mPFC), and temporal gyrus (Xue et al., 2013; Wang et al., 2016; Li et al., 2017). Additionally, a significant correlation could be found between migraine pain intensity and disease duration, with the thalamus and mPFC (Coppola et al., 2018; Qin et al., 2020b). For example, functional connectivity of mPFC and thalamus had a negative correlation with pain intensity of migraine. Moreover, studies previously demonstrated nociceptive stimulation would activate TCC and modulate the endogenous pain circuitry which associated the ascending trigeminal spinal-thalamo-cortical pathways with the migraineur's pain sensitivity (Marciszewski et al., 2018; Lim et al., 2021). All of the above studies have revealed a potential central mechanism of migraine, involving TCC, thalamus, mPFC, and temporal gyrus, which could be the targets of migraine treatment.

In terms of these brain regions, our previous studies have investigated the effects of tVNS in treating migraines. For example, the effects of tVNS could be associated with the mPFC and thalamus, which had a negative correlation with migraine attacks days after tVNS treatment (Luo et al., 2020b; Zhang et al., 2021). Furthermore, our recent study also suggested that tVNS would increase functional connectivity between the middle cingulate cortex (MCC) and periaqueductal gray which were involved in descending pain modulation system (DPMS) (Cao et al., 2021). All of those studies indicated that tVNS would treat migraines by modulating these pain-related regions, which reflected the intrinsic characteristics of migraineurs and their relationship with clinical manifestations. Nevertheless, potential biomarkers still have not been applied to migraines. The goals of current studies mainly concentrate on identifying

neuroimaging measures related to phenotypic measures, which often does not generalize to novel individuals, thus, it results in inadequate clinical utility (Bisenius et al., 2017; Scheinost et al., 2019).

As a data-driven technique, multivariate pattern analysis (MVPA) plays an important role in analyzing neuroimaging data and is expected to help solve this problem, due to which it is sensitive to the fine-grained spatial discriminative patterns and exploration of inherent multivariate nature from high-dimensional neuroimaging data and also could provide novel insight into the differences between two groups because it allows the identification of features which contribute the most to individual classification or prediction (Khosla et al., 2019; Rocca et al., 2020). Several sensitive and specific neuroimaging potential biomarkers have been explored by machine learning in psychiatric and neurologic diseases, expected to instruct diagnosis and treatment (Yang et al., 2019; Huang et al., 2020; Luo et al., 2020a; Ma et al., 2020; Schneider et al., 2020; Chiarelli et al., 2021). Some previous studies have used machine learning combined with fMRI to identify migraineurs from healthy controls (HCs) with an accuracy of 83.33 to 91.4% (Chong et al., 2017; Tu et al., 2020; Yin et al., 2020; Chen et al., 2021). Furthermore, previous studies have predicted the efficacy of acupuncture for migraines before treatment in individuals, which developed a personalized medicine strategy based on the predictive model (Tu et al., 2020; Yin et al., 2020). Those studies have indicated that the combination of fMRI and machine learning might be used to diagnose specific patients and predict individual responses to clinical therapy.

Thus, the present study aimed to explore the neuroimaging biomarker which can be used to discriminate migraineurs and predict the efficacy of tVNS for migraines. In this study, we selected fractional amplitudes of low-frequency fluctuations (fALFF) as the feature to construct the biomarker, which could reflect the local spontaneous fluctuation of the fMRI BOLD signal (Zou et al., 2008). The advantage of fALFF is that it does not require a prior hypothesis which strengthens test-retest reliability. Moreover, compared with ALFF, fALFF has higher sensitivity and specificity in detecting regional spontaneous brain activity (Zou et al., 2008). In study 1, we calculated the fALFF of each voxel and imported the fALFF value into support vector machine (SVM) to construct the discriminative model which could discriminate migraineurs and healthy controls (HCs). We assessed the performance of model by analyzing the accuracy of the discriminative model. In addition, extracted the mask of discriminative features for further research. In study 2, we used the mask from study 1 and support vector regression (SVR) to construct the predicting model to predict the reduction of migraine attack frequency after tVNS treatment. Finally, we tested the correlation between regions of interest and the reduction of migraine attack frequency.

Materials and methods

Participants

MWoA patients with matched healthy controls (HC) were recruited between May 2017 and May 2019. The study was approved by the Institutional Review Board of the Second Affiliation Hospital, Guangzhou University of Chinese Medicine. This study protocol was registered on the Chinese Clinical Trial Registry (ChiCTR-INR-17010559, February 7, 2017, http://www.chictr.org.cn/hvshowproject.aspx?id=11101). Informed consent was obtained from all participants.

This study was an advanced exploration based on our previously published article (Zhang et al., 2021). Thus, eligible criteria and intervention protocol of MWoA patients would not be listed in detail.

In brief, in study 1, patients diagnosed with MWoA by the International Classification of Headache Disorders, the Second Edition (ICHD-2), were included. The patients were asked to fulfill a 4-week (Weeks 1–4) migraine diary including attack frequency, intensity of each attack, and emotion evaluation. Attack frequency was defined as the International Headache Society Clinical Trials Committee recommended (Diener et al., 2020). The intensity of the attack was assessed by a visual analog scale (VAS) of 0 to 100. A higher score meant more severe pain. Patients were asked to record each intensity of head attack using VAS and the average VAS score of each subject was included in the final analysis. Migraine Specific Quality of Life Questionnaire (MSQ) was used to assess the life quality of migraineurs. Self-rating anxiety scale (SAS) and self-rating depression scale (SDS) were used to evaluate emotion. Then, patients and age, sex-matched HCs were required to complete once MRI scanning. We used the demographics and fMRI data of patients and HCs to construct a discriminate model.

In study 2, patients with MWoA were randomly divided into the real group and sham group, receiving a 4-week (Weeks 5–8) treatment according to the treatment protocol. The real tVNS group was applied at the left cymba concha (the true stimulation site), while the sham tVNS group was stimulated on the left tail of the helix. During the treatment, patients were required to complete another 4-week migraine diary as well as MRI scanning as a post-treatment assessment. We constructed predicting model, using the difference in attack frequency between baseline (Weeks 1–4) and post-treatment (Weeks 5–8) of the real group as a label, and discriminative features of the discriminating model generated in study 1 as inputs.

Demographic and clinical outcomes statistics analysis

Demographic and clinical outcomes were conducted by SPSS 24.0. T-student analysis and Chi-square analysis were

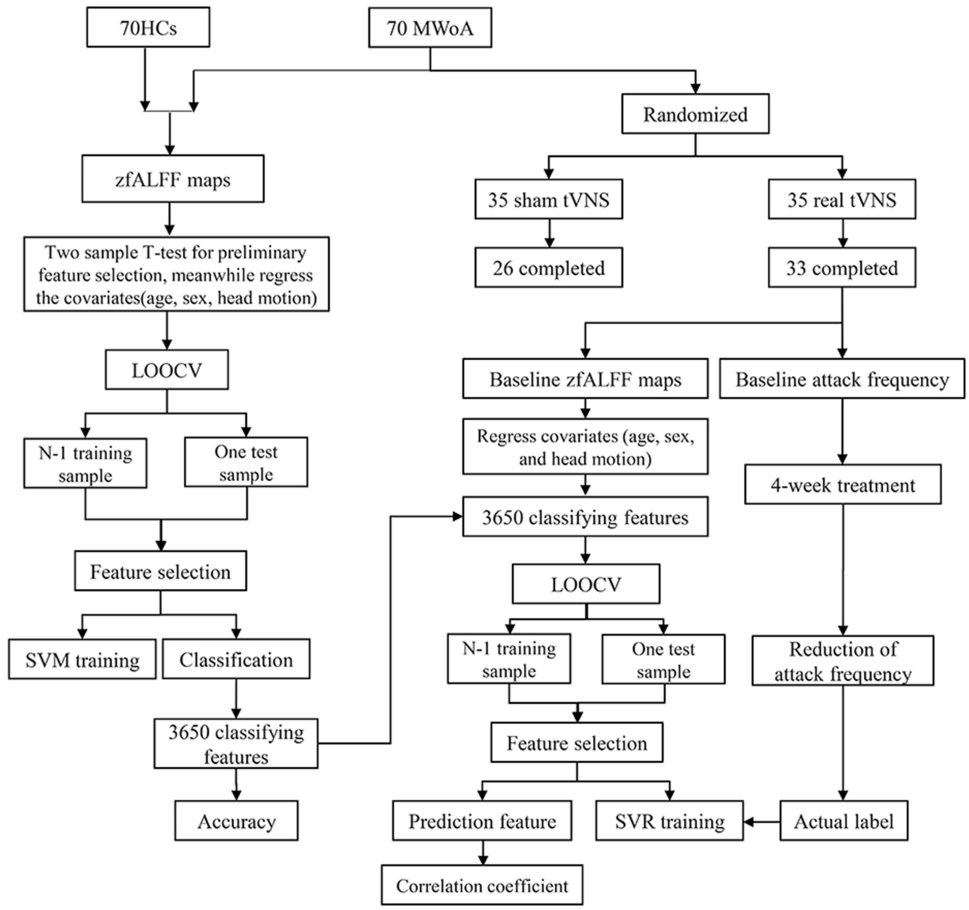

FIGURE 1
The flow diagram of classification and prediction. A total of 70 HCs and 70 MWOA were used to construct the classification model and the top 3,650 discriminative features could discriminate MWoA with the highest accuracy. Then, the 3,650 discriminative features were used to construct predicting model and 70 predictive features could predict the reduction of migraine attack frequency with the highest correlation coefficient. HCs, healthy controls; MWOA, migraine without aura; LOOCV, leave-one-out cross-validation; SVM, support vector machine; SVR, support vector regression.

used for continuous and counting variables individually. The significance threshold was set to $p < 0.05$ (two-tailed).

Image acquisition

A 3.0 T Siemens MRI scanner (Siemens MAGNETOM Verio 3.0 T, Erlangen, Germany) was conducted to scan all participants with a 24-channel phased-array head coil. To minimize head movement and scanner noise, foam padding and earplugs were applied. All of them were required to remain motionless, sober with eyes closed, and avoid thinking of anything in particular. All patients participated in the identical functional MRI (fMRI) scanning sessions before and after 4 weeks of treatment in the interval period (If MWoA patients had a headache attack within 48 h before and after scanning, we would make another appointment for fMRI scanning), while HCs completed only one. Resting-state fMRI encompassing the whole brain was acquired with the following parameters: (1) T1-weighted structural images: TR = 1900 ms, TE = 2.27 ms, flip angle = 9°, FOV = 256 × 256 mm, matrix = 256 × 256, and slice thickness = 1.0 mm. (2) Resting-state fMRI images: repetition time (TR) = 2,000 ms, echo time (TE) = 30 ms, field of view (FOV) = 224 × 224 mm, matrix = 64 × 64, flip angle = 90°, slice thickness = 3.5 mm, interslice ga$p$ = 0.7 mm, 31 axial slices paralleled, and 240 time points.

fMRI preprocessing and fALFF analysis

The fMRI data were preprocessed in Data Processing and Analysis for Brain Imaging 3.0 (DPABI 3.0) (Yan et al., 2016). The main steps were as follow: (1) The first 10 volumes were discarded, followed by slice timing and realignment.

(2) After head motion correction, structural images were segmented into grey matter, white matter and cerebrospinal fluid. (3) Functional images were coregistered to the structural images. (4) Functional and structural images were standardized into Montreal Neurological Institute (MNI) space. (5) After correcting head motion with Friston 24, linear trending, white matter, and cerebrospinal fluid were conducted. (6) Finally, a 6-mm Gaussian kernel was used to spatially smooth it and a 3-mm voxel resolution was adopted to further analysis.

Before fALFF calculation, the data were filtered by a frequency window of 0.01 to 0.10 HZ. Fast Fourier transform changed the time series to the frequency series. Each voxel computed and averaged the square root of the power spectrum. Then, fALFF was used to calculate the ratio of the power of each frequency at the low-frequency range (0.01–0.08 Hz) to that of the entire frequency range (0–0.25 Hz). Finally, to make the statistics conveniently, the data transformed into z-maps.

Classification of MWoA and HCs

First, we performed a group-level two-sample *t*-test on fALFF values between HCs and MWoA, with age, sex, and head motion as covariates. Significant differences for fALFF were assessed with a threshold of $p < 0.05$ and false discovery rate (FDR) correction. Features showing significant differences were retained for the subsequent analyses to construct the discriminating model. Second, a leave-one-out cross-validation (LOOCV) was used in the model to avoid the risk of overfitting. Thus, the analyses were unbiased in the sense that the training features were selected independently of each test case. It was used to obtain the best classifier using a linear SVM algorithm combining a feature selection of F-Score. We took all meaningful voxels with the highest ranks to calculate the accuracy, setting the step until incorporating all features. The performance of a classifier was evaluated by accuracy, sensitivity, and specificity. To measure the robustness of the model, a non-parametric permutation test was performed. More specifically, we randomly permuted the labels and repetitively executed the CV procedure 5,000 times. If the accuracy of the classifier on real class labels was more significant than the accuracies of the classifiers trained on randomly relabeled class labels, this classifier was considered to be well-performing. The significance threshold was set to $p < 0.05$ (two-tailed). After obtaining the best-performing model, we extracted all discriminative features of the model for visualizing the results. Then, we identified brain regions by setting the threshold to > 30% of the maximum weight vector scores for visualizing the results of classification.

Prediction of the efficacy of tVNS

We still chose LOOCV as the validationmethod. We also regressed sex, age, and headmotion. The prediction model was constructed by support vector regression (SVR) combining with feature select of weight based on the LIBSVM toolbox. The prediction model was trained using the discriminative feature set gained from study 1. The correlation coefficient was calculated to assess the fitting between predictive and actual values. The significance was measured by permutation testing (permutation times = 1,000). The significance threshold was set to $p < 0.05$ (two-tailed). After obtaining the best-performing model, we extracted all predicting features of the model and identified brain regions by visualizing the results of prediction. To investigate the association between fALFF change (post-per treatment) of predict weight brain regions and clinical outcomes (frequency of migraine attack) in the real group, we extracted the average z values of the brain regions. We then performed a partial correlation analysis between the fALFF z value change and the clinical outcomes (frequency of migraine attack), using age, sex, SAS, SDS, and MSQ as covariates. A threshold of $p < 0.05$ false discovery rate (FDR) corrected was applied for multiple comparisons.

See Figure 1 for the flow diagram of classification and prediction.

Results

Clinical characteristics

A total of 70 patients with MWoA and 70 HCs participated in the study. They all completed the first scanning fMRI. Nine patients were dropped out in the sham tVNS group (2 for change of residency, 2 for familial dissenting opinion, 2 for time restriction, and 3 for unsatisfied with treatment). Two patients were dropped out in the real tVNS group (1 for time restriction and 1 for unsatisfied with treatment). Consequently, 33 patients with real tVNS and 26 patients with sham tVNS finally completed studies with two times scanning fMRI. Table 1 showed the demographic of participants and migraine characteristics of patients with MWoA. Age and sex between HCs and patients were balanced. After a 4-week treatment, patients in real group indicated a significant reduction in migraine attack frequency ($t = 3.341$, $p = 0.002$), VAS score of attack intensity ($t = 4.614$, $p < 0.001$), SAS ($t = 4.627$, $p < 0.001$), SDS ($t = 3.900$, $p < 0.001$), and MSQ ($t = 6.603$, $p < 0.001$).

Classification results

With the number of features increasing, the accuracy changed dynamically (Figure 2). The top 3,650 meaningful features showed the best classification ability (79.3% accuracy, 78.6% sensitivity, 80.0% specificity, and 83.35% AUC), which suggested well performance of the result in machine learning. The permutation analysis conducted 5,000 times showed that the classifier with 3,650 meaningful features was superior

TABLE 1 Characteristics of each subject group.

Study 1

	MWoA ($n = 70$)	HCs ($n = 70$)	t/χ^2	p value
Age (years)	30.34 ± 7.20	28.07 ± 6.71	1.93	0.056
Sex (males/females)	16/54	25/45	2.794	0.095
Attack frequency (times/month)	3.81 ± 2.39			
VAS	49.77 ± 15.45			
SAS	43.39 ± 5.64			
SDS	44.88 ± 5.97			
MSQ	57.23 ± 9.94			

Study 2 ($n = 33$)

	Before	After	t/χ^2	p value
Age (years)	29.94 ± 6.30			
Sex (males/females)	10/23			
Attack frequency (times/month)	4.0 ± 2.3	2.55 ± 2.25	3.341	0.002
VAS	49.98 ± 14.67	32.23 ± 21.26	4.614	<0.001
SAS	43.30 ± 6.15	40.27 ± 6.98	4.627	<0.001
SDS	43.94 ± 6.14	41.0 ± 6.09	3.900	<0.001
MSQ	57.12 ± 9.68	70.76 ± 10.62	6.063	<0.001

VAS, visual analog scale; SAS, self-rating anxiety scale; SDS, self-rating depression scale; MSQ, Migraine Specific Quality of Life Questionnaire.

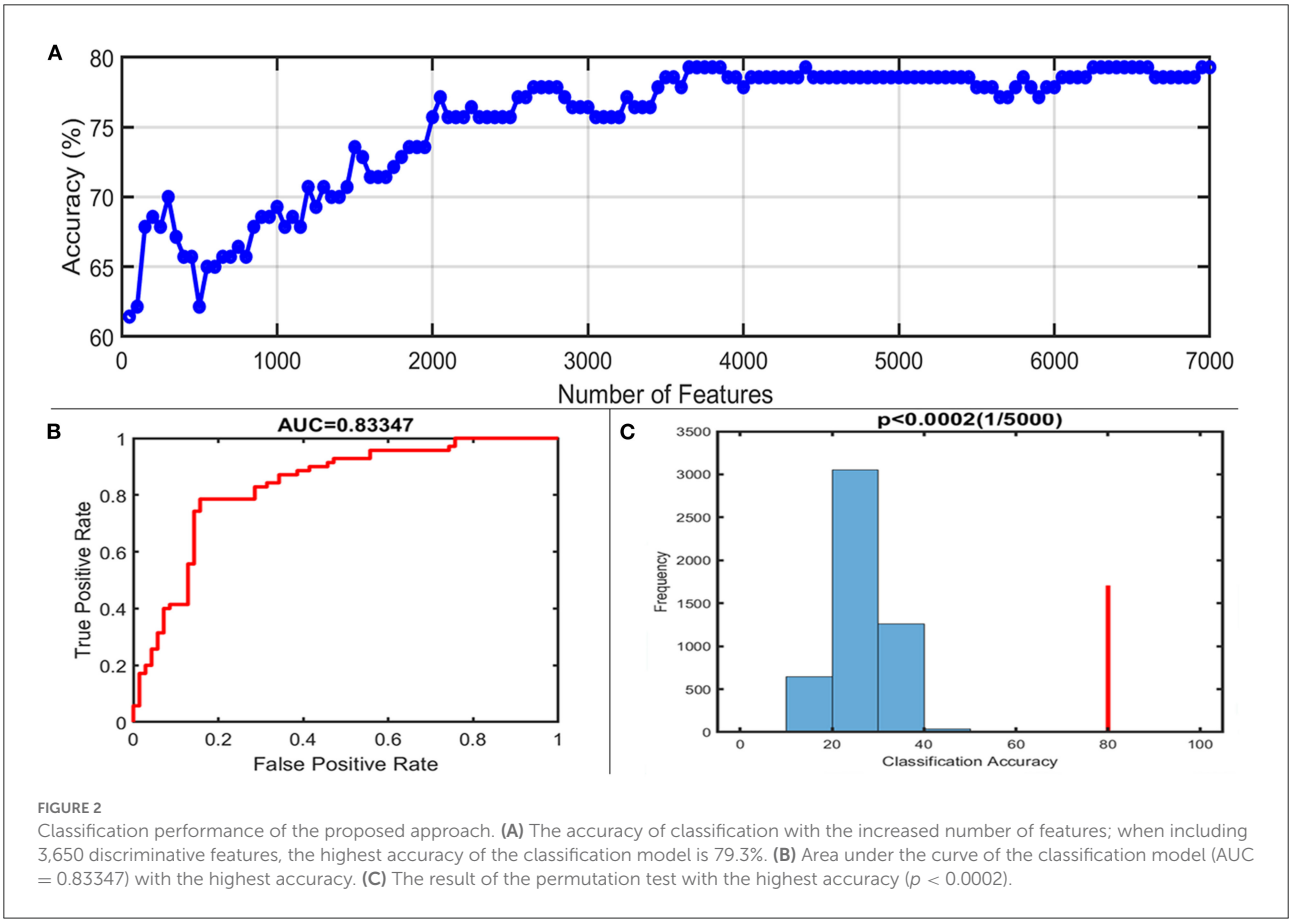

FIGURE 2
Classification performance of the proposed approach. **(A)** The accuracy of classification with the increased number of features; when including 3,650 discriminative features, the highest accuracy of the classification model is 79.3%. **(B)** Area under the curve of the classification model (AUC = 0.83347) with the highest accuracy. **(C)** The result of the permutation test with the highest accuracy ($p < 0.0002$).

TABLE 2 Discriminative features to discriminate MWoA from HCs.

Weight	Cluster voxels	Brain region	Peak intensity	MNI coordinates		
				X	Y	Z
Postive weight	16	TCC/RVMS_Bi	0.006254	3	−30	−48
	22	Frontal_Sup_L	0.010727	−21	30	57
	13	Temporal_pole_sup_L	0.007674	−39	6	−18
	10	Temporal_Sup_L	0.007724	−51	−15	−6
	18	Supp_Motor_Area_R_L	0.005871	6	21	66
	5	Occipital_Sup_L	0.005292	−12	−93	36
	6	ParaHipppcampal_R	0.005616	21	−12	−30
	11	Cerebelum_Crus1_L	0.008698	−51	−63	−33
Negative weight	8	Thalamus_R	−0.007086	6	−9	9
	24	Cingulum_Mid and Post_Bi	−0.005831	−6	−45	33
	9	Frontal_Sup_Medial_L	−0.008821	−3	42	54
	12	Frontal_Sup_Medial_R	−0.009839	6	51	24
	12	Frontal_ Mid_L	−0.006807	−42	15	45
	9	Frontal_Orb_L	−0.010326	−21	24	−18
	30	Frontal_Sup_R	−0.006637	21	−12	63
	14	Paracentral Lobule_L	−0.008106	−6	−18	66
	12	Postcentral_L	−0.007618	−39	−21	54
	18	Precuneus_L	−0.007034	−3	−69	63

Bi, bilateral; Inf, inferior; L, left; Mid, middle; Orb, orbit; R, right; RVM, rostral ventromedial medulla; Sup, superior; TCC, trigeminal cervical complex.

to the random classifiers ($p < 0.002$). After extracting the discriminative features, we found the classification results of 3650 voxels were enormous and very unfavourable for results demonstrating. According to previous study (Li et al., 2014), we identified brain regions by setting the threshold to >30% of the maximum weight vector scores for visualizing the results of classification (Table 2). The voxels were found in bilateral TCC/rostral ventromedial medulla (TCC/RVM), bilateral mPFC, bilateral MCC, right thalamus, temporal gyrus, right precuneus, and postcentral gyrus, which were involved in trigeminal spinal-thalamo-cortical pathways, default mode network (DMN), auditory network, and DPMS (Figure 3).

Prediction of tVNS efficacy

A total of 3,650 discriminative features from study 1 were used to construct the predicting model. We found that 70 of 3,650 discriminative features contributed significantly to predicting the reduction of attacks after a 4-week tVNS treatment ($r = 0.36$, $p = 0.03$) (Figure 4). After extracting the predictive features, we analyzed the distribution of discriminative voxels (Table 3). The voxels are mainly distributed in TCC/RVM, mPFC, and temporal gyrus (Figure 5). Moreover, a paired *t*-test showed significant fALFF changes in right TCC/RVM, bilateral mPFC, right superior temporal gyrus/middle temporal gyrus (TSG/TMG), left insula, and left MCC. The reduction of migraine attack frequency had a positive correlation with TCC/RVM ($r = 0.433$, $p = 0.021$), bilateral mPFC ($r = 0.419$, $p = 0.029$), and left MCC ($r = 0.451$, $p = 0.016$), and negative with left insula ($r = -0.473$, $p = 0.011$) and right TSG/TMG ($r = -0.684$, $p < 0.001$), respectively (Figure 6, Figure 7).

Discussion

As an advanced exploration of the previous study (Zhang et al., 2021), by performing complicated machine learning with fMRI, we investigated the potential of spontaneous brain activity in individual diagnosis and treatment in the present study. Our results not only confirmed that the aberrant fALFF patterns served the possibility to be a neuroimaging biomarker with high accuracy (79.3%), sensitivity (78.6%), and specificity (80.0%) in classifying MWoA but also further extended the clinical value of the classification model for predicting the efficacy of tVNS with moderate correlation ($r = 0.36$). The results indicated that TCC/RVM, MCC, mPFC, and temporal gyrus are the main brain regions in discriminating migraine and predicting the efficacy of tVNS treatment which can be involved in trigeminal spinal-thalamo-cortical pathways, DMN, AN, and DPMS, respectively. Meanwhile, fALFF of the above brain regions had high correlations with the reduction of

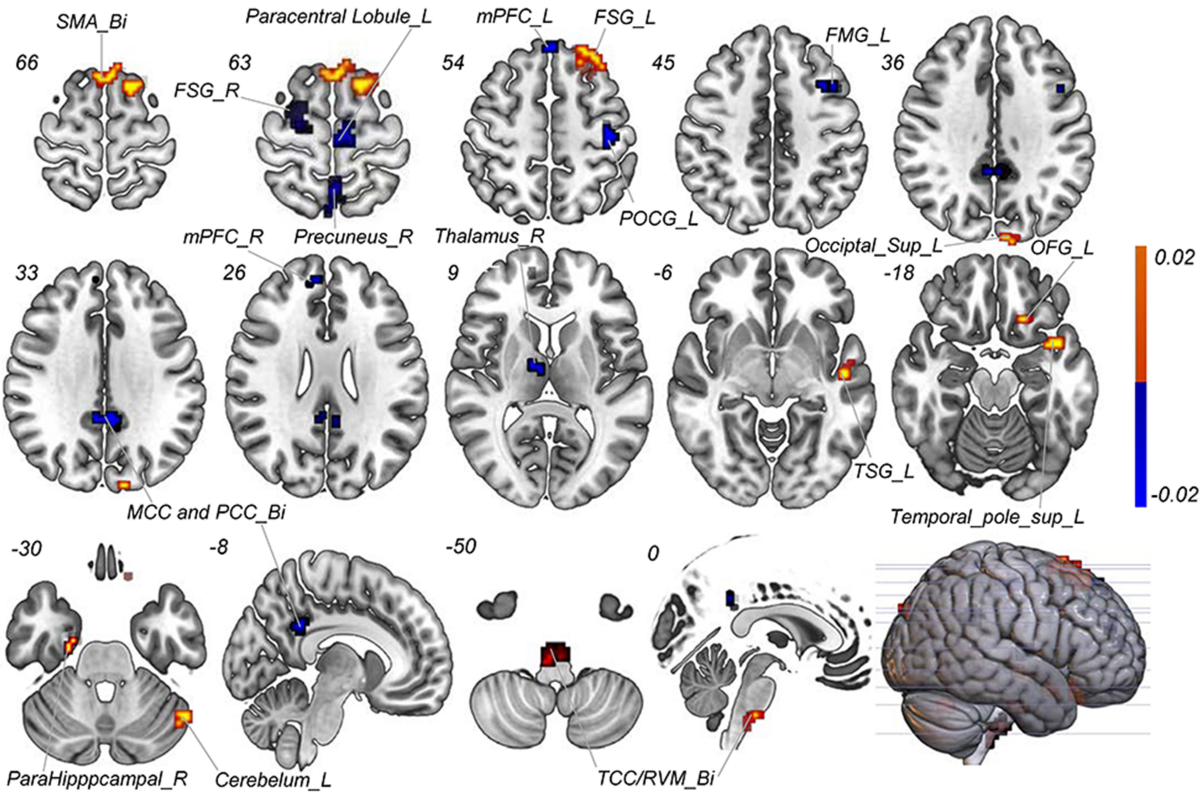

FIGURE 3
Discriminative features to discriminate MWoA patients and HCs. Red means positive weight and blue means negative weight. The main weight brain areas were located in TCC/RVM, thalamus, PFC, and TSG/TMG. Bi, bilateral; FMG, frontal middle gyrus; FSG, frontal superior gyrus; L, left; MCC, middle cingulate cortex; mPFC, medial prefrontal gyrus; OFG, orbitofrontal gyrus; PCC, post cingulate cortex; POCG, postcentral gyrus; R, right; RVM, rostral ventromedial medulla; SMA, supplementary motor area; Sup, superior; TCC, trigeminal cervical complex; TMG, middle temporal gyrus; TSG, superior temporal gyrus.

migraine attack frequency after tVNS treatment. Taken together, our results linked disrupted spontaneous brain activity to migraine and enhanced the comprehension of pathophysiology and treatment of migraine.

In the study, we demonstrated that both TCC/RVM and thalamus could help to identify MWoA which extended previous findings that the thalamus could be used for identifying migraineurs (Chong et al., 2017; Tu et al., 2020). As well-known, TCC and thalamus are the first and second order of trigeminal spinal-thalamo-cortical pathways, cooperating in migraine attacks (Ashina, 2020; Lim et al., 2021). On one hand, TCC is the trigger of sensitization and activation of nociception transmitting nociception from the periphery to the central (Bartsch and Goadsby, 2003; Weir and Cader, 2011; Akerman and Romero-Reyes, 2013; Luz et al., 2019). On the other hand, through comprehensive processing in the selection, amplification, and prioritization, the thalamus could handle the nociceptive inputs from TCC, and then, projected to higher centers inducing pain response (Schwedt et al., 2013; Noseda et al., 2017; Tolner et al., 2019; Tu et al., 2019). The synergistic effect of TCC and thalamus in nociception transmission might be a crucial key to pain response and analgesia.

Consequently, in terms of its important position in the pathophysiology and treatment of migraine, researchers have investigated the aberrant brain alternation of trigeminal spinal-thalamo-cortical pathways in migraineurs. A recent study found a greater BOLD signal variability of the trigeminal spinal-thalamo-cortical pathways in migraineurs than HCs which may amplify nociception processing in migraineurs (Lim et al., 2021). Meanwhile, Meylakh et al. found increasing ALFF of thalamus, TCC/RVM, dorsal pons, and thalamus in migraineurs before an attack (Meylakh et al., 2018). This evidence suggested that TCC and thalamus were coupled to the progress of headache attack events. Moreover, two studies revealed that TCC had a negative correlation with migraine attack intensity, whereas the thalamus had a positive correlation (Hodkinson et al., 2016; Li et al., 2017), revealing a close relationship between the trigeminal spinal-thalamo-cortical pathway and migraine. Further studies suggested that acupuncture, triptans, and tVNS could regulate the disrupted functional connectivity

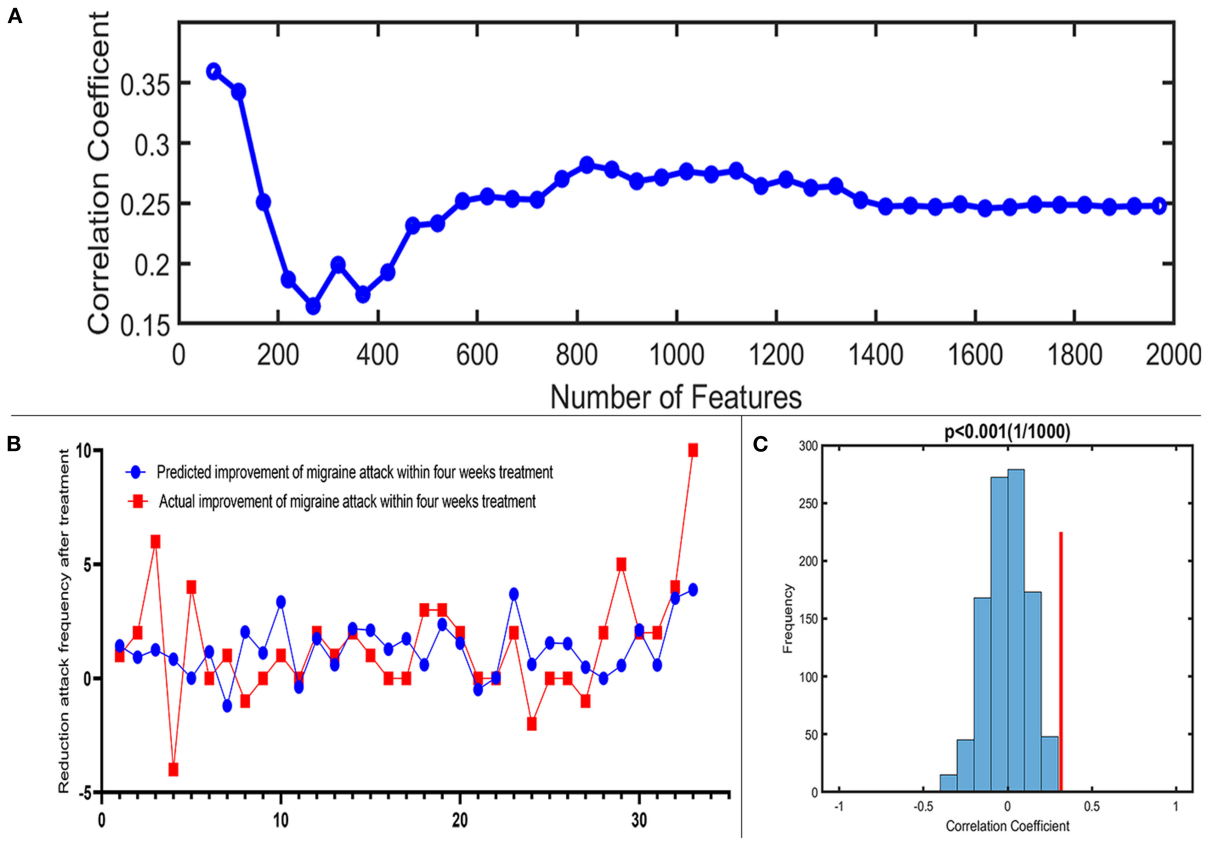

FIGURE 4
Prediction performance of the proposed approach. **(A)** Line chart reflecting actual label and predictive label; when including 70 predictive features, the highest correlation coefficient is 0.36. **(B)** Correlation coefficient between the actual label and predictive label with the increased number of features in the reduction of migraine attack frequency. **(C)** The result of the permutation test with the highest correlation coefficient ($p < 0.001$).

TABLE 3 Predictive features to predict the efficacy of tVNS.

Weight	Cluster voxels	Brain region	Peak intensity	MNI coordinates		
				X	Y	Z
Positive weight	5	TCC/RVM_R	0.20958	9	−30	−51
	5	Frontal_Sup_Medial_L_R	0.117150	0	63	24
	7	Cingulum_Mid_L	0161300	−3	−42	36
	7	Frontal_Mid_ L	0.189710	−27	24	54
	4	Frontal_Sup_R	0.003670	21	42	51
	8	Temporal_Inf_R	0.067577	57	−51	−30
Negative weight	9	Precuneus_R	−0.109190	21	−63	24
	6	Temporal_Sup_R	−0.222620	51	−12	−6
	3	Insula_L	−0.039590	−42	−12	0

Inf, inferior; L, left; Mid, middle; Orb, orbitofrontal; R, right; RVM, rostral ventromedial medulla; Sup, superior; TCC, trigeminal cervical complex.

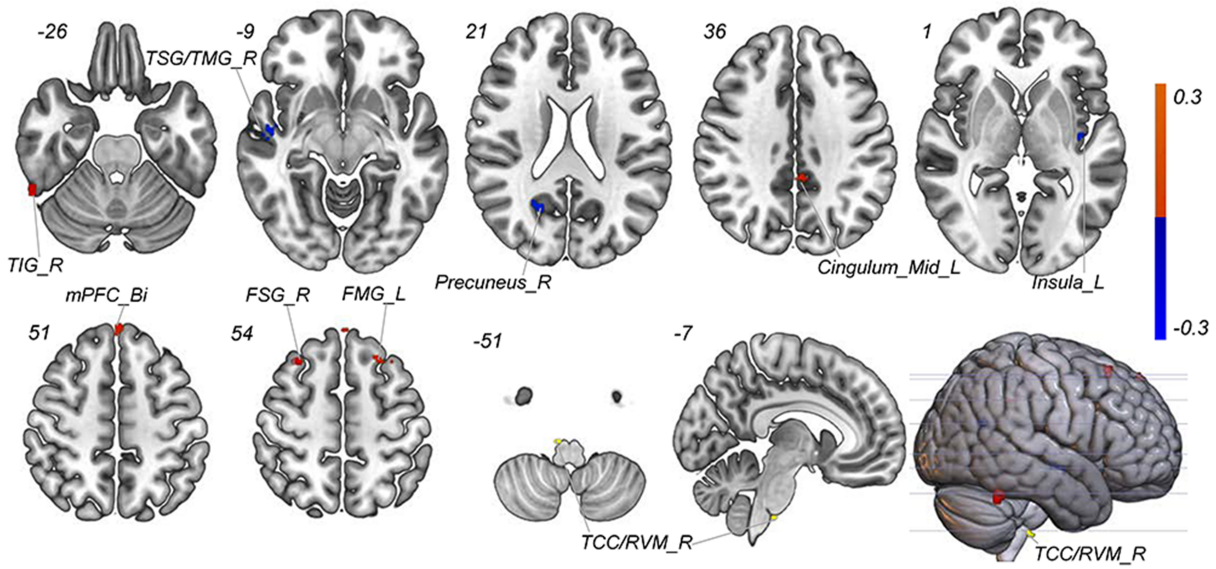

FIGURE 5
Predictive features to predict the efficacy of tVNS based on the classification model. Red means positive weight and blue means negative weight. The main weight brain areas were located in TCC/RVM, mPFC, TSG, and Insula. Bi, bilateral; FSG, frontal superior gyrus; FMG, frontal middle gyrus; L, left; mPFC, medial prefrontal gyrus; R, right; RVM, rostral ventromedial medulla; TCC, trigeminal cervical complex; TSG, superior temporal gyrus.

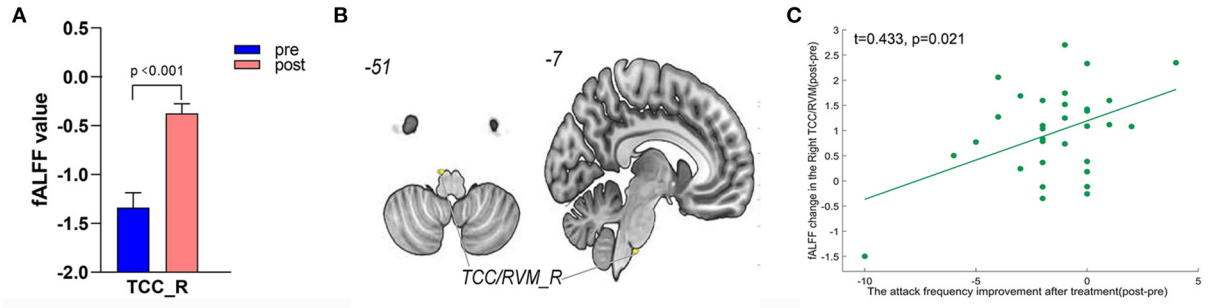

FIGURE 6
Treatment response of fALFF (mean±sem) and correlation between fALFF changes and migraine frequency changes (FDR corrected). **(A)** Significant increase of fALFF in right TCC/RVM after tVNS treatment. **(B)** Location of TCC/RVM, **(C)** a positive correlation between right TCC and the reduction of migraine attack frequency ($r = 0.433$, $p = 0.021$). R, right; RVM, rostral ventromedial medulla; TCC, trigeminal cervical complex.

and spontaneous activity within the trigeminal spinal-thalamo-cortical pathways in migraines (Kroger and May, 2015; Moller et al., 2020; Chang et al., 2021; Zhang et al., 2021). And modulation of trigeminal spinal-thalamo-cortical pathways is considered a crucial strategy for the management of migraine (Goadsby et al., 2009). Therefore, it may not be a coincidence that trigeminal spinal-thalamo-cortical pathways could be applied to discriminate migraineurs in our studies.

Another notable finding is that TCC/RVM could predict the efficacy of tVNS, which also had a positive correlation with the reduction of attack frequency. Animal experiments have revealed that tVNS could block the sensitization of TCC by direct and indirect pathways (Lyubashina et al., 2012; Lerman et al., 2019; Sclocco et al., 2019; Vila-Pueyo et al., 2019). Neuroimaging studies provided more straight evidence that tVNS could activate the signal of TCC in participants (Frangos et al., 2015; Frangos and Komisaruk, 2017). All the above studies suggested that tVNS could modulate the function of TCC supporting it as the meaningful brain region in predicting the efficacy of tVNS. Nonetheless, the result did not take the thalamus into the prediction model. But considering its crucial structural connection and synergetic effect with other brain regions of the trigeminal spinal-thalamo-cortical pathways and

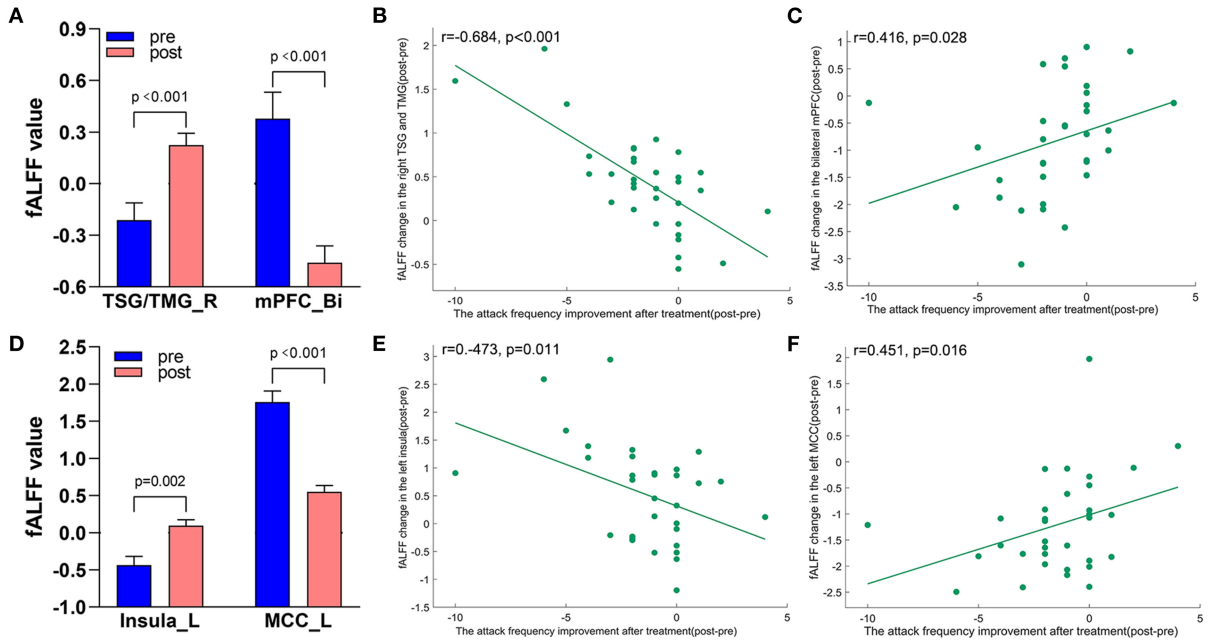

FIGURE 7
Treatment response of fALFF (mean ± sem) and correlation between fALFF changes and migraine frequency changes (FDR corrected). **(A)** Significant decrease and increase of fALFF in bilateral mPFC and right TSG/TMG after tVNS treatment. **(B)** A negative correlation between right TSG/TMG and the reduction of migraine attack frequency ($r = -0.684$, $p < 0.001$). **(C)** A positive correlation between bilateral mPFC and the reduction of migraine attack frequency ($r = 0.416$, $p = 0.028$). **(D)** Significant increase and decrease of fALFF in the left insula and left MCC after tVNS treatment. **(E)** A negative correlation between left Insula and the reduction of migraine attack frequency ($r = -0.473$, 0.011). **(F)** A positive correlation between left MCC and the reduction of migraine attack frequency ($r = 0.451$, $p = 0.016$). Bi, bilateral; L, left; MCC, middle cingulate cortex; mPFC, medial prefrontal gyrus; R, right; TMG, middle temporal gyrus; TSG, superior temporal gyrus.

response to the tVNS (Noseda et al., 2011; Luo et al., 2020b; Lim et al., 2021; Zhang et al., 2021), the effect of thalamus in predicting the efficacy of tVNS in treating migraine could not be neglected.

In addition to the trigeminal spinal-thalamo-cortical pathways, the DMN is another significant brain network in the study consisting of mPFC and precuneus. Consistent with other studies (Tu et al., 2020; Chen et al., 2021), our result showed that DMN could discriminate migraineurs from HCs. The DMN is a network related to individual stressful experiences which could respond to the environment in a predictive manner (Buckner et al., 2008; McEwen and Gianaros, 2011). Nevertheless, the abnormalities in DMN in migraineurs lead to information transfer and multimodal integration dysfunction (Xue et al., 2012; Tessitore et al., 2013; Zhang et al., 2016; Yu et al., 2017). Particularly, researchers have suggested that DMN had a negative correlation with pain intensity, attack frequency, and duration years of migraine (Xue et al., 2012; Gao et al., 2016; Yu et al., 2017; Coppola et al., 2020; Qin et al., 2020a), highlighting the role of DMN in migraine. Additionally, as the integration center, mPFC receives inputs from the thalamus and limbic system, modulating the pain response directly as well as impacting pain management indirectly by regulating emotion and cognition (Ong et al., 2019; Thompson and Neugebauer, 2019; Xu et al., 2019). In short, all the above studies confirmed the critical role of DMN in the pathogenesis of migraine which supported using the DMN to discriminate migraineurs from HCs.

Previous studies have revealed that tVNS could regulate the function of DMN in both physiological and pathological conditions (Kraus et al., 2007; Badran et al., 2018; Wang et al., 2018; Yap et al., 2020; Yakunina and Nam, 2021). Further studies suggested that tVNS would inhibit pain response through the DMN (Usichenko et al., 2017; Guo et al., 2020). What's more, our recent studies demonstrated the changes in the DMN in migraineurs after tVNS treatment (Luo et al., 2020b; Zhang et al., 2021). These findings implicated that DMN might be a potential target of tVNS treatment for migraine. Interestingly, the current study verified that DMN could predict the efficacy of tVNS in migraine management. Especially, fALFF of the mPFC had a positive correlation with the migraine attack frequency. These results expanded our understanding of the important role of the DMN in tVNS treatment for migraine.

Moreover, another finding of the study is that temporal gyrus, MCC, and insula also played important role in discrimination and prediction, whose fALFF value had a

correlation with the reduction of attack frequency. These brain regions have two main roles in migraine. For one thing, migraineurs often complain about phonophobia and tinnitus, especially suffering migraine attacks. A reasonable explanation is that MWoA may have a more vulnerable temporal gyrus that is susceptible to external stimuli, causing concomitant symptoms associated with auditory (Langguth et al., 2015; Goadsby et al., 2017). For another, MCC and insula would be responsible for coding pain perception and termination and integrating interoceptive information with emotional salience (Zhao et al., 2020). Acute nociceptive stimuli would consistently activate MCC and insula, affecting a subjective impression of our bodily state, tricking the body into making the wrong decision (Vogt, 2016; Uddin et al., 2017). Although we have provided a robust framework for neural markers in MWoA, there are still several limitations. First, we only recruited subjects suffering from MWoA, lacking comparisons in different subtypes of migraines. Admittedly, a comparison of various subtypes of migraine by machine learning would improve the performance of our model, but considering the morbidity, we thought MWoA should take priority. Second, we only adapted the SVM algorithm without comparing the differences in other algorithms. What needs illustration is that SVM is a growing popularity algorithm for its relative simplicity within the neuroimaging community (Campbell et al., 2020). Third, although we did not perform the sample size estimation, we determined it according to the previously published similar article (Yin et al., 2020). Further larger scale study will be conducted to enhance the data reliability (Page 18, line 431). Finally, it is a single-center study without external validation. Thus, further multi-center research should be carried out to verify the repeatability and generalization of the models.

Conclusion

In summary, the study preliminarily demonstrated that fALFF features (infra-slow oscillations) at baseline have good potential for classifying the MwoA with the HCs and predicting the individualized treatment response of tVNS. And we provided a pattern for selecting patients to respond well to tVNS for migraine which could optimize the allocation of medical resources. TCC/RVM, thalamus, mPFC, and temporal gyrus are the potential targets both in the classification and prediction model.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by Guangdong Provincial Hospital of Chinese Medicine. The patients/participants provided their written informed consent to participate in this study.

Author contributions

CF: investigation, conceptualization, and writing—original draft. YZ: conceptualization, methodology, formal analysis, and writing—original draft. YY: data curation, supervision, and project administration. XH: methodology and formal analysis. ZW: software, writing—review, and editing. ZY: resources and supervision. WL: data curation and supervision. MF: investigation and data curation. BL: conceptualization, supervision, project administration, and writing—review and editing. All authors approval of the version of the manuscript to be published.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Akerman, S., and Romero-Reyes, M. (2013). Insights into the pharmacological targeting of the trigeminocervical complex in the context of treatments of migraine. *Expert. Rev. Neurother.* 13, 1041–1059. doi: 10.1586/14737175.2013.827472

Arnold, M. (2018). Headache classification committee of the international headache society (ihs) the international classification of headache disorders. *Cephalalgia* 38, 1–211. doi: 10.1177/0333102417738202

Ashina, M. (2020). Migraine. *N. Engl. J. Med.* 383, 1866–1876. doi: 10.1056/NEJMra1915327

Ashina, S., Bentivegna, E., Martelletti, P., and Eikermann-Haerter, K. (2021). Structural and functional brain changes in migraine. *Pain Ther.* 10, 211–223. doi: 10.1007/s40122-021-00240-5

Badran, B. W., Dowdle, L. T., Mithoefer, O. J., LaBate, N. T., Coatsworth, J., Brown, J. C., et al. (2018). Neurophysiologic effects of transcutaneous auricular vagus nerve stimulation (taVNS) via electrical stimulation of the tragus: A concurrent taVNS/fMRI study and review. *Brain Stimul.* 11, 492–500. doi: 10.1016/j.brs.2017.12.009

Bartsch, T., and Goadsby, P. J. (2003). The trigeminocervical complex and migraine: current concepts and synthesis. *Curr. Pain Headache Rep.* 7, 371–376. doi: 10.1007/s11916-003-0036-y

Bisenius, S., Mueller, K., Diehl-Schmid, J., Fassbender, K., Grimmer, T., Jessen, F., et al. (2017). Predicting primary progressive aphasias with support vector machine approaches in structural MRI data. *Neuroimage Clin.* 14, 334–343. doi: 10.1016/j.nicl.2017.02.003

Blech, B., Starling, A. J., Marks, L. A., Wingerchuk, D. M., and O'Carroll, C. B. (2020). Is noninvasive vagus nerve stimulation a safe and effective alternative to medication for acute migraine control? *Neurologist* 25, 97–100. doi: 10.1097/NRL.0000000000000274

Buckner, R. L., Andrews-Hanna, J. R., and Schacter, D. L. (2008). The brain's default network: anatomy, function, and relevance to disease. *Ann. N Y Acad Sci.* 1124, 1–38. doi: 10.1196/annals.1440.011

Campbell, J. M., Huang, Z., Zhang, J., Wu, X., Qin, P., Northoff, G., et al. (2020). Pharmacologically informed machine learning approach for identifying pathological states of unconsciousness via resting-state fMRI. *Neuroimage* 206, 116316. doi: 10.1016/j.neuroimage.2019.116316

Cao, J., Zhang, Y., Li, H., Yan, Z., Liu, X., Hou, X., et al. (2021). Different modulation effects of 1 Hz and 20 Hz transcutaneous auricular vagus nerve stimulation on the functional connectivity of the periaqueductal gray in patients with migraine. *J. Transl. Med.* 19, 354. doi: 10.1186/s12967-021-03024-9

Chang, C. M., Yang, C. P., Yang, C. C., Shih, P. H., and Wang, S. J. (2021). Evidence of potential mechanisms of acupuncture from functional MRI data for migraine prophylaxis. *Curr. Pain Headache Rep.* 25, 49. doi: 10.1007/s11916-021-00961-4

Chen, H., Qi, G., Zhang, Y., Huang, Y., Zhang, S., Yang, D., et al. (2021). Altered dynamic amplitude of low-frequency fluctuations in patients with migraine without aura. *Front. Hum. Neurosci.* 15, 636472. doi: 10.3389/fnhum.2021.636472

Chiarelli, A. M., Sestieri, C., Navarra, R., Wise, R. G., and Caulo, M. (2021). Distinct effects of prematurity on MRI metrics of brain functional connectivity, activity, and structure: Univariate and multivariate analyses. *Hum. Brain Mapp.* 42:3593–607. doi: 10.1002/hbm.25456

Chong, C. D., Gaw, N., Fu, Y., Li, J., Wu, T., and Schwedt, T. J. (2017). Migraine classification using magnetic resonance imaging resting-state functional connectivity data. *Cephalalgia* 37, 828–844. doi: 10.1177/0333102416652091

Coppola, G., Di Renzo, A., Petolicchio, B., Tinelli, E., Di Lorenzo, C., Serrao, M., et al. (2020). Increased neural connectivity between the hypothalamus and cortical resting-state functional networks in chronic migraine. *J. Neurol.* 267, 185–191. doi: 10.1007/s00415-019-09571-y

Coppola, G., Di Renzo, A., Tinelli, E., Di Lorenzo, C., Scapeccia, M., Parisi, V., et al. (2018). Resting state connectivity between default mode network and insula encodes acute migraine headache. *Cephalalgia* 38, 846–854. doi: 10.1177/0333102417715230

Cornelison, L. E., Woodman, S. E., and Durham, P. L. (2020). Inhibition of trigeminal nociception by non-invasive vagus nerve stimulation: investigating the role of gabaergic and serotonergic pathways in a model of episodic migraine. *Front. Neurol.* 11, 146. doi: 10.3389/fneur.2020.00146

Diener, H. C., Goadsby, P. J., Ashina, M., Al-Karagholi, M. A., Sinclair, A., Mitsikostas, D., et al. (2019). Non-invasive vagus nerve stimulation (nVNS) for the preventive treatment of episodic migraine: The multicentre, double-blind, randomised, sham-controlled PREMIUM trial. *Cephalalgia* 39, 1475–1487. doi: 10.1177/0333102419876920

Diener, H. C., Tassorelli, C., Dodick, D. W., Silberstein, S. D., Lipton, R. B., Ashina, M., et al. (2020). Guidelines of the International Headache Society for controlled trials of preventive treatment of migraine attacks in episodic migraine in adults. *Cephalalgia* 40, 1026–1044. doi: 10.1177/0333102420941839

Frangos, E., Ellrich, J., and Komisaruk, B. R. (2015). Non-invasive access to the vagus nerve central projections via electrical stimulation of the external ear: fMRI evidence in humans. *Brain Stimul.* 8, 624–636. doi: 10.1016/j.brs.2014.11.018

Frangos, E., and Komisaruk, B. R. (2017). Access to vagal projections via cutaneous electrical stimulation of the neck: fmri evidence in healthy humans. *Brain Stimul.* 10, 19–27. doi: 10.1016/j.brs.2016.10.008

Gao, Q., Xu, F., Jiang, C., Chen, Z., Chen, H., Liao, H., et al. (2016). Decreased functional connectivity density in pain-related brain regions of female migraine patients without aura. *Brain Res.* 1632, 73–81. doi: 10.1016/j.brainres.2015.12.007

Goadsby, P. J., Charbit, A. R., Andreou, A. P., Akerman, S., and Holland, P. R. (2009). Neurobiology of migraine. *Neuroscience* 161, 327–341. doi: 10.1016/j.neuroscience.2009.03.019

Goadsby, P. J., Holland, P. R., Martins-Oliveira, M., Hoffmann, J., Schankin, C., and Akerman, S. (2017). Pathophysiology of migraine: a disorder of sensory processing. *Physiol. Rev.* 97, 553–622. doi: 10.1152/physrev.00034.2015

Guo, X., Zhao, Y., Huang, F., Li, S., Luo, M., Wang, Y., et al. (2020). Effects of transcutaneous auricular vagus nerve stimulation on peripheral and central tumor necrosis factor alpha in rats with depression-chronic somatic pain comorbidity. *Neural. Plast.* 2020, 8885729. doi: 10.1155/2020/8885729

Hodkinson, D. J., Wilcox, S. L., Veggeberg, R., Noseda, R., Burstein, R., Borsook, D., et al. (2016). Increased amplitude of thalamocortical low-frequency oscillations in patients with migraine. *J. Neurosci.* 36, 8026–8036. doi: 10.1523/JNEUROSCI.1038-16.2016

Huang, J., Li, Y., Xie, H., Yang, S., Jiang, C., Sun, W., et al. (2020). Abnormal intrinsic brain activity and neuroimaging-based fMRI classification in patients with herpes zoster and postherpetic neuralgia. *Front. Neurol.* 11, 532110. doi: 10.3389/fneur.2020.532110

Khosla, M., Jamison, K., Ngo, G. H., Kuceyeski, A., and Sabuncu, M. R. (2019). Machine learning in resting-state fMRI analysis. *Magn. Reson. Imaging* 64, 101–121. doi: 10.1016/j.mri.2019.05.031

Kraus, T., Hosl, K., Kiess, O., Schanze, A., Kornhuber, J., and Forster, C. (2007). BOLD fMRI deactivation of limbic and temporal brain structures and mood enhancing effect by transcutaneous vagus nerve stimulation. *J. Neural. Transm.* 114, 1485–1493. doi: 10.1007/s00702-007-0755-z

Kroger, I. L., and May, A. (2015). Triptan-induced disruption of trigemino-cortical connectivity. *Neurology* 84, 2124–2131. doi: 10.1212/WNL.0000000000001610

Langguth, B., Hund, V., Busch, V., Jurgens, T. P., Lainez, J. M., Landgrebe, M., et al. (2015). Tinnitus and Headache. *Biomed. Res. Int.* 2015, 797416. doi: 10.1155/2015/797416

Launer, L. J., Terwindt, G. M., and Ferrari, M. D. (1999). The prevalence and characteristics of migraine in a population-based cohort: the GEM study. *Neurology* 53, 537–542. doi: 10.1212/WNL.53.3.537

Lerman, I., Davis, B., Huang, M., Huang, C., Sorkin, L., Proudfoot, J., et al. (2019). Noninvasive vagus nerve stimulation alters neural response and physiological autonomic tone to noxious thermal challenge. *PLoS ONE* 14, e0201212. doi: 10.1371/journal.pone.0201212

Li, F., Huang, X., Tang, W., Yang, Y., Li, B., Kemp, G. J., Gong, Q. (2014). Multivariate pattern analysis of DTI reveals differential white matter in individuals with obsessive-compulsive disorder. *Hum Brain Mapp.* 35, 2643–2651. doi: 10.1002/hbm.22357

Li, Z., Zeng, F., Yin, T., Lan, L., Makris, N., Jorgenson, K., et al. (2017). Acupuncture modulates the abnormal brainstem activity in migraine without aura patients. *Neuroimage Clin.* 15, 367–375. doi: 10.1016/j.nicl.2017.05.013

Lim, M., Jassar, H., Kim, D. J., Nascimento, T. D., and DaSilva, A. F. (2021). Differential alteration of fMRI signal variability in the ascending trigeminal somatosensory and pain modulatory pathways in migraine. *J. Headache Pain* 22, 4. doi: 10.1186/s10194-020-01210-6

Luo, J., Yang, R., Yang, W., Duan, C., Deng, Y., Zhang, J., et al. (2020a). Increased amplitude of low-frequency fluctuation in right angular gyrus and left superior occipital gyrus negatively correlated with heroin use. *Front. Psychiat.* 11, 492. doi: 10.3389/fpsyt.2020.00492

Luo, W., Zhang, Y., Yan, Z., Liu, X., Hou, X., Chen, W., et al. (2020b). The instant effects of continuous transcutaneous auricular vagus nerve stimulation at

acupoints on the functional connectivity of amygdala in migraine without aura: a preliminary study. *Neural. Plast.* 2020, 8870589. doi: 10.1155/2020/8870589

Luz, L. L., Fernandes, E. C., Dora, F., Lukoyanov, N. V., Szucs, P., and Safronov, B. V. (2019). Trigeminal Adelta- and C-afferent supply of lamina I neurons in the trigeminocervical complex. *Pain* 160, 2612–2623. doi: 10.1097/j.pain.0000000000001659

Lyubashina, O. A., Sokolov, A. Y., and Panteleev, S. S. (2012). Vagal afferent modulation of spinal trigeminal neuronal responses to dural electrical stimulation in rats. *Neuroscience* 222, 29–37. doi: 10.1016/j.neuroscience.2012.07.011

Ma, X., Lu, F., Chen, H., Hu, C., Wang, J., Zhang, S., et al. (2020). Static and dynamic alterations in the amplitude of low-frequency fluctuation in patients with amyotrophic lateral sclerosis. *PeerJ.* 8, e10052. doi: 10.7717/peerj.10052

Marciszewski, K. K., Meylakh, N., Di Pietro, F., Mills, E. P., Macefield, V. G., Macey, P. M., et al. (2018). Changes in Brainstem Pain Modulation Circuitry Function over the Migraine Cycle. *J Neurosci* 38, 10479–10488. doi: 10.1523/JNEUROSCI.1088-18.2018

McEwen, B. S., and Gianaros, P. J. (2011). Stress- and allostasis-induced brain plasticity. *Annu. Rev. Med.* 62, 431–445. doi: 10.1146/annurev-med-052209-100430

Meylakh, N., Marciszewski, K. K., Di Pietro, F., Macefield, V. G., Macey, P. M., and Henderson, L. A. (2018). Deep in the brain: Changes in subcortical function immediately preceding a migraine attack. *Hum. Brain Mapp.* 39, 2651–2663. doi: 10.1002/hbm.24030

Moller, M., Mehnert, J., Schroeder, C. F., and May, A. (2020). Noninvasive vagus nerve stimulation and the trigeminal autonomic reflex: An fMRI study. *Neurology* 94, e1085–e1093. doi: 10.1212/WNL.0000000000008865

Morais, A., Liu, T. T., Qin, T., Sadhegian, H., Ay, I., Yagmur, D., et al. (2020). Vagus nerve stimulation inhibits cortical spreading depression exclusively through central mechanisms. *Pain* 161, 1661–1669. doi: 10.1097/j.pain.0000000000001856

Mu, J., Chen, T., Quan, S., Wang, C., Zhao, L., and Liu, J. (2020). Neuroimaging features of whole-brain functional connectivity predict attack frequency of migraine. *Hum. Brain Mapp.* 41, 984–993. doi: 10.1002/hbm.24854

Noseda, R., Borsook, D., and Burstein, R. (2017). Neuropeptides and Neurotransmitters That Modulate Thalamo-Cortical Pathways Relevant to Migraine Headache. *Headache* 57, 97–111. doi: 10.1111/head.13083

Noseda, R., Jakubowski, M., Kainz, V., Borsook, D., and Burstein, R. (2011). Cortical projections of functionally identified thalamic trigeminovascular neurons: implications for migraine headache and its associated symptoms. *J. Neurosci.* 31, 14204–14217. doi: 10.1523/JNEUROSCI.3285-11.2011

Ong, W. Y., Stohler, C. S., and Herr, D. R. (2019). Role of the prefrontal cortex in pain processing. *Mol. Neurobiol.* 56, 1137–1166. doi: 10.1007/s12035-018-1130-9

Ozge, A., Aydinlar, E., and Tasdelen, B. (2015). Grey zones in the diagnosis of adult migraine without aura based on the International Classification of Headache Disorders-III beta: exploring the covariates of possible migraine without aura. *Pain Res. Manag.* 20, e1–7. doi: 10.1155/2015/234193

Qin, Z., Su, J., He, X. W., Ban, S., Zhu, Q., Cui, Y., et al. (2020a). Disrupted functional connectivity between sub-regions in the sensorimotor areas and cortex in migraine without aura. *J. Headache Pain.* 21, 47. doi: 10.1186/s10194-020-01118-1

Qin, Z. X., Su, J. J., He, X. W., Zhu, Q., Cui, Y. Y., Zhang, J. L., et al. (2020b). Altered resting-state functional connectivity between subregions in the thalamus and cortex in migraine without aura. *Eur. J Neurol.* 27, 2233–2241. doi: 10.1111/ene.14411

Rocca, M. A., Harrer, J. U., and Filippi, M. (2020). Are machine learning approaches the future to study patients with migraine? *Neurology* 94, 291–292. doi: 10.1212/WNL.0000000000008956

Scheinost, D., Noble, S., Horien, C., Greene, A. S., Lake, E. M., Salehi, M., et al. (2019). Ten simple rules for predictive modeling of individual differences in neuroimaging. *Neuroimage* 193, 35–45. doi: 10.1016/j.neuroimage.2019.02.057

Schneider, I., Schmitgen, M. M., Boll, S., Roth, C., Nees, F., Usai, K., et al. (2020). Oxytocin modulates intrinsic neural activity in patients with chronic low back pain. *Eur. J. Pain* 24, 945–955. doi: 10.1002/ejp.1543

Schwedt, T. J., Schlaggar, B. L., Mar, S., Nolan, T., Coalson, R. S., Nardos, B., et al. (2013). Atypical resting-state functional connectivity of affective pain regions in chronic migraine. *Headache* 53, 737–751. doi: 10.1111/head.12081

Sclocco, R., Garcia, R. G., Kettner, N. W., Isenburg, K., Fisher, H. P., Hubbard, C. S., et al. (2019). The influence of respiration on brainstem and cardiovagal response to auricular vagus nerve stimulation: A multimodal ultrahigh-field (7T) fMRI study. *Brain Stimul.* 12, 911–921. doi: 10.1016/j.brs.2019.02.003

Straube, A., Ellrich, J., Eren, O., Blum, B., and Ruscheweyh, R. (2015). Treatment of chronic migraine with transcutaneous stimulation of the auricular branch of the vagal nerve (auricular t-VNS): a randomized, monocentric clinical trial. *J. Headache Pain* 16, 543. doi: 10.1186/s10194-015-0543-3

Tassorelli, C., Grazzi, L., de Tommaso, M., Pierangeli, G., Martelletti, P., Rainero, I., et al. (2018). Noninvasive vagus nerve stimulation as acute therapy for migraine: The randomized PRESTO study. *Neurology* 91, e364–e373. doi: 10.1212/WNL.0000000000005857

Tessitore, A., Russo, A., Giordano, A., Conte, F., Corbo, D., De Stefano, M., et al. (2013). Disrupted default mode network connectivity in migraine without aura. *J. Headache Pain* 14, 89. doi: 10.1186/1129-2377-14-89

Thompson, J. M., and Neugebauer, V. (2019). Cortico-limbic pain mechanisms. *Neurosci. Lett.* 702, 15–23. doi: 10.1016/j.neulet.2018.11.037

Tolner, E. A., Chen, S. P., and Eikermann-Haerter, K. (2019). Current understanding of cortical structure and function in migraine. *Cephalalgia* 39, 1683–1699. doi: 10.1177/0333102419840643

Tu, Y., Fu, Z., Zeng, F., Maleki, N., Lan, L., Li, Z., et al. (2019). Abnormal thalamocortical network dynamics in migraine. *Neurology* 92, e2706–e2716. doi: 10.1212/WNL.0000000000007607

Tu, Y., Zeng, F., Lan, L., Li, Z., Maleki, N., Liu, B., et al. (2020). An fMRI-based neural marker for migraine without aura. *Neurology* 94, e741–e751. doi: 10.1212/WNL.0000000000008962

Uddin, L. Q., Nomi, J. S., Hébert-Seropian, B., Ghaziri, J., and Boucher, O. (2017). Structure and Function of the Human Insula. *J. Clin. Neurophysiol.* 34, 300–306. doi: 10.1097/WNP.0000000000000377

Usichenko, T., Laqua, R., Leutzow, B., and Lotze, M. (2017). Preliminary findings of cerebral responses on transcutaneous vagal nerve stimulation on experimental heat pain. *Brain Imaging Behav.* 11, 30–37. doi: 10.1007/s11682-015-9502-5

Vila-Pueyo, M., Strother, L. C., Kefel, M., Goadsby, P. J., and Holland, P. R. (2019). Divergent influences of the locus coeruleus on migraine pathophysiology. *Pain* 160, 385–394. doi: 10.1097/j.pain.0000000000001421

Vogt, B. A. (2016). Midcingulate cortex: Structure, connections, homologies, functions and diseases. *J. Chem. Neuroanat.* 74, 28–46. doi: 10.1016/j.jchemneu.2016.01.010

Wang, J. J., Chen, X., Sah, S. K., Zeng, C., Li, Y. M., Li, N., et al. (2016). Amplitude of low-frequency fluctuation (ALFF) and fractional ALFF in migraine patients: a resting-state functional MRI study. *Clin. Radiol.* 71, 558–564. doi: 10.1016/j.crad.2016.03.004

Wang, Z., Fang, J., Liu, J., Rong, P., Jorgenson, K., Park, J., et al. (2018). Frequency-dependent functional connectivity of the nucleus accumbens during continuous transcutaneous vagus nerve stimulation in major depressive disorder. *J. Psychiatr. Res.* 102, 123–131. doi: 10.1016/j.jpsychires.2017.12.018

Weir, G. A., and Cader, M. Z. (2011). New directions in migraine. *BMC Med.* 9, 116. doi: 10.1186/1741-7015-9-116

Xu, P., Chen, A., Li, Y., Xing, X., and Lu, H. (2019). Medial prefrontal cortex in neurological diseases. *Physiol. Genomics* 51, 432–442. doi: 10.1152/physiolgenomics.00006.2019

Xue, T., Yuan, K., Cheng, P., Zhao, L., Zhao, L., Yu, D., et al. (2013). Alterations of regional spontaneous neuronal activity and corresponding brain circuit changes during resting state in migraine without aura. *NMR Biomed.* 26, 1051–1058. doi: 10.1002/nbm.2917

Xue, T., Yuan, K., Zhao, L., Yu, D., Zhao, L., Dong, T., et al. (2012). Intrinsic brain network abnormalities in migraines without aura revealed in resting-state fMRI. *PLoS ONE* 7, e52927. doi: 10.1371/journal.pone.0052927

Yakunina, N., and Nam, E. C. (2021). Direct and Transcutaneous Vagus Nerve Stimulation for Treatment of Tinnitus: A Scoping Review. *Front. Neurosci.* 15, 680590. doi: 10.3389/fnins.2021.680590

Yan, C. G., Wang, X. D., Zuo, X. N., and Zang, Y. F. (2016). DPABI: Data Processing and Analysis for (Resting-State) Brain Imaging. *Neuroinformatics* 14, 339–351. doi: 10.1007/s12021-016-9299-4

Yang, X., Hu, X., Tang, W., Li, B., Yang, Y., Gong, Q., et al. (2019). Multivariate classification of drug-naive obsessive-compulsive disorder patients and healthy controls by applying an SVM to resting-state functional MRI data. *BMC Psychiatry* 19, 210. doi: 10.1186/s12888-019-2184-6

Yap, J. Y. Y., Keatch, C., Lambert, E., Woods, W., Stoddart, P. R., and Kameneva, T. (2020). Critical review of transcutaneous vagus nerve stimulation: challenges for translation to clinical practice. *Front. Neurosci.* 14, 284. doi: 10.3389/fnins.2020.00284

Yin, T., Sun, G., Tian, Z., Liu, M., Gao, Y., Dong, M., et al. (2020). The spontaneous activity pattern of the middle occipital gyrus predicts the clinical efficacy of acupuncture treatment for migraine without aura. *Front. Neurol.* 11, 588207. doi: 10.3389/fneur.2020.588207

Yu, D., Yuan, K., Luo, L., Zhai, J., Bi, Y., Xue, T., et al. (2017). Abnormal functional integration across core brain networks in migraine without aura. *Mol. Pain.* 13, 1744806917737461. doi: 10.1177/1744806917737461

Zhang, J., Su, J., Wang, M., Zhao, Y., Yao, Q., Zhang, Q., et al. (2016). Increased default mode network connectivity and increased regional homogeneity in migraineurs without aura. *J. Headache Pain.* 17, 98. doi: 10.1186/s10194-016-0692-z

Zhang, Y., Chen, H., Zeng, M., He, J., Qi, G., Zhang, S., et al. (2020). Abnormal whole brain functional connectivity pattern homogeneity and couplings in migraine without aura. *Front. Hum. Neurosci.* 14, 619839. doi: 10.3389/fnhum.2020.619839

Zhang, Y., Huang, Y., Li, H., Yan, Z., Zhang, Y., Liu, X., et al. (2021). Transcutaneous auricular vagus nerve stimulation (taVNS) for migraine: an fMRI study. *Reg. Anesth. Pain Med.* 46, 145–150. doi: 10.1136/rapm-2020-102088

Zhao, Y., Liu, R., Zhang, J., Luo, J., and Zhang, W. (2020). Placebo effect on modulating empathic pain: reduced activation in posterior insula. *Front. Behav. Neurosci.* 14, 8. doi: 10.3389/fnbeh.2020.00008

Zou, Q. H., Zhu, C. Z., Yang, Y., Zuo, X. N., Long, X. Y., Cao, Q. J., et al. (2008). An improved approach to detection of amplitude of low-frequency fluctuation (ALFF) for resting-state fMRI: fractional ALFF. *J. Neurosci. Methods* 172, 137–141. doi: 10.1016/j.jneumeth.2008.04.012

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Transcutaneous auricular vagal nerve stimulation releases extrapineal melatonin and reduces thermal hypersensitivity in Zucker diabetic fatty rats

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Type 2 diabetes (T2D) is the most common comorbidity of COVID-19, and both are related to the lack of circulating melatonin. In addition, chronic pain is a common sequela of both COVID-19 and T2D. Using a neuropathic pain model produced by sciatic nerve chronic constriction injury in Zucker diabetic fatty rats, a verified preclinical genetic T2D neuropathy animal model, this study aimed to show that transcutaneous auricular vagal nerve stimulation (taVNS) could elevate plasma melatonin concentration, upregulate the expression of melatonin receptors (MTRs) in the amygdala, and relieve peripheral neuropathic pain. Furthermore, taVNS would restore melatonin levels and relieve pain even in pinealectomized rats. On the contrary, intraperitoneally injected luzindole, a melatonin receptor antagonist, would attenuate the antinociceptive effects of taVNS. In conclusion, the mechanism of the therapeutic effect of taVNS on chronic pain involves the release of extrapineal melatonin and the positive regulation of the expression of central MTRs. This beneficial efficacy should be considered during COVID-19 rehabilitation in individuals with diabetes.

KEYWORDS

extrapineal melatonin secretion, transcutaneous auricular vagal nerve stimulation, chronic pain relief, Zucker diabetic fatty rats, COVID-19 rehabilitation

Introduction

Affecting up to 77% of patients infected with SARS-CoV-2, chronic pain is the most common sequela of COVID-19 (Kemp et al., 2020). Angiotensin-converting enzyme 2 (ACE2) is a transmembrane protein that converts angiotensin II into angiotensin 1–7 to balance the renin-angiotensin system (Rahman et al., 2021). It has been recently verified that ACE2 is the receptor and the entry port for the SARS-CoV-2 to infect host cells. Once the virus binds to ACE2, it will lead to a downregulated expression of this enzyme

(Pawlikowski and Winczyk, 2021). Physiologically, ACE2 is also the escort of tryptophan transporter protein and is necessary for the successful absorption of tryptophan from the digestive tract (Pawlikowski and Winczyk, 2021). Since tryptophan is the precursor of melatonin (5-methoxy-*N*-acetyltryptamine), a neurohormone that has a short half-life of 0.56–20 min in the blood, the patient would soon exhaust it.

A growing number of studies demonstrated that melatonin modulates glucose metabolism through receptor-dependent influences on glucagon and insulin secretion (Peschke et al., 2007; Bähr et al., 2011; Nagorny et al., 2011). In pancreatic islets, the melatonin receptor type 1 (MT1) is expressed on α-cells while the type 2 (MT2) on β-cells (Peschke et al., 2007; Nagorny et al., 2011). At a physiological concentration, melatonin increases glucagon production from pancreatic α-cells (Bähr et al., 2011) but inhibits insulin production in β-cells, but with a functional phase shift following the binding of melatonin to the MT2 receptor (Stumpf et al., 2009). In the Type 2 diabetes condition, insulin secretion may lose part of this negative regulatory mechanism and result in hyperinsulinemia (Frese et al., 2009; McMullan et al., 2013). In a clinical setting, with the expectation to restore the melatonin level, physicians may prescribe melatonin tablets or injections to patients. Unfortunately, at this time, it is not known what melatonin dosage and administration time we should give to patients, mainly due to the fact that an ultra-physiological concentration of melatonin will induce a negative effect on glucose metabolism (Gerdin et al., 2004). In addition, it is necessary for melatonin to be secreted in a fluctuant manner to regulate blood glucose concentration, which is hard to reach by administrated melatonin (Ferlazzo et al., 2020).

Decreased pineal melatonin synthesis is reported in both rodents and patients with diabetes (Wajid et al., 2020). Our previous study demonstrated that transcutaneous auricular vagal nerve stimulation (taVNS) could effectively enhance central serotonergic function and reduce pain sensitivity in diabetic rats (Li et al., 2018). Since both pain sensitivity and insulin resistance are related to low plasma melatonin concentrations, it might be beneficial to elevate the circulating melatonin. It is now clear that there exist vagal efferent branches in the auricular concha. Pulses applied to the vagal branches will eventually reach the vagal afferent branches, innervating the digestive tract and stimulating the secretion of melatonin from pheochromocytes here (manuscript in preparing). Using a rodent model of diabetic neuropathic pain induced by sciatic nerve chronic constriction injury (CCI) in Zucker diabetic fatty (ZDF; *fa*/*fa*) rats, in this study, we show that taVNS would be an approach to enhance melatonin secretion in diabetic individuals with COVID-19 and the mechanism is, at least partially, due to an enhancement of extrapineal melatonin release and an upregulation of the expression of melatonin receptors (MTRs) type 1 (MT1) in the brain.

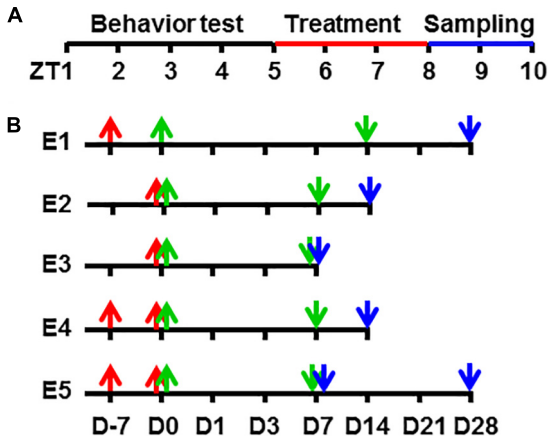

FIGURE 1
A schematic diagram of experimental procedures in zeitgeber time and time points. Showing the zeitgeber time of procedures **(A)** and the time points in each experimental group **(B)** in this study. ZT, zeitgeber time in hour [ZT0, light turn on time (7 AM)]. D, time in day; E1, Experiment 1; E2, Experiment 2, and so on.

Materials and methods

General methodology

Diabetic animal model

Male ZDF (*fa*/*fa*) rats were used as the diabetic animal models and were purchased from VitalRiver Laboratories International Inc. (Beijing, China). The animal room was artificially lighted from 7 a.m. (zeitgeber time, 0, ZT0) to 7 p.m. (ZT12; **Figure 1A**). Littermates from the same or from a foster mother were housed in large cages until they were ready to enter the experiment procedure at 8 weeks of age. We used only mature male ZDF rats for a further detailed study to avoid a possible confounding effect from gender differences on the endogenous melatonin level and other possible hormone variations. The experimental protocol was approved by the Institutional Animal Care and Use Committee in China Academy of Chinese Medical Sciences.

Neuropathic pain model

The sciatic nerve CCI model was produced by loosely ligating a common sciatic nerve referring to the method of Bennett and Xie (1988). Briefly, under 2% isoflurane-inhaling anesthesia, the left side sciatic nerve was separated from surrounding tissue. Four chromic suture circles were loosely ligated around the sciatic nerve with an interval

of 1 mm to each other so that only the superficial vessels of the nerve trunk were interrupted. The nerve was then put back and the wound closed with a suture clipper. The rats exhibiting postoperative neurological deficits (e.g., paralysis) or poor grooming were excluded from further experiments.

Electroacupuncture and time points

All the time points recorded in this study were in accordance with the taVNS occurrences, i.e., the beginning of taVNS was Day 0, and accidents that happened before this time point was recorded as the minus day. For example, D-7 meant 7 days before the beginning of taVNS, and D14 meant 14 days counted from the beginning of taVNS (**Figure 1B**).

For auricular concha electroacupuncture, under 2% isoflurane inhalation anesthesia, two opposite magnetic electrodes were placed over the auricular concha region of an ear, inside and outside, respectively. A procedure of 30-min transcutaneous electric nerve stimulation at a frequency of 2/15 Hz (2 and 15 Hz, switched every second) and an intensity of 2 mA was administered *via* an electrical stimulator (HANS-100, Nanjing, China). These stimulus parameters and sites have been repeatedly demonstrated to be effective in our previous study. The procedures were given in the afternoon (ZT5-8) after blood sample collection, beginning from Day 0. The acupoint selected in this study was the auricular concha of both sides, and the auricular margin was used as the sham acupoint.

Intraperitoneal injection

Vehicle, melatonin (60 mg/kg) or luzindole (0.01 μmol), was administered one time daily in the afternoon (ZT5-8) after blood sample collection. The dose of melatonin was based on our previous experiment and the dose of luzindole on the previous experiment. Melatonin and luzindole were purchased from Sigma Chemical Co. (St. Louis, United States) and dissolved in 5% ethanol saline (v/v) immediately before use.

Behavior tests

The animals were habituated to the test environment daily (a 60-min session) for 2 days before baseline testing. The testing procedure for thermal hyperalgesia was performed according to a previously published method (Hargreaves et al., 1988). The temperature was set to have the baseline latency of 12–14 s and a cutoff of 20 s. Mechanical allodynia was examined by applying a set of von Frey filaments to the plantar surface of each hindpaw, up and down depending on the withdrawal responses of the paw (Tal and Bennett, 1994). The cutoff force was 26 gm. All behavior testing was conducted between 8 a.m. and 12 p.m. (ZT1-5) before any daily drug or taVNS treatment.

Collection of plasma

For analyzing the concentration of melatonin in plasma, the rats were anesthetized by inhalation of 2% isoflurane in oxygen, and 0.1-ml blood samples were collected from one of the tail veins at each time point between 3 and 5 p.m. (ZT8-10) before daily treatments. The blood sample was centrifuged for 10 min at 1,000 rpm, and the plasma was collected. All plasma samples were stored at -80° until use. The animals were sacrificed after the last collection of blood, and brain samples were harvested.

Enzyme-linked immunosorbent assay

The concentration of plasma melatonin was analyzed using an enzyme-linked immunosorbent assay (ELISA) kit (Lot# DZE30014, R&D System, Beijing, China) by Huanya Biomedicine Technology Co. Ltd. The results were read using a microplate reader (Multiskan MK3, Thermo Scientific, Beijing, China) at a wavelength of 450 nm. The plasma melatonin concentration was calculated based on the standard curve and presented in nanograms per liter (ng/L).

Immunohistochemical staining

The rats were anesthetized with pentobarbital (60 mg/kg, i.p.) and transcardially perfused with 200 ml of saline, followed by 400 ml of 4% paraformaldehyde in a 0.1-M phosphate buffer (PB). The brains were dissected, postfixed for 2 h, and kept in 30% sucrose in 0.1-M PB in a cold room until they sank to the bottom. Tissues were then mounted in an OCT compound and frozen on dry ice. The brain (30 μm) sections were cut on a cryostat, mounted serially onto microscope slides, and stored at -80°C. Immunohistochemical staining was used to detect MT1 (1:1,000, rabbit polyclonal; Abbiotec, San Diego, CA, United States). Sections were blocked with 1% goat serum in 0.3% Triton × 100 for 1 h at room temperature and incubated overnight at 4°C with the primary antibody. For controls, the primary antibody was omitted. The sections were then incubated for 1 h at room temperature with a corresponding Cy3-conjugated secondary antibody (1:200; JacksonImmunoResearch, West Grove, PA, United States). Brain sections were read using a LEXT OLS4000 3D Laser Measuring Microscope (Olympus), recorded using a digital camera, and processed using Adobe Photoshop.

Western blot

The rats were decapitated under anesthesia. Amygdala samples were collected separately and homogenized in an SDS buffer containing a mixture of proteinase inhibitors (Sigma). Protein samples were separated on SDS-PAGE gel (4–15% gradient gel; Bio-Rad, Hercules, CA, United States) and transferred to polyvinylidene difluoride filters (Millipore, Bedford, MA, United States). The filters were blocked with 3% milk and incubated overnight at 4°C with an MT1 primary antibody (40 kD, rabbit polyclonal, 1:500, Millipore, Billerica, MA, United States) and for 1 h at room temperature with an HRP-conjugated secondary antibody

(1:10,000; Abcam, Cambridge, MA, United States). The blots were visualized in ECL solution (NEN, Boston, MA, United States) for 1 min and exposed onto hyperfilms (Amersham Biosciences) for 1–10 min. The blots were then incubated in a stripping buffer (67.5-mM Tris; pH, 6.8; 2% SDS; and 0.7% β-mercaptoethanol) for 30 min at 50°C and reprobed with a polyclonal rabbit anti-β-actin antibody (1:20,000; Alpha Diagnostic International, San Antonio, TX, United States) as the loading control. The Western analysis was made in triplicate. The density and the size of the bands were measured with a computer-assisted imaging analysis system and normalized against loading controls.

Statistical analysis

By running GraphPadInStat version 3.10 for Windows (La Jolla, CA, United States), raw data from behavior tests, ELISA, and Western blots were analyzed by using repeated measures ANOVA across testing time points to detect overall differences among treatment groups and across treatment groups to examine overall differences among testing time points. Differences were considered to be statistically significant at the level of $\alpha = 0.05$.

Experiment 1: Evaluate the therapeutic effects of transcutaneous auricular vagal nerve stimulation on chronic pain, melatonin secretion, and melatonin receptors expression

To find out whether taVNS is effective in relieving fully established neuropathic pain in ZDF rats, nociceptive behavior was evaluated in five groups of rats ($n = 6$ each): (1) naive, (2) sham CCI 1 week followed by taVNS, (3) CCI 1 week, (4) CCI 1 week followed by taVNS, and (5) CCI 1 week followed by auricular margin electroacupuncture (AMEA). Blood samples were taken at different time points for ELISA detection of the plasma melatonin level, and brain samples were collected upon sacrifice at Day 28 for Western blot detection of MT1 expression at the protein level.

Experiment 2: Evaluate the preventive effects of transcutaneous auricular vagal nerve stimulation on chronic pain, melatonin secretion, and melatonin receptors expression

To find out whether taVNS can prevent the development of neuropathic pain after CCI and the effect of electroacupuncture on nociception behavior, melatonin secretion, and MT1 expression during the progress of neuropathic pain, five groups of ZDF rats were used ($n = 6$ each): (1) sham operation, (2) sham operation plus taVNS, (3) CCI, (4) CCI plus taVNS, and (5) CCI plus AMEA. The blood samples were collected as mentioned above for ELISA, and the brain samples taken on Day 14 for Western blot detections.

Experiment 3: Evaluate the involvement of melatonin receptors in the function of transcutaneous auricular vagal nerve stimulation

There are mainly three types of MTRs, namely, MT1, MT2, and MT3. However, MT3 is expressed in the nucleus, while MT1 and MT2 are expressed on the cellular membrane, and MT1 is reported to be involved in the neuronal functions of the brain (Dubocovich et al., 2010). To find out whether MTRs mediate the function of taVNS and whether the blockade of MTRs modulates the release of melatonin and the expression of MTRs itself, the competitive melatonin receptor MT1/MT2 antagonist luzindole was administrated, and its effect on pain behaviors, plasma melatonin concentration, and the expression levels of MT1 were examined in four groups of ZDF rats ($n = 6$ each), including (1) a naive plus vehicle, (2) naive plus luzindole, (3) CCI, a taVNS plus vehicle, and (4) CCI, taVNS plus luzindole. Each agent or treatment was given one time daily (i.p.) in the afternoon 30–60 min before taVNS for 7 consecutive days beginning on Day 0. The blood samples were collected on Days 0 (baseline), 1, 3, and 7 after the behavior test but before any treatment. The brain samples were taken on Day 7 for Western blot detections.

Experiment 4: Compare the effects of transcutaneous auricular vagal nerve stimulation with melatonin on pain behavior

The efficacy of taVNS was compared with that of melatonin on established and developing neuropathic pain, melatonin secretion, and MTR expression. Four groups of ZDF rats were used ($n = 6$ each): (1) CCI operation followed by taVNS 7 days later, (2) CCI operation followed by melatonin i.p. administration 7 days later, (3) CCI operation followed by taVNS immediately, and (4) CCI operation followed by melatonin i.p. administration immediately. All treatment lasted for 1 week. The rats were kept for another week to watch the lasting effects of taVNS. The brain samples were collected on Day 14 for Western blot.

Experiment 5: Evaluate the involvement of the pineal gland in the effect of transcutaneous auricular vagal nerve stimulation

To find out the role of the pineal gland in taVNS and to explore whether there are other melatonin sources, the pain behaviors were examined in pinealectomized (Px) rats with established neuropathic pain and in rats expected to develop neuropathic pain after CCI. The pineal gland was removed from the rats according to a reported method (Maganhin et al., 2009). For the Px sham control, the skull was opened over the pineal gland but the gland was not removed. The rats exhibiting postoperative neurological deficits (e.g., paralysis) or poor grooming were excluded from further experiments. Five groups of ZDF were used (n = 6 each): (1) Px sham operation; (2) Px alone; (3) CCI and Px; (4) CCI and Px followed by taVNS 7 days after operation; and (5) CCI and Px followed by taVNS immediately after operation. The taVNS lasted for 7 days. Half of the rats from each group were sacrificed after the behavior test on Day 7; the brain samples were taken for Western analysis. Another half were sacrificed on Day 28 to see the long-term effects of taVNS on pain behavior and plasma melatonin in Px rats.

Results

The transcutaneous auricular vagal nerve stimulation was effective on both pain relief and pain progression

We watched the antinociceptive effect of taVNS on established pain first. The hyperalgesia to thermal stimulation was demonstrated 7 days after CCI operation. The taVNS treatment alleviated hyperalgesia immediately and lasted for another 2 weeks after 2 week of daily treatment (**Figure 2A**). The plasma melatonin concentrations and the MT1 protein expression in amygdala were both increased corresponding to the taVNS treatment (**Figures 2C,E**). The melatonin concentration reached a high platform at Day 14 and lasted for another 2 weeks, possibly longer, even without further taVNS session after the first 2-week daily trials. Consecutive daily taVNS sessions would not only relieve the established pain but also could prevent the development of pain after CCI if administrated starting immediately after CCI operation (**Figure 2B**). Similar to the efficiency in established pain, taVNS also promoted efficiency in the secretion of melatonin and in the expression of MT1 during the development of neuropathic pain (**Figures 2D,F**). Although, at a lower amplitude, AMEA also showed a partial analgesic effect on both established and developing pain as demonstrated by Day 14.

Central melatonin receptor type 1 was involved in the function of transcutaneous auricular vagal nerve stimulation treatment

An immunofluorescent study found that MT1 was expressed in various brain regions. There were more MT1 positive neurons in the amygdala of the ZDF rats treated with taVNS than that in the rats treated with AMEA (**Figures 2G,H**).

Melatonin receptors are necessary for the therapeutic effect of taVNS as seen in **Figure 3**. Luzindole treatments not only reduced nociceptive thresholds to thermal stimulation in naive rats as compared with vehicle treatments but also attenuated the antinociceptive effect of taVNS. This indicates that MTRs are necessary for mediating the antinociceptive effect of taVNS. Additionally, the plasma melatonin concentration and the expression of central MT1 were also declined by luzindole treatment. This indicates that more melatonin uncombined with MTRs was eliminated and that, although luzindole competes with melatonin to combine with MTRs, it does not have the ligand-dependent positive modulation effect on the expression of MTRs as melatonin does.

As compared with exogenous melatonin, taVNS effectively relieved both established and developing pain, immediately and for a long term. Exogenous melatonin, on the other side, showed a higher effect on the prevention of pain development but reduced effect on the relieving of established pain from the beginning. This might be due to a downregulated expression of MT1 during the development of pain (**Figure 4**).

Transcutaneous auricular vagal nerve stimulation was effective in pain relief in pinealectomized animals

Pinealectomized rats were used to evaluate the involvement of the pineal gland in the function of taVNS (**Figure 5**). Px alone in the ZDF rats lowered the nociceptive threshold as compared to the sham-Px rats. taVNS reduced the pain in Px or Px plus CCI rats, beginning immediately or 1 week after CCI operation (**Figure 5A**). Px dramatically decreased the plasma melatonin concentration as detected at all time points in this study. However, taVNS in the Px rats restored the plasma melatonin level to the normal level immediately and even higher at 2–3 weeks after taVNS termination (**Figure 5B**). In addition, Px negatively modulated the expression of MT1 (**Figure 5C**). taVNS would positively regulate the expression of MT1 not only in the ZDF rats with an intact pineal gland (**Figures 5A–C**) but also in the Px ZDF rats.

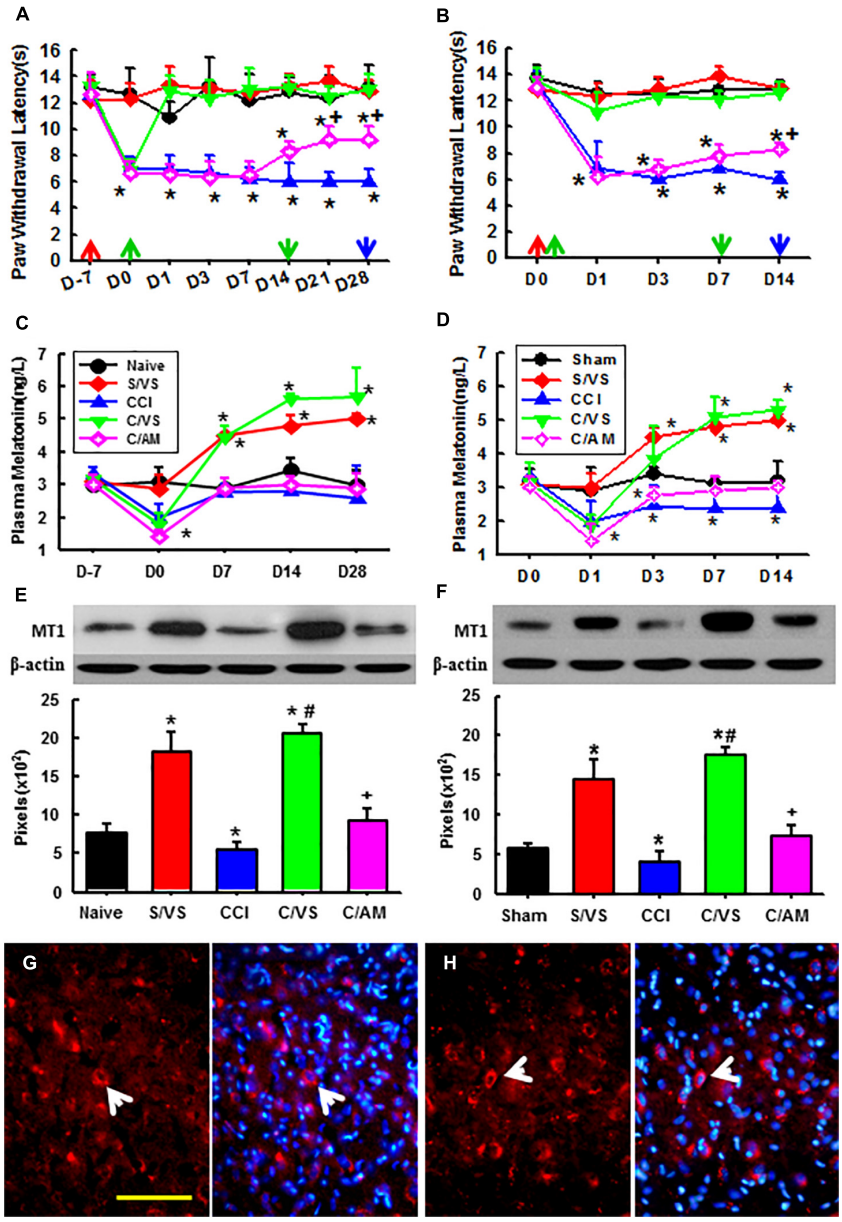

FIGURE 2
Effects of taVNS on pain behavior, melatonin level, and MT1 expression. Showing the effect of taVNS on established **(A)** and developing **(B)** thermal hyperalgesia, corresponding plasma melatonin concentration **(C,D)**, and MT1 protein expression at the last time point **(E,F)**. D, day; red arrow, CCI or sham operation; the green up arrow, treatments start; the green down arrow, treatments end; the blue arrow, sacrifice; S/VS, CCI sham operation plus taVNS; CCI, sciatic nerve chronic constriction injury; C/VS, CCI + taVNS; C/AM, CCI + AMEA. *$p < 0.05$, as compared with naive or sham groups on the same day and the baseline of the same group; +$p < 0.05$ as compared with S/VS, CCI, and C/VS groups; and #$p < 0.05$ as compared with CCI and C/AM groups. **(G)** The MT1 positive neurons (the arrow) in the amygdala of ZDF rats, CCI treated with AMEA, on Day 28. **(H)** The MT1 positive neurons (the arrow) in the amygdala of the ZDF rats, CCI treated with taVNS, on Day 14. Bar, 100 μm.

Discussion

In our body, there are parasympathetic and sympathetic autonomic nerves systems. In a night-sleeping state, the balance of the counterparts will be shifted toward an enhanced parasympathetic activity, concomitant with a reduction of the sympathetic tone (DeBenedittis et al., 1994). This autonomic imbalance can also be induced by vagus nerve stimulation (VNS), which stimulates the afferent vagus fibers, especially in the auricular concha area (La Marca et al., 2010). Although no report showed that stimulation to the parasympathetic system can enhance melatonin secretion,

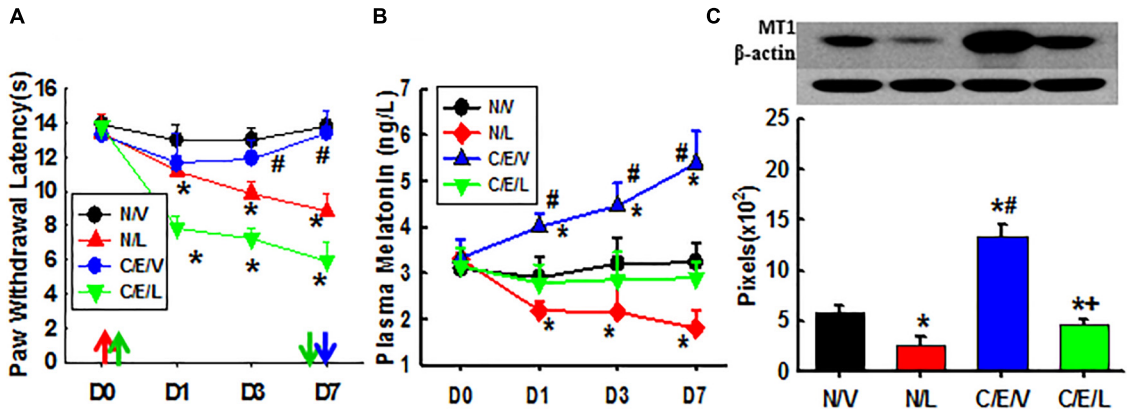

FIGURE 3
Effects of luzindole on pain behavior, the melatonin level, and MT1 expression. Showing the effect of luzindole on nociceptive thresholds to thermal stimulation and on the efficacy of taVNS **(A)**, plasma melatonin concentration **(B)**, and the expression of MT1 in amygdala (Day 7; **C)**. D, day; the red arrow, CCI operation; the green up arrow, treatments start; the green down arrow, treatments end (Day 6); the blue arrow, sacrifice; N/V, a naive plus vehicle; N/L, naive plus luzindole; C/E/V, CCI plus a taVNS plus vehicle; C/E/L, CCI plus taVNS plus luzindole. *$p < 0.05$, as compared with naive or the N/V group on the same day and with the baseline of the same group; #$p < 0.05$, as compared with N/L and C/E/L groups; and +$p < 0.05$, as compared with N/L.

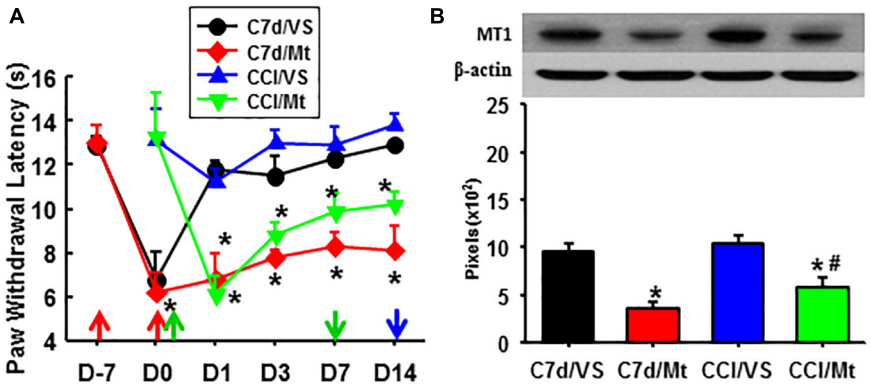

FIGURE 4
Comparison of the effects between taVNS and exogenous melatonin. **(A)** A hyperalgesia threshold to thermal stimulation. *$P < 0.05$ vs. CCI/VS at the same testing time points. **(B)** Expression of MT1 in the amygdala (Day 14). *$P < 0.05$ vs. C7d/VS, #$P < 0.05$ vs. CCI/VS. D, day; the red arrow, CCI operation; the green up arrow, treatments start; the green down arrow, treatments end; the blue arrow, sacrifice; C7d/VS, C7d/Mt, CCI operation followed by taVNS or melatonin i.p. injection 7 days later, respectively; CCI/VS, CCI/Mt, CCI operation followed by taVNS or melatonin i.p. injection immediately.

some pieces of evidence do demonstrate that light increases the sympathetic activity and simultaneously suppresses the vagal parasympathetic activity; both can be dose dependently attenuated by melatonin (Mutoh et al., 2003). Since bright light during the day represses melatonin secretion and due to the fact that melatonin is naturally secreted during the night, it is very likely that VNS can increase melatonin secretion. Additionally, increased sympathetic and decreased parasympathetic tones have been shown in the leptin receptor deficient (*db/db*) mice (Goncalves et al., 2009), which are genetically equivalent to the leptin receptor-deficient ZDF (*fa/fa*) rats used in this study. Combining the literature with our study results that the taVNS functionally increases the plasma melatonin concentration in ZDF rats, we conclude that taVNS stimulates the parasympathetic system and promotes the release of melatonin.

Melatonin is believed to be secreted only by the pineal gland. Interestingly, taVNS even stimulated the melatonin secretion in the Px rats. It implies that taVNS functions through the modulation of melatonin secretion from sources other than the pineal gland. According to recent reports, melatonin can be secreted also from the retina, the digestive tract, the bone marrow, the skin, kidneys, the ovaries, the testis, circulating leukocytes, and many other sources in

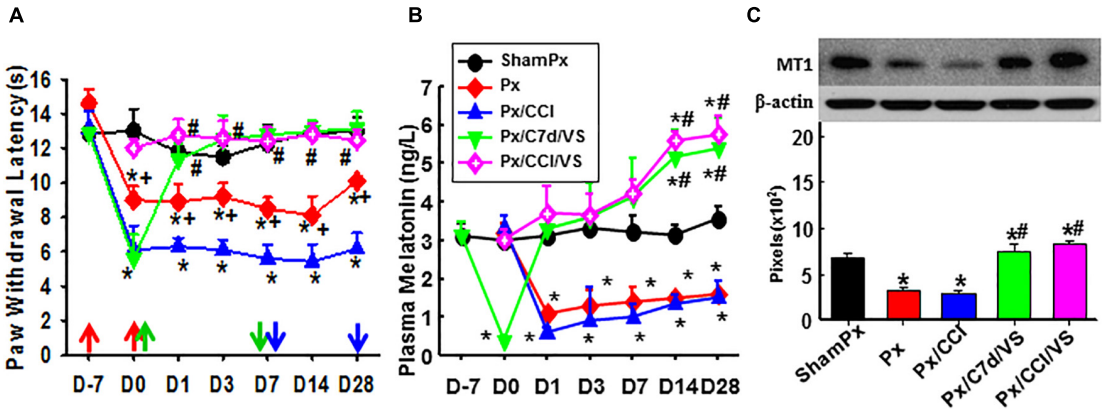

FIGURE 5
Involvement of the pineal gland in the function of taVNS. Showing the effect of taVNS on nociceptive thresholds **(A)**, plasma melatonin concentration **(B)**, and the expression of MT1 in the amygdala (Day 7; **C)** in Px rats. D, day; the red arrow, CCI, Px, or sham operation; the green up arrow, treatments start; the green down arrow, treatments end; the blue arrow, sacrifice; ShamPx, sham Px operation; Px, pinealectomized; Px/CCI, Px, and CCI; Px/C7d/VS, Px, and CCI operation followed by taVNS treatments 7 days later; Px/CCI/VS, Px, and CCI operation followed by taVNS treatments immediately. *$p < 0.05$, as compared with the ShamPx group on the same day and the baseline of the same group; #$p < 0.05$, as compared with Px or Px/CCI groups; and +$p < 0.05$, as compared with the Px/CCI group.

the vertebrate species (Esposito and Cuzzocrea, 2010). It is noteworthy that these extrapineal melatonin sources have a large total volume, and some of them have high levels of melatonin, such as the melatonin pools of the gastrointestinal tract (Bubenik, 2002) and the bone marrow (Conti et al., 2000). The concentration of melatonin in both pools surpasses blood melatonin levels by 2 to 3 orders of magnitude, and the gastrointestinal tract alone secretes over 400 as much melatonin as the pineal gland (Werbach, 2008). In the gastrointestinal tract, melatonin secretes from the enterochromaffin cells, which contain precursors of melatonin (5-oxytryptophane, tryptamine, serotonin, and mexamine) and an increase in numbers after pinealectomy (Kim et al., 2012). For the release of melatonin from these pools upon and after taVNS, another beneficial key factor is that these extrapineal sources are innervated by the vegetative nervous system, and their release of melatonin is independent of photoperiodic direct regulation (Conti et al., 2000; Bubenik, 2002; Werbach, 2008).

If the therapeutic effect of taVNS on chronic pain is functionally mediated through the enhanced secretion of extrapineal melatonin as shown in the current study, one may expect that other melatonin enhancement methodologies also have therapeutic effects on pain or other illnesses lacking melatonin and that complimentarily administered melatonin has the same therapeutic effects as taVNS. The hypothesis may be partially true. On the one hand, melatonin and acupuncture are both effective for the treatment of circadian phase disorders that affect sleep (Gooneratne, 2008). Acupuncture and acupressure are also beneficial in ameliorating insomnia (Nordio and Romanelli, 2008) and in improving circadian rhythms of blood pressure in patients (Kim et al., 2012). On the other hand, exogenous melatonin may not relieve chronic pain as effectively as taVNS does for the reasons of affinity and metabolism property of both MTRs and melatonin – the MTRs are high-affinity binding sites, but the expression of these G-protein-coupled 7 transmembrane subunits may be ligands dependent; any free melatonin that is unbounded to MTRs will be rapidly distributed (serum half-life, 0.5–5.6 min) and eliminated (Iguchi et al., 1982; Paul et al., 1999). In this study, the taVNS session was 30 min daily. If we suppose that only melatonin secretion is involved, the efficacy of taVNS may also be short term. The long-term beneficial efficiency of taVNS may be attributed to a lasting release of high-level melatonin from extrapineal pools and the upregulated expression of MTRs. However, if the MTRs are combined with luzindole, taVNS-promoted extra melatonin may be eliminated quickly and may not work properly to the hyperalgesia and the expression of MTRs, as shown in our results.

Taken together, a cascade of events may occur upon taVNS stimulation: (i) taVNS stimulates the vagal afferent terminals located in the auricular concha, thus exciting the parasympathetic system; (ii) the parasympathetic signal conducts, directly or relayed through the central nervous system, to the efferent fibers innervating the gastrointestinal tract or other extrapineal melatonin sources, thus promoting the synthesis and release of extrapineal melatonin; (iii) the increased secretion of melatonin, in turn, excites the parasympathetic system, thus forming a beneficial cycle; and (iv) accumulated effect of taVNS one time daily for consecutive 7 days or over reliefs of chronic pain. Although we verified that taVNS is effective in diabetic and neuropathic chronic pain animal models (Li et al., 2018), it may be effective for all chronic pain since melatonin is a strong antioxidant in general and has an analgesic effect on chronic pain by itself (Wang et al., 2012).

All these beneficial effects might be helpful to individuals with diabetes as well as individuals with COVID-19 lacking circulating melatonin. We believe that all of the results to date about the VNS and its enhancing effect on melatonin release are obtained in preclinical studies in which animals were anesthetized. Although anesthesia is helpful in precluding unwanted factors such as anxiety and distress, it raises the question of whether melatonin releases can be enhanced in a conscious state.

In this study, electrostimulation at the auricular margin elicited partial efficacy of taVNS. The reason may be that some of the stimulated currents and/or electromagnetic fields acted at the vagal terminals nearby in the auricular concha.

The ZDF rats are genetically deficient in leptin receptor expression. Both the leptin and the leptin receptor are involved in nociceptive modulation through the activation of *N*-methyl-*D*-aspartic acid (NMDA)-induced currents and through the positive modulation of NMDA receptor subunit 1 (NR1) expression (Tian et al., 2011). The plasma leptin concentration of normal humans is about 8.0 ng/ml, whereas, in the case of leptin receptor deficiency, which is the equivalent to the leptin receptor-deficient *db/db* mice, the value may reach 500–700 ng/ml (Sone and Osamura, 2001). This may explain why the ZDF rats are more sensitive to nociceptive stimulation. However, in normal rats and in high fat-fed induced obesity rats, melatonin counteracted the secretion and function of leptin (Ríos-Lugo et al., 2010). In this study, the taVNS-enhanced central melatoninergic function by promoting extrapineal melatonin release and upregulating central MT1 expression, thus might, in turn, relieve chronic pain by counteracting the function of leptin, inhibiting the NMDA-induced current, and downregulating the expression of NMDA receptor NR1 (Tian et al., 2011).

Data availability statement

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by Chinese Academy of Chinese Medicine.

Author contributions

XZ, SL, JH, LL, XH, and WL carried out the experiments and collected the data. SW and PR contributed to the conception of idea, design of experiments, and analysis and interpretation of data, wrote the manuscript, and revised and approved the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Bähr, I., Mühlbauer, E., Schucht, H., and Peschke, E. (2011). Melatonin stimulates glucagon secretion in vitro and in vivo. *J. Pineal Res.* 50, 336–344.

Bennett, G. J., and Xie, Y. K. (1988). A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* 33, 87–107.

Bubenik, G. A. (2002). Gastrointestinal melatonin: Localization, function, and clinical relevance. *Dig. Dis. Sci.* 47, 2336–2348.

Conti, A., Conconi, S., Hertens, E., Skwarlo-Sonta, K., Markowska, M., and Maestroni, J. M. (2000). Evidence for melatonin synthesis in mouse and human bone marrow cells. *J. Pineal Res.* 28, 193–202.

DeBenedittis, G., Cigada, M., Bianchi, A., Signorini, M. G., and Cerutti, S. (1994). Autonomic changes during hypnosis: A heart rate variability power spectrum analysis as a marker of sympatho-vagal balance. *Int. J. Clin. Exp. Hypn.* 42, 140–152. doi: 10.1080/00207149408409347

Dubocovich, M. L., Delagrange, P., Krause, D. N., Sugden, D., Cardinali, D. P., and Olcese, J. (2010). International Union of Basic and Clinical Pharmacology. LXXV. Nomenclature, classification, and pharmacology of G protein-coupled melatonin receptors. *Pharmacol. Rev.* 62, 343–380. doi: 10.1124/pr.110.002832

Esposito, E., and Cuzzocrea, S. (2010). Antiinflammatory activity of melatonin in central nervous system. *Curr. Neuropharmacol.* 8, 228–242.

Ferlazzo, N., Andolina, G., Cannata, A., Costanzo, M. G., Rizzo, V., Currò, M., et al. (2020). Is melatonin the cornucopia of the 21st century? *Antioxidants* 9, 1088–1117. doi: 10.3390/antiox9111088

Frese, T., Bach, A. G., Mühlbauer, E., Pönicke, K., Brömme, H. J., Welp, A., et al. (2009). Pineal melatonin synthesis is decreased in type 2 diabetic Goto-Kakizaki rats. *Life Sci.* 85, 526–533.

Gerdin, M. J., Masana, M. I., and Dubocovich, M. L. (2004). Melatonin-mediated regulation of human MT(1) melatonin receptors expressed in mammalian cells. *Biochem. Pharmacol.* 67, 2023–2030.

Goncalves, A. C., Tank, J., Diedrich, A., Hilzendeger, A., Plehm, R., Bader, M., et al. (2009). Diabetic hypertensive leptin receptor-deficient db/db mice develop cardioregulatory autonomic dysfunction. *Hypertension* 53, 387–392. doi: 10.1161/HYPERTENSIONAHA.108.124776

Gooneratne, N. S. (2008). Complementary and alternative medicine for sleep disturbances in older adults. *Clin. Geriatr. Med.* 24, 121–138.

Hargreaves, K., Dubner, R., Brown, F., Flores, C., and Joris, J. (1988). A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain* 32, 77–88.

Iguchi, H., Kato, K. I., and Ibayashi, H. (1982). Melatonin serum levels and metabolic clearance rate in patients with liver cirrhosis. *J. Clin. Endocrinol. Metab.* 254, 1025–1027. doi: 10.1210/jcem-54-5-1025

Kemp, H. I., Corner, E., and Colvin, L. A. (2020). Chronic pain after COVID-19: Implications for rehabilitation. *Br. J. Anaesth.* 125, 436–440.

Kim, H. M., Cho, S. Y., Park, S. U., Sohn, I. S., Jung, W. S., Moon, S. K., et al. (2012). Can acupuncture affect the circadian rhythm of blood pressure? A randomized, double-blind, controlled trial. *J. Altern. Complement. Med.* 18, 918–923. doi: 10.1089/acm.2011.0508

La Marca, R., Nedeljkovic, M., Yuan, L., Maercker, A., and Elhert, U. (2010). Effects of auricular electrical stimulation on vagal activity in healthy men: Evidence from a three-armed randomized trial. *Clin. Sci.* 118, 537–546. doi: 10.1042/CS20090264

Li, S., Sun, C., Rong, P., Zhai, X., Zhang, J., Baker, M., et al. (2018). Auricular vagus nerve stimulation enhances central serotonergic function and inhibits diabetic neuropathy development in Zucker fatty rats. *Mol. Pain.* 14:1744806918787368. doi: 10.1177/1744806918787368

Maganhin, C. C., Simões, R. S., Fuchs, L. F., Oliveira-Filho, R. M., SimõesMde, J., EvêncioNeto, J., et al. (2009). Rat pinealectomy: A modified direct visual approach. *Acta Cir. Bras.* 24, 321–324. doi: 10.1590/s0102-86502009000400013

McMullan, C. J., Schernhammer, E. S., Rimm, E. B., Hu, F. B., and Forman, J. P. (2013). Melatonin secretion and the incidence of type 2 diabetes. *JAMA* 309, 1388–1396.

Mutoh, T., Shibata, S., Korf, H. W., and Okamura, H. (2003). Melatonin modulates the light-induced sympathoexcitation and vagal suppression with participation of the suprachiasmatic nucleus in mice. *J. Physiol.* 547, 317–332. doi: 10.1113/jphysiol.2002.028001

Nagorny, C. L., Sathanoori, R., Voss, U., Mulder, H., and Wierup, N. (2011). Distribution of melatonin receptors in murine pancreatic islets. *J. Pineal Res.* 50, 412–417. doi: 10.1111/j.1600-079X.2011.00859.x

Nordio, M., and Romanelli, F. (2008). Efficacy of wrists overnight compression (HT 7 point) on insomniacs: Possible role of melatonin? *Minerva Med.* 99, 539–547.

Paul, P., Lahaye, C., Delagrange, P., Nicolas, J. P., Canet, E., and Boutin, J. A. (1999). Characterization of 2-[$_{125}$I]iodomelatonin binding sites in Syrian hamster peripheral organs. *J. Pharmacol. Exp. Ther.* 290, 334–340.

Pawlikowski, M., and Winczyk, K. (2021). Endocrine and metabolic aspects of COVID-19. *Endokrynol. Pol.* 72, 256–260.

Peschke, E., Stumpf, I., Bazwinsky, I., Litvak, L., Dralle, H., and Mühlbauer, E. (2007). Melatonin and type 2 diabetes - a possible link? *J. Pineal Res.* 42, 350–358.

Rahman, M. M., Hasan, M., and Ahmed, A. (2021). Potential detrimental role of soluble ACE2 in severe COVID-19 comorbid patients. *Rev. Med. Virol.* 31, 1–12. doi: 10.1002/rmv.2213

Ríos-Lugo, M. J., Cano, P., Jiménez-Ortega, V., Fernández-Mateos, M. P., Scacchi, P. A., Cardinali, D. P., et al. (2010). Melatonin effect on plasma adiponectin, leptin, insulin, glucose, triglycerides and cholesterol in normal and high fat-fed rats. *J. Pineal Res.* 49, 342–348. doi: 10.1111/j.1600-079X.2010.00798.x

Sone, M., and Osamura, R. Y. (2001). Leptin and the pituitary. *Pituitary* 4, 15–23.

Stumpf, I., Bazwinsky, I., and Peschke, E. (2009). Modulation of the cGMP signaling pathway by melatonin in pancreatic beta-cells. *J. Pineal Res.* 46, 140–147.

Tal, M., and Bennett, G. J. (1994). Extra-territorial pain in rats with a peripheral mononeuropathy: Mechano-hyperalgesia and mechano-allodynia in the territory of an uninjured nerve. *Pain* 57, 375–382. doi: 10.1016/0304-3959(94)90013-2

Tian, Y., Wang, S., Ma, Y., Lim, G., Kim, H., and Mao, J. (2011). Leptin enhances NMDA-induced spinal excitation in rats: A functional link between adipocytokine and neuropathic pain. *Pain* 152, 1263–1271. doi: 10.1016/j.pain.2011.01.054

Wajid, F., Poolacherla, R., Mim, F. K., Bangash, A., and Rutkofsky, I. H. (2020). Therapeutic potential of melatonin as a chronobiotic and cytoprotective agent in diabetes mellitus. *J. Diabetes Metab. Disord.* 19, 1797–1825. doi: 10.1007/s40200-020-00585-2

Wang, S., Tian, Y., Song, L., Lim, G., Tan, Y., You, Z., et al. (2012). Exacerbated mechanical hyperalgesia in rats with genetically predisposed depressive behavior: Role of melatonin and NMDA receptors. *Pain* 153, 2448–2457. doi: 10.1016/j.pain.2012.08.016

Werbach, M. R. (2008). Melatonin for the treatment of gastroesophageal reflux disease. *Altern. Ther. Health Med.* 14, 54–58.

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Acupuncture modulates the frequency-specific functional connectivity density in primary dysmenorrhea

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Background: The study aimed to investigate how acupuncture modulates brain activities across multiple frequency bands to achieve therapeutic effects in PDM.

Methods: A total of 47 patients with PDM were randomly assigned to the verum acupuncture group and sham acupuncture group with three menstrual cycles of the acupuncture course. The fMRI scans, visual analog scale (VAS) scores, and other clinical evaluations were assessed at baseline and after three menstrual-cycles treatments. The global functional connectivity density (gFCD) analyses were performed between the pre-and post-acupuncture course of two groups at full-low frequency band, Slow-3 band, Slow-4 band, and Slow-5 band.

Results: After the acupuncture treatments, the patients with PDM in the verum acupuncture group showed significantly decreased VAS scores ($p < 0.05$). The frequency-dependent gFCD alternations were found in the verum acupuncture group, altered regions including DLPFC, somatosensory cortex, anterior cingulate cortex (ACC), middle cingulate cortex (MCC), precuneus, hippocampus, and insula. The sham acupuncture modulated regions including angular gyrus, inferior frontal gyrus, and hippocampus. The gFCD alternation in DLPFC at the Slow-5 band was negatively in the patients with PDM following verum acupuncture, and S2 at the Slow-4 band was positively correlated with VAS scores.

Conclusion: These findings supported that verum acupuncture could effectively modulate frequency-dependent gFCD in PDM by influencing

abnormal DLPFC at Slow-5 band and hippocampus at the Slow-3 band. The outcome of this study may shed light on enhancing the potency of acupuncture in clinical practice.

KEYWORDS

primary dysmenorrhea, acupuncture, resting-state fMRI, functional connectivity density, frequency band

Introduction

Primary dysmenorrhea (PDM), characterized by persistent menstrual discomfort in the absence of pelvic abnormalities, is a common gynecological condition affecting between 17 and 90% of females of reproductive age. However, the pathogenesis of PDM is not fully understood. Recently, neuroimaging studies showed that the prolonged PDM alternates brain structure and function, providing a new viewpoint on PDM diagnosis and treatment.

Resting-state functional magnetic resonance imaging (rs-fMRI) is a non-invasive imaging technique that provides a reliable representation of the brain's spontaneous functional activity (Fox and Raichle, 2007; Baliki et al., 2011a). It offers new insights into the underlying spontaneous brain activity associated with chronic pain. The previous studies identified the activation of primary (S1) and second somatosensory cortex (S2), anterior cingulate cortex (ACC), insula (INS), dorsolateral prefrontal cortex (DLPFC), and hippocampus in patients with chronic pain (Liu and Chen, 2009; Fomberstein et al., 2013; Schmidt-Wilcke, 2015; Schmidt-Wilcke and Diers, 2017). Furthermore, the frequency-dependent alternations of brain activities in chronic pain were also demonstrated (Baliki et al., 2011a; Kilpatrick et al., 2014; Alshelh et al., 2016).

With the global functional connectivity density (gFCD) analysis, a graph-based and data-driven approach that quantifies the number of whole-brain connections over the whole-brain (Tomasi et al., 2016), our previous study first confirmed that the patients with PDM exhibited frequency-dependent distribution patterns of brain activity. Specifically, these altered brain areas, especially in the central executive network (CEN), default mode network (DMN), sensorimotor network (SMN), and the hippocampus (Yu et al., 2021). Notably, we observed a decreased gFCD in DLPFC at the Slow-5 band and an increased gFCD in the hippocampus at the Slow-3 band, indicating that the patients with PDM showed aberrant pain processing. As a result, we confirmed that the patients with PDM showed distinct functional brain activity frequency specific.

Acupuncture is a non-pharmacological intervention for analgesia that has been recognized (Berman et al., 2004; Witt et al., 2008; Hinman et al., 2014; Hershman et al., 2018). Over the last two decades, fMRI has been utilized to investigate the underlying brain mechanism of acupuncture, and studies have revealed that acupuncture significantly modulates cortical/subcortical brain areas involved in pain processing, cognition, and emotional processing (Bai and Lao, 2013; Cai et al., 2018; Zhang et al., 2020). Consequently, it is a reliable method to use fMRI to reveal the imaging mechanism of acupuncture for PDM.

In this study, we hypothesized that acupuncture might normalize the brain regions previously identified (Yu et al., 2021) as aberrant in PDM for therapeutic purposes, and this modulation was of frequency specific. First, we investigated how acupuncture modulates brain activity by analyzing gFCD changes across multiple frequency bands before and after treatment. Second, we evaluated the differences in brain regions modulated by verum acupuncture (VA) vs. sham acupuncture (SA) to determine if modulation of acupuncture on brain activity in PDM is a therapeutic effect. In addition, we performed a correlation analysis to determine the relationship between acupuncture-induced gFCD changes and clinical results.

Abbreviations: AG, angular gyrus; ACC, anterior cingulate cortex; aIFG, anterior inferior frontal gyrus; CAU, caudate nucleus; CEN, central executive network; CMSS, Cox Menstrual Symptom Scale; CMSS-s, Cox Menstrual Symptom Scale-severity; CMSS-t, Cox Menstrual Symptom Scale-time; DMN, default mode network; DLPFC, dorsolateral prefrontal cortex; TE, echo time; FOV, field of view; FLF, full low frequency; FCD, functional connectivity density; gFCD, global functional connectivity density; PHG, hippocampal gyrus; mPFC, medial prefrontal cortex; MCC, middle cingulate cortex; MNI, Montreal Neurological Institute; NAC, nucleus accumbens; PCU, precuneus; PDM, primary dysmenorrhea; S1, primary somatosensory cortex; RMANOVA, repeated measurement of analysis of variance; TR, repetition time; rTMS, repetitive transcranial magnetic stimulation; rs-fMRI, resting-state functional magnetic resonance imaging; S2, second somatosensory cortex; SMN, sensorimotor network; SA, sham acupuncture; SMA, supplementary motor area; SAS, the Zung Self-Rating Anxiety Scale; SDS, the Zung Self-Rating Depression Scale; VA, verum acupuncture; VAS, visual analog scale.

Material and methods

Study design

This was a two-arm, randomized rs-fMRI clinical trial. The patients with PDM were randomized to VA and SA groups

using a random number table. The patients with PDM were required to fulfill the dysmenorrhea diary to characterize their PDM symptoms in altogether 6 months.

Participants

The inclusion and exclusion criteria in this study were similar to our previous study (Yu et al., 2021). The patients with PDM were recruited primarily through the gynecology outpatient and inpatient sections at the Affiliated Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu University of Traditional Chinese Medicine, Sichuan University, Southwest University of Finance and Economics, Southwest Jiaotong University, and Southwest University for Nationalities. The recruitment period was from December 2015 to July 2018. Well-informed consent was obtained from all involved participants. The study protocol was approved by the medical ethics review of the Sichuan Regional Ethics Review Committee of Traditional Chinese Medicine (No. 2013KL-033).

The patients with PDM were enrolled fulfilling the following inclusion criteria: (1) Women aged 18–30 years, right-handed; (2) those with PDM diagnosed using the Society of Obstetricians and Gynecologists of Canada's diagnostic criteria (Lefebvre et al., 2005); (3) patients with regular menstrual cycle (27–32 days); (4) patients with at least 1-year history of PDM; and (5) no exogenous hormones or centrally acting medicine in the previous 6 months; (6) pain assessed by visual analog scale (VAS) averaged over four points in the last 3 months. The exclusion criteria were as follows: Patients with (1) diagnosis of secondary dysmenorrhea due to organic pathology by ultrasound or gynecological examination; (2) suffering from other chronic pain conditions; (3) severe basic disorders; (4) mental illness, or with severe psychiatric disorders; (5) pregnant, preparing for pregnancy or breastfeeding; (6) treated with acupuncture in the past 3 months or have been taking analgesic medication for a long period; (7) contraindications to MRI examination; and (8) severe cranial anatomical asymmetry or well-defined lesions found on MRI scans.

Acupuncture manipulations

Acupuncture treatments were performed by qualified practitioners who had undergone extensive clinical and operational training. The selection of the VA and SA was identical to the previous study (Wang et al., 2021). The VA acupoints were bilateral SP6, which were known to alleviate PDM well (Abaraogu et al., 2016; Yu et al., 2017). The SA acupoints were located bilaterally at the midpoint between SP6 and the Bladder meridian without known clinical effects. The location of the acupoints is shown in **Supplementary Figure 1**. The patients with PDM in the VA group were required to obtain deqi sensation (a complex phenomenon including soreness, numbness, heaviness, swelling, or dull pain achieved by pricking the needle 5–15 mm subcutaneously and gently manipulating) (Kong et al., 2007). The duration of the VA or SA was 30 min, once a day, during which the needles were retained without any manipulation. All patients received three menstrual cycles of acupuncture treatment, beginning 5–7 days before menstruation and ending until the onset of menstruation.

Clinical outcomes

All clinical outcomes were assessed at Month 0 (baseline) and Month 3 (post-treatment). The primary outcome was VAS (McCormack et al., 1988) scores, in which the score from 0 to 10 indicates the pain level from low to high. In addition, Cox Menstrual Symptom Scale (CMSS) (Cox and Meyer, 1978) was utilized to assess the severity (CMSS-s) and time (CMSS-t) of PDM symptoms on 18 items. To assess anxiety and depressive symptoms, the Zung Self-Rating Anxiety Scale (SAS) (Zung, 1971) and the Zung Self-Rating Depression Scale (SDS) (Zung et al., 1965) were employed. Noteworthy, retrospective scoring (VAS, CMSS, SAS, and SDS) of 3 months before the enrollment was used as the baseline.

Functional magnetic resonance imaging acquisition

This study used a 3.0-tesla magnetic resonance scanner (Discovery MR750, General Electric, Milwaukee, WI, United States) to perform scans. The participants were instructed to keep their eyes closed and awake during the scan. All subjects underwent MRI scans within 72 h of menstruation, with a total of two scans (including three scans in the baseline period and three scans in the treatment period).

First, a conventional three-plane localization was performed. The T1-weighted fast spoiled gradient–echo sequence was applied with the following parameters: Repetition time (TR) = 2.53 ms, echo time (TE) = 3.39 ms, field of view (FOV) = 256 mm × 256 mm, flip angle = 7°, slice thickness = 1 mm, and resolution = 256 × 256 × 188. The gradient–echo T2-weighted echo–planar imaging sequence was applied for resting-state fMRI, with parameters: TR = 2,000 ms, TE = 30 ms, FOV = 240 mm × 240 mm, flip angle = 90°, intra-layer resolution = 64 × 64, layer thickness = 4 mm, and number of slices = 32. Totally 250 scan time point were obtained.

Data analysis

Clinical outcomes

Statistics analyses were performed using the SPSS 20.0 statistical software (SPSS Inc., Chicago, IL). Comparison

of baseline characteristics (continuous variables) between the VA and SA group was statistically performed by the independent sample *t*-test. The variations of clinical outcomes (VAS scores, CMSS-t scores, CMSS-s scores, SAS scores, and SDS scores) before and after the treatment in the two groups were statistically determined by paired samples *t*-test. A repeated measurement of analysis of variance (RMANOVA) was performed on the values of changes in clinical outcomes before and after VA compared to SA. The significance level for statistical analysis of two-tailed testing was $p < 0.05$. The Pearson correlation analysis was applied to correlate the changes in VAS scores, CMSS-t scores, CMSS-s scores, SAS scores, and SDS scores before and after the treatment within the group. The significance level for statistical analysis of two-tailed testing was $p < 0.05$.

Functional magnetic resonance imaging data preprocessing

The data preprocessing for resting-state fMRI was performed by the Statistical Parametric Mapping (SPM12)[1] in MATLAB 2014a (Mathworks, Inc., Natick, MA, United States). The first 10-time points were discarded, slice-timing correction, head motion estimation, normalization to standard Montreal Neurological Institute (MNI) EPI template and spatial smoothing with a 6-mm, and full-width-at-half-maximum Gaussian kernel were performed for the remaining 240-time points. Nuisance covariates regression was applied including six-direction head motion parameters, white matter, and cerebrospinal fluid (Ciric et al., 2017; Tu et al., 2019). The full low frequency (FLF) of 0.01–0.08 Hz was performed for functional connectivity analysis. Based on the study of Rogachov et al. (2018) and our previous study (Yu et al., 2021) on frequency-related neuroimaging studies of chronic pain, three different frequency band-based filters were selected for analysis, including Slow-5 band (0.01–0.027 HZ), Slow-4 band (0.027–0.073 Hz), and Slow-3 band (0.073–0.198 Hz).

Global functional connectivity density calculation

The FCD was calculated by the BRANT toolkit[2] in MATLAB 2014a. The FCD of each voxel was calculated according to the method described by Tomasi and Volkow (Tomasi and Volkow, 2010, 2011, 2014). The gFCD value for a given voxel is the total number of active functional connections possessed by the voxel. Fisher *Z*-transformed version of correlation coefficient was the normalization method for FCD matrix. Pearson linear correlation analysis was performed to calculate the linear correlation between a given voxel (*i*) and all other voxels in the whole-brain as the number of global functional connections *k* (*i*), at a given voxel (*i*). Voxel pairs with a correlation coefficient of $r_0 > 0.6$ were considered a significant connection. The gFCD calculations were limited to the cerebral gray matter mask (N_{voxels}) region, setting a signal-to-noise ratio greater than 50% to minimize the adverse effects of signal loss and artifacts associated with magnetic sensitivity (Tomasi and Volkow, 2010).

Group analysis was applied using a random-effects model at different frequency bands. First, a voxel-based paired *t*-test was performed to measure the change in gFCD before and after the treatment in the VA or SA groups. Second, the brain regions that decreased or increased significantly after the treatment in the VA group compared with the SA group were explored by RMANOVA. Age was considered as a covariate in the statistics. For brain regions explicitly associated with pain in the previous studies that could not be corrected by family-wise error (FWE), a small-volume (anatomical structure) correction based 3dClustSim was taken by AFNI version 18.0.25 (Worsley et al., 1996).[3] The threshold of voxel-wise $p < 0.005$ and $p < 0.05$ FWE corrected at cluster level (more than 20 consecutive voxels) was applied for all the analyses.

Correlation analysis

We conducted a Pearson linear correlation analysis to explore the clinical relevance of gFCD changes in brain regions identified in the previous study (Yu et al., 2021) by extracting the average *Z*-score values of the significantly altered gFCD clusters. The SPSS 20.0 statistical software was used to conduct the analysis.

Results

A total of 58 patients with PDM were recruited in the study. Among the 47 patients with PDM who completed baseline clinical observations and fMRI scans, 41 patients (22 in the VA group, 19 in the SA group) completed 3-month treatment. Seven patients were excluded from the data processing for incomplete fMRI data (1 in the SA group and 2 in the VA group) and head movement exceeding 1.5 mm (1 in the VA group and 3 in the SA group). The flow chart was shown in **Supplementary Figure 2**.

Clinical outcomes

Baseline characteristics

Table 1 showed the baseline and clinical characteristics in the statistics. Twenty patients with PDM (aged 24.70 ± 2.11 years) in the VA group and 14 patients with PDM (aged 24.29 ± 1.90 years) in the SA group were ultimately included in the statistical analysis. Of note, a moderate

1 http://www.fil.ion.ucl.ac.uk/spm

2 http://www.brainnetome.org/toolkit/bf/

3 https://afni.nimh.nih.gov/pub/dist/doc/program_help/3dClustSim.html

TABLE 1 Demographic and pain assessment of baseline and after treatment.

Items	Conditions	Verum acupuncture group ($n = 20$)	Sham acupuncture group ($n = 14$)	T	p^*
Age (years)		24.70 ± 2.11	24.29 ± 1.90	0.59	0.56
Duration of PDM (years)		7.59 ± 2.91	7.84 ± 2.73	−0.26	0.80
Height (cm)		161.45 ± 4.06	160.71 ± 4.29	0.51	0.62
Weight (kg)		50.95 ± 3.81	49.75 ± 4.10	0.88	0.39
VAS scores	Pre-treatment	6.18 ± 0.99	5.93 ± 1.21	0.65	0.52
	Post-treatment	3.35 ± 1.50	5.39 ± 1.39	–	–
	Post-pre	−2.83 ± 1.57	−0.54 ± 1.55	−4.21	<0.001
CMSS-t scores	Pre-treatment	17.85 ± 8.36	18.50 ± 5.24	−0.26	0.80
	Post-treatment	13.20 ± 7.05	15.21 ± 5.28	–	–
	Post-pre	−4.65 ± 6.68	−3.29 ± 4.29	−0.67	0.51
CMSS-s scores	Pre-treatment	18.50 ± 7.74	14.43 ± 3.34	1.84	0.07
	Post-treatment	12.88 ± 7.19	14.50 ± 6.37	–	–
	Post-pre	−5.63 ± 7.80	0.07 ± 6.49	−2.24	0.03
SAS scores	Pre-treatment	41.88 ± 4.77	40.68 ± 8.08	0.54	0.59
	Post-treatment	35.44 ± 4.12	37.38 ± 5.48	–	–
	Post-pre	−6.44 ± 4.65	−3.30 ± 6.57	−1.63	0.11
SDS scores	Pre-treatment	40.36 ± 6.61	44.20 ± 9.97	−1.35	0.19
	Post-treatment	31.75 ± 5.34	39.91 ± 7.26	–	–
	Post-pre	−14.96 ± 4.27	−12.27 ± 7.30	−1.36	0.18

Values are mean ± standard deviation (SD); "post-pre" means changes in clinical outcomes before and after treatment; *p < 0.05 is considered statistically significant. PDM, primary dysmenorrhea; VAS, visual analog scale; SAS, self-anxiety scale; SDS, self-depression scale; CMSS-t, Cox Menstruation Symptom Scale-time subscale; CMSS-s, Cox Menstruation Symptom Scale-severity subscale.

menstrual pain was experienced with VAS scores of 6.18 ± 0.99 in the VA group, and 5.93 ± 1.21 in the SA group. There was no significant difference in age, duration of PDM, and the height and weight between the two groups ($p > 0.05$). No significant differences were found between the two groups in VAS scores, CMSS-t scores, CMSS-s scores, SAS, or SDS scores for the three menstrual cycles at baseline ($p > 0.05$).

Clinical outcomes

The VA group showed a significant decrease in VAS scores, CMSS-t scores, CMSS-s scores, SAS scores, and SDS scores after the treatment ($p < 0.05$). In contrast, paired t-sample tests revealed no significant changes in VAS scores, SAS scores, and CMSS-s scores ($p < 0.05$), a significant decrease in CMSS-t scores ($p < 0.05$) and an approach significant change in SDS ($p = 0.05$) after manipulating SA. RMANOVA suggested that the changes in VAS scores and CMSS-s scores were more significant in the VA group compared with the SA group; there were no significant differences in the changes in SAS, SDS, and CMSS-t scores between the two groups (see Table 1).

Frequency-specific global functional connectivity density alternation

Post-pre-global functional connectivity density alternations

The more pronounced frequency-specific gFCD changes were observed in VA compared to SA after the treatment. The VA group showed significant gFCD decreases in the bilateral hippocampus at FLF. At Slow-5 band, the gFCD reduced in the hippocampus and right middle cingulate cortex (MCC)/supplementary motor area (SMA) bilaterally and increased in the right ACC, right DLPFC and right anterior inferior frontal gyrus (aIFG). An increased gFCD was seen in the S2 bilaterally within Slow-4 band. The Slow-3 band resulted in a decreased gFCD in the left precuneus (PCU) and an increased gFCD in the bilateral INS (see Figure 1 and Table 2).

The SA group showed a decreased gFCD in the left angular gyrus (AG) and an increased gFCD in the right IFG at FLF. Also, a decreased gFCD was located in the left hippocampus at Slow-5 band. The gFCD in the bilateral medial prefrontal cortex (mPFC) reduced within the Slow-3 band (see Figure 2 and Table 3).

Comparison of global functional connectivity density within groups

The RMANOVA explored the more significant-altered brain regions at different frequency bands in VA compared with SA. The VA increased more gFCD in the left DLPFC at Slow-5 band and in the left MCC at Slow-4 band (Supplementary Figure 3 and Supplementary Table 1). Compared with SA, VA decreased more gFCD in the left caudate nucleus (CAU), nucleus accumbens (NAC), the right hippocampus and hippocampal gyrus (PHG) by VA at FLF; in the left CAU, NAC, and the aIFG was significant at Slow-4 band; on the left hippocampus and right SMA at Slow-3 band (Supplementary Figure 3 and Supplementary Table 1).

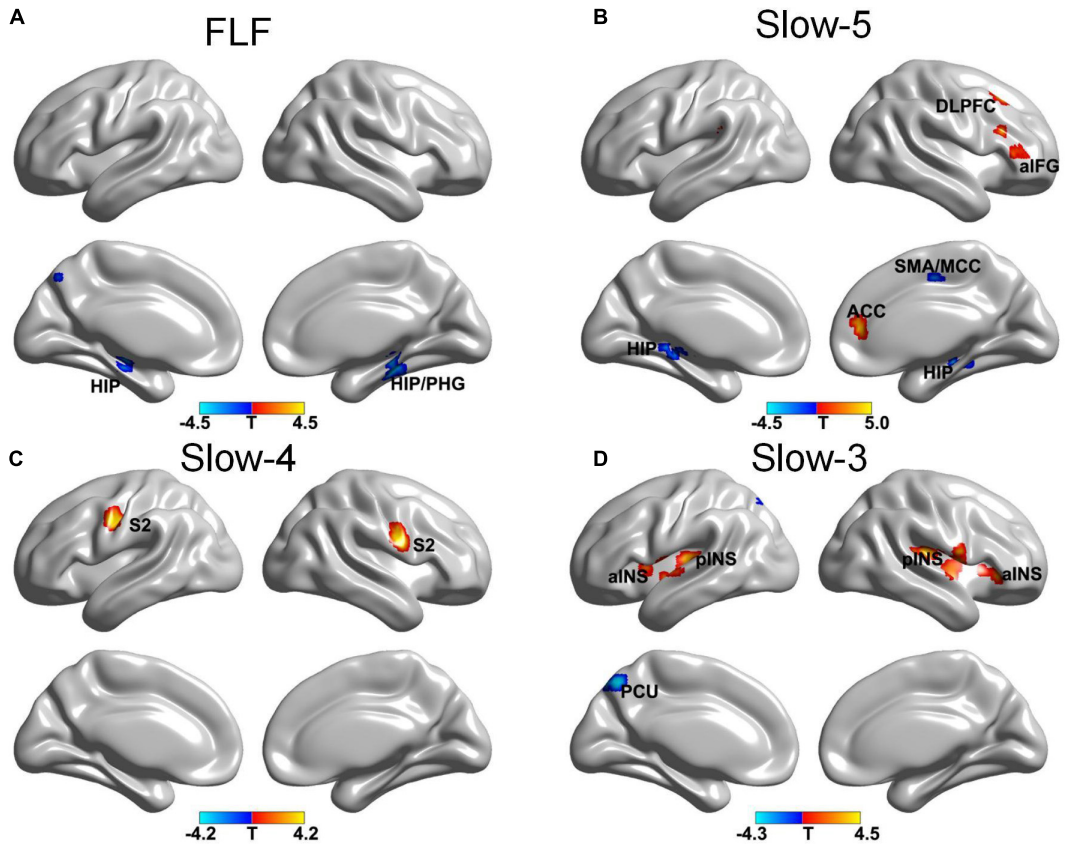

FIGURE 1
The gFCD distribution patterns at multiple frequency-bands following verum acupuncture (voxel-level $p < 0.005$, cluster-level $p < 0.05$, cluster size > 20 voxels, the small volume corrected and FWE corrected). **(A)** A decreased gFCD in HIP/PHG at FLF; **(B)** A decreased gFCD in HIP, MCC/SMA and an increased gFCD in ACC, DLPFC, and aIFG at Slow-5 band; **(C)** An increased gFCD in S2 at Slow-4 band. **(D)** A decreased gFCD in the PCU, and an increased gFCD in INS. gFCD, global functional connectivity density; HIP, hippocampus; PHG, parahippocampal gyrus; SMA, supplementary motor area; MCC, middle cingulate cortex; DLPFC, dorsolateral prefrontal cortex; S2, second somatosensory cortex; aINS, anterior INS; pINS, posterior INS; PCU, precuneus.

Correlation analysis results

We investigated the clinical significance of gFCD alterations with acupuncture in the VA group only. The change in gFCD values at Slow-5 band of the left DLPFC in patients with PDM treated by VA was negatively correlated with the change in VAS scores ($r = -0.508$, $p = 0.022$). Moreover, the gFCD changes at Slow-4 band of the S2 was positively correlated with the change in VAS scores ($r = 0.587$, $p = 0.006$) (see **Figure 3**).

Discussion

This study investigated the frequency-dependent gFCD alternations before and after VA and SA treatment. Generally, the Slow-5 band and Slow-4 band indicated gray-matter-related brain function changes (Zuo et al., 2010), whereas a spontaneous brain activity at the Slow-3 band can predict the lower frequency node activity (Bajaj et al., 2013). The VA group demonstrated a substantial reduction in pain intensity and improvement in PDM symptoms when compared to the SA group. Remarkably, the VA group raised gFCD in the DLPFC at the Slow-5 band and decreased gFCD in the hippocampus at the Slow-3 band, which was in contrast to the direction of pathological gFCD brain function changes in the DLPFC and hippocampus observed in our earlier study (Yu et al., 2021). The results also indicated that VA modulated a more comprehensive range of brain regions than SA. The correlation analysis showed gFCD change in DLPFC at Slow-5 band was negatively correlated with the change of VAS scores in patients with PDM following VA. As a result, the DLPFC and hippocampus may be therapeutic targets for re-establishing normal brain activity in patients with PDM.

The result showed an altered gFCD in the VA group located in S2, CEN (including the DLPFC, ACC, MCC, and aIFG), DMN (including PCU), INS, hippocampus, and PHG. The result is consistent with the previously reported brain responses to VA, down-regulating hippocampus, SMA, and PCU and up-regulating S2, INS, ACC, and DLPFC were observed (Ming-Ting et al., 1999; Bai and Lao, 2013). As a result, brain adaption to VA

TABLE 2 Frequency-specific gFCD alternations in the VA group.

Frequency band	Contrast	Cluster regions	L/R	Cluster size	MNI coordinates			*Z*-score
					x	*y*	*z*	
Full low frequency	Post > Pre	–						
	Pre > Post	HIP	L	20	0	−63	48	3.63
		HIP/PHG	R	36	27	−30	−18	3.44
Slow-5	Post > Pre	ACC	R	26	9	42	9	3.36
		DLPFC	R	20	33	24	45	3.73
		aIFG	R	32	51	39	9	3.63
	Pre > Post	HIP	L	43	−15	−33	−6	3.52
		HIP	R	30	36	−27	−15	3.22
		SMA/MCC	R	26	12	−12	48	3.54
Slow-4	Post > Pre	S2	L	59	−54	−6	39	3.46
		S2	R	58	63	3	18	3.30
	Pre > Post	–						
Slow-3	Post > Pre	aINS	L	37	−39	12	3	4.07
		aINS	R	22	39	30	−3	3.05
		pINS	L	148	−51	−6	3	4.09
		pINS	R	162	69	−3	9	3.76
	Pre > Post	PCU	L	34	−9	−66	51	3.52

Voxel level, $p < 0.005$, cluster level, $p < 0.05$, cluster size > 20 voxels; the small volume correction was applied in case the pain-related brain regions (cluster size less than or equal to 20) were not significant by FWE test. HIP, Hippocampus; PHG, Parahippocampal Gyrus; ACC, Anterior Cingulate Cortex; DLPFC, Dorsolateral Prefrontal Cortex; aIFG, Anterior Inferior Frontal Gyrus; SMA, Supplementary Motor Area; MCC, Middle Cingulate Cortex; S2, Second Somatosensory Cortex; aINS, Anterior Insula; pINS, Posterior insula; PCU, Precuneus.

overlapped with several core networks, involving sensory and cognitive function in pain processing progress, which indicated the non-specific modulation mechanism of acupuncture.

The hippocampus and DLPFC were identified as frequency-specific therapeutic targets for specific acupuncture-based PDM modulation. Our prior study showed a decreased gFCD in DLPFC at Slow-5 band and an increased gFCD in hippocampus at Slow-3 band in patients with PDM when compared with healthy controls (Yu et al., 2021). In contrast, the current findings indicated ascending DLPFC at Slow-5 band and descending hippocampus at Slow-3 band following VA. Both DLPFC and hippocampus are crucial nodes of pain processing. The DLPFC regulates top–down pain pathways in the human brain, which is intimately connected to the individual's executive function (Eippert et al., 2009; Kong et al., 2013). As a result, DLPFC is capable of exerting cognitive control over the perception of pain. Fierro et al. (2010) performed a non-invasive short-duration high-frequency repetitive transcranial magnetic stimulation (rTMS) of the left DLPFC considerably diminish the pain perception generated by capsaicin stimulation. Additionally, studies have revealed a link between pain self-control and DLPFC downregulation in patients with PDM (Lorenz et al., 2003). Therefore, the overlap of DLPFC in this study implied that acupuncture could alleviate pain by modulating cognition to reach individual pain control in PDM. The deficits in hippocampus volume, neurogenesis, and synaptic plasticity are connected with abnormal emotional functioning associated with various types of chronic pain (Mutso et al., 2012; Grilli, 2017). These findings suggested that the hippocampus may regulate pain by modulating pain perception and emotional memory (Rolls, 2015). The PDM has been exacerbated by increased emotional stress caused by aberrant feedback from the hippocampus to the hypothalamus (Jacobson and Sapolsky, 1991; Wang et al., 2004; Ulrich-Lai et al., 2006).

Consistent with the results of the previous studies, we found that acupuncture could down-regulate the hippocampus (Gao et al., 2021; Pang et al., 2021). We speculated that acupuncture could help diminish hippocampal sensitivity and reduce unpleasant memories of pain in PDM (Egorova et al., 2015).

Moreover, S2 is involved in the regulation of nociception and the encoding of painful experiences (Schreckenberger et al., 2005). Consistent with prior research, VA activated S2 (Maeda et al., 2013). Additionally, VA was consistently demonstrated resting-state down-regulation of the DMN subregion (Jung et al., 2015; Makary et al., 2018). Intriguingly, we discovered that PCU was deactivated following VA in our investigation. The PCU is recognized as the hub of the DMN, modulating a broad range of highly interconnected functions and regulating pain, affection, and empathy (Cavanna and Trimble, 2006). The consistent down-regulation in DMN was also shown in the study of Zou et al. (2019) that FC within the DMN (between left superior prefrontal cortex and left PCU) after acupuncture decreased to HC levels. The INS plays an essential role in pain

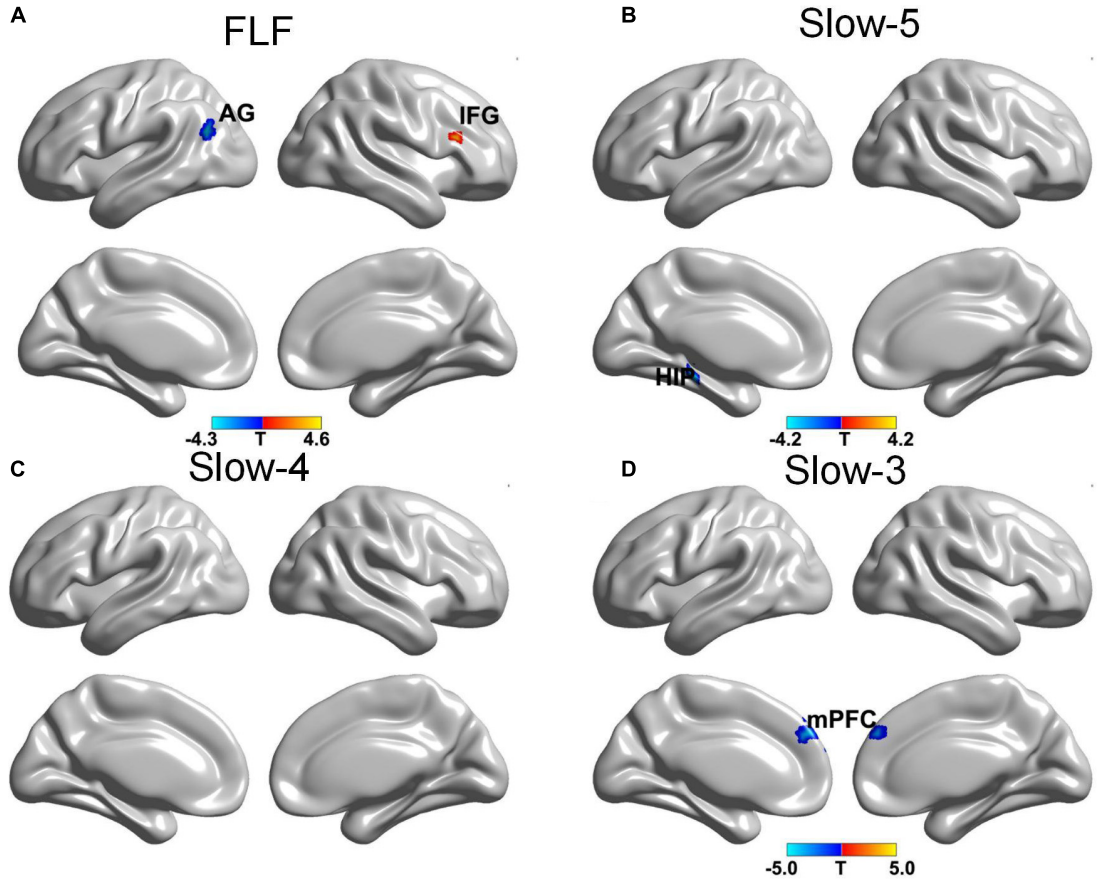

FIGURE 2
The gFCD distribution patterns at multiple frequency-bands following sham acupuncture (voxel-level $p < 0.005$, cluster-level $p < 0.05$, cluster size > 20 voxels, the small volume corrected and FWE corrected). **(A)** A decreased gFCD in AG, and an increased gFCD in IFG at FLF; **(B)** A decreased gFCD in HIP at Slow-5 band; **(C)** No significant change at Slow-4 band; **(D)** A decreased gFCD in mPFC at Slow-3 band. gFCD, global functional connectivity density; AG, angular gyrus; IFG, frontal inferior gyrus; HIP, hippocampus; mPFC, medial prefrontal cortex.

intensity coding. Studies have revealed a decreased gray matter density in the INS of patients with PDM (Tan et al., 2017). Spontaneous brain activity at the Slow-3 band is associated with structural correlation (Bajaj et al., 2013). Our study up-regulated INS in Slow-3, suggesting that VA may modulate INS structure. Although the current research has confirmed the modulation of INS function by acupuncture, the modulation of INS structure by acupuncture requires more investigation (Jung et al., 2015; Dun et al., 2017; Guo et al., 2019; Duan et al., 2021). In addition, the modulation of the cingulate cortex (ACC, MCC) and IFG by acupuncture are associated with modulation of pain perception (Fuchs et al., 2014) and empathic processing of pain (Li et al., 2021).

The results showed that VA and SA had distinct effects on the brain responses of patients with PDM. The prior studies have identified the distinctions and consistency in the brain response to VA compared to SA (Fang et al., 2009; Harris et al., 2009; Chae et al., 2013; Maeda et al., 2017). As with VA on brain modulation, SA down-regulates gFCD in the left hippocampus and up-regulates gFCD in the right IFG. The result also showed that SA reduced gFCD in the anterior DMN. The result implied that SA, similar to VA, could modulate pain emotions in patients with PDM. The studies on SA or phantom acupuncture have concluded that SA can specifically activate DLPFC to provide the placebo effect (Makary et al., 2018; Shi et al., 2021). However, this study suggested that SA did not exhibit a placebo effect associated with self-control in pain. The medial prefrontal lobe is primarily involved in the modulation of visceral motor output of the individual's internal experience and is associated with pain rumination (Kucyi et al., 2014). The SA modulation of the medial prefrontal cortex suggests that the placebo effect of SA may be mediated by improving individual pain rumination.

We preliminarily investigated the modulation of acupuncture on PDM at different frequency bands. The gFCD provided a mapping of the functional connectivity between the brain regions throughout the brain and is more sensitive to identifying the individual differences (Tomasi and Volkow, 2010, 2011). The result showed

TABLE 3 Frequency-specific gFCD alternations in the SA group.

Frequency band	Contrast	Cluster regions	L/R	Cluster size	MNI coordinates			Z-score
					x	*y*	*z*	
Full low frequency	Post > Pre	IFG	R	26	54	27	24	3.66
	Pre > Post	AG	L	26	−51	−69	24	3.33
Slow-5	Post > Pre	–						
	Pre > Post	HIP	L	34	−24	−30	−18	3.27
Slow-4	Post > Pre	–						
	Pre > Post	–						
Slow-3	Post > Pre	–						
	Pre > Post	mPFC	L/R	95	−3	51	39	3.66

Voxel level, $p < 0.005$, cluster level, $p < 0.05$, cluster size > 20 voxels; the small volume correction was applied in case the pain-related brain regions (cluster size less than or equal to 20) were not significant by FWE test. Abbreviations: IFG, Inferior Frontal Gyrus; AG, Angular Gyrus; HIP, Hippocampus; mPFC, Medial Prefrontal Cortex.

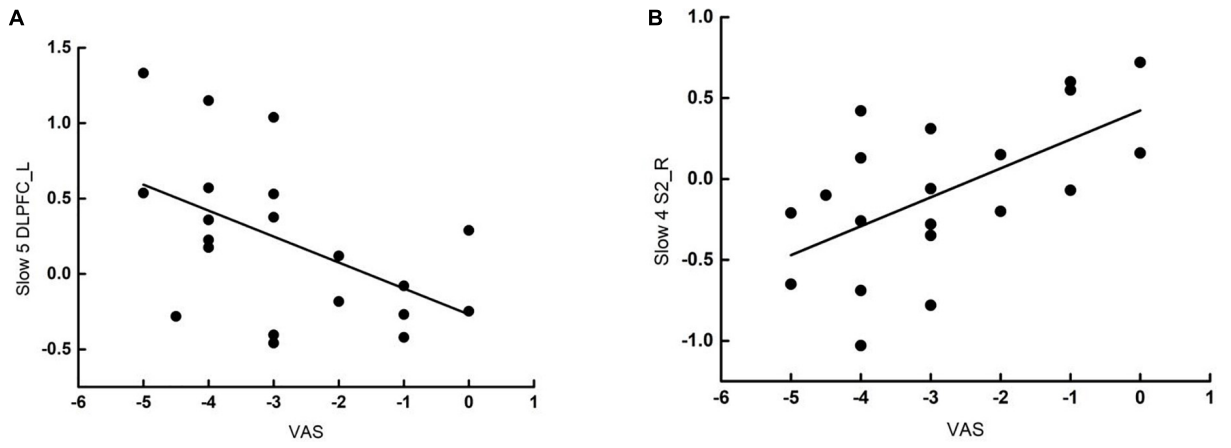

FIGURE 3
Correlation analysis following verum acupuncture and VAS scores of PDM. **(A)** The gFCD changes at Slow-5 band of the left DLPFC in patients with PDM was negatively correlated with the change in VAS scores ($r = -0.508$, $p = 0.022$). **(B)** The gFCD changes at Slow-4 band of the right S2 in patients with PDM was positively correlated with the change in VAS scores ($r = -0.587$, $p = 0.006$). gFCD, global functional connectivity density; VAS, visual analog scale; S2, second somatosensory cortex; DLPFC, dorsolateral prefrontal cortex; pINS, posterior INS; aINS, anterior INS; ACC, anterior cingulate gyrus.

that VA modulated more comprehensive frequency than SA. At the Slow-5 band, VA on the left DLPFC increased significantly compared to SA. Similar to a previous longitudinal study, reduced amplitude of low-frequency fluctuation (ALFF)/fALFF in DLPFC patients with trigeminal neuralgia was observed exclusively at the Slow-5 band (Zhang et al., 2019). At FLF and Slow-3 band, decreased gFCD was more significant in the hippocampus in VA rather than SA. Above results suggested that modulation in different frequency bands is more significant in VA than in SA, and there is a frequency-specific sensitivity of the same modulated brain regions. Notably, abnormal BOLD signal fluctuations occur in different frequency bands ranging from 0.1 to 0.25 Hz under different chronic pain conditions (Baliki et al., 2011b; Alshelh et al., 2016). However, the neurophysiological mechanisms behind the different frequency bands are largely unknown, which needs further exploration (Buzsaki and Draguhn, 2004).

This study found that the gFCD changes of left DLPFC at Slow-5 band were negatively correlated with the change in pain VAS scores, suggesting that VA could increase the cognitive modulation of DLPFC to improve the control of pain in individuals (Lorenz et al., 2003). Our study found that more gFCD changes in DLPFC correspond to more reduction in VAS scores. This suggests that the changes in gFCD of DLPFC are consistent with improvements in VAS and that VA may increase individual control in pain intensity by improving the cognitive modulation of DLPFC. The increased gFCD of S2 after VA also associated with the pain relief of PDM. Therefore, the response of DLPFC and S2 after VA may be an objective biomarker that can predict the efficacy of acupuncture in the treatment of PDM.

Limitations

There are still limitations to the study. First, the study design was small sample size, and patient compliance was influenced by the schedule of the MRI scan (3 days before the onset of menstruation). Second, this study focused exclusively on the altered FCD of the PDM brain network, and more substantial structural and metabolic brain changes need to be investigated. Third, the results showed that acupuncture normalized the brain activity of DLPFC in PDM. However, no relevant behavioral scales in this study were designed to assess individual executive function in this study.

Conclusion

In summary, we re-verified that VA is significantly more effective than SA in treating PDM. The findings supported the hypothesis that acupuncture can restore normalcy to the DLPFC and hippocampus, which had previously been identified as abnormal in PDM. The results elucidated the brain targets of acupuncture for PDM and may facilitate the development of brain stimulation methods to facilitate the therapeutic response to acupuncture.

Data availability statement

All data are presented in this article, and the raw data are not available to the public. Requests to access the datasets should be directed to JY, jenny_yang_jie@126.com.

Ethics statement

The study protocol was approved by the Medical Ethics Review of the Sichuan Regional Ethics Review Committee of Traditional Chinese Medicine (No. 2013KL-033). The patients/participants provided their written informed consent to participate in this study.

Author contributions

JY and S-YY: experimental design. L-YL, XL, Z-LT, and QZ: data collection. S-YY and Z-FS: data analysis. L-YL, S-YY, and Z-LT: manuscript preparation and revision. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2022.917721/full#supplementary-material

References

Abaraogu, U. O., Igwe, S. E., and Tabansi-Ochiogu, C. S. (2016). Effectiveness of SP6 (Sanyinjiao) acupressure for relief of primary dysmenorrhea symptoms: A systematic review with meta- and sensitivity analyses. *Complement. Ther. Clin. Pract.* 25, 92–105. doi: 10.1016/j.ctcp.2016.09.003

Alshelh, Z., Di Pietro, F., Youssef, A. M., Reeves, J. M., Macey, P. M., Vickers, E. R., et al. (2016). Chronic Neuropathic Pain: It's about the Rhythm. *J. Neurosci.* 36, 1008–1018. doi: 10.1523/JNEUROSCI.2768-15.2016

Bai, L., and Lao, L. (2013). Neurobiological foundations of acupuncture: the relevance and future prospect based on neuroimaging evidence. *Evid. Based Complement. Alternat. Med.* 2013:812568. doi: 10.1155/2013/812568

Bajaj, S., Adhikari, B. M., and Dhamala, M. (2013). Higher frequency network activity flow predicts lower frequency node activity in intrinsic low-frequency BOLD fluctuations. *PLoS One* 8:e64466. doi: 10.1371/journal.pone.0064466

Baliki, M. N., Baria, A. T., and Apkarian, A. V. (2011a). The cortical rhythms of chronic back pain. *J. Neurosci.* 31, 13981–13990. doi: 10.1523/JNEUROSCI.1984-11.2011

Baliki, M. N., Schnitzer, T. J., Bauer, W. R., and Apkarian, A. V. (2011b). Brain morphological signatures for chronic pain. *PLoS One* 6:e26010. doi: 10.1371/journal.pone.0026010

Berman, B. M., Lao, L., Langenberg, P., Lee, W. L., Gilpin, A. M., and Hochberg, M. C. (2004). Effectiveness of acupuncture as adjunctive therapy in osteoarthritis of the knee: a randomized, controlled trial. *Ann. Intern. Med.* 141, 901–910. doi: 10.7326/0003-4819-141-12-200412210-00006

Buzsaki, G., and Draguhn, A. (2004). Neuronal oscillations in cortical networks. *Science* 304, 1926–1929. doi: 10.1126/science.1099745

Cai, R. L., Shen, G. M., Wang, H., and Guan, Y. Y. (2018). Brain functional connectivity network studies of acupuncture: a systematic review on resting-state fMRI. *J. Integr. Med.* 16, 26–33. doi: 10.1016/j.joim.2017.12.002

Cavanna, A. E., and Trimble, M. R. (2006). The precuneus: a review of its functional anatomy and behavioural correlates. *Brain* 129, 564–583. doi: 10.1093/brain/awl004

Chae, Y., Chang, D. S., Lee, S. H., Jung, W. M., Lee, I. S., Jackson, S., et al. (2013). Inserting needles into the body: a meta-analysis of brain activity associated with acupuncture needle stimulation. *J. Pain* 14, 215–222. doi: 10.1016/j.jpain.2012.11.011

Ciric, R., Wolf, D. H., Power, J. D., Roalf, D. R., Baum, G. L., Ruparel, K., et al. (2017). Benchmarking of participant-level confound regression strategies for the control of motion artifact in studies of functional connectivity. *Neuroimage* 154, 174–187. doi: 10.1016/j.neuroimage.2017.03.020

Cox, D. J., and Meyer, R. G. (1978). Behavioral treatment parameters with primary dysmenorrhea. *J. Behav. Med.* 1, 297–310. doi: 10.1007/BF00846681

Duan, G., Chen, Y., Pang, Y., Feng, Z., Liao, H., Liu, H., et al. (2021). Altered fractional amplitude of low-frequency fluctuation in women with premenstrual syndrome via acupuncture at Sanyinjiao (SP6). *Ann. Gen. Psychiat.* 20:29. doi: 10.1186/s12991-021-00349-z

Dun, W. H., Yang, J., Yang, L., Ding, D., Ma, X. Y., Liang, F. L., et al. (2017). Abnormal structure and functional connectivity of the anterior insula at pain-free periovulation is associated with perceived pain during menstruation. *Brain Imag. Behav.* 11, 1787–1795. doi: 10.1007/s11682-016-9646-y

Egorova, N., Gollub, R. L., and Kong, J. (2015). Repeated verum but not placebo acupuncture normalizes connectivity in brain regions dysregulated in chronic pain. *Neuroimag. Clin.* 9, 430–435. doi: 10.1016/j.nicl.2015.09.012

Eippert, F., Bingel, U., Schoell, E. D., Yacubian, J., Klinger, R., Lorenz, J., et al. (2009). Activation of the opioidergic descending pain control system underlies placebo analgesia. *Neuron* 63, 533–543. doi: 10.1016/j.neuron.2009.07.014

Fang, J., Jin, Z., Wang, Y., Li, K., Kong, J., Nixon, E. E., et al. (2009). The salient characteristics of the central effects of acupuncture needling: limbic-paralimbic-neocortical network modulation. *Hum. Brain Mapp.* 30, 1196–1206. doi: 10.1002/hbm.20583

Fierro, B., De Tommaso, M., Giglia, F., Giglia, G., Palermo, A., and Brighina, F. (2010). Repetitive transcranial magnetic stimulation (rTMS) of the dorsolateral prefrontal cortex (DLPFC) during capsaicin-induced pain: modulatory effects on motor cortex excitability. *Exp. Brain Res.* 203, 31–38. doi: 10.1007/s00221-010-2206-6

Fomberstein, K., Qadri, S., and Ramani, R. (2013). Functional MRI and pain. *Curr. Opin. Anaesthesiol.* 26, 588–593. doi: 10.1097/01.aco.0000433060.59939.fe

Fox, M. D., and Raichle, M. E. (2007). Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat. Rev. Neurosci.* 8, 700–711. doi: 10.1038/nrn2201

Fuchs, P. N., Peng, Y. B., Boyette-Davis, J. A., and Uhelski, M. L. (2014). The anterior cingulate cortex and pain processing. *Front. Integr. Neurosci.* 8:35. doi: 10.3389/fnint.2014.00035

Gao, L., Zhang, J. F., Williams, J. P., Yan, Y. N., Xiao, X. L., Shi, W. R., et al. (2021). Neuropathic Pain Creates Systemic Ultrastructural Changes in the Nervous System Corrected by Electroacupuncture but Not by Pregabalin. *J. Pain Res.* 14, 2893–2905. doi: 10.2147/JPR.S322964

Grilli, M. (2017). Chronic pain and adult hippocampal neurogenesis: translational implications from preclinical studies. *J. Pain Res.* 10, 2281–2286. doi: 10.2147/JPR.S146399

Guo, J., Yang, M., Biswal, B. B., Yang, P., Liao, W., and Chen, H. (2019). Abnormal functional connectivity density in post-stroke aphasia. *Brain Topogr.* 32, 271–282. doi: 10.1007/s10548-018-0681-4

Harris, R. E., Zubieta, J. K., Scott, D. J., Napadow, V., Gracely, R. H., and Clauw, D. J. (2009). Traditional Chinese acupuncture and placebo (sham) acupuncture are differentiated by their effects on mu-opioid receptors (MORs). *Neuroimage* 47, 1077–1085. doi: 10.1016/j.neuroimage.2009.05.083

Hershman, D. L., Unger, J. M., Greenlee, H., Capodice, J. L., Lew, D. L., Darke, A. K., et al. (2018). Effect of acupuncture vs sham acupuncture or waitlist control on joint pain related to aromatase inhibitors among women with early-stage breast cancer: A randomized clinical trial. *Jama* 320, 167–176. doi: 10.1001/jama.2018.8907

Hinman, R. S., McCrory, P., Pirotta, M., Relf, I., Forbes, A., Crossley, K. M., et al. (2014). Acupuncture for chronic knee pain: A randomized clinical trial. *Jama* 312, 1313–1322. doi: 10.1001/jama.2014.12660

Jacobson, L., and Sapolsky, R. (1991). The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. *Endocr. Rev.* 12, 118–134. doi: 10.1210/edrv-12-2-118

Jung, W. M. I, Lee, S., Wallraven, C., Ryu, Y. H., Park, H. J., and Chae, Y. (2015). Cortical activation patterns of bodily attention triggered by acupuncture stimulation. *Sci. Rep.* 5:12455. doi: 10.1038/srep12455

Kilpatrick, L. A., Kutch, J. J., Tillisch, K., Naliboff, B. D., Labus, J. S., Jiang, Z., et al. (2014). Alterations in resting state oscillations and connectivity in sensory and motor networks in women with interstitial cystitis/painful bladder syndrome. *J. Urol.* 192, 947–955. doi: 10.1016/j.juro.2014.03.093

Kong, J., Gollub, R., Huang, T., Polich, G., Napadow, V., Hui, K., et al. (2007). Acupuncture de qi, from qualitative history to quantitative measurement. *J. Altern. Complement. Med.* 13, 1059–1070. doi: 10.1089/acm.2007.0524

Kong, J., Jensen, K., Loiotile, R., Cheetham, A., Wey, H. Y., Tan, Y., et al. (2013). Functional connectivity of the frontoparietal network predicts cognitive modulation of pain. *Pain* 154, 459–467. doi: 10.1016/j.pain.2012.12.004

Kucyi, A., Moayedi, M., Weissman-Fogel, I., Goldberg, M. B., Freeman, B. V., Tenenbaum, H. C., et al. (2014). Enhanced medial prefrontal-default mode network functional connectivity in chronic pain and its association with pain rumination. *J. Neurosci.* 34, 3969–3975. doi: 10.1523/JNEUROSCI.5055-13.2014

Lefebvre, G., Pinsonneault, O., Antao, V., Black, A., Burnett, M., Feldman, K., et al. (2005). Primary dysmenorrhea consensus guideline. *J. Obstet. Gynaecol. Can.* 27, 1117–1146. doi: 10.1016/s1701-2163(16)30395-4

Li, Y., Li, W., Zhang, T., Zhang, J., Jin, Z., and Li, L. (2021). Probing the role of the right inferior frontal gyrus during Pain-Related empathy processing: Evidence from fMRI and TMS. *Hum. Brain Mapp.* 42, 1518–1531. doi: 10.1002/hbm.25310

Liu, M. G., and Chen, J. (2009). Roles of the hippocampal formation in pain information processing. *Neurosci. Bull.* 25, 237–266. doi: 10.1007/s12264-009-0905-4

Lorenz, J., Minoshima, S., and Casey, K. L. (2003). Keeping pain out of mind: the role of the dorsolateral prefrontal cortex in pain modulation. *Brain* 126, 1079–1091. doi: 10.1093/brain/awg102

Maeda, Y., Kettner, N., Lee, J., Kim, J., Cina, S., Malatesta, C., et al. (2013). Acupuncture evoked response in contralateral somatosensory cortex reflects peripheral nerve pathology of carpal tunnel syndrome. *Med. Acupunct.* 25, 275–284. doi: 10.1089/acu.2013.0964

Maeda, Y., Kim, H., Kettner, N., Kim, J., Cina, S., Malatesta, C., et al. (2017). Rewiring the primary somatosensory cortex in carpal tunnel syndrome with acupuncture. *Brain* 140, 914–927. doi: 10.1093/brain/awx015

Makary, M. M., Lee, J., Lee, E., Eun, S., Kim, J., Jahng, G. H., et al. (2018). Phantom acupuncture induces placebo credibility and vicarious sensations: A parallel fmri study of low back pain patients. *Sci. Rep.* 8:930. doi: 10.1038/s41598-017-18870-1

McCormack, H. M., Horne, D. J., and Sheather, S. (1988). Clinical applications of visual analogue scales: a critical review. *Psychol. Med.* 18, 1007–1019. doi: 10.1017/s0033291700009934

Ming-Ting, W., Hsieh, J.-C., and Xiong, J. (1999). Central nervous pathway for acupuncture stimulation: Localization of processing with functional MR imaging of the brain—preliminary experience. *Radiology* 212, 133–141. doi: 10.1148/radiology.212.1.r99jl04133

Mutso, A. A., Radzicki, D., Baliki, M. N., Huang, L., Banisadr, G., Centeno, M. V., et al. (2012). Abnormalities in hippocampal functioning with persistent pain. *J. Neurosci.* 32, 5747–5756. doi: 10.1523/JNEUROSCI.0587-12.2012

Pang, Y., Liao, H., Duan, G., Feng, Z., Liu, H., Zou, Z., et al. (2021). Regulated aberrant amygdala functional connectivity in premenstrual syndrome via electroacupuncture stimulation at sanyinjiao acupoint(SP6). *Gynecol. Endocrinol.* 37, 315–319. doi: 10.1080/09513590.2020.1855633

Rogachov, A., Cheng, J. C., Hemington, K. S., Bosma, R. L., Kim, J. A., Osborne, N. R., et al. (2018). Abnormal low-frequency oscillations reflect trait-like pain ratings in chronic pain patients revealed through a machine learning approach. *J. Neurosci.* 38, 7293–7302. doi: 10.1523/JNEUROSCI.0578-18.2018

Rolls, E. T. (2015). Limbic systems for emotion and for memory, but no single limbic system. *Cortex* 62, 119–157. doi: 10.1016/j.cortex.2013.12.005

Schmidt-Wilcke, T. (2015). Neuroimaging of chronic pain. *Best Pract. Res. Clin. Rheumatol.* 29, 29–41. doi: 10.1016/j.berh.2015.04.030

Schmidt-Wilcke, T., and Diers, M. (2017). New insights into the pathophysiology and treatment of fibromyalgia. *Biomedicines* 5:22. doi: 10.3390/biomedicines5020022

Schreckenberger, M., Siessmeier, T., Viertmann, A., Landvogt, C., Buchholz, H. G., Rolke, R., et al. (2005). The unpleasantness of tonic pain is encoded by the insular cortex. *Neurology* 64, 1175–1183. doi: 10.1212/01.WNL.0000156353.17305.52

Shi, Y., Cui, S., Zeng, Y., Huang, S., Cai, G., Yang, J., et al. (2021). Brain network to placebo and nocebo responses in acute experimental lower back pain: A multivariate granger causality analysis of fMRI data. *Front. Behav. Neurosci.* 15:696577. doi: 10.3389/fnbeh.2021.696577

Tan, L. L., Pelzer, P., Heinl, C., Tang, W., Gangadharan, V., Flor, H., et al. (2017). A pathway from midcingulate cortex to posterior insula gates nociceptive hypersensitivity. *Nat. Neurosci.* 20, 1591–1601. doi: 10.1038/nn.4645

Tomasi, D., Shokri-Kojori, E., and Volkow, N. D. (2016). Temporal changes in local functional connectivity density reflect the temporal variability of the amplitude of low frequency fluctuations in gray matter. *PLoS One* 11:e0154407. doi: 10.1371/journal.pone.0154407

Tomasi, D., and Volkow, N. D. (2010). Functional connectivity density mapping. *Proc. Natl. Acad. Sci. U. S. A.* 107, 9885–9890. doi: 10.1073/pnas.1001414107

Tomasi, D., and Volkow, N. D. (2011). Functional connectivity hubs in the human brain. *Neuroimage* 57, 908–917. doi: 10.1016/j.neuroimage.2011.05.024

Tomasi, D., and Volkow, N. D. (2014). Mapping small-world properties through development in the human brain: disruption in schizophrenia. *PLoS One* 9:e96176. doi: 10.1371/journal.pone.0096176

Tu, Y., Jung, M., Gollub, R. L., Napadow, V., Gerber, J., Ortiz, A., et al. (2019). Abnormal medial prefrontal cortex functional connectivity and its association with clinical symptoms in chronic low back pain. *Pain* 160, 1308–1318. doi: 10.1097/j.pain.0000000000001507

Ulrich-Lai, Y. M., Xie, W., Meij, J. T., Dolgas, C. M., Yu, L., and Herman, J. P. (2006). Limbic and HPA axis function in an animal model of chronic neuropathic pain. *Physiol. Behav.* 88, 67–76. doi: 10.1016/j.physbeh.2006.03.012

Wang, L., Wang, X., Wang, W., Chen, C., Ronnennberg, A. G., Guang, W., et al. (2004). Stress and dysmenorrhoea: a population based prospective study. *Occup. Environ. Med.* 61, 1021–1026. doi: 10.1136/oem.2003.012302

Wang, Y., Xu, J., Zhang, Q., Zhang, Q., Yang, Y., Wei, W., et al. (2021). Immediate analgesic effect of acupuncture in patients with primary dysmenorrhea: A fMRI study. *Front. Neurosci.* 15:647667. doi: 10.3389/fnins.2021.647667

Witt, C. M., Reinhold, T., Brinkhaus, B., Roll, S., Jena, S., and Willich, S. N. (2008). Acupuncture in patients with dysmenorrhea: a randomized study on clinical effectiveness and cost-effectiveness in usual care. *Am. J. Obstet. Gynecol.* 198, 166.e1–8. doi: 10.1016/j.ajog.2007.07.041

Worsley, K. J., Marrett, S., Neelin, P., Vandal, A. C., Friston, K. J., and Evans, A. C. (1996). A unified statistical approach for determining significant signals in images of cerebral activation. *Hum. Brain Mapp.* 4, 58–73. doi: 10.1002/(SICI)1097-019319964:1<58::AID-HBM4<3.0.CO;2-O

Yu, S., Xu, J., Shen, Z., Wang, Y., Wei, W., Guo, X., et al. (2021). Frequency-specific alterations in brain function in patients with primary dysmenorrhea. *Pain Med.* 23, 902–911. doi: 10.1093/pm/pnab225

Yu, S. Y., Lv, Z. T., Zhang, Q., Yang, S., Wu, X., Hu, Y. P., et al. (2017). Electroacupuncture is beneficial for primary dysmenorrhea: The evidence from meta-analysis of randomized controlled trials. *Evid. Based Complement. Alternat. Med.* 2017:1791258. doi: 10.1155/2017/1791258

Zhang, J., Zhang, Y., Hu, L., Huang, X., Liu, Y., Li, J., et al. (2020). Global trends and performances of magnetic resonance imaging studies on acupuncture: A bibliometric analysis. *Front. Neurosci.* 14:620555. doi: 10.3389/fnins.2020.620555

Zhang, Y., Mao, Z., Pan, L., Ling, Z., Liu, X., Zhang, J., et al. (2019). Frequency-specific alterations in cortical rhythms and functional connectivity in trigeminal neuralgia. *Brain Imaging Behav.* 13, 1497–1509. doi: 10.1007/s11682-019-00105-8

Zou, Y., Tang, W., Li, X., Xu, M., and Li, J. (2019). Acupuncture reversible effects on altered default mode network of chronic migraine accompanied with clinical symptom relief. *Neural. Plast.* 2019:5047463. doi: 10.1155/2019/5047463

Zung, W. W. (1971). A rating instrument for anxiety disorders. *Psychosomatics* 12, 371–379. doi: 10.1016/S0033-3182(71)71479-0

Zung, W. W., Richards, C. B., and Short, M. J. (1965). Self-rating depression scale in an outpatient clinic. Further validation of the SDS. *Arch. Gen. Psychiat.* 13, 508–515. doi: 10.1001/archpsyc.1965.01730060026004

Zuo, X. N., Di Martino, A., Kelly, C., Shehzad, Z. E., Gee, D. G., Klein, D. F., et al. (2010). The oscillating brain: complex and reliable. *Neuroimage* 49, 1432–1445. doi: 10.1016/j.neuroimage.2009.09.037

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Electroacupuncture for the treatment of frozen shoulder: A systematic review and meta-analysis

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Background: Electroacupuncture (EA) has reportedly been successful in controlling pain, but there have been no systematic reviews examining the impact of EA on patients with frozen shoulder (FS). The purpose of this review is to provide evidence on the safety and efficacy of EA for pain management in patients with FS.

Methods: We searched 11 databases from their inception: EMBASE, the Cochrane Library, PubMed, AMED, one Chinese medical database, and six Korean medical databases. Two researchers independently performed the study selection, data extraction, and assessment. Bias-related risk was evaluated using the Cochrane risk-of-bias assessment tool.

Results: This review included thirteen studies involving 936 patients. The EA group exhibited improvements in FS pain (*MD* −1.11, 95% CI −1.61 to −0.61, $p < 0.0001$, $I^2 = 97\%$), function (SMD 2.02, 95% CI 0.36–3.69, $p < 0.00001$, $I^2 = 97\%$), and response rates (RR 1.16, 95% CI 1.07–1.25; $p = 0.0002$; $I^2 = 0\%$) over the manual acupuncture (MA) group. As an adjunct treatment, EA improved FS pain (SMD −1.12, 95% CI −1.52 to −0.71, $P < 0.00001$, $I^2 = 0$) compared to the control treatments. No adverse effects were reported.

Conclusion: EA is reported to improve FS pain and function compared with control treatments. Additionally, EA can be used as an adjunct therapy for FS pain. EA could emerge as a potent intervention against FS.

Systematic review registration: [http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42021247090], identifier [CRD42021247090]

KEYWORDS

frozen shoulder, electroacupuncture, systematic review, pain, acupuncture

Introduction

In the multifactorial disease frozen shoulder (FS), patients often have shoulder pain with limited active and passive mobility of the shoulder (1). The prevalence of frozen shoulder ranges from 2 to 5%, and most cases occur between the ages of 40 and 65 years (2, 3). The patient's symptoms may appear suddenly and usually have a slow recovery (4). It takes anywhere between 1 and 4 years for FS to heal completely (5). The long morbidity period of the disease is burdensome for patients and profoundly affects their quality of life by causing issues such as sleep disturbance and restriction of daily activities (6).

For patients with FS, the use of intra-articular corticosteroids is linked with greater benefits than other interventions, including better pain reduction and range of motion (ROM) (7). However, the duration of this impact is limited (8). Acupuncture can serve as an alternative treatment. Acupuncture is mainly widely used in Asia for managing a variety of conditions, including cardiovascular diseases, infertility, pain and mental health (9–11). According to a meta-analysis of chronic pain (12), the effect of acupuncture did not decrease significantly over 12 months. Electroacupuncture (EA) is considered to enhance acupuncture-induced analgesia. It is possible that EA will have a lasting impact on FS with minimal side effects. In EA, a small current is passed through pairs of acupuncture needles. Needles are inserted into the same acupoints, and several pairs of needles are simultaneously stimulated. When standard operating procedures are followed, EA is a safe and easily sustained mode of treatment that does not exceed patients' tolerance (13).

EA Analgesia, the mechanism by which EA controls pain, involves activation of the nervous system as well as induction of bioactive chemicals. Basically, EA treatment sends neuroimmune, and neuroinflammatory signals. In response to EA, sensory nerve fibers express calcitonin gene-related peptides and substance P, which bind to neurokinin 1 in mast cells, release serotonin, and exert analgesic effects (14). By activating the immune system, it also regulates the production of interleukin-2 (IL-2), interleukin-17 (IL-17), and interferon gamma (IFN-γ) through differentiation and activation of splenic T cells (15). In addition, treatment with EA inhibits sensory and affective components acting through peripheral, spinal, and supraspinal mechanisms. Bioactive molecules such as opioids, N/OFQ, serotonin, norepinephrine, glutamate receptors and transporters, cytokines, and signaling molecules play important roles. Opioids desensitize peripheral nociceptors, reduce the amount of proinflammatory cytokines in the periphery, and decrease cytokine and substance P levels in the spinal cord (16). In addition, the neurotransmitters serotonin and norepinephrine activate the descending inhibitory system, reduce GluN1 phosphorylation, and prevent pain (17). In addition, treatment with EA blocks the expression of inflammatory cytokines by releasing norepinephrine and acetylcholine from the adrenal gland *via* the HPA axis, sympathetic nervous system, and vagus nerve, creating a neuroimmune and neuroendocrine modulatory circuit. In addition, the HPA axis, sympathetic nervous system, and vagus nerve interact with immune cells and nociceptive neurons to create a feedback loop and suppress inflammation (18).

Although a meta-analysis on acupuncture for FS has been undertaken in the past (19), EA is not interchangeable with manual acupuncture (MA), since EA delivers stronger stimulation. One study (13) has shown that EA has greater analgesic effects than MA on several different types of pain. Therefore, pooling the results of MA and EA lowers the homogeneity of studies on acupuncture effects in systematic reviews (20). To the best of our knowledge, no systematic reviews on the impact of EA treatment for FS have been carried out. The purpose of this study was to review and meta-analyze the evidence from randomized controlled trials (RCTs) regarding the safety and efficacy of EA for pain management in patients with FS.

Methods

This protocol was registered on PROSPERO (CRD42021247090) and published (5). The reporting of this review adheres to the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (21).

Search strategy

The following electronic databases were searched from inception to June 2022: EMBASE, MEDLINE, CINII, the Cochrane Central Register of Controlled Trials (CENTRAL), one Chinese database [China National Knowledge Infrastructure (CNKI)], and six Korean databases [The Korean Traditional Knowledge Portal, KoreaMed, Oriental Medicine Advanced Searching Integrated System (OASIS), DBpia, the Research Information Service System, and the Korean Studies Information Service System]. We scanned the reference lists and retrieved any ongoing or recently completed studies that were not in the initial search results. Furthermore, the World Health Organization International Clinical Trials Registry Platform[1] and Google Scholar[2] were searched. To find unpublished trials, we searched the

1 http://apps.who.int/trialsearch/

2 http://scholar.google.co.kr/

ClinicalTrials.gov registry.[3] Our search strategy involved keywords such as "frozen shoulder," "electroacupuncture," "periarthritis of shoulder," and "adhesive capsulitis" written in the languages of the databases (English, Chinese, Japanese, and Korean). **Supplementary Material 1** lists the search terms for each database.

Criteria for considering studies

Types of studies

This review included only prospective RCTs. Observational studies, cohort studies, case series, case–control studies, uncontrolled trials, qualitative studies, and laboratory studies were excluded. No language restriction was imposed.

Types of participants

Patients with FS were eligible regardless of age, sex, or race. We included only those studies that applied an external set of criteria to screen participants for FS.

Types of interventions and controls

The review included studies assessing any form of invasive acupuncture with electrical stimulation. The control interventions could include many different treatments, such as general conventional care (drugs, exercise), MA, waiting-list conditions, or sham treatment (interventions that mimic "true" EA/true treatment but deviate in at least one element deemed important by EA theory, such as correct point location or skin penetration). The acceptability of sham acupuncture as a valid control is highly controversial (22–24), and we planned to analyze the results using subgroup and sensitivity analyzes. In this review, trials comparing EA plus another active treatment to the same active treatment in isolation were also included. However, RCTs wherein one type of EA was compared to another type were not included.

Outcome measures

Pain intensity was the primary outcome measure. It was rated on a numerical rating scale and a visual analog scale (VAS). The secondary outcome measures were variables reflecting functional status [e.g., total effective rate, adverse effects (AEs), Constant–Murley score (CMS), and range of motion].

3 https://clinicaltrials.gov/ct2/home

Data collection, extraction, and assessment

Study selection

Two independent reviewers (JWH and JHJ) searched and screened EMBASE, MEDLINE, CINII, CENTRAL, CNKI, and six Korean databases to find RCTs. Both the titles and the abstracts of the search results were screened, followed by an evaluation of criteria for study inclusion, after which the decisions were recorded in accordance with predefined criteria. The third reviewer (JIK, the corresponding author) resolved any lack of agreement or consensus in the study selection.

Data extraction

Two reviewers (JWH and JHJ) read all the articles that remained after the above steps and extracted data from the articles based on predefined criteria. The data that were tabulated for future analysis included the following: name(s) of author(s), country where the study was performed, year of publication, age, sample size, sex distribution of participants, control intervention, EA intervention, main outcomes, and AEs. To create a summary table of findings, the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) software was used to ascertain the quality of evidence on the basis of the Cochrane Handbook for Systematic Reviews of Interventions (25). When the reported data were unclear or insufficient, an author established contact with the first author or corresponding author of that paper by e-mail or telephone to seek clarity or request missing data.

Evaluating risk of bias

The risk-of-bias assessment tool from the Cochrane Handbook for Systematic Reviews of Interventions was used to perform a quality assessment (26). The characteristics that were examined included generation of a random allocation sequence, concealment of allocation, blinding of both participants and personnel, blinding of the outcome assessment, incomplete data on outcomes, selective reporting of outcomes, and other sources of bias (baseline imbalance was evaluated). This review utilized "L," "U," and "H" as grades for these assessments, where "U" ("unclear") indicated that the risk of bias was uncertain, "H" ("high") indicated a high risk of bias, and "L" ("low") indicated a low risk of bias. In the event of disagreement, the authors reached a consensus by discussion. Information about the risk-of-bias assessment for the aforementioned studies is presented in a table. A critical discussion of the results and their implications is provided.

Data analysis

Cochrane Collaboration's software Review Manager (RevMan), v. 5.4.1 for Windows (The Nordic Cochrane Center, Copenhagen, Denmark) was used to conduct all statistical analyses. This was followed by an evaluation of the differences between the intervention and control groups. In the analysis of clinical efficacy, the assessment of categorical data considered the risk ratios and continuous data with respect to the mean difference (MD). Both categorical and continuous variables are expressed as efficacy values with 95% confidence intervals (CIs). In cases where outcome variables had different scales, the standardized MD was preferred over the weighted MD. If heterogeneity (defined by the results of statistical heterogeneity testing, with $p < 0.1$ on the chi-square test and Higgins' $I^2 \geq 50\%$) was detected, the cause of clinical heterogeneity was assessed by performing subgroup analyses. To assess combined effect sizes from efficacy variables, we used a random-effects model. Notably, we expected substantial clinical heterogeneity across the studies that were included based on the study designs, the diversity of interventions, and other conditions. If more than ten studies were available, publication bias was evaluated by drawing funnel plots (27).

Results

Search results

The 11 database searches yielded 268 studies. Fourteen studies (28–41) met the inclusion criteria (**Figure 1**). **Tables 1**, **2** shows the primary features of the 14 included studies.

Included studies' characteristics

All of the included studies (28–41) were carried out in China. Thirteen studies were written in Chinese (28, 29, 31–41), and only one study (30) was published in English.

Five studies (28–32) compared EA with MA. Three studies compared EA plus Western medicine (WM) to WM in isolation, including joint mobilization (33), arthrolysis (34) and general rehabilitation (35). Two studies compared EA plus frequency therapy (FT) to FT in isolation, including TDP (36) and intermediate frequency therapy (37). One study (38) compared EA plus ultra-short-wave therapy with joint mobilization to ultra-short-wave therapy with joint mobilization. Three studies compared EA plus CM with CM in isolation, including electromoxibustion (EM) (39) and tuina (40, 41). Eight studies (28, 30–32, 34, 38, 39, 41) utilized dense-dispersed waves for EA, five studies (28, 30–32, 39) applied 2 Hz/100 Hz, one study (38) applied 50 Hz/100 Hz, one study (34) applied 2 Hz/15 Hz or 100 Hz, two studies (33, 35) used a continuous frequency, and one study (36) applied 2–20 Hz (**Table 2**). No mention of the frequency was made in the other four articles (29, 37, 40, 41).

Risk of bias

Six studies (28, 29, 32, 33, 35, 40) utilized a random number table, and their risk of bias from random number sequence generation was found to be low (**Figure 2**). Eight studies (31–33, 35, 36, 38, 40, 41) did not clarify whether they employed a random number generation method, which means that they had an ambiguous risk of bias for the generation of random allocation. All studies (28–41) failed to describe the method of allocation concealment and thus had an unclear risk of bias in this respect. The investigator did not perform blinding in one study (30); however, it was determined that blinding would not impact the assessment of results, and so the risk of detection bias was low. As for the other 13 studies (28, 29, 31–41), a decision could not be made as to whether the outcome assessor was blinded. For this reason, these studies had an ambiguous risk of detection bias. Twelve studies (28, 29, 31–38, 40, 41) were found to have a low risk of attrition bias owing to low dropout rates. Higher dropout rates were reported in two papers (30, 39), but they did not impact the results; for this reason, the risk of attrition bias was considered low. Notably, due to the lack of registered protocols, all studies (28–41) were found to have an unclear risk of reporting bias; these studies may not have prespecified the variables of interest and the anticipated values of these variables. Eight studies (28–31, 36–38, 40) were found to have a low risk of other forms of bias due to the absence of a significant difference in baseline information between the groups. The other six studies (32–35, 39, 41) had an ambiguous risk of other forms of bias due to the potential for additional biases; the articles did not provide sufficient information to rule out this possibility.

Effect of interventions

Electroacupuncture vs. manual acupuncture

Five RCTs compared the effects of EA and MA on FS symptoms (28–32). Four RCTs (28, 30–32) compared the effects of EA and MA on VAS pain scores, and the meta-analysis showed that EA effectively reduced pain (MD −1.11, 95% CI −1.61 to −0.61, $p < 0.0001$, **Figure 3A**), although there was high heterogeneity ($I^2 = 97\%$).

Three RCTs (28, 30, 31) also reported the positive effects of EA over MA on the improvement of function, whereas one RCT (32) failed to do so. Our meta-analysis showed favorable effects of EA on the improvement of FS function (SMD 2.02, 95% CI 0.36–3.69, $p = 0.02$, **Figure 3B**), but there was a high degree of heterogeneity ($I^2 = 97\%$).

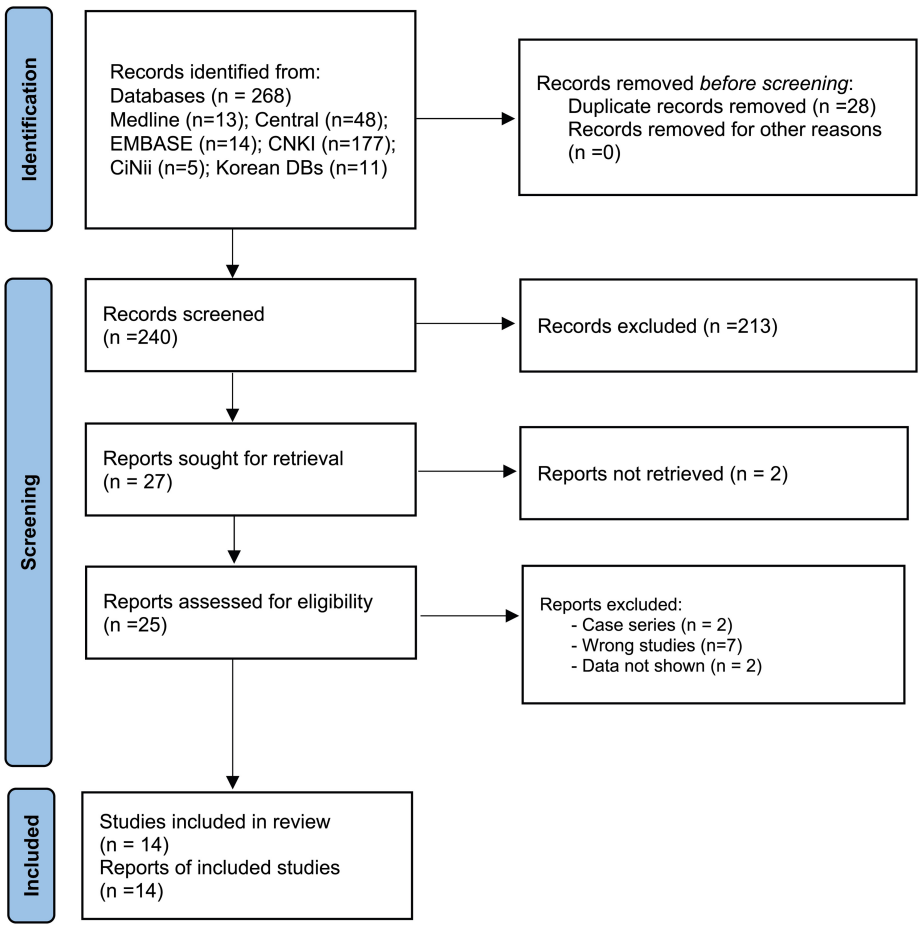

FIGURE 1
Study flow chart. A flowchart of the patient selection process.

Four RCTs (28, 29, 31, 32) showed equivalent effects of EA and MA on the response rate, and one RCT (30) showed positive effects of EA compared to MA. Meta-analysis showed favorable effects of EA on the response rate (RR 1.16, 95% CI 1.07–1.25, $p = 0.0002$, $I^2 = 0\%$; **Figure 3C**).

Electroacupuncture plus western medicine vs. western medicine

Three RCTs tested the effects of EA plus WM compared with WM alone on the symptoms of FS. Two of them (33, 34) reported positive effects of EA plus WM on pain, and the meta-analysis also showed favorable effects of EA plus WM (SMD −1.12, 95% CI −1.52 to −0.71, $p < 0.00001$, $I^2 = 0\%$; **Figure 3D**). Only one RCT assessed the effects of EA plus MA on function; that study reported positive effects of EA plus MA.

One RCT (35) showed a favorable effects of EA plus WM on response rate, while the other 2 RCTs (33, 34) failed to do so. The meta-analysis also failed to show any superior effect of EA plus WM on the response rate (RR 1.12 95% CI 0.95–1.31, $p = 0.18$, $I^2 = 67\%$; **Figure 3E**).

Electroacupuncture plus frequency therapy vs. frequency therapy

Two RCTs (36, 37) tested the effect of EA plus FT compared with FT alone on the response rate. The meta-analysis failed to show a superior effect of EA plus FT on the response rate (RR 1.16, 95% CI 0.87–1.54, $p = 0.30$, **Figure 3E**). These studies had high heterogeneity ($I^2 = 82\%$).

One RCT (38) investigated the effect of EA plus FT and joint mobilization compared to FT plus joint mobilization alone on function and response rate. The results showed favorable effects of EA plus FT and JM on both function and response rate.

Electroacupuncture plus CM therapies vs. CM therapies

Three RCTs compared FS symptoms after EA plus CM vs. CM alone. The CMs included EM (39) and tuina (40, 41). EA plus EM showed positive effects on function and pain but not on response rate compared with EM alone. EA plus tuina showed

TABLE 1 Summary of the characteristics of the included studies.

References	FS duration Sex (M/F) Age	Intervention	Control	Outcome measures	Main results	Authors' conclusion
Lin et al. (28)	A: 52.1; B: 52.9 days A: 16/24; B: 18/22 A: 55.4; B: 57.3	(A) EA (30 min, $n = 40$)	(B) MA (30 min, $n = 40$)	1) Pain (VAS†) 2) Function (Constant–Murley‡) 3) Response rate	1) MD −0.65 [−1.25, −0.05], $P < 0.05$ 2) MD 7.45 [4.58, 10.32], $P < 0.001$ 3) RR 1.19 [1.00, 1.41], $P < 0.05$	"EA. . .reduces shoulder pain, improves shoulder joint mobility, . . ."
Shao (29)	A: 6.85; B: 6.82 days A: 13/16; B: 12/17 A: 54.5; B: 53.8	(A) EA (30 min, $n = 20$)	(B) MA (30 min, $n = 29$)	Response rate	RR 1.12 [0.95, 1.32], NS	"EA has a reliable curative effect. . ."
Shi et al. (30)	A: 3.11; B: 2.97 months A: 23/34; B: 18/38 A: 54.9; B: 52.1	(A) EA (30 min, $n = 57$)	(B) MA (30 min, $n = 56$)	1) Pain (VAS†) 2) Function (neck and shoulder pain‡) 3) Response rate	1) MD −1.50 [−1.57, −1.43], $P < 0.001$ 2) MD 17.87 [16.49, 19.25], $P < 0.001$ 3) RR 1.18 [1.01, 1.38], $P < 0.05$	". . .EA. . . [is] superior. . ."
Cong et al. (31)	A: 2.66; B: 2.49 months A: 14/13; B: 10/18 A: 56; B: 52	(A) EA (30 min, $n = 27$)	(B) MA (30 min, $n = 28$)	1) Pain (VAS†) 2) Function (neck and shoulder pain‡) 3) Response rate	1) MD −1.61 [−1.77, −1.45], $P < 0.001$ 2) MD 15.48 [11.32, 19.64], $P < 0.001$ 3) RR 1.17 [0.97, 1.41], NS	". . .EA. . .[was] better than MA"
Cong et al. (32)	A: 2.66; B: 2.05 months A: 11/11; B: 17/11 A: 52.3; B: 55.0	(A) EA (60 min, $n = 22$)	(B) MA (60 min, $n = 28$)	1) Pain (VAS†) 2) Function (neck and shoulder pain‡) Response rate	1) MD −0.56 [−0.73, −0.39], $P < 0.001$ 2) MD 4.05 [−2.29, 10.39], NS 3) RR 1.11 [0.89, 1.38], NS	No clear conclusion given
Huang (33)	n.r. A: 13/14; B: 10/11 A: 56.2; B: 54.5	(A) EA, plus B ($n = 27$)	(B) Joint mobilization ($n = 21$)	1) Pain (VAS†) 2) Response rate	1) MD −1.21 [−1.94, −0.48], $P < 0.01$ 2) RR 1.06 [0.91, 1.25], NS	". . .[the relationship of EA]. . . with joint mobilization. . .is definite."
Yang et al. (34)	1–6 months n.r. n.r.	(A) EA, plus B ($n = 31$)	(B) Arthrolysis ($n = 31$)	1) Pain (MRMC‡) 2) Function (MRMC‡) 3) Response rate	1) MD 7.82 [4.78, 10.86], $P < 0.001$ 2) MD 5.79 [4.72, 6.86], $P < 0.001$ 3) RR 1.03 [0.92, 1.16], NS	". . .arthrolysis. . . with EA . . . is better. . ."
Li et al. (35)	n.r. n.r. n.r.	(A) EA, plus B ($n = 31$)	(B) general rehabilitation ($n = 31$)	1) Pain (VAS†) 2) Function (aROM‡) 3) Response rate	1)-2) details n.r., $P < 0.05$ 3) RR 1.36 [1.08, 1.72], $P < 0.01$	". . .[general rehabilitation] combined with EA is beneficial."
Huang et al. (36)	A: 6.1; B: 6 months A: 32/20; B: 14/12 n.r.	(A) EA, plus B ($n = 52$)	(B) TDP ($n = 26$)	Response rate	RR 1.05 [0.95, 1.15], NS	". . .EA. . . with TDP. . .is better than. . .TDP. . .alone"
He (37)	A: 4.03; B: 3.85 months A: 18/14; B: 14/16 A: 48.1; B: 49.0	(A) EA, plus B ($n = 32$)	(B) Intermediate frequency (4∼6 kHz) ($n = 30$)	Response rate	RR 1.32 [1.06, 1.65], NS	"EA. . .with intermediate frequency has a significant curative effect"
Ke et al. (38)	A: 3.3; B: 3.6 weeks A: 8/22; B: 9/21 A: 48.4; B: 50.2	(A) EA, plus B ($n = 30$)	(B) Very high frequency + joint mobilization ($n = 30$)	1) Function (Constant–Murley‡) 2) Response rate	1) MD 17.30 [6.59, 28.01], $P < 0.01$ 2) RR 1.33 [1.04, 1.72], $P < 0.05$	"Treatment. . .with EA . . .improved [outcomes]."
Li et al. (39)	n.r. A: 14/16; B: 15/15 n.r.	(A) EA, plus B ($n = 30$)	(B) Electromoxibustion (Fuyang pot warming) ($n = 30$)	1) Pain (VAS†) 2) Function (Constant–Murley‡; ASES‡) 3) Response rate	1)-2) details n.r., $P < 0.01$ 3) A vs. B: RR 1.08 [0.88, 1.32], NS	"Fuyang pot warming. . .with EA. . .has. . .[a greater] curative effect . . . than EA. . . or Fuyang pot warming therapy alone"
Huang et al. (41)	A: 6.53; B: 7.03 months A: 13/17; B: 11/19 A: 52.0; B: 53.6	(A) EA, plus B ($n = 30$)	(B) Tuina ($n = 30$)	1) Function (Constant–Murley‡) 2) Response rate	1) MD 11.80 [8.72, 14.88], $P < 0.001$ 2) RR 1.50 [1.09, 2.06], $P < 0.01$	"EA. . . with tuina. . . [was] superior. . . [for] improving range of motion. . . [and] alleviating clinical symptoms"
Li (40)	A: 7.85; B: 7.85 days A: 29/45; B: 32/42 A: 54.4; B: 50.2	(A) EA, plus B ($n = 74$)	(B) Tuina ($n = 74$)	Response rate	RR 1.13 [1.01, 1.27], $P < 0.05$	"Tuina. . . [with] EA has a reliable curative effect"

†A lower score indicates better condition; ‡a higher score indicates better condition.

A, intervention group; B, comparison group.

ASES, American Shoulder and Elbow Surgeons; AEs, adverse effects; EA, electroacupuncture; FS, frozen shoulder; MA, manual acupuncture; MD, mean difference; MRMC, Michael Reese Medical Center; n.r., not reported; NS, not significant; RCT, randomized controlled trial; aROM, active range of motion; RR, risk ratio; SD, standard deviation; TDP, tending diancibo pu; VAS, visual analog scale.

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TABLE 2 Summary of the regimens used in the included studies.

References	Acupuncture points	Medium (model, manufacturer)	Wave (Hz)	Intensity	Treatment session and interval
Lin et al. (28)	EA: Ashi points, LI15, TE14, EX-UE70, SI9, LI11, TE5, LI4, SI3 MA: Ashi points, LI15, TE14, EX-UE70, SI9, LI11, TE5, LI4, SI3	n.r.	Dense-dispersed wave (2 Hz/100 Hz)	Tolerance level	14 times (once daily)
Shao (29)	EA: EX-UE70, LI15, TE14, SI11, LI4, GB34 MA: X-UE70, LI15, TE14, SI11, LI4, GB34	n.r.	n.r.	n.r.	20 times (once daily)
Shi et al. (30)	EA: EX-UE70, TE14, LI15, SI10, TE5, LI MA: EX-UE70, TE14, LI15, SI10, TE5, LI4	HANS LH-202H	Dense-dispersed wave (2 Hz/100 Hz)	(3 ± 2) mA	5 times (once every other day)
Cong et al. (31)	EA: EX-UE70, LI15 or TE14, SI10, TE5, LI4 MA: EX-UE70, LI15 or TE14, SI10, TE5, LI4	HANS LH-202H	Dense-dispersed wave (2 Hz/100 Hz)	n.r.	5 times (once every other day)
Cong et al. (32)	EA: EX-UE70, LI15, TE14, SI10, TE5, LI4 MA: EX-UE70, LI15, TE14, SI10, TE5, LI4	HANS LH-202H	Dense-dispersed wave (2 Hz/100 Hz)	(1 ± 2) mA	5 times (once every other day)
Huang (33)	(Ashi points, LI15, TE14, LI11, LI4/Flexion restriction: LI14, Tai Jian Extension restriction: TE13, SI10 Abduction restriction: Nao Shang, Cheng Feng External rotation restriction: SI11, SI9 Internal rotation restriction: Jian Nei Ling) + joint mobilization	n.r.	Continuous (high frequency 10 min − > low frequency 10 min)	n.r.	10 times (once daily)
Yang et al. (34)	(LI15, SI9, EX-UE70, LI14, PC3, LI11, LU5, GB34, SP8, SI11, TE5/GB12, BL60, LI4, ST38, TE3, LU5, LU7, TE5)	n.r.	Dense-dispersed wave (2 Hz/15 Hz OR 100 Hz)	n.r.	5 times (once daily)
Li et al. (35)	(LI meridian, SI meridian, TE meridian, LI15, Ashi points, TE14)	KWD808-I	Continuous (0–10 Hz)	2–4 mA	20 times (once daily)
Huang et al. (36)	(Ashi points, LI15, SI9, TE14, EX-UE70)	G6805	2–20 Hz	2–3 mA	10 times (once daily)
He (37)	(Ashi points, LI15, EX-UE70, SI9, LI11, LI4, SP9, LI3, TE3, SI3)	G6805	n.r.	Tolerance level	20 times (once daily)
Ke et al. (38)	SI14, EX-UE70, LI15, TE14, SI9, LI11, TE5, LI4 + Very high frequency (frequency 40.68 mHz, wavelength 7.37 m, output 200 W)	G6805-II	Dense-dispersed wave (50 Hz/100 Hz)	n.r.	21 times (once daily)
Li et al. (39)	(Ashi points, GB20, GV14, GB21, LI15, LI14, ST39, BL57) + Fuyang pot warming (Ashi points, GB20, GV14, GB21, LI15, SI9, LU1, SI11)	HANS LH-202H	Dense-dispersed wave (2 Hz/100 Hz)	1–1.5 mA	12 times (three times/week)
Huang et al. (41)	EA: Ashi points, LI15, TE14, EX-UE70, SI9	SDZ II, Hwato	Dense-dispersed wave (n.r.)	Tolerance level	21 times (once daily)
Li (40)	(SI9, Tai jian, LI15, TE14, SI11, LI4)	n.r.	n.r.	n.r.	20 times (once daily)

EA, electroacupuncture; MA, manual acupuncture; n.r., not reported.

TABLE 3 Summary of findings.

Patient or population: Patients with FSIntervention: EA or EA + WMComparison: MA or WM

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	No of participants (Studies)	Certainty of the evidence (GRADE)
	Risk with MA	Risk with EA			
EA vs. MA					
Pain (VAS)	The mean pain (as measured by the VAS) ranged from −1.61 to −0.61	MD 1.11 lower (1.61 lower to 0.61 lower)	–	298 (4 RCTs)	⊕◯◯◯ Very low[a,b,c]
Function	–	SMD 2.02 SD higher (0.36 higher to 3.69 higher)	–	298 (4 RCTs)	⊕◯◯◯ Very low[a,b,c]
Response rate	812 per 1,000	942 per 1,000 (869–1,000)	RR 1.16 (1.07–1.25)	356 (5 RCTs)	⊕⊕◯◯ Low[a,d]
EA + WM vs. WM					
Pain intensity	–	SMD 1.12 SD lower (1.52 lower to 0.71 lower)	–	110 (2 RCTs)	⊕⊕◯◯ Low[a,b]

[a]Most information is unclear (random number generation and allocation concealment).
[b]Serious limitation of inconsistency: Unexplained high heterogeneity ($I^2 > 50\%$).
[c]Total participants < 400.
[d]Total participants < 300.
*The risk in the intervention group (along with its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI). CI, confidence interval; MD, mean difference; RR, risk ratio; SMD, standardized mean difference; WM, Western medicine.
GRADE Working Group grades of evidence. High certainty: we are very confident that the true effect lies close to the estimate of the effect. Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different. Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect. Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

positive effects of EA on function and response rate compared with tuina alone. However, the meta-analysis failed to show a superior effect of EA plus tuina on the response rate (RR 1.26, 95% CI 0.94–169, $p = 0.13$, Figure 3E). These studies had high heterogeneity ($I^2 = 69\%$).

Safety of interventions

Thirteen studies failed to report whether any AEs took place (28–34, 36–41). An absence of AEs was reported by Li et al. (35). Since 13 of the 14 studies failed to record whether any AEs took place, more studies are needed regarding the safety of EA.

Summary of findings

The certainty of the evidence comparing the impacts of EA and MA on pain was downgraded from high to very low (three levels) owing to the severity of concerns about imprecision, inconsistency, and risk of bias (Table 3). The certainty of the evidence comparing the impacts of EA and MA on function was downgraded from high to very low (three levels) owing to the severity of concerns regarding imprecision, inconsistency, and risk of bias. The certainty of the evidence comparing the impacts of EA and MA on response rates was downgraded from high to low (two levels) owing to the severity of concerns pertaining to imprecision and the risk of bias. The certainty of the evidence that compared the impact of EA treatment plus WM vs. WM alone on pain was downgraded from high to low (two levels) owing to serious concerns pertaining to the risk of bias and imprecision.

Discussion

EA was found to be an efficacious method for treating FS in this review. The meta-analysis showed that EA led to a greater reduction of FS pain than MA did, although with a very low certainty of evidence. In comparison to MA, EA led to a superior degree of functional improvement in FS patients, with a very low certainty of evidence. In comparison to MA, EA enhanced the response rate of FS, again with a low certainty of evidence. Compared with WM used in isolation, EA plus WM reduced FS pain with a low certainty of evidence. Four RCTs compared the efficacy of EA and MA as treatments for FS (29, 33, 36, 39). Three RCTs reported favorable impacts on functional scores in FS patients treated with EA compared to those treated with MA (29, 30, 36). However, one trial showed no favorable effects of EA on function compared to MA (32), which might be attributable to the treatment duration of 60 min. Generally, the longer the stimulation time, the more effective the stimulation. A shorter stimulation time translates

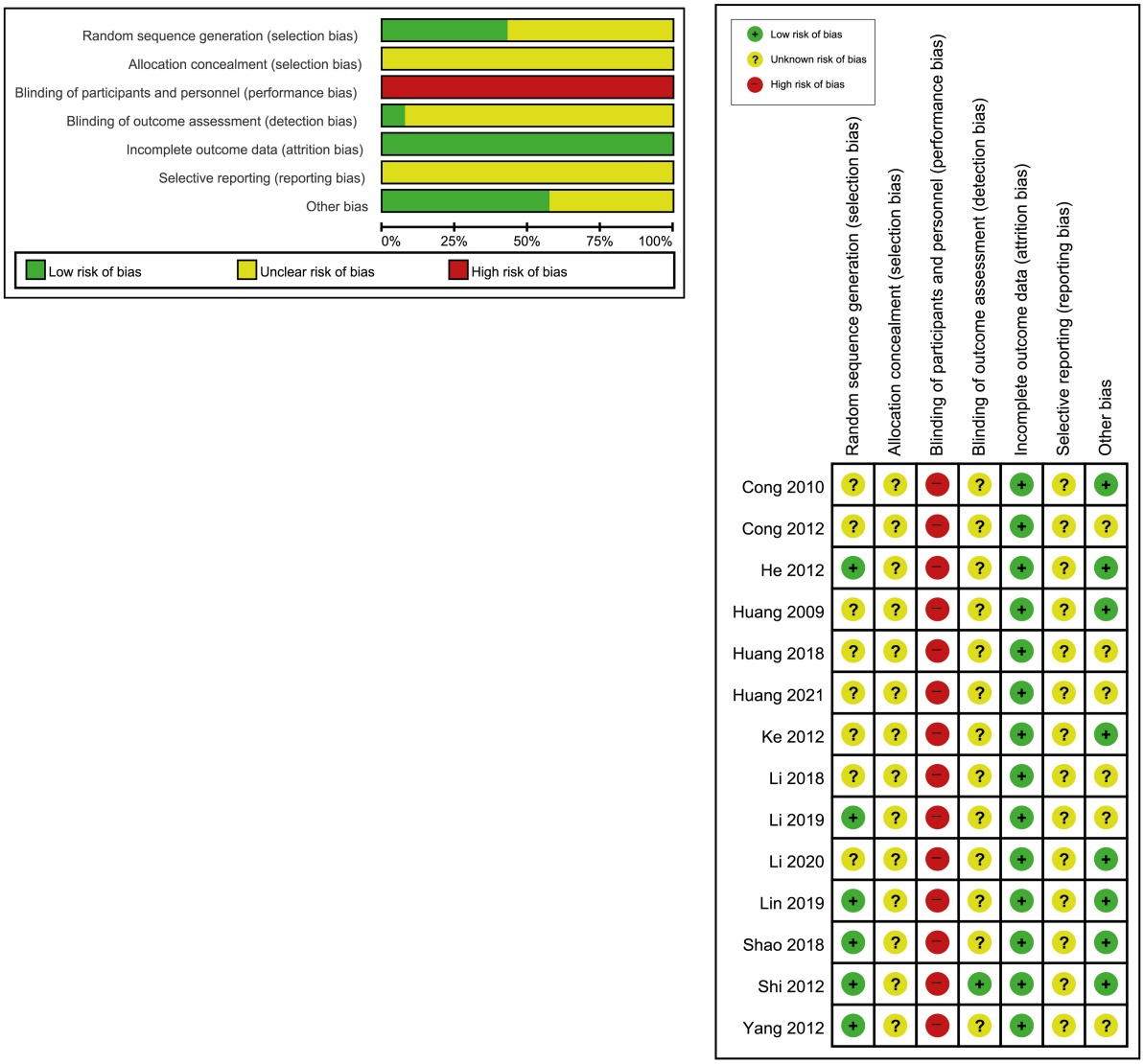

FIGURE 2
(A) Risk-of-bias graph and **(B)** risk-of-bias summary: The present authors' judgments regarding the risk of each form of bias in all included studies.

to less effective stimulation. However, if the stimulation time is extended indefinitely, the effective stimulation worsens or becomes invalid (42). Therefore, EA may have led to functional improvement in FS patients if an appropriate treatment time had been set. Additionally, when using EA for FS, it would be prudent to enhance the patient's function by selecting a target treatment area that would focus on the muscles impacting the shoulder joint's ROM, for example, the elevator muscle of the scapula, as opposed to considering only the Ashi points. Therefore, in future RCTs exploring the use of EA for FS, it could be worthwhile to choose the treatment area by focusing on the muscle that is restricting shoulder movement.

This review included only one study where AEs were linked with EA. Despite being a relatively safe treatment tool, EA is not free of risks. Therefore, it is necessary to conduct more studies to evaluate the risk of AEs.

According to our assessment, allocation concealment, appropriate randomization, blinding of outcome assessment, and selective reporting were not mentioned in the various RCTs included in this review. Notably, random number generation was reported in only six studies (28, 29, 32, 33, 35, 40), and the blinding of the outcome assessment was referred to in only one study (30). This indicates a high risk of bias in all the included studies, which could potentially

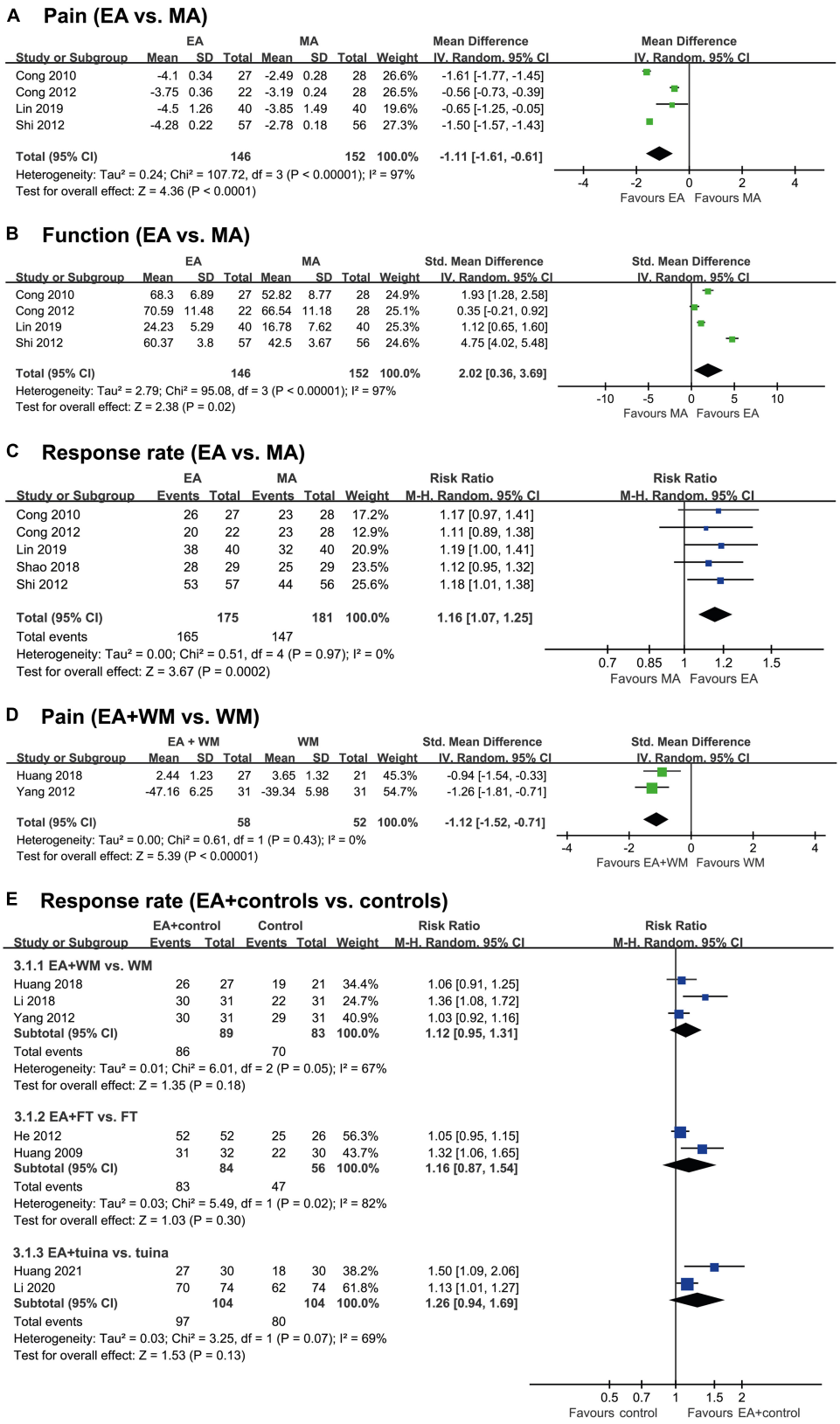

FIGURE 3
Forest plot of each outcome according to the comparison made. **(A)** Pain (EA vs. MA), **(B)** Function (EA vs. MA), **(C)** Response rate (EA vs. MA), **(D)** Pain (EA + WM vs. WM) and **(E)** Response rate (EA + controls vs. controls). EA, electroacupuncture; MA, manual acupuncture; FT, frequency therapy; WM, Western medicine.

result in false positives. Moreover, since all the studies (28–41) were performed in China, it is important to conduct independent studies in different countries to determine the generalizability of the results.

In terms of the therapeutic effect for FS, no systematic review thus far has focused only on EA. One systematic review (19) examined the impacts of acupuncture on FS in five studies (30, 43–46). EA was utilized as an intervention in some of those studies, but three (44–46) were excluded from the present study because they failed to satisfy its inclusion criteria, and no meta-analysis was carried out on the other two studies (30, 43). Thus, no strong recommendation can be made for the use of EA in FS. Unlike prior reviews, ours has demonstrated the efficacy of EA in FS compared with MA. Furthermore, the efficacy of EA as an adjunct therapy for FS has been established.

This study has certain limitations. First, since the sample size of the meta-analysis was small, one must be cautious about generalizing the results. The reason for the small sample size was because the trials used inconsistent outcome measures, with only a small number of trials actually being eligible for the meta-analysis of each measure. Second, the treatment regimen used in different trials varied in several aspects, such as the selection of acupuncture points and the treatment frequency. Thus, future studies to assess treatment effects should use a consistent acupuncture treatment regimen. Third, our study's findings should be interpreted cautiously owing to the high risk of bias within the included studies. The fourth limitation is the high heterogeneity between the included trials. The included studies used different standards to measure effectiveness through the response rate, and the clinical characteristics of the patients and their treatments were also different, including the dose and type of intervention and origin of shoulder pain. The pooled results also showed high statistical heterogeneity. Clearly, these potential confounding factors may reduce the comparability of the final results; therefore, the results need to be interpreted with caution.

To enable RCTs and pilot trials designed as precursors for appropriate RCTs, future studies on FS treatment with EA should emphasize appropriate and uniform methods. It will also be necessary to conduct long-term studies to determine the duration of the treatment effects. Furthermore, a cost analysis needs to be conducted.

In conclusion, the results from this systematic review and meta-analysis suggest that EA is more effective than MA for managing FS, with larger effect sizes in terms of pain (particularly after 30 min of treatment), function, and response rate. Additionally, this systematic review and meta-analysis provides suggestive evidence for the superiority of EA as an adjunct therapy to reduce FS pain. However, given the high risk of bias, the differences in treatment regimens, and the small sample size, the level of evidence is low. To confirm the effect of EA on FS, it will be necessary to conduct well-designed research studies with larger sample sizes.

Data availability statement

The original contributions presented in the study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author/s.

Author contributions

J-IK: conceptualization. J-WH and J-HJ: data curation and methodology. J-WH, J-HJ, J-JL, and J-IK: formal analysis. J-WH, J-HJ, HK, and T-YC: investigation. MSL and J-IK: project administration. J-JL and HK: resources. J-WH, MSL, and J-IK: software. HK, T-YC, MSL, and J-IK: supervision. J-WH: writing—original draft. J-WH, J-HJ, J-JL, HK, T-YC, MSL, and J-IK: writing—review and editing. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.928823/full#supplementary-material

References

1. Abrassart S, Kolo F, Piotton S, Chiu JC-H, Stirling P, Hoffmeyer P, et al. 'Frozen shoulder'is ill-defined. How can it be described better? *Efort Open Rev.* (2020) 5:273–9. doi: 10.1302/2058-5241.5.190032

2. Kingston K, Curry EJ, Galvin JW, Li X. Shoulder adhesive capsulitis: epidemiology and predictors of surgery. *J Shoulder Elbow Surg.* (2018) 27:1437–43. doi: 10.1016/j.jse.2018.04.004

3. Hodgetts C, Walker B. Epidemiology, common diagnoses, treatments and prognosis of shoulder pain: a narrative review. *Int J Osteopathic Med.* (2021) 42:11–9. doi: 10.1016/j.ijosm.2021.10.006

4. Mezian K, Coffey R, Chang K-V. *Frozen Shoulder*. Tampa, FL: StatPearls Publishing (2021).

5. Heo J-W, Jo J-H, Lee J-J, Kang H, Choi T-Y, Lee M-S, et al. Electroacupuncture for the treatment of frozen shoulder: a protocol for systematic review and meta-analysis. *Medicine.* (2021) 100:e28179. doi: 10.1097/MD.0000000000028179

6. Asheghan M, Aghda AK, Hashemi E, Hollisaz M. Investigation of the effectiveness of acupuncture in the treatment of frozen shoulder. *Materia Soc Med.* (2016) 28:253. doi: 10.5455/msm.2016.28.253-257

7. Challoumas D, Biddle M, McLean M, Millar NL. Comparison of treatments for frozen shoulder: a systematic review and meta-analysis. *JAMA Netw Open.* (2020) 3:e2029581–2029581. doi: 10.1001/jamanetworkopen.2020.29581

8. Favejee M, Huisstede B, Koes B. Frozen shoulder: the effectiveness of conservative and surgical interventions—systematic review. *Br J Sports Med.* (2011) 45:49–56. doi: 10.1136/bjsm.2010.071431

9. Vickers AJ, Cronin AM, Maschino AC, Lewith G, MacPherson H, Foster NE, et al. Acupuncture for chronic pain: individual patient data meta-analysis. *Arch Internal Med.* (2012) 172:1444–53. doi: 10.1001/archinternmed.2012.3654

10. Pan W, Li F-X, Wang Q, Huang Z-Q, Yan Y-M, Zhao L, et al. A randomized sham-controlled trial of manual acupuncture for infertile women with polycystic ovary syndrome. *Integrat Med Res.* (2022) 11:100830. doi: 10.1016/j.imr.2021.100830

11. Qi W-C, Fu H-J, Sun R-R, Li X, Cai D-J, Wang C, et al. Effectiveness and safety of acupuncture for angina pectoris: an overview of systematic reviews. *Integrat Med Res.* (2022) 11:100864. doi: 10.1016/j.imr.2022.100864

12. MacPherson H, Vertosick E, Foster N, Lewith G, Linde K, Sherman K, et al. The persistence of the effects of acupuncture after a course of treatment: a meta-analysis of patients with chronic pain. *Pain.* (2017) 158:784. doi: 10.1097/j.pain.0000000000000747

13. Ulett GA, Han S, Han J-S. Electroacupuncture: mechanisms and clinical application. *Biol Psychiat.* (1998) 44:129–38. doi: 10.1016/S0006-3223(97)00394-6

14. Chen L-Z, Kan Y, Zhang Z-Y, Wang Y-L, Zhang X-N, Wang X-Y, et al. Neuropeptide initiated mast cell activation by transcutaneous electrical acupoint stimulation of acupoint LI4 in rats. *Sci Rep.* (2018) 8:1–12. doi: 10.1038/s41598-018-32048-3

15. Chen L, Xu A, Yin N, Zhao M, Wang Z, Chen T, et al. Enhancement of immune cytokines and splenic CD4+ T cells by electroacupuncture at ST36 acupoint of SD rats. *PLoS One.* (2017) 12:e0175568. doi: 10.1371/journal.pone.0175568

16. Binder W, Mousa SA, Sitte N, Kaiser M, Stein C, Schäfer M. Sympathetic activation triggers endogenous opioid release and analgesia within peripheral inflamed tissue. *Eur J Neurosci.* (2004) 20:92–100. doi: 10.1111/j.1460-9568.2004.03459.x

17. Zhang R, Lao L, Ren K, Berman BM. Mechanisms of acupuncture–electroacupuncture on persistent pain. *Anesthesiology.* (2014) 120:482–503. doi: 10.1097/ALN.0000000000000101

18. Yuan L, Mingxiao Y, Fan W, Ke C, Haiyong C, Xueyong S, et al. Mechanism of electroacupuncture on inflammatory pain: neural-im mune-endocrine interactions. *JTCM.* (2019) 39:740–9.

19. Ben-Arie E, Kao PY, Lee YC, Ho WC, Chou LW, Liu HP. The effectiveness of acupuncture in the treatment of frozen shoulder: a systematic review and meta-analysis. *Evid Based Complement Alternat Med.* (2020) 2020:9790470. doi: 10.1155/2020/9790470

20. Langevin HM, Schnyer R, MacPherson H, Davis R, Harris RE, Napadow V, et al. Manual and electrical needle stimulation in acupuncture research: pitfalls and challenges of heterogeneity. *Evid Based Complement Alternat Med.* (2015) 21:113–28. doi: 10.1089/acm.2014.0186

21. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Int J Surg.* (2010) 8:336–41. doi: 10.1016/j.ijsu.2010.02.007

22. Birch S, Lee MS, Kim T-H, Alraek T. On defining acupuncture and its techniques: a commentary on the problem of sham. *Integrat Med Res.* (2022) 11:100834. doi: 10.1016/j.imr.2022.100834

23. Birch S, Lee MS, Kim T-H, Alraek T. Historical perspectives on using sham acupuncture in acupuncture clinical trials. *Integrat Med Res.* (2022) 11:100725. doi: 10.1016/j.imr.2021.100725

24. Lee I-S, Chae Y. Cognitive components of acupuncture treatment. *Integrat Med Res.* (2021) 10:100754. doi: 10.1016/j.imr.2021.100754

25. Group GW. Grading quality of evidence and strength of recommendations. *BMJ.* (2004) 328:1490. doi: 10.1136/bmj.328.7454.1490

26. Higgins JP, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ.* (2011) 343:d5928. doi: 10.1136/bmj.d5928

27. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ.* (1997) 315:629–34. doi: 10.1136/bmj.315.7109.629

28. Lin L, Liu K, Yuan H, Yang Z. Clinical observation on treating acute scapulohumeral periarthritis by electric-acupuncture encircling needling. *Clin J Chin Med.* (2019) 11:92–5.

29. Shao X. Clinical experience of electroacupuncture treatment of frozen shoulder of wind-cold-dampness type. *Chin Manipul Rehabil Med.* (2018) 9:23–4.

30. Shi H, Fang JQ, Li BW, Cong WJ, Zhang Y, Chen L. Efficacy assessment for different acupuncture therapies in the treatment of frozen shoulder. *World J Acupunct Moxibust.* (2012) 22:6-11. doi: 10.1016/S1003-5257(12)60020-8

31. Cong W, Fang J, Wang C, Li B, Shi H. Comparison of the therapeutic effects of filiform needle-based different acupuncture methods on scapulohumeral periarthritis. *Shanghai J Acupunct Moxibust.* (2010) 29:713–5.

32. Cong W, Fang J, Wang C, Shi H, Li B. Comparison study on therapeutic efficacy of different acupuncture methods treating 60min on pain in frozen shoulder. *Chin Arch Tradit Chin Med.* (2012) 30:140–2.

33. Huang J. Analysis of the clinical effect of electroacupuncture and joint mobilization in the treatment of frozen shoulder. *Contemp Med Sympos.* (2018) 16:188–90.

34. Yang J, Jiang J, Xiong D, Luo Y, Zhang D. Therapeutic effect of painless arthrolysis combined with electroacupuncture for scapulohumeral periarthritis. *Chin J Pain Med.* (2012) 18:8–12.

35. Li M, Fu L, Wang J. Effective observation on treating 62 cases of periarthritis by acupuncture plus electroacupuncture. *Clin J Chin Med.* (2018) 10:90–1.

36. Huang N, Ma H, Zheng C. Treatment of 52 cases of frozen shoulder with electroacupuncture and TDP irradiation. *J Clin Acupunct Moxibust.* (2009) 25:29–30.

37. He Z. Thiry-two cases of periarthritis treated with electro-acupuncture and intermediate frequency. *J Clin Acupunct Moxibust.* (2012) 28:28–9.

38. Ke Z, Ye F, Xiao S, Han X. Observation on therapeutic effect of electroacupuncture on frozen shoulder. *Chin J Rehabilitat.* (2012) 27:31–2.

39. Li YH, Li M, Li Q, Guo Y, Liao XH, Wang SQ, et al. Treatment of scapulohumeral periarthritis by Fuyang-pot warming combined with electroacupuncture stimulation. *Zhen Ci Yan Jiu.* (2019) 44:610–4. doi: 10.13702/j.1000-0607.180332

40. Li Y. Clinical observation on the treatment of frozen shoulder by tuina combined with electric acupuncture. *Chin Manipul Rehabil Med.* (2020) 11:8–9.

41. Huang Y, Liu H, Zheng X, Zheng W. Clinical observation of electroacupuncture combined with massage in treating primary periarthritis of shoulder. *China's Naturopathy.* (2021) 29:25–8.

42. Romita VV, Suk A, Henry JL. Parametric studies on electroacupuncture-like stimulation in a rat model: effects of intensity, frequency, and duration of stimulation on evoked antinociception. *Brain Res Bull.* (1997) 42:289–96. doi: 10.1016/s0361-9230(96)00264-x

43. Lo MY, Wu CH, Luh JJ, Wang TG, Fu LC, Lin JG, et al. The effect of electroacupuncture merged with rehabilitation for frozen shoulder syndrome: a single-blind randomized sham-acupuncture controlled study. *J Formos Med Assoc.* (2020) 119:81–8. doi: 10.1016/j.jfma.2019.03.012

44. Cheing GL, So EM, Chao CY. Effectiveness of electroacupuncture and interferential electrotherapy in the management of frozen shoulder. *J Rehabil Med.* (2008) 40:166–70. doi: 10.2340/16501977-0142

45. Lin J-G, Chen C-T, Lu T-W, Lin Y-S, Chen H-L, Chen Y-S. Quantitative evaluation of the motion of frozen shoulders treated with acupuncture by puncturing from Tiaokou (St. 38) towards Chengshan (UB 57). *Biomed Engine.* (2005) 17:31–7. doi: 10.4015/S1016237205000068

46. Zhengen F, Zhengqiu C. Two-hundred and ten cases of shoulder periarthritis treated by needling lingxia and sanjian. *J Tradit Chin Med.* (2003) 23: 201–2.

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Acupuncture for menstruation-related migraine prophylaxis: A multicenter randomized controlled trial

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Objective: The aim of this study was to evaluate the efficacy of acupuncture, an alternative medicine therapy, as a preventive treatment for menstruation-related migraine (MRM).

Patients and methods: This was a prospective, multicenter, double-dummy, participant-blinded, randomized controlled clinical trial conducted in China between 1 April 2013, and 30 April 2014. The participants were enrolled from four study centers and randomized to into either the acupuncture group, which received 24 sessions of acupuncture at traditional acupoints plus placebo, or the medication group, which received sham acupuncture plus naproxen. The primary endpoint was change from the baseline average number of migraine days per perimenstrual period over cycles 1–3. The secondary endpoints included changes from the baseline average number of migraine days outside the perimenstrual period, mean number of migraine hours during and outside the perimenstrual period, mean visual analog scale score during and outside the perimenstrual period, ≥50% migraine responder rate, and the proportion of participants who used acute pain medication over cycles 1–3 and 4–6.

Results: A total of 172 women with MRM were enrolled; 170 in the intention-to-treat analyses. Our primary outcome reported a significant between-group difference that favored the acupuncture group (95% CI, 0.17–0.50; $P < 0.001$), with the average reduction of migraine days per perimenstrual period from

the baseline was 0.94 (95% CI, 0.82–1.07) in the acupuncture group and 0.61 (95% CI, 0.50–0.71) in the medication group over cycles 1–3.

Conclusion: This study showed that compared to medication, acupuncture reduces the number of migraine days experienced by patients with MRM. For patients who received the acupuncture treatment over three cycles, the preventive effect of the therapy was sustained for six cycles.

Clinical trial registration: [https://www.isrctn.com/ISRCTN57133712], identifier [ISRCTN15663606].

KEYWORDS

acupuncture, alternative medicine, efficacy, menstruation-related migraine, prophylaxis, safety

Introduction

Migraine affects 18.9% of women globally (Collaborators GBDH, 2018), and its occurrence in women is frequently associated with menstruation. Menstrual migraine affects 20% of female migraineurs and most of the attacks are migraines without aura (Vetvik et al., 2014). Menstrual migraine can be divided into two subtypes: pure menstrual migraine and menstruation-related migraine (MRM), with most of the cases being MRM. Approximately 60% of female migraineurs report an association between migraine and menstruation (Pavlović et al., 2015). According to the International Classification of Headache Disorders (ICHD) (versions II, III beta, and III), MRM is defined as attacks of migraine without aura, occurring between two days before the onset of menstruation and the third day of menstruation (five-day window) in at least two out of three menstrual cycles, with additional attacks of migraine at other times of the cycles (Headache Classification Subcommittee of the International Headache Socitey [IHS], 2004; Headache Classification Committee of the International Headache Society [IHS], 2013; Headache Classification Committee of the International Headache Society [IHS], 2018). MRM causes a significant public health burden, particularly during women's reproductive years (Pavlović et al., 2015).

The clinical management of MRM is challenging. The migraine attacks that occur during the perimenstrual period tend to be more painful, longer lasting, more disabling, and accompanied by more severe nausea than those that occur outside the perimenstrual period (Vetvik et al., 2015). In addition, these attacks are less responsive to medication than non-menstrual migraines (Granella et al., 2004; MacGregor et al., 2010; Pinkerman and Holroyd, 2010). MRM treatments are classified as acute, short-term prophylaxis, or daily prevention treatment. Pharmacotherapy, including administration of drugs such as aspirin, naproxen, triptans, estrogen, magnesium, and dihydroergotamine, is recommended for the prevention of MRM (Allais et al., 2018). Non-steroidal anti-inflammatory drugs, which are considered the first-line treatment option for migraines, have been widely used for short-term prophylaxis of MRM (Newman and Yugrakh, 2014; Maasumi et al., 2017) owing to their high efficacy and ability to prevent other forms of perimenstrual pain, such as dysmenorrhea (Pringsheim et al., 2008; Allais et al., 2012; Newman and Yugrakh, 2014; Maasumi et al., 2017). However, the use of non-steroidal anti-inflammatory drugs or other preventive treatments are often associated with an increased risk of adverse events (AEs), including cardiovascular disorders, gastrointestinal bleeding, menstrual irregularity, and diarrhea (MacGregor, 2015). In addition, excessive use of analgesics or specific anti-migraine treatments may cause medication-overuse headaches and an increase in the frequency of headaches (Bigal and Lipton, 2008; Bigal et al., 2009). Owing to these limitations associated with conventional treatments, efforts have been made to identify other effective and low-risk interventions for MRM.

Acupuncture is a commonly researched and widely accepted complementary and alternative medicine therapy used for the treatment of migraine (Linde K. et al., 2005; Diener et al., 2006; Wang et al., 2011; Li et al., 2012; Coeytaux and Befus, 2016; Linde et al., 2016; Zhao et al., 2017; Xu et al., 2020). However, only one former rigorous clinical trials by Linde et al. reported the efficacy of acupuncture treatment for MRM, which reminds the lack of researching in both acupuncturing efficiency and MRM (Linde M. et al., 2005). Therefore, we conducted a prospective, randomized, clinical trial under conditions similar

Abbreviations: AE, adverse events; CI, confidence interval; ITT, intention-to-treat; ICHD, International Classification of Headache Disorders; LOCF, last observation carried forward; MCID, minimal clinically important difference; MRM, menstruation-related migraine; PP, per-protocol; VAS, visual analog scale.

to those of routine care to evaluate the efficacy of acupuncture as a preventive treatment for MRM.

Materials and methods

Ethical considerations, protocol approvals, and registrations

The protocol of this clinical trial was registered in International Standard Randomized Controlled Trial Number (ISRCTN) with registry no. ISRCTN57133712, and ethical approval was obtained from the Research Ethical Committee of Beijing Hospital of Traditional Chinese Medicine (ref: 201212) prior to the commencement of the trial. The study was conducted in accordance with the principles of the Declaration of Helsinki, and reported according to the guidelines of the Consolidated Standards of Reporting Trials (Moher et al., 2010), as well as the Standards For Reporting Interventions In Controlled Trials Of Acupuncture (MacPherson et al., 2002) guidelines. The methods are fully reported in the published protocol (**Supplementary material 1**; Zhang et al., 2013). All the included patients provided written informed consent prior to participation.

Study design and patients

This was a prospective, multicenter, double-dummy, participant-blinded, randomized controlled clinical trial that consisted of three phases: a baseline phase (cycle -3 to cycle 0), a treatment phase (cycle 1 to cycle 3), and a follow-up phase (cycle 4 to cycle 6). The study was conducted at the departments of acupuncture and pain management of the following centers in China: Beijing Hospital of Traditional Chinese Medicine, Peking University Third Hospital, Beijing Tiantan Hospital, and Xiyuan Hospital. One experienced neurologist in each center assessed the eligibility of all potential participants according to predefined inclusion/exclusion criteria and provided detailed explanation of the trial design to the participants.

The inclusion criteria were as follows: (Collaborators GBDH, 2018) patients diagnosed with MRM according to the diagnostic criteria of the ICHD, version II (Headache Classification Subcommittee of the International Headache Socitey [IHS], 2004; Vetvik et al., 2014) patients with regular menstrual cycles (25—35 days); (Pavlović et al., 2015) patients who can predict the onset of menstruation and perimenstrual migraine attacks within three days prior; (Headache Classification Subcommittee of the International Headache Socitey [IHS], 2004) patients who experience repeated migraine attacks, with the frequency of non-menstrual migraine being more than once a month; and (Headache Classification Committee of the International Headache Society [IHS], 2013) patients who provided written informed consent. The exclusion criteria were as follows: (Collaborators GBDH, 2018) patients with chronic migraine, tension headache, cluster headache, and other primary headaches; (Vetvik et al., 2014) patients with secondary headache and other neurological diseases; (Pavlović et al., 2015) patients with relatively severe systemic diseases (cardiovascular disease, acute infectious disease, hematopathy, endocrinopathy, and allergies); (Headache Classification Subcommittee of the International Headache Socitey [IHS], 2004) patients with headache caused by otorhinolaryngological diseases or intracranial pathological changes; (Headache Classification Committee of the International Headache Society [IHS], 2013) patients taking oral contraceptives and pregnant or lactating patients; (Headache Classification Committee of the International Headache Society [IHS], 2018) patients who used prophylactic migraine medication in the past 3 months; and (Vetvik et al., 2015) patients involved in other clinical trials.

Study procedures

Eligible participants were randomly assigned into two treatment groups: the acupuncture group, which received acupuncture at traditional acupoints plus placebo naproxen, and the medication group, which received sham acupuncture at non-effective acupoints plus naproxen. Participants were allowed to take acute pain medication during the entire trial and were required to record the details of the medication taken.

Randomization and blinding

Eligible participants were randomly assigned at a 1:1 ratio to the acupuncture or medication groups. Central randomization, conducted using an online or messaging system, was performed by the Research Center of Clinical Epidemiology affiliated to Peking University. Randomization was stratified according to centers with a fixed block size of four.

The placebo medication was made to be identical to actual naproxen tablets in terms of taste, smell, and appearance. We used a sham acupuncture method that produced the same stimulation as true acupuncture to ensure that all the participants were blinded to their group allocation. Participants in different groups were treated separately and blinded to the type of acupuncture they received. All outcome assessors and trial statisticians were blinded to the group allocations throughout the duration of the trial. Only the acupuncturists who administered true or sham acupuncture treatment were aware of the participants' group allocation. The investigators, researchers, and participants were all blinded during the study until the randomization code was broken at the end of the trial or if serious adverse events occurred during the study period. A standard operating procedure and relevant documents were provided to all trial centers to ensure consistency in terms of blinding.

Clinical assessments

Participants were required to complete a headache diary from the baseline phase to the end of the follow-up phase. The participants recorded the details of their migraine attacks, including the time the headaches started and ceased, the intensity, frequency, location (the forehead, top, temporal, and back of the head), and cause of the headache, and the concomitant symptoms of each migraine attack. Additionally, participants were required to record information regarding their menstruation and the dates they received acupuncture treatments in their headache diaries. If acute pain medications were taken, participants were required to document the name and dosage of the medicine, the time it was taken, time of pain relief, and the side effects experienced. The headache diary for each cycle was collected by researchers who were blinded to the participants' group allocation.

The participants were also required to report any AEs they experienced. The causality of the AEs and their association with acupuncture or the trial medication were determined by the trial clinicians.

Interventions

The intervention scheme of this trial was determined according to the consensus of experts and the results of a previous pilot study (Li et al., 2011). The acupuncture treatment methods were developed based on the information in classical and modern literature (Yang, 1995; Deng and Huang, 2004) and the results of previous research on the treatment of migraine using acupuncture (Wang et al., 2011). All acupuncture treatments (true or sham) were administered by acupuncturists who are registered with the Ministry of Health of the People's Republic of China and have more than 20 years of clinical experience. In each trial center, only one acupuncturist administered all the acupuncture treatments to ensure consistency. Prior to the commencement of the trial, all the acupuncturists received training on the purpose and design of the trial, treatment strategies, and quality control.

Naproxen sustained-release tablets (250 mg/tablet) and the placebo medication were provided by the Diao Group Chengdu Pharmaceutical LTD., Chengdu, China. During the three-cycle treatment phase, participants started to take naproxen or placebo (two tablets, once per day) three days before each predicted onset of menstruation and continued until the end of each menstrual cycle. If menstruation began later than predicted, the treatment was not adjusted. If MRM occurred earlier than predicted, participants were asked to begin taking naproxen (or placebo) immediately and one day earlier in the next cycle to provide prophylactic coverage. Considering that the duration of menstruation may vary, the timing of the treatments of the participants was determined based on the information collected from their baseline headache diaries. Variability in the onset of menstruation and migraine during the treatment phase was not taken into account. In addition, participants were allowed to take acute pain medication during the entire trial and required to record the details of the medication.

Ten to twelve sterile disposable steel needles (Hwato Needles, made in Suzhou, China; gauge and size: 0.25 mm × 25 mm for head points, 0.3 mm × 40 mm for limb and abdomen points) were used in each session of the true and sham acupuncture treatments. At each point, the needle was inserted 10 mm to 15 mm into the skin and manipulated using rotation methods to produce a characteristic sensation known as "de qi" (tenseness around the needle felt by the practitioner and numbness, distension, soreness, and heaviness around the point felt by the patient).

Treatment of the acupuncture group

In the acupuncture group, participants were administered with true acupuncture plus placebo naproxen. The acupuncture therapy consisted of preventive treatment (two sessions each week) and premenstrual conditioning treatment (at least three sessions during the 10 days before the predicted onset of each menstruation), administered for three cycles. Each session lasted for 30 min. The acupoints (**Supplementary Figure 1** in **Supplementary material 2**) used for preventive treatment included both standard and additional points. The standard points were GV20 (Baihui), GV24 (Shenting), GB13 (Benshen), GB8 (Shuaigu), TE20 (Jiaosun), and GB20 (Fengchi). Additional points were chosen individually depending on the syndrome differentiation of meridians in the headache region. The additional points were TE5 (Waiguan) and GB34 (Yanglingquan) for Shaoyang headache, LI4 (Hegu) and ST44 (Neiting) for Yangming headache, BL60 (Kunlun) and SI3 (Houxi) for Taiyang headache, LR3 (Taichong) and GB40 (Qiuxu) for Jueyin headache, PC6 (Neiguan) for nausea and vomiting, and LR3 (Taichong) for dysphoria and susceptibility to rage. For premenstrual conditioning, each participant received treatment at the standard acupoints KI12 (Dahe), CV3 (Zhongji), and ST29 (Guilai). All the selected acupoints were determined based on the findings of our previous research (Wang et al., 2011).

Treatment of the medication group

Participants assigned to the medication group received sham acupuncture treatment plus true naproxen. Sham acupuncture was administered using the same methods used for true acupuncture but on non-effective acupoints. The selection of non-effective acupoints was based on the following rules: (Collaborators GBDH, 2018) acupoints defined as unrelated to headache or menstruation based on the information in a vast amount of Chinese medicine reference books (26 ancient Chinese books of acupuncture, three Chinese acupuncture textbooks, and more than 100 acupuncture research literatures); (Vetvik et al., 2014) 15 acupoints (**Supplementary Table 1** in **Supplementary material 2**) in the vicinity of the elbow and

knee joints were selected, whereas the acupoints on the head, hands, feet, and trunk were excluded. To mimic the nature of selecting points based on syndrome differentiation, the 15 sham points in the vicinity of the elbow and knee joints were further randomly assigned into three subgroups, B, C, and D. Each subgroup had two points on the arms and three points on the legs (**Supplementary Table 2** in **Supplementary material 2**). The participants in the medication group were further randomly assigned into one of these three subgroups through a central randomization system.

Outcome measures

The primary outcome measure was change from the baseline average number of migraine days per perimenstrual period over cycles 1−3. The secondary outcome measures were changes from the baseline average number of migraine days outside the perimenstrual period, mean number of migraine hours during and outside the perimenstrual period, mean visual analogue scale (VAS) score recorded during and outside the perimenstrual period, ≥50% migraine responder rate, which was defined as the proportion of participants who achieved ≥50% reduction in the number of migraine days, and the proportion of participants who used acute pain medication over cycles 1−3 and 4−6.

Data regarding migraine days, migraine hours, and VAS scores were extracted from the completed headache diaries for each cycle from the start of the baseline phase to the end of the follow-up phase. In the diary, participants documented migraine days, migraine hours, and the intensity and time of each attack. For cycles 1−3 and 4−6, migraine days were calculated as the cycle average, whereas mean migraine hours and mean VAS scores were calculated as the daily average.

Safety assessments

At the baseline assessment and the end of the treatment phase, all the participants underwent general medical and neurological examinations carried out by clinicians, in addition to clinical laboratory tests for full blood, liver function, kidney function, and chemistry evaluations. During the trial period, participants were required to document the AEs they experienced in their headache diaries. Medication- or acupuncture-related AEs were documented with full details, and clinicians, including neurologists and acupuncturists, evaluated the severity of each AE. If serious adverse events occurred, the data safety and monitoring committee, which had the right to terminate the trial, adjudicated the severity of the AE reported by the investigators.

Credibility of the blinding test

To confirm the blinding of the participants, a blinding test questionnaire was administered to all the participants in the middle of the treatment phase (cycle 1.5) and at the end of the treatment phase (cycle 3). The participants were asked to guess which group they were allocated to.

Statistical analysis

Based on the information from a previous pilot study (Li et al., 2011), we estimated that the number of migraine days over 12 weeks would be 3.1 (standard deviation [SD], 2.7) days in the acupuncture group and 5.2 (SD, 4.4) days in the medication group. With a two-sided significance level of 5% and a power of 90%, 68 participants would be required for each group, as calculated using PASS 2008 software (NCSS, Kaysville, UT, United States). Considering an estimated loss-to-follow-up rate of 20%, we planned to enroll a total of 172 participants (86 participants per group) in the study.

The analysis plan was determined before the study was conducted (**Supplementary material 1**). The baseline characteristics and clinical outcomes described were based on the intention-to-treat (ITT) population, which included participants who received at least one treatment and had at least one primary outcome measure (n = 170). We performed sensitivity analyses using the per-protocol (PP) set, which included all randomized participants who had no major protocol deviation. We also performed safety analysis using the safety set, which included all randomized participants who received at least one session of acupuncture.

The primary outcome was analyzed according to the ITT principle. The change from the baseline average number of migraine days per perimenstrual period over cycles 1−3 was analyzed by fitting a mixed-effects model using the baseline value as a covariate, treatment as a fixed effect, and center as a random effect. The same approach was used for the analysis of the secondary outcomes, which included the changes from the baseline average number of migraine days outside each perimenstrual period, mean number of migraine hours during and outside the perimenstrual period, and the mean VAS score recorded during and outside the perimenstrual period. For other continuous variables, comparisons between treatment groups were assessed using the t-test or Wilcoxon rank-sum test as appropriate. Categorical variables were compared using the chi-square test or Fisher's exact test as appropriate. Kappa analysis was used to determine whether participants correctly guessed their group allocation at a higher rate than would be expected by chance.

Missing data on the primary and secondary outcomes were imputed using the last observation carried forward (LOCF) method. To examine the sensitivity of the LOCF method, we performed a sensitivity analysis using multiple imputation methods (**Supplementary Appendix 1** and **Supplementary Table 3** in **Supplementary material 2**) under the "missing at random" assumption for missing primary outcome data (**Supplementary Appendix 2** in **Supplementary material 2**).

There are between- and within-woman variations in menstrual cycle lengths that could inherently confound the outcomes. To evaluate this potential confounder, we performed a sensitivity analysis using menstrual cycle length as a control

variable in a mixed-effects model (**Supplementary Appendix 3** in **Supplementary material 2**).

An independent statistician who was blinded to the group allocation performed all the statistical analyses using SAS statistical software (SAS Institute, Cary, NC, United States). For both continuous and categorical variables, 95% confidence intervals (CI) were calculated as appropriate. All the statistical comparisons were two sided, and $P < 0.05$ considered significant.

Results

Between April 2013 and April 2014, a total of 364 female migraineurs provided informed consent and were screened for eligibility for this study. After exclusion of 192 ineligible patients with detailed reasons recorded and reported (**Supplementary Figure 1** and **Supplementary Table 7** in **Supplementary material 2**), a total of 172 participants were randomized into the acupuncture and medication groups. Two participants in the medication group were excluded because they withdrew their consent and did not receive treatment. Thus, 170 participants (86 in the acupuncture group, 84 in the medication group) were included in the ITT population (**Figure 1**). During the treatment phase, 13 participants dropped out of the study (dropout rate, 7.65%; acupuncture group: $n = 6$ [6.98%], medication group: $n = 7$ [8.33%]). The main reasons for dropout were time restrictions, change in residential location, dissatisfaction with the treatment, and fear of needling (**Supplementary Table 12** in **Supplementary material 2**). We

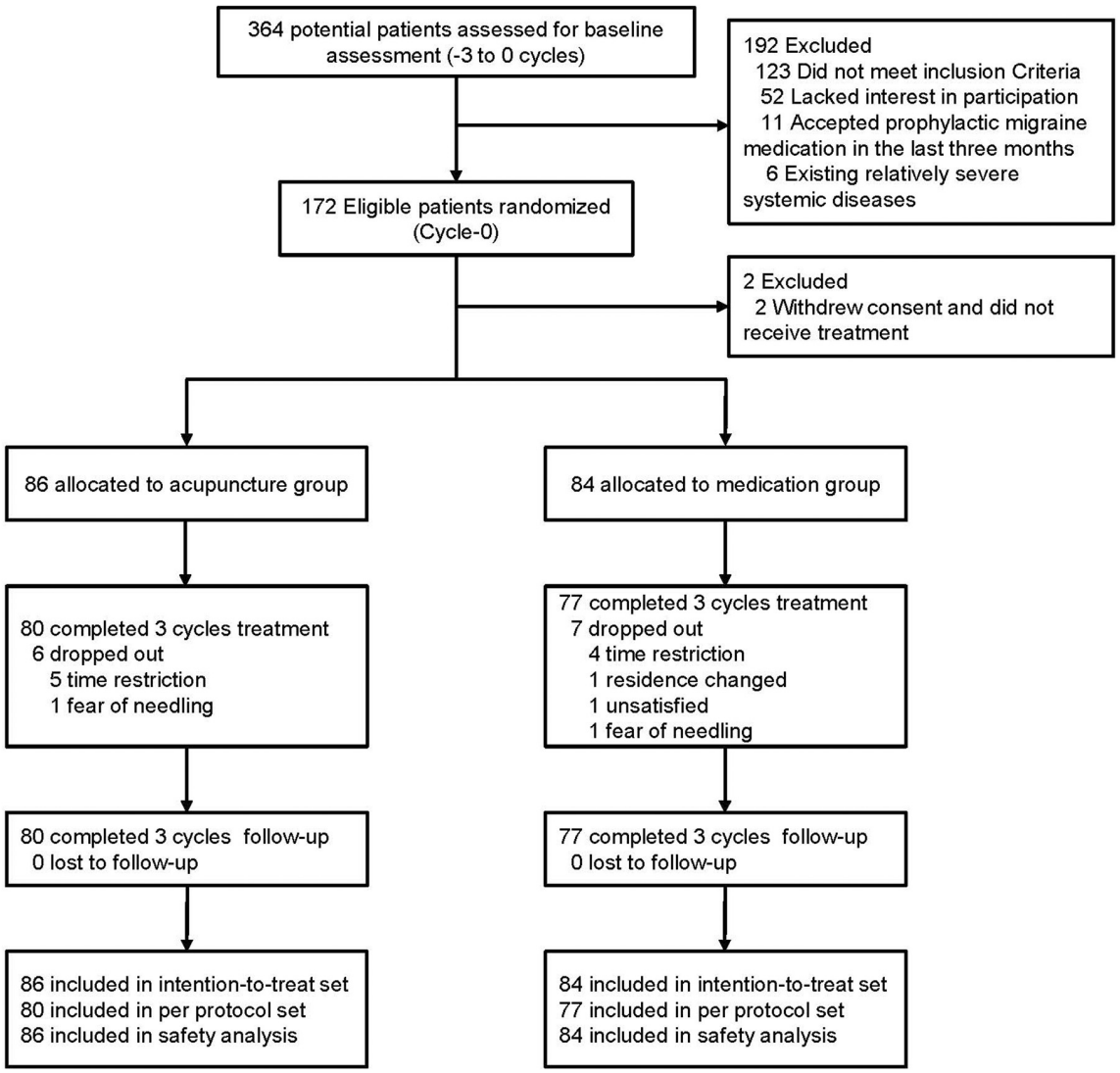

FIGURE 1
Flowchart of the screening, enrollment, randomization, and follow-up of patients.

did not lose contact with any of the participants during the follow-up phase. Details of the trial procedure are presented in Figure 1 in accordance with the Standards For Reporting Interventions In Controlled Trials Of Acupuncture guidelines (MacPherson et al., 2002).

The participants in acupuncture group received an average of 22.95 sessions of treatment, whereas those in the medication group received an average of 22.71 treatment sessions. Regarding the degree of participation, 93.02% of the participants in the acupuncture group and 91.67% of those in the medication group received at least 20 (≥80%) of the planned acupuncture treatment sessions. By tablet count, 93.02% of the participants in the acupuncture group and 91.67% of those in the medication group achieved at least 80% of the planned medication adherence.

The demographic characteristics and baseline comparable of the ITT population are summarized in Table 1. The mean age of the participants was 35.81 years old, with 53.53% of the patients being > 35 years old. The mean menstrual cycle length and mean menstruation length were 28.99 and 5.51 days, respectively. Migraine history was well balanced, with no clinically relevant differences between the two groups. The average number of migraine days during and outside the perimenstrual period over the three-cycle baseline phase were 1.75 and 1.94, respectively; approximately 67.65% of the participants used acute pain medication during this period. The baseline characteristics of per-protocol (PP) population were similar to those of the ITT population (Supplementary Table 5 in Supplementary material 2).

Efficacy findings

Primary outcome measure

Figure 2 and Table 2 show the primary analysis performed using ITT data. The average number of migraine days recorded per perimenstrual period was 1.83 (95% confidence interval [CI], 1.68–1.99) at baseline and 0.89 (95% CI, 0.74–1.05) over cycles 1−3 in the acupuncture group, and 1.67 (95% CI, 1.55–1.79) at baseline and 1.06 (95% CI, 0.95–1.17) over cycles 1−3 in the medication group. The reduction from the baseline average number of migraine days per perimenstrual period over cycles 1−3 was 0.94 (95% CI, 0.82–1.07) in the acupuncture group and 0.61 (95% CI, 0.50–0.71) in the medication group, with a 0.33 between-group difference that favored the acupuncture group (95% CI, 0.17–0.50; $P < 0.001$) (Table 2). Similar between-group differences were observed in the PP population (Supplementary Table 6 in Supplementary material 2), the sensitivity analysis performed using a multiple imputation method with missing data (Supplementary Table 4 in Supplementary material 2), and the sensitivity analysis performed for controlling for individual menstrual cycle lengths in the mixed-effects model (Supplementary Appendix 3 and Supplementary Tables 8–11 in Supplementary material 2).

Key secondary outcome measures

The acupuncture group achieved a significantly greater decrease in the average number of migraine days outside each perimenstrual period and the mean number of migraine hours during and outside the perimenstrual period over cycles

TABLE 1 Demographic and baseline characteristics of the 170 patients included in the intention-to-treat analysis.

Characteristics	Acupuncture group (n = 86)	Medication group (n = 84)	Total (n = 170)
Mean (SD) age, y	36.44 (6.74)	35.15 (6.73)	35.81 (6.75)
Mean (SD) duration from the time of migraine diagnosis to baseline, y	8.24 (7.06)	8.35 (5.72)	8.30 (6.42)
Family history, n (%)	28 (32.56)	27 (32.14)	55 (32.35)
Accompanying symptoms			
Nausea or vomiting, n (%)	82 (95.35)	80 (95.24)	162 (95.29)
Photophobia or phonophobia, n (%)	57 (66.28)	52 (61.90)	109 (64.12)
Others, n (%)	22 (25.58)	15 (17.86)	37 (21.76)
Dysmenorrhea, n (%)	28 (32.56)	21 (25.00)	49 (28.82)
Mean (SD) length of menstrual cycle, day	29.24 (2.32)	28.74 (2.15)	28.99 (2.24)
Mean (SD) length of menstruation, day	5.53 (1.15)	5.49 (1.19)	5.51 (1.16)
Mean (SD) number of migraine days during the perimenstrual period	1.83 (0.73)	1.67 (0.55)	1.75 (0.65)
Mean (SD) number of migraine days outside the perimenstrual period	2.07 (1.43)	1.81 (0.99)	1.94 (1.23)
Mean (SD) number of migraine hours during the perimenstrual period	21.03 (12.25)	19.77 (11.18)	20.41 (11.71)
Mean (SD) number of migraine hours outside the perimenstrual period	14.72 (8.85)	14.15 (8.27)	14.44 (8.55)
Mean (SD) pain VAS score recorded during the perimenstrual period	7.30 (1.45)	7.27 (1.41)	7.28 (1.43)
Mean (SD) pain VAS score recorded outside the perimenstrual period	6.13 (1.45)	6.04 (1.48)	6.08 (1.46)
Use of acute pain medication, n (%)	60 (69.77)	55 (65.48)	115 (67.65)

SD, standard deviation; VAS, visual analog scale.

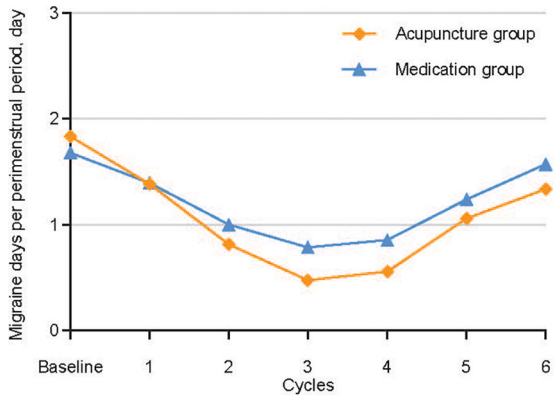

FIGURE 2
Primary outcomes throughout the trial.

1−3 and 4−6 than the medication group. The between-group difference in the reduction of the average number of migraine days outside the perimenstrual period was 0.59 (95% CI, 0.37–0.80; $P < 0.001$) for cycles 1−3, and 0.64 (95% CI, 0.41–0.87; $P < 0.001$) for cycles 4−6. The between-group difference in the reduction of the mean number of migraine hours during the perimenstrual period was 2.60 (95% CI, 0.79–4.41; $P = 0.006$) for cycles 1−3, and 2.55 (95% CI, 0.97–4.13; $P = 0.002$) for cycles 4−6. The between-group difference in the reduction of the mean number of migraine hours outside the perimenstrual period was 1.97 (95% CI, 0.82–3.12; $P = 0.001$) for cycles 1−3, and 2.01 (95% CI, 0.95–3.07; $P < 0.001$) for cycles 4−6.

The acupuncture group showed greater decrease in the mean VAS score recorded during and outside the perimenstrual period over cycles 4−6 (not over cycles 1−3) than the medication group (VAS score during the perimenstrual period: between-group difference, 0.38) (95% CI, −0.10–0.85, $P = 0.12$); VAS score outside the perimenstrual period: between-group difference, 0.36 (95% CI, −0.12–0.85, $P = 0.15$). The between-group difference in the reduction of the mean VAS score during and outside the perimenstrual period was 1.08 (95% CI, 0.69–1.48; $P < 0.001$) and 0.50 (95% CI, 0.17–0.83; $P = 0.004$), respectively, for cycles 4−6.

The ≥50% migraine responder rates in the acupuncture and medication groups were 62.8% and 42.9% at the end of the treatment phase, and 58.1% and 38.1% at the end of follow-up phase, respectively. (Table 2). The between-group difference in the responder rates for the treatment and the follow-up phases were 19.9% (95% CI, 5.22–34.64; $P = 0.009$) and 20.0% (95% CI, 5.33–34.76; $P = 0.009$), respectively. Data from the acupuncture group demonstrated that ≥50% migraine responder rates were sustained throughout the six-cycle interval.

Changes from the baseline mean VAS score during and outside the perimenstrual period over cycles 1−3 ($P > 0.05$ for all) did not differ between the two groups. We did not note any between-group difference in the proportion of participants that used acute pain medication during cycles 1−3 and 4−6 ($P > 0.05$).

Safety

A total of 22 participants (12.94%) experienced at least one treatment-emergent adverse event (TEAE), but none of them required special medical interventions. Eight (9.30%) participants in the acupuncture group and 14 (16.67%) in the medication group had TEAEs, which mainly included dermorrhagia, numbness, and lassitude for acupuncture-related AEs, and nausea, palpitations, dyspepsia, upper abdominal pain, heartburn, somnolence, dizziness, and sweating attack for medication-related AEs (Table 3). These events were mild or moderate. The incidence of TEAEs was generally balanced between the acupuncture and medication groups ($P = 0.15$).

In the acupuncture group, three participants ($n = 3$ [3.49%]) reported dermorrhagia (without formation of subcutaneous hematoma) at a few acupoints after needle removal. These AEs were classified as a cause relevant to acupuncture. Five participants reported gastrointestinal, central nervous system, and dermatologic events (dyspepsia: $n = 2$ [2.33%]; upper abdominal pain: $n = 1$ [1.16%]; somnolence: $n = 1$ [1.16%]; sweating attack: $n = 1$ [1.16%]). These AEs were classified as a cause relevant to placebo medication. In the medication group, 10 participants reported gastrointestinal, cardiovascular, central nervous system, and dermatologic events (nausea: $n = 2$ [2.38%]; palpitations: $n = 2$ [2.38%]; dyspepsia: $n = 1$ [1.19%]; upper abdominal pain: $n = 1$ [1.19%]; heartburn: $n = 1$ [1.19%]; somnolence: $n = 1$ [1.19%]; dizziness: $n = 1$ [1.19%]; sweating attack: $n = 1$ [1.19%]). These AEs were considered to be related to the side effects of naproxen. Four participants reported acupuncture-related AEs (dermorrhagia: $n = 2$ [2.38%]; numbness: $n = 1$ [1.19%]; lassitude: $n = 1$ [1.19%]). The participants who reported these AEs fully recovered and continued the trial.

Credibility of blinding

At cycle 1.5, 61 of 80 participants in the acupuncture group and 53 of 77 participants in the medication group guessed their treatment to be true acupuncture plus placebo naproxen (kappa coefficient: 0.075 [−0.07, 0.22]; $P = 0.30$). At cycle 3, the numbers were 62/80 participants in the acupuncture group and 49/77 participants in the medication group (kappa coefficient: 0.14 [−0.00, 0.28]; $P = 0.06$) (Table 4). There was no significant between-group difference in the proportion of participants who guessed "true acupuncture plus placebo naproxen" when asked if they received true acupuncture plus placebo naproxen or sham acupuncture plus naproxen treatment at cycles 1.5 and 3.

TABLE 2 Primary and secondary outcomes (intention-to-treat population)[a].

Outcome	Acupuncture group (n = 86)	Medication group (n = 84)	Between-group difference	
			Value (95% CI)	P-value[b]
Primary outcome				
Average number of migraine days per perimenstrual period[c], cycles 1−3, mean (95% CI)				
	0.89 (0.74 to 1.05)	1.06 (0.95 to 1.17)	0.17 (−0.24 to 0.36)	0.0862
Change from the baseline average number of migraine days per perimenstrual period[d], cycles 1−3, mean (95% CI)[e]				
	0.94 (0.82 to 1.07)	0.61 (0.50 to 0.71)	0.33 (0.17 to 0.50)	0.0001
Secondary outcomes				
Change from the baseline average number of migraine days per perimenstrual period, cycles 4−6, mean (95% CI)[e]				
	0.84 (0.72 to 0.97)	0.44 (0.32 to 0.56)	0.41(0.24 to 0.58)	<0.0001
Change from the baseline average number of migraine days outside the perimenstrual period, mean (95% CI)[e]				
Cycles 1−3	1.08 (0.89 to 1.27)	0.49 (0.39 to 0.60)	0.59 (0.37 to 0.80)	<0.0001
Cycles 4−6	0.95 (0.75 to 1.16)	0.31 (0.21 to 0.41)	0.64 (0.41 to 0.87)	<0.0001
Change from the baseline mean number of migraine hours during the perimenstrual period[f], mean (95% CI)[e]				
Cycles 1−3	7.61 (6.22 to 9.02)	5.01 (3.83 to 6.20)	2.60 (0.79 to 4.41)	0.0055
Cycles 4−6	5.65 (4.36 to 6.93)	3.09 (2.14 to 4.05)	2.55 (0.97 to 4.13)	0.0019
Change from the baseline mean number of migraine hours outside the perimenstrual period[f], mean (95% CI)[e]				
Cycles 1−3	4.56 (3.56 to 5.66)	2.59 (1.99 to 3.19)	1.97 (0.82 to 3.12)	0.0010
Cycles 4−6	3.52 (2.66 to 4.37)	1.51 (0.86 to 2.15)	2.01 (0.95 to 3.07)	0.0003
Change from the baseline mean VAS score during the perimenstrual period, mean (95% CI)[e]				
Cycles 1−3	2.52 (2.15 to 2.90)	2.14 (1.84 to 2.44)	0.38 (−0.10 to 0.85)	0.1221
Cycles 4−6	2.39 (2.05 to 2.73)	1.30 (1.09 to 1.52)	1.08 (0.69 to 1.48)	<0.0001
Change from the baseline mean VAS score outside the perimenstrual period, mean (95% CI)[e]				
Cycles 1−3	2.17 (1.76 to 2.57)	1.81 (1.53 to 2.09)	0.36 (−0.12 to 0.85)	0.1462
Cycles 4−6	1.45 (1.20 to 1.69)	0.95 (0.71 to 1.18)	0.50 (0.17 to 0.83)	0.0039
50% migraine responder rate, participants, n (%)[g]				
Cycles 1−3	54 (62.8%)	36 (42.9%)	19.9 (5.22 to 34.64)	0.0092
Cycles 4−6	50 (58.1%)	32 (38.1%)	20.0 (5.33 to 34.76)	0.0089
Use of acute pain medication, participants, n (%)[g]				
Cycles 1−3	24 (27.9%)	35 (41.7%)	13.8 (−27.94 to 0.42)	0.0595
Cycles 4−6	40 (46.5%)	46 (54.8%)	8.3 (−23.23 to 6.73)	0.2821

[a]Six participants in the acupuncture group and seven in the medication group were missing information on the average number of migraine days during/outside the perimenstrual period, mean number of migraine hours during/outside the perimenstrual period, and mean VAS score during/outside the perimenstrual period over cycles 1−3. Six participants in the acupuncture group and seven in the medication group were missing these details in cycles 4−6. The missing data of participants who dropped out were replaced using the last observation carried forward method. The number of participants with imputed data: 6 (7.0%) in the acupuncture group and 7 (8.3%) in the medication group.
[b]All tests were two-sided. P-value < 0.05 was considered significant.
[c]The perimenstrual period is starts from the two days before the onset of menstruation to first three days of menstruation.
[d]Baseline was calculated as the cycle average of the three-cycle screening phase prior to the start of treatment.
[e]Analyzed by fitting a mixed-effect model using the baseline value as a covariate, treatment as a fixed effect, and center as a random effect.
[f]Baseline was calculated the daily average of the three-cycle screening phase prior to the start of treatment.
[g]Analyzed using the chi-square test.
CI, confidence interval; VAS, visual analog scale.

Discussion

This double-dummy, randomized, controlled trial was conducted to evaluate the efficacy of acupuncture as a preventive treatment for MRM. The results indicated that the acupuncture group showed significantly greater reductions in the number of migraine days and hours from baseline and greater ≥50% migraine responder rate than the medication group during the treatment (cycles 1−3) and follow-up (cycles 4−6) phases. Moreover, the acupuncture group reported relatively better alleviation of headache intensity during the follow-up phase than the medication group, but not during the treatment phase. However, the proportion of participants who used acute pain medication did not differ between the two groups.

The reduction in the average number of migraine days per perimenstrual period in the acupuncture group was

TABLE 3 Treatment-emergent adverse events (safety population)[a].

Adverse events	Acupuncture group (n = 86)		Medication group (n = 84[b])	
	Participants, N (%)	Events, N	Participants, N(%)	Events, N
Total	8 (9.30%)	8	14 (16.67%)	16
Related to acupuncture overall	3 (3.49%)[c]	3	4 (4.76%)[d]	4
Dermorrhagia	3 (3.49%)	3	2 (2.38%)	2
Numbness	0	0	1 (1.19%)	1
Lassitude	0	0	1 (1.19%)	1
Related to medication overall	5 (5.81%)[e]	5	10 (11.90%)[f]	12
Nausea	0	0	2 (2.38%)	2
Palpitations	0	0	2 (2.38%)	2
Dyspepsia	2 (2.33%)	2	1 (1.19%)	2
Upper abdominal pain	1 (1.16%)	1	1 (1.19%)	1
Heartburn	0	0	1 (1.19%)	2
Somnolence	1 (1.16%)	1	1 (1.19%)	1
Dizziness	0	0	1 (1.19%)	1
Sweating attack	1 (1.16%)	1	1 (1.19%)	1

[a]Adverse events were analyzed in all participants who received at least one session of treatment. Adverse events experienced by the same participant were counted according to type rather than frequency. Adverse events of different types experienced by a single participant were defined as independent adverse events. Multiple occurrences of an adverse event in a single participant was defined as one adverse event.
[b]Two participants in the medication group did not receive treatment.
[c]Adverse events related to true acupuncture.
[d]Adverse events related to sham acupuncture.
[e]Adverse events related to placebo naproxen.
[f]Adverse events related to naproxen.

TABLE 4 Credibility of blinding.

	Treatment guess, No. (%)	Acupuncture group (n = 80[a])	Medication group (n = 77[b])	Kappa Coefficient (95% CI)	P[c]
Cycle 1.5	True acupuncture plus placebo naproxen	61 (76.2%)	53 (68.8%)	0.075 (−0.07, 0.22)	0.30
	Sham acupuncture plus naproxen	19 (23.8%)	24 (31.2%)		
Cycle 3	True acupuncture plus placebo naproxen	62 (77.5)	49 (63.6%)	0.14 (−0.00, 0.28)	0.06
	Sham acupuncture plus naproxen	18 (22.5%)	28 (36.4%)		

[a]Six participants in the acupuncture group were not recorded at Cycles 1.5 and 3 because they dropped out.
[b]Seven participants in the medication group were not recorded at Cycles 1.5 and 3 because they dropped out.
[c]P was calculated from a kappa analysis.
CI, confidence interval.

0.94 for cycles 1−3 and 0.84 for cycles 4−6. Both results were greater than the minimal clinically important difference (MCID) of 0.5 days (National Clinical Guideline Centre, 2012). Nevertheless, the between-group difference in these reductions was 0.33 days for cycles 1−3 and 0.41 days for cycles 4−6, which do not meet the MCID. This demonstrates that the difference in the reduction of the number of migraine days between the acupuncture and medication groups may be statistically significant but does not indicate a clinically meaningful difference in the improvement of migraine frequency during the perimenstrual period.

The sensitivity analyses of the PP population (between-group difference: 0.37 days) (**Supplementary Table 6** in **Supplementary material 2**), conducted using a multiple imputation method with missing data (between-group difference: 0.35 days) (**Supplementary Table 4** in **Supplementary material 2**) and controlling for individual variations in menstrual cycle length (between-group difference: 0.33 days) (**Supplementary Table 11** in **Supplementary material 2**), were similar to the ITT analysis performed using the LOCF method. The between-group differences in the reduction of the average number of migraine days outside the perimenstrual period over cycles 1−3 and cycles 4−6 were greater than the MCID of 0.5 days. This suggests that acupuncture may have a statistically significant and clinically meaningful preventive effect by reducing the frequency of migraine outside the perimenstrual period over six cycles. Our findings are inconsistent with those of a previous study, which

indicated that there were no significant differences between the treatment ($n = 15$) and control ($n = 13$) groups during treatment or follow-up 3 and 6 months later (Linde M. et al., 2005). This variation in findings may be associated with differences in the number of participants included and in the acupoints chosen in both studies. The total number of participants included in the present study was 172, whereas only 28 participants were included in the former one study by Linde et al.; thus, it was easier for them to get non-significant results. Besides, according to the theories of traditional Chinese medicine, the efficacy of acupuncture intervention is affected by the acupoints used. The acupoints used in the present study and in the former study are different, partially accounting for the discrepancy between the results of the two studies.

Visual analog scale score is widely accepted as one of the major measures of headache intensity. In this trial, there was no significant difference between the VAS scores of the acupuncture and the medication groups. However, over cycles 4−6, the VAS score recorded during and outside the perimenstrual period reduced by 2.39 and 1.45 points, respectively, in the acupuncture group, compared with a reduction of 1.30 or 0.95, respectively, in the medication group, with a between-group difference of 1.08 and 0.50 points (VAS during the perimenstrual period, $P < 0.0001$; VAS outside the perimenstrual period, $p = 0.0039$). In the study by Linde M. et al., 2005, the reduction of VAS score at week 24 was 1.0 for women who received acupuncture, and −0.2 for those who received sham acupuncture ($P>0.05$). The sample size of the present study differs from that of the study by Linde et al. (n = 172 vs. 28). Consequently, the differences between the VAS scores of the treatment and control groups in the two studies are probably comparable and are not clinically meaningful. A mean reduction in baseline VAS score of 3.5 points 2 hours after the intake of medication for moderate and severe headache has been reported as the MCID (Aicher et al., 2012). Similar to the results of the average number of migraine days per perimenstrual period, the reductions of VAS scores over cycles 4−6 suggest that acupuncture was more effective than medication in reducing the intensity of headache; however, the between-group difference was smaller than the MCID. Although the pain reduction reported by the two groups during the treatment phase was not significantly different, the results indicated that patients in the acupuncture group achieved greater pain reduction during the follow-up phase than those in the medication group. Such results indicate that acupuncture yielded a long-term therapeutic effect.

To a certain extent, we did not observe any difference between the acupuncture and medication groups in terms of the proportion of participants that used acute pain medication. However, the between-group difference in the reduction of the number of migraine days during and outside the perimenstrual period was statistically significant. Since we recorded the proportion of participants who used acute pain medication, but not the dose of medication used, it was not feasible to compare actual medication usage between the groups. This should be considered in future research.

Interestingly, although changes from the baseline number of migraine days and VAS score decreased over time (baseline, cycle 3, and cycle 6) in both the acupuncture and medication groups, the between-group differences in the changes during the perimenstrual period became stronger over time. The abovementioned results may be caused by a markedly decreased tendency to changes from the baseline number of migraines days and VAS score in the medication group. Although these results share similarities with those of the former one, there are some differences between them. In the study by Linde M. et al., 2005, the number of migraine days decreased after 12 weeks of acupuncture treatment and 12 weeks of follow up, but stayed relatively the same at 36 weeks. These discrepancies may be caused by variations in the duration of follow-up, sham acupuncture design, and number of acupuncture sessions.

In present study, the sham acupuncture method was performed by choosing non-disease-related acupoints; however, the stimulation experienced by the patient was same as that induced by real acupuncture. Present studies have focused on the effects due to acupoint specificity. Repetitive results of Yang et al. reported the different mode of activated brain metabolism within the disease-related acupoint and non-disease-related acupoint groups, convincing a superiority of acupoint specificity in pain relieving (Yang et al., 2012, 2014). Thus, this type of sham acupuncture has been found to have more therapeutic effects than non-penetrating sham acupuncture and is considered not totally inert (MacPherson et al., 2014) due to the physiological effect it produces. The activation of ergoreceptors, which deliver the information in A-delta or type II or III afferents into the spinal cord, leads to the afferent stimulation produced by acupuncture (Andersson and Lundeberg, 1995; Hui et al., 2000). Moreover, the activities of acupuncture are associated with the sensory and affective components of pain through the activation of descending pain-inhibiting pathways and the deactivation of the limbic structures, respectively (Han, 2003; Hui et al., 2005). Recently, several studies have demonstrated that even a light touch on the skin can trigger activity in the insular region, but not in the somatosensory cortex, by stimulating mechanoreceptors coupled to slow-conducting unmyelinated (C) afferents, which induces a "limbic touch" response, leading to the emotional and hormonal reactions commonly seen following caressing. Therefore, as neither the sham acupuncture method used in the present study nor non-penetrating sham acupuncture are totally inert, they cannot be considered "real placebo" because they activate C tactile afferents, resulting in the alleviation of unpleasantness and the re-establishment of the patient's sense of self-esteem and wellbeing (Damasio, 1999; Olausson et al., 2002; Mohr et al., 2005). The application of blunt needles on effective acupoints was not used in this

trial as a control intervention because patients in China are very familiar with the sensations caused by acupuncture and may easily identify the sensation caused by blunt needles. Most physiological mechanisms proposed for acupuncture may activate unmyelinated afferent nerves, which can influence pain perception, as mentioned above (Lund and Lundeberg, 2006). Meanwhile, different types of placebos may have different placebo effects (Finniss et al., 2010). Acupuncture—with its repeated sessions, intense provider contact, slightly painful procedure, an often "exotic" model of symptom explanation, and associated relaxation during sessions—may maximize such placebo effects (Linde et al., 2016). Sham acupuncture is associated with greater placebo effects than a placebo pill or other non-pharmacological sham interventions (Oken, 2008; Zhang et al., 2008). Therefore, the placebo effects of placebo medication and sham acupuncture on participants may be different. However, this difference is less likely to cause between-group differences than the real difference between acupuncture and medication.

Generalizability of the results of this trial may be limited by the following factors: low baseline number of migraine days (mean baseline number of migraine days during the perimenstrual period: 1.83 and 1.67 days in the acupuncture and medication groups, respectively; mean baseline number or migraine days outside the perimenstrual period: 2.07 and 1.81 days in the acupuncture and medication groups, respectively), limited and monoethnic study population (all Chinese), lack of assessments of the participants' expectations (Mao et al., 2007) at baseline and treatment satisfaction (Trutnovsky et al., 2018) at cycles 3 and 6, lack of medication usage records, and lack of evaluation of other quality of life outcomes, e.g., the Migraine-Specific Quality of Life Questionnaire score (Cole et al., 2007) and the Migraine Disability Assessment Score (Stewart et al., 2001). More appropriate sham acupuncture, such as non-penetrating needles, should be used for a minimal therapeutic effect as high-qualified sham control. In addition, this study was supposed to be a 2 × 2 design with four groups (true acupuncture plus naproxen, true acupuncture plus placebo, sham acupuncture plus naproxen, and sham acupuncture plus placebo) had sufficient funding support and time been available.

Conclusion

In this study, participants with MRM who received a three-cycle acupuncture treatment experienced significantly greater reductions in the number of migraine days over six cycles than those who received medication, indicating that acupuncture is effective for preventing MRM and has a sustained treatment effect over six cycles. Considering these promising results, it indicates that MRM patients with an inter-individual difference in drug response or those who are unwilling to accept possible drug-induced adverse events may benefit from acupuncture treatment. Consequently, acupuncture as a complementary and alternative medicine therapy can be a more potent and safe treatment for MRM prophylaxis.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Research Ethical Committee of Beijing Hospital of Traditional Chinese Medicine in Beijing. The patients/participants provided their written informed consent to participate in this study.

Author contributions

L-PW and H-LL designed the study protocol. L-PW, X-HJ, and BL conceived and designed the study and revised the manuscript for intellectual content. T-LL, L-PZ, M-NW, Z-YQ, L-MN, JG, X-ZZ, and Y-HL had an effective role in the implementation of this study and the data collection. LL, C-SZ, FH, and LZ performed the statistical analyses and prepared the figures and tables. LL wrote the manuscript. K-LW contributed to a thorough revision of the manuscript. All authors revised and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2022.992577/full#supplementary-material

References

Aicher, B., Peil, H., Peil, B., and Diener, H. C. (2012). Responsiveness of efficacy endpoints in clinical trials with over the counter analgesics for headache. *Cephalalgia* 32, 953–962. doi: 10.1177/0333102412452047

Allais, G., Castagnoli Gabellari, I., Mana, O., and Benedetto, C. (2012). Treatment strategies for menstrually related migraine. *Womens Health* 8, 529–541. doi: 10.2217/whe.12.37

Allais, G., Chiarle, G., Sinigaglia, S., and Benedetto, C. (2018). Menstrual migraine: A review of current and developing pharmacotherapies for women. *Expert Opin Pharmacother.* 19, 123–136. doi: 10.1080/14656566.2017.1414182

Andersson, S., and Lundeberg, T. (1995). Acupuncture–from empiricism to science: Functional background to acupuncture effects in pain and disease. *Med. Hypotheses* 45, 271–281. doi: 10.1016/0306-9877(95)90117-5

Bigal, M. E., Borucho, S., Serrano, D., and Lipton, R. B. (2009). The acute treatment of episodic and chronic migraine in the USA. *Cephalalgia* 29, 891–897. doi: 10.1111/j.1468-2982.2008.01819.x

Bigal, M. E., and Lipton, R. B. (2008). Excessive acute migraine medication use and migraine progression. *Neurology* 71, 1821–1828. doi: 10.1212/01.wnl.0000335946.53860.1d

Coeytaux, R. R., and Befus, D. (2016). Role of Acupuncture in the Treatment or Prevention of Migraine, Tension-Type Headache, or Chronic Headache Disorders. *Headache* 56, 1238–1240. doi: 10.1111/head.12857

Cole, J. C., Lin, P., and Rupnow, M. F. (2007). Validation of the Migraine-Specific Quality of Life Questionnaire version 2.1 (MSQ v. 2.1) for patients undergoing prophylactic migraine treatment. *Qual. Life Res.* 16, 1231–1237. doi: 10.1007/s11136-007-9217-1

Collaborators GBDH (2018). Global, regional, and national burden of migraine and tension-type headache, 1990-2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* 17, 954–976. doi: 10.1016/S1474-4422(18)30322-3

Damasio, A. R. (1999). *The feeling of what happens: Body and emotion in the making of consciousness*. New York, NY: Harcourt Brace.

Deng, L. Y., and Huang, L. X. (2004). *Chinese acupuncture and Moxibustion Zheng Zhi Tong Jian*. Qingdao,China: Qingdao Press.

Diener, H. C., Kronfeld, K., Boewing, G., Lungenhausen, M., Maier, C., Molsberger, A., et al. (2006). Efficacy of acupuncture for the prophylaxis of migraine: A multicentre randomised controlled clinical trial. *Lancet Neurol.* 5, 310–316. doi: 10.1016/S1474-4422(06)70382-9

Finniss, D. G., Kaptchuk, T. J., Miller, F., and Benedetti, F. (2010). Biological, clinical, and ethical advances of placebo effects. *Lancet* 375, 686–695. doi: 10.1016/S0140-6736(09)61706-2

Granella, F., Sances, G., Allais, G., Nappi, R. E., Tirelli, A., Benedetto, C., et al. (2004). Characteristics of menstrual and nonmenstrual attacks in women with menstrually related migraine referred to headache centres. *Cephalalgia* 24, 707–716. doi: 10.1111/j.1468-2982.2004.00741.x

Han, J. S. (2003). Acupuncture: Neuropeptide release produced by electrical stimulation of different frequencies. *Trends Neurosci.* 26, 17–22. doi: 10.1016/s0166-2236(02)00006-1

Headache Classification Committee of the International Headache Society [IHS] (2013). The International Classification of Headache Disorders, 3rd edition (beta version). *Cephalalgia* 33, 629–808. doi: 10.1177/0333102413485658

Headache Classification Committee of the International Headache Society [IHS] (2018). The International Classification of Headache Disorders, 3rd edition. *Cephalalgia* 38, 1–211. doi: 10.1177/0333102417738202

Headache Classification Subcommittee of the International Headache Socitey [IHS] (2004). The International Classification of Headache Disorders: 2nd edition. *Cephalalgia* 24, 9–160. doi: 10.1111/j.1468-2982.2003.00824.x

Hui, K. K., Liu, J., Makris, N., Gollub, R. L., Chen, A. J., Moore, C. I., et al. (2000). Acupuncture modulates the limbic system and subcortical gray structures of the human brain: Evidence from fMRI studies in normal subjects. *Hum. Brain Mapp.* 9, 13–25. doi: 10.1002/(sici)1097-019320009:1<13::aid-hbm2<3.0.co;2-f

Hui, K. K., Liu, J., Marina, O., Napadow, V., Haselgrove, C., Kwong, K. K., et al. (2005). The integrated response of the human cerebro-cerebellar and limbic systems to acupuncture stimulation at ST 36 as evidenced by fMRI. *Neuroimage* 27, 479–496. doi: 10.1016/j.neuroimage.2005.04.037

Li, C., Liu, H., and Yang, C. (2011). Clinical observation of acupuncture as prophylaxis for menstrually related migraine (MRM). *Beijing J. Tradit. Chin. Med.* 30, 617–618.

Li, Y., Zheng, H., Witt, C. M., Roll, S., Yu, S. G., Yan, J., et al. (2012). Acupuncture for migraine prophylaxis: A randomized controlled trial. *CMAJ* 184, 401–410. doi: 10.1503/cmaj.110551

Linde, K., Allais, G., Brinkhaus, B., Fei, Y., Mehring, M., Vertosick, E. A., et al. (2016). Acupuncture for the prevention of episodic migraine. *Cochrane Database Syst. Rev.* 2016:CD001218. doi: 10.1002/14651858.CD001218.pub3

Linde, K., Streng, A., Jürgens, S., Hoppe, A., Brinkhaus, B., Witt, C., et al. (2005). Acupuncture for patients with migraine: A randomized controlled trial. *JAMA* 293, 2118–2125. doi: 10.1001/jama.293.17.2118

Linde, M., Fjell, A., Carlsson, J., and Dahlöf, C. (2005). Role of the needling per se in acupuncture as prophylaxis for menstrually related migraine: A randomized placebo-controlled study. *Cephalalgia* 25, 41–47. doi: 10.1111/j.1468-2982.2004.00803.x

Lund, I., and Lundeberg, T. (2006). Are minimal, superficial or sham acupuncture procedures acceptable as inert placebo controls? *Acupunct. Med.* 24, 13–15. doi: 10.1136/aim.24.1.13

Maasumi, K., Tepper, S. J., and Kriegler, J. S. (2017). Menstrual Migraine and Treatment Options: Review. *Headache* 57, 194–208. doi: 10.1111/head.12978

MacGregor, E. A. (2015). Migraine Management During Menstruation and Menopause. *Continuum* 21, 990–1003. doi: 10.1212/CON.0000000000000196

MacGregor, E. A., Victor, T. W., Hu, X., Xiang, Q., Puenpatom, R. A., Chen, W., et al. (2010). Characteristics of menstrual vs nonmenstrual migraine: A post hoc, within-woman analysis of the usual-care phase of a nonrandomized menstrual migraine clinical trial. *Headache* 50, 528–538. doi: 10.1111/j.1526-4610.2010.01625.x

MacPherson, H., Vertosick, E., Lewith, G., Linde, K., Sherman, K. J., Witt, C. M., et al. (2014). Influence of control group on effect size in trials of acupuncture for chronic pain: A secondary analysis of an individual patient data meta-analysis. *PLoS One* 9:e93739. doi: 10.1371/journal.pone.0093739

MacPherson, H., White, A., Cummings, M., Jobst, K. A., Rose, K., Niemtzow, R. C., et al. (2002). Standards for Reporting Interventions in Controlled Trials of Acupuncture: The STRICTA recommendations. *J. Altern. Complement. Med.* 8, 85–89. doi: 10.1089/107555302753507212

Mao, J. J., Armstrong, K., Farrar, J. T., and Bowman, M. A. (2007). Acupuncture expectancy scale: Development and preliminary validation in China. *Explore* 3, 372–377. doi: 10.1016/j.explore.2006.12.003

Moher, D., Hopewell, S., Schulz, K. F., Montori, V., Gøtzsche, P. C., Devereaux, P. J., et al. (2010). CONSORT 2010 explanation and elaboration: Updated guidelines for reporting parallel group randomised trials. *BMJ* 340:c869. doi: 10.1136/bmj.c869

Mohr, C., Binkofski, F., Erdmann, C., Büchel, C., and Helmchen, C. (2005). The anterior cingulate cortex contains distinct areas dissociating external from self-administered painful stimulation: A parametric fMRI study. *Pain* 114, 347–357. doi: 10.1016/j.pain.2004.12.036

National Clinical Guideline Centre (2012). *Headaches: Diagnosis and Management of Headaches in Young People and Adults*. London: Royal College of Physicians.

Newman, L. C., and Yugrakh, M. S. (2014). Menstrual migraine: Treatment options. *Neurol. Sci.* 35, 57–60. doi: 10.1007/s10072-014-1743-3

Oken, B. S. (2008). Placebo effects: Clinical aspects and neurobiology. *Brain* 131, 2812–2823. doi: 10.1093/brain/awn116

Olausson, H., Lamarre, Y., Backlund, H., Morin, C., Wallin, B. G., Starck, G., et al. (2002). Unmyelinated tactile afferents signal touch and project to insular cortex. *Nat. Neurosci.* 5, 900–904. doi: 10.1038/nn896

Pavlović, J. M., Stewart, W. F., Bruce, C. A., Gorman, J. A., Sun, H., Buse, D. C., et al. (2015). Burden of migraine related to menses: Results from the AMPP study. *J. Headache Pain* 16:24. doi: 10.1186/s10194-015-0503-y

Pinkerman, B., and Holroyd, K. (2010). Menstrual and nonmenstrual migraines differ in women with menstrually-related migraine. *Cephalalgia* 30, 1187–1194. doi: 10.1177/0333102409359315

Pringsheim, T., Davenport, W. J., and Dodick, D. (2008). Acute treatment and prevention of menstrually related migraine headache: Evidence-based review. *Neurology* 70, 1555–1563. doi: 10.1212/01.wnl.0000310638.54698.36

Stewart, W. F., Lipton, R. B., Dowson, A. J., and Sawyer, J. (2001). Development and testing of the Migraine Disability Assessment (MIDAS) Questionnaire to assess headache-related disability. *Neurology* 56, S20–S28. doi: 10.1212/wnl.56.suppl_1.s20

Trutnovsky, G., Schneeweiss, M., Pregartner, G., Reif, P., Lang, U., and Ulrich, D. (2018). Prenatal acupuncture: Women's expectations and satisfaction and influence on labor. *Birth* 45, 210–216. doi: 10.1111/birt.12336

Vetvik, K. G., Benth, J., MacGregor, E. A., Lundqvist, C., and Russell, M. B. (2015). Menstrual versus non-menstrual attacks of migraine without aura in women with and without menstrual migraine. *Cephalalgia* 35, 1261–1268. doi: 10.1177/0333102415575723

Vetvik, K. G., Macgregor, E. A., Lundqvist, C., and Russell, M. B. (2014). Prevalence of menstrual migraine: A population-based study. *Cephalalgia* 34, 280–288. doi: 10.1177/0333102413507637

Wang, L. P., Zhang, X. Z., Guo, J., Liu, H. L., Zhang, Y., Liu, C. Z., et al. (2011). Efficacy of acupuncture for migraine prophylaxis: A single-blinded, double-dummy, randomized controlled trial. *Pain* 152, 1864–1871. doi: 10.1016/j.pain.2011.04.006

Xu, S., Yu, L., Luo, X., Wang, M., Chen, G., Zhang, Q., et al. (2020). Manual acupuncture versus sham acupuncture and usual care for prophylaxis of episodic migraine without aura: Multicentre, randomised clinical trial. *BMJ* 368:m697. doi: 10.1136/bmj.m697

Yang, J., Zeng, F., Feng, Y., Fang, L., Qin, W., Liu, X., et al. (2012). A PET-CT study on the specificity of acupoints through acupuncture treatment in migraine patients. *BMC Complement. Altern. Med.* 12:123. doi: 10.1186/1472-6882-12-123

Yang, J. Z. (1995). *Compendium of acupuncture and Moxibustion*. Beijing: People's Medical Publishing House.

Yang, M., Yang, J., Zeng, F., Liu, P., Lai, Z., Deng, S., et al. (2014). Electroacupuncture stimulation at sub-specific acupoint and non-acupoint induced distinct brain glucose metabolism change in migraineurs: A PET-CT study. *J. Transl. Med.* 12:351. doi: 10.1186/s12967-014-0351-6

Zhang, W., Robertson, J., Jones, A. C., Dieppe, P. A., and Doherty, M. (2008). The placebo effect and its determinants in osteoarthritis: Meta-analysis of randomised controlled trials. *Ann. Rheum. Dis.* 67, 1716–1723. doi: 10.1136/ard.2008.092015

Zhang, X. Z., Zhang, L., Guo, J., Zeng, L., Yang, Y., Zhang, T., et al. (2013). Acupuncture as prophylaxis for menstrual-related migraine: Study protocol for a multicenter randomized controlled trial. *Trials* 14:374. doi: 10.1186/1745-6215-14-374

Zhao, L., Chen, J., Li, Y., Sun, X., Chang, X., Zheng, H., et al. (2017). The Long-term Effect of Acupuncture for Migraine Prophylaxis: A Randomized Clinical Trial. *JAMA Intern. Med.* 177, 508–515. doi: 10.1001/jamainternmed.2016.9378

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Potential mechanisms of acupuncture for neuropathic pain based on somatosensory system

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Neuropathic pain, caused by a lesion or disease of the somatosensory system, is common and distressing. In view of the high human and economic burden, more effective treatment strategies were urgently needed. Acupuncture has been increasingly used as an adjuvant or complementary therapy for neuropathic pain. Although the therapeutic effects of acupuncture have been demonstrated in various high-quality randomized controlled trials, there is significant heterogeneity in the underlying mechanisms. This review aimed to summarize the potential mechanisms of acupuncture on neuropathic pain based on the somatosensory system, and guided for future both foundational and clinical studies. Here, we argued that acupuncture may have the potential to inhibit neuronal activity caused by neuropathic pain, through reducing the activation of pain-related ion channels and suppressing glial cells (including microglia and astrocytes) to release inflammatory cytokines, chemokines, amongst others. Meanwhile, acupuncture as a non-pharmacologic treatment, may have potential to activate descending pain control system *via* increasing the level of spinal or brain 5-hydroxytryptamine (5-HT), norepinephrine (NE), and opioid peptides. And the types of endogenously opioid peptides was influenced by electroacupuncture-frequency. The cumulative evidence demonstrated that acupuncture provided an alternative or adjunctive therapy for neuropathic pain.

KEYWORDS

neuropathic pain, acupuncture, ion channels, glial cells, descending pain control system, somatosensory system

Introduction

Neuropathic pain, caused by a lesion or disease of the somatosensory system, is one of the most intractable human complaints (Jensen et al., 2011). Patients commonly experienced spontaneous pain and/or evoked pain. The former is described as shooting, lancinating or burning pain and the latter is characterized by hyperalgesia to mechanical

and cold stimulus (Bouhassira, 2019; Bannister et al., 2020). In addition to the obvious pain-related suffering, neuropathic pain may lead to negative effects such as depression, anxiety, and reduce the quality of life in patients (Laumet et al., 2015). Epidemiological studies have shown that more than 7% of the general population who undergo neuropathic pain, accounting for 20 to 25% of individuals with chronic pain (Torrance et al., 2006; Bouhassira et al., 2008). The high morbidity brought enormous psychological and economic burden to patients, families, and society. However, treatment of neuropathic pain has been extremely challenging, and treatment options are often unmanageable and limited due to the side effects and tolerability (Vranken, 2012; Finnerup et al., 2015). Conventional options to manage neuropathic pain leave much to be desired and more complementary therapies are sorely needed.

The somatosensory system consists of a number of neural pathways that carry various senses from the starting point in skin, muscles, tendons, and internal organs to the central nervous system and ultimately to consciousness (Gomez-Ramirez et al., 2016). Neuropathic pain is a direct consequence of alterations in the somatosensory system (Laedermann et al., 2013). Acupuncture as a non-invasive strategy for nerve stimulation, was a valuable therapy to improve neuropathic pain with a low incidence of adverse events (Barnes et al., 2008; Kelly and Willis, 2019). The somatosensory system mediates the sensation of *de qi* in acupuncture and plays an important role in the analgesic mechanism of acupuncture (Su et al., 2014). Aβ, Aδ, and C fibers are the most important types of primary afferent nerves in transmitting the acupuncture signal (Huo R. et al., 2020). The pain relief effects of acupuncture are also mediated by activity in the brain and spinal cord (Han, 2004). Along with the increasing application of acupuncture, the analgesic mechanisms of acupuncture through the somatosensory system has been increasingly discovered and progressively confirmed. This review synthesized relevant studies to gain a comprehensive understanding of the production, transmission and processing of acupuncture-like signals from the periphery to the central nervous system.

Materials and methods

Search strategy

We searched PubMed, Web of Science, and Embase for available information describing issues related to acupuncture and neuropathic pain. The searches identified English language papers published from 2,000 up to the present time. Keywords included ["Acupuncture" or "Electroacupuncture" or "EA"] and ["Neuropathic pain" or "Peripheral neuropathic pain" or "Chronic neuropathic pain after peripheral nerve injury" or "Trigeminal neuralgia" or "Painful radiculopathy" or "Postherpetic neuralgia" or "painful diabetic neuropathy" or "Neuropathy after radiotherapy" or "Neuropathy after chemotherapy" or "Central post-stroke pain" or "Spinal cord injury pain" or "Multiple sclerosis pain" or "Parkinson's disease pain"].

Literature selection

The inclusion criteria were designed as follows: (1) Research focused on acupuncture for neuropathic pain. (2) The intervention measures of the treatment group were acupuncture (manual acupuncture or electroacupuncture). (3) Literatures contained clinical research, systematic review/meta-analysis, and basic research. Meanwhile, studies that did not meet the above mentioned criteria were excluded.

Searches retrieved 918 articles. After careful evaluation of the data, we sought to summarize the information identified through the literature search. The search was performed by screening the reference lists of articles that met our inclusion criteria based on the titles and abstracts. Of these articles, we excluded 191 articles due to the absence of the full text, leaving 727 articles. Then, we excluded 287 articles including 77 case reports, 121 study protocols, and 89 review articles. Through the title and abstract, we excluded 52 articles that intervention measures were special acupuncture methods other than manual acupuncture or electroacupuncture. Finally, 388 articles were included after reading the full text, including 22 clinical studies, 59 meta-analyses/systematic reviews, and 307 basic studies. A flow chart of the search and filter process is shown in **Figure 1**.

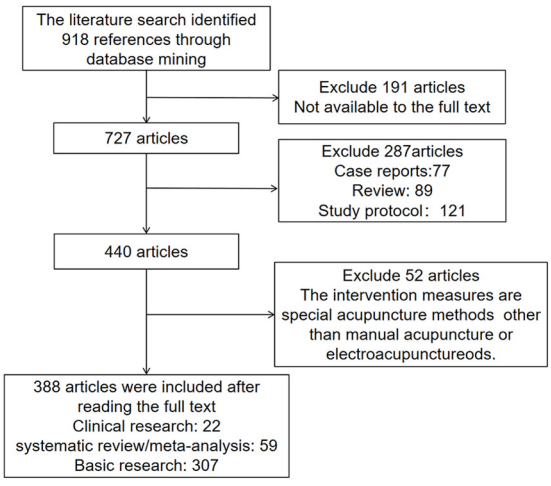

FIGURE 1
Flow chart of search and filter process.

Application of acupuncture for neuropathic pain

Acupuncture manipulations

Manual acupuncture (MA) and electroacupuncture (EA) were the two most common interventions for pain with acupuncture. In MA, the acupuncture needles are inserted into the acupoints and twisted up and down by hand. MA emphasizes the occurrence of *de qi* sensations, which can be induced by correct and effective manual manipulation (Spaeth et al., 2013). In EA, stimulating current is delivered to acupoints *via* the needle connected to an electrical stimulator. The therapeutic benefit of EA depends on the frequency, current amplitude, and pulse width of stimulation. Changing manipulations of MA or parameters of EA may produce different therapeutic effects (Xu et al., 2020). Comparing the changes in pain thresholds for different frequencies of rotational MA, strong stimulation of MA (4 r/s MA) was more effective than mild MA (2 r/s MA) on pain model rats, which was associated with C fiber activation (Song et al., 2021). The analgesia induced by 2 Hz EA was mediated by the endomorphin and that of 100 Hz EA by dynorphin (Han, 2004). In the neuropathic pain model rats, low-frequency (2 Hz) EA had a considerably greater effect on mechanical and thermal pain than high-frequency (100 Hz) EA (Sun et al., 2002). 2/100 Hz (at 2 Hz and 100 Hz frequencies alternately) stimulation increased the release of both endomorphin and dynorphin. It was thus obvious that a proper combination of different frequencies might produce a maximal release of a cocktail of neuropeptides for better therapeutic effects (Han, 2003). Despite of evidence supported the effectiveness of acupuncture for neuropathic pain and showed benefits of MA and EA. It was controversial whether EA does more effective than MA. EA results in more reproducible stimulation and may be substantially more advantageous than MA in continuous stimulation and reducing response times (Zhao et al., 2019). However, another study suggested that EA was not superior to MA treatment. Both therapies had similar efficacy in reducing chronic pain (Comachio et al., 2020).

Acupoints for neuropathic pain

There are several ways to select acupoints for neuropathic pain: local, regional, and distal points (Millstine et al., 2017). Common local points are where patients feel the most intense pain or pressure (or both) along the pain distribution, in light of the fact that the location of these acupoints are anatomically identical to those of humans. E.g., trigeminal neuralgia take Yuyao (Ex-HN 4), Cuanzhu (BL 2), and Yangbai (GB 14) (**Figure 2A**), which are located around the eyes (Gao et al., 2019); radiculalgia (lumbar) take Dachangshu (BL 25), and Guanyuanshu (BL 26) (**Figure 2C**) which parallel to the fourth and fifth lumbar spinous processes (Yu et al., 2021). Regional points are taken by following the meridians or according to the nerve distribution characteristics of the pain area. For trigeminal neuralgia, maxillary branch includes Quanliao (SI 18), Sibai (ST 2), and Juliao (ST 3) (**Figure 2A**), and mandibular branch includes Xiaguan (ST 7) and Jiache (ST 6) (**Figure 2B**), which all belonged to the stomach meridian of foot-yangming (Yu et al., 2021). Depending on the distribution of pain, such as persistent lower limb pain caused by nerve root compression, pain confined to the side of the affected leg will be treated through acupoints on the gallbladder meridian, including Huantiao (GB 30), Fengshi (GB 31), Xiyangguan (GB 33), Yanglingquan (GB 34), and Xuanzhong (GB 39) (**Figures 2C,D**). Pain confined to the posterior part of the affected leg will be treated through points on the bladder meridian, including Zhibian (BL 54), Chengfu (BL 36), Weizhong (BL 40), Chengshan (BL 57), and Kunlun (BL 60) (**Figures 2C,D**; Yu et al., 2021). Distal regions, such as Neiting (ST 44), Hegu 4 (LI 4), and Sanjian (LI 3) (**Figure 2D**), are taken for trigeminal neuralgia (Gao et al., 2019). It is an acupoint selection method based on traditional Chinese medicine theory. The location of acupoint is determined using basic theoretical frameworks (e.g., traditional Chinese medicine, TCM) or anatomical structures (e.g., innervation). Currently, acupuncturists tend to use a hybrid approach when providing TCM-based acupoint localization, and they may combine their practice with localized treatments based on current anatomical knowledge, such as the principles of acupoint selection mentioned earlier.

Clinical evidence for acupuncture on neuropathic pain

A variety of diseases including peripheral and/or central nerve injury, neuropathic pain induced by diabetes, anticancer chemotherapy, post-stroke pain and Parkinson's have been used to study the effect of acupuncture on neuropathic pain (**Figure 3**; Becker et al., 2017; Fan et al., 2018; Zhi et al., 2018; Lovaglio et al., 2019; Yu et al., 2019; Wang et al., 2020; Edwards and Shaw, 2021; Zhang et al., 2021; Sollie et al., 2022). **Table 1** listed acupuncture treatments for neuropathic pain caused by different diseases. In addition to the difference in the treatment of acupoints, the depth of acupuncture, the needle manipulation and the course of treatment may also be varied. These methods were not the only ones that physicians also made determinations based on their clinical experience. With the deepening of clinical research, the efficacy of acupuncture on neuropathic pain has been confirmed by several systemic reviews and meta-analyses, which were listed in **Table 2** (Heo et al., 2013; Dimitrova et al., 2017; Hu et al., 2019; Liu et al., 2019; Pei et al., 2019; Yun et al., 2020; Cui et al., 2021; Yu et al., 2021).

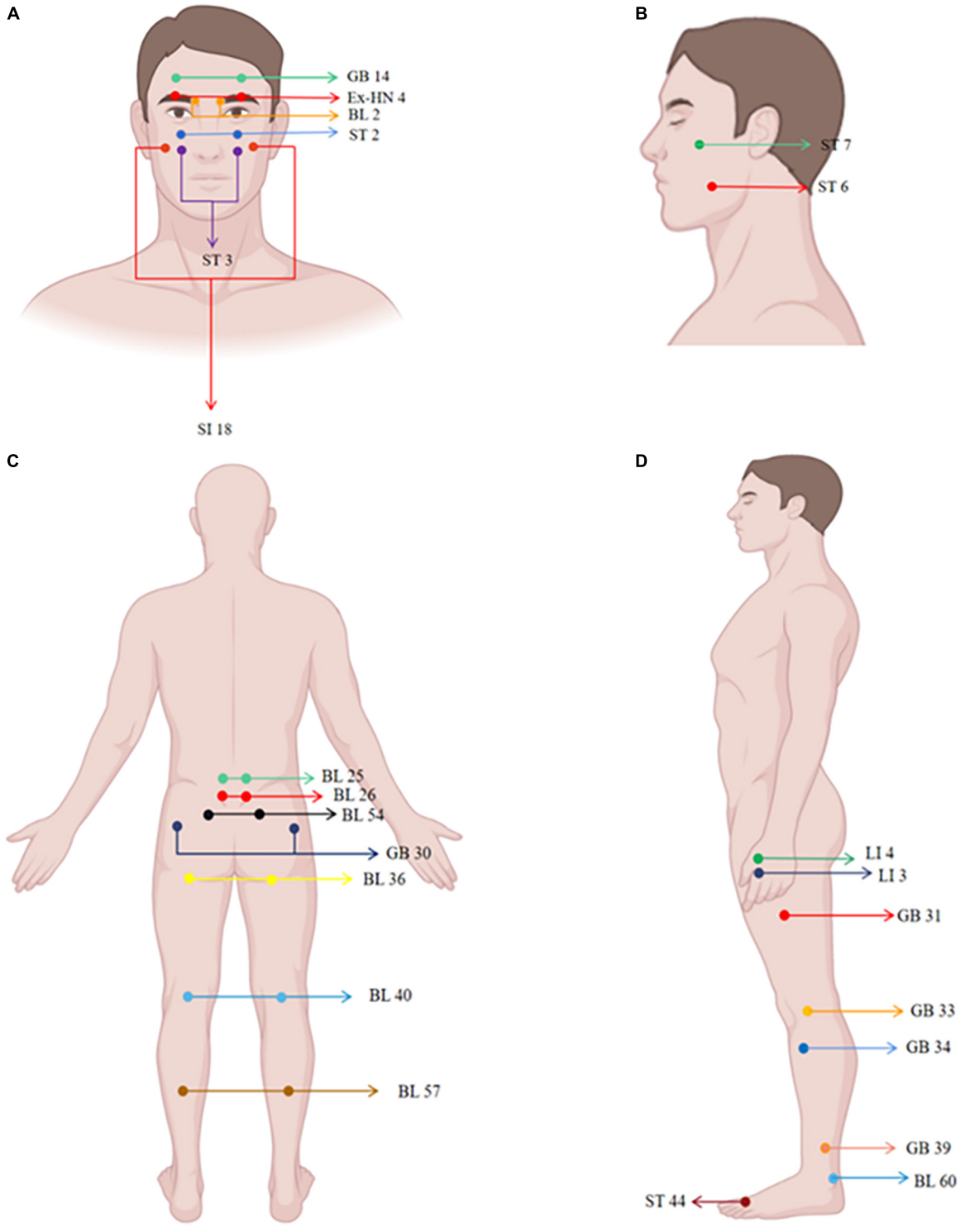

FIGURE 2
Human maps of acupoints used in neuropathic pain studies. The locations of acupoints are marked in the figure.

A study described the effectiveness and safety of acupuncture for the treatment of chemotherapy-induced peripheral neuropathy. After 8 weeks of treatment and follow-up, the acupuncture group showed a greater reduction in pain score than the vitamin B1 or gabapentin group. Moreover, the nerve conduction study was improved best in the acupuncture group and no adverse

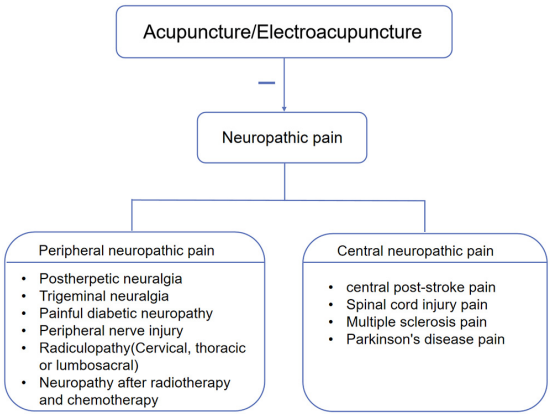

FIGURE 3
Acupuncture inhibits neuropathic pain induced by various diseases.

events were observed (Iravani et al., 2020). Based on currently available evidences, acupuncture appears more effective than pharmacotherapy or surgery with high degree of safety for improving neuropathic pain (Huang Z. et al., 2019; Yu et al., 2019; Iravani et al., 2020; Lee et al., 2020).

A majority of acupuncturists emphasized *de qi* which is a feeling of numbness, fullness, and sometimes soreness, when they performed acupuncture treatments. It seemed that acupuncture analgesia was manifest when the *de qi* feeling occured in patients following manipulation of acupuncture (Hui et al., 2005). The complete somatosensory system was the prerequisite for the appearance of *de qi* (Cao, 2002). Previous research confirmed that the *de qi* sensation and pressure pain threshold increased according to the depth and rotation of acupuncture (Choi et al., 2013). Treatment for chronic neuropathy pain usually lasts more than 4 weeks. Acupuncture may be a slow-acting agent and has a specific pattern of the dynamics for the entire coupled nervous system. The therapeutic effect of acupuncture is a gradual accumulation process, that is, as the number of acupuncture courses increases, the therapeutic effect gradually increases (Bai et al., 2009). Therefore, the above factors should be considered in acupuncture analgesia.

Animal models of neuropathic pain

Animal models of neuropathic pain are critical for understanding the underlying mechanism and further development of acupuncture therapy. A variety of neuropathic pain animal models which induced by central or peripheral nerve injury have been used to study the acupuncture mechanism (**Table 3**; Zhang Y. et al., 2018; Li et al., 2019b; Huo B.B. et al., 2020; Du et al., 2021; Jang et al., 2021; Jiang et al., 2021; Wei et al., 2021; Xu et al., 2021; Zou et al., 2021). In addition to animal models of nerve ligation, there are injectable chemotherapy drug-induced neuropathy, post-herpetic neuralgia, and diabetes-induced peripheral nerve injury (Colleoni and Sacerdote, 2010). The former is caused by a primary injury or dysfunction of the nervous system, while the latter is caused by diseases such as diabetes, shingles, and cancer chemotherapy. Although every model possesses its own unique characteristics, different etiologies of neuropathic pain appear to lead to similar behavioral endpoints.

Acupuncture mechanisms on neuropathic pain

Neuropathic pain is divided into two major categories: peripheral and central, depending on the location of the lesion or disease (Finnerup et al., 2016; Scholz et al., 2019; **Figure 4**). At the peripheral level, alterations in receptors and ion channels impact neuronal function, resulting in spontaneous (ectopic) activity and pain (Khan et al., 2019). The pathological hallmarks of different types of peripheral nerve lesions have individual characteristics. Some may damage to the entire nerve, causing axonal neuropathy; others may damage to part of the axon or myelin sheath, causing demyelinating neuropathy (Hoffmann et al., 2008; Held et al., 2019). In the central nervous system, there are a variety of conditions that can cause central neuralgia, including damage to the spinal cord or brain, such as trauma, ischemic stroke, cerebral hemorrhage, or multiple sclerotic plaques (Apkarian et al., 2005; Siddall and Middleton, 2015). The mechanism of acupuncture for neuropathic pain is mediated by the somatosensory system (**Figure 4**). Acupuncture modulated the alterations of receptors and ion channels, inhibited activation of protein kinases and glia and activated the descending pain control system (Goldman et al., 2010; Li et al., 2013; Xu et al., 2020). This review synthesized these studies to provide a comprehensive understanding of how acupuncture alleviates pain through the somatosensory system.

Acupuncture modulates the receptors and ion channels in the periphery

Peripheral nerve endings perceive nociceptive stimuli and activate pain pathways. In order for this interaction to happen, mechanical or other stimuli may affect the cytoplasmic membrane potential of axon which as soon exceeds a certain threshold level triggering action potentials. Nociceptors sense thermal, mechanical, and chemical stimuli through the expression of different ion channels such as the transient receptor potential (TRP) family of ion channels as well as ATP-gated purinergic channels (P2X). At this point, diverse types of voltage-gated sodium channels come into play to amplify

TABLE 1 Acupuncture methods for neuropathic pain caused by different diseases.

Condition	Acupoints	Acupuncture methods	Duration of treatment	References
Trigeminal neuralgia	Neiting (ST 44), Hegu 4 (LI 4), Sanjian (LI 3), ophthalmic branch (Yuyao, Ex-HN 4; Cuanzhu, B 2; Yangbai, GB 14), maxillary branch (Quanliao, Sl 18; Sibai, ST 2; Juliao, ST 3), and mandibular branch (Xiaguan, ST 7; Jiache, ST 6; Extraordinary point, Ex)	The penetration depth was 25 to 50 mm in the muscle.	The retention time for the needles was 20 min and one session per week for a total of 10 weekly sessions.	Gao et al., 2019
Sciatica	The bilateral acupoints of Dachangshu (BL 25), Shenshu (BL 23), Weizhong (BL 40), and Chengshan (BL 57)	The needles were inserted 30–70 mm into the acupoints slowly and vertically. Twirling, lifting, and thrusting manipulates were performed tenderly and evenly three times in order to reach the *de qi* sensation.	12 sessions of treatment (30 min each) for 4 weeks (three times a week).	Huang Z. et al., 2019
Painful diabetic neuropathy	Zusanli (ST 36), Feishu (BL 13), Pishu (BL 20), Sanyinjiao (SP 6), and Yinlingquan (SP 9), Xuanzhong (GB 39), Taichong (LR 3) and Zulinqi (GB 41)	Needles will be inserted perpendicularly and stimulated only in the beginning to achieve a *de qi* sensation and then will be left in place.	12 sessions administered over a period of 8 weeks (preferably 2 sessions in each of the first 4 weeks, followed by 1 session per week in the remaining 4 weeks).	Lee S. et al., 2013
Postherpetic neuralgia	Jiaji (Ex-B2) and Ashi points	A filiform needle, 0.25–0.30 mm in diameter, 25–40 mm in length, is stimulated with an electrical current	20–30 min in each session	Ruengwongroj et al., 2020
Spinal cord injury pain	Changqiang (GV 14), Jiaji (Ex-B2), et al.	Needles were inserted to a depth of 15 to 30 mm and left in place for 20 min	15 sessions of acupuncture over a $7^{1/2}$-week period	Nayak et al., 2001
Chemotherapy-induced peripheral neuropathy	Qihai (CV 6), Baihui (GV 20), Bilateral Zusanli (ST 36), Sanyinjiao (SP 6), Hegu (LI 4), Quchi (LI 11), and Taichong (LR 3) as the general points and bilateral Bafeng (EX-LE 10) and Baxie (EX-UE 9)	Needles (0.25×0.40 mm) were inserted perpendicularly at the depth of 5–15 mm acupoints, with proper needling manipulation to induce "de qi" (the arrival of qi).	3 times per week for 4 weeks (20 min in each session)	Iravani et al., 2020

transient receptor potentials and thus reach depolarization levels sufficient to initiate action potentials (Bannister et al., 2020; Sharif et al., 2020). Transient receptor potential vanilloid 1 (TRPV1) belongs to the family of TRP, that are intensively expressed in the peripheral nervous system and involved in a variety of physiological and pathophysiological processes in mammals (Nilius et al., 2007; Mickle et al., 2015). There is pharmacological evidence that blocking TRPV1 channel, alleviates neuropathic hypersensitivity in rodent models (Basso and Altier, 2017). P2X, specifically the C-fiber localized P2X3 receptor (P2X3R) subtypes, are expressed in the dorsal root ganglion (DRG) and involved in the initiation and maintenance of neuropathic pain (Tang Y. et al., 2016; Khan et al., 2019). Besides, P2X4 and P2X7 in DRG were also involved in thermal nociceptive hypersensitivity (Masoodifar et al., 2021). Voltage-gated sodium channels $Na_v1.1$, $Na_v1.6$, $Na_v1.7$, $Na_v1.8$, and

TABLE 2 Efficacy of acupuncture for neuropathic pain.

Authors	Journal	Type of study	Sample size	Disease types	Conclusion
Cui et al. (2021)	Complement Med Res	Systematic review and meta-analysis	915	Trigeminal neuralgia	Acupuncture may be effective for patients with trigeminal neuralgia.
Yu et al. (2021)	J Clin Pharm Ther	Systematic reviews	8378	Diabetic peripheral neuropathy	Acupuncture appears to have an effect on painful diabetic neuropathy, effectively improving nerve conduction and clinical symptoms.
Pei et al. (2019)	J Pain Res	Meta-analysis	498	Postherpetic neuralgia	Acupuncture may reduce pain intensity, relieve anxiety and improve quality of life in patients with postherpetic neuralgia.
Liu et al. (2019)	Front Neurol	Systematic review and meta-analysis	3,184	Central post stroke pain	Adding acupuncture to conventional rehabilitation treatment for post-stroke pain is superior to rehabilitation treatment alone.
Dimitrova et al. (2017)	J Altern Complement Med	Meta-analysis	680	Peripheral neuropathy	Acupuncture is beneficial in some peripheral neuropathies.
Ma et al. (2015)	J Neurotrauma	Systematic review and meta-analysis	35	Spinal cord injury pain	Acupuncture may be an effective treatment for reducing chronic pain in patients with spinal cord injury.

TABLE 3 Animal models of neuropathic pain.

Model	Mode of induction	Disease types	Peripheral/Central
partial sciatic nerve ligation (PSNL)	By ligation of 1/3 or 1/2 of sciatic nerve	Peripheral nerve injury	Peripheral
chronic constriction injury (CCI)	By ligation of sciatic nerve	Peripheral nerve injury	Peripheral
spinal nerve ligation (SNL)	By ligation of L5/L6 of spinal cord	Peripheral nerve injury	Peripheral
Spared nerve injury (SNI)	By ligation of peroneal and tibial nerves	Peripheral nerve injury	Peripheral
Brachial plexus avulsion injury (BPAI)	By damaging the dorsal and ventral nerve rootlets of C5-T1	Peripheral nerve and spinal cord injury	Peripheral and Central
Spinal cord injury (SCI)	By transecting T10 spinal cord	Spinal cord injury pain	Central
Chemotherapy-induced peripheral neuropathy (CIPN)	By i.p. injecting anti-cancer agents (vincristine, cisplatin,oxaliplatin, paclitaxel)	Neuropathy after chemotherapy	Peripheral
Postherpetic neuralgia (PHN) model	By injecting 10 μL PLVX-IRES-Zsgreen1-Mir-223-3p into the lumbar spine between L5 and L6	Postherpetic neuralgia	Peripheral
chronic constriction injury to infra-orbital nerve	By ligation of infra-orbital nerve	Trigeminal Neuralgia	Peripheral
Diabetes-induced neuropathic pain	By persistenting hyperglycemia-induced changes in the nerves	Painful diabetic neuropathy	Peripheral

$Na_v1.9$ are expressed in peripheral sensory neurons in different patterns and function as key regulators of sensory nerve excitability (Bennett et al., 2019). Mutations in voltage-gated sodium channels are associated with a variety of pain disorders. In neuropathic conditions, $Na_v1.8$ is most highly expressed in small-diameter neuron subtypes (Dib-Hajj and Waxman, 2019). Another family of excitatory channels associated with neuropathic pain is hyperpolarization-activated and cyclic nucleotide-gated (HCN) channels. HCN2 was specifically deleted in nociceptors expressing $Na_v1.8$ in mice, but nerve

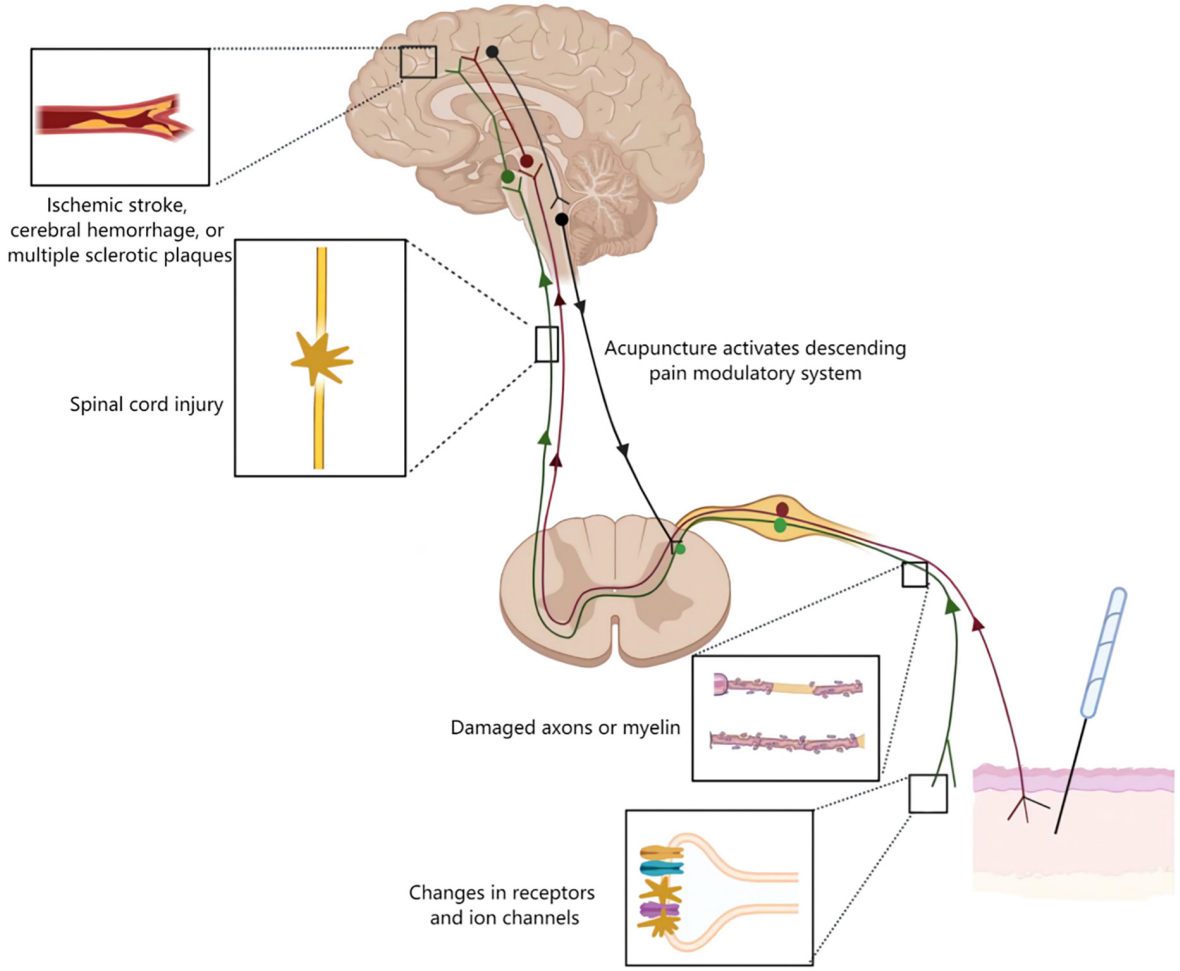

FIGURE 4
Acupuncture mechanisms on neuropathic pain. Neurological damage from peripheral to the cortical brain may lead to neuropathic pain. The figure shows four typical examples of lesions. Alterations in receptors and ion channels impact neuronal function, resulting in spontaneous (ectopic) activity and pain. Along the peripheral nerve different types of lesions may damage either the entire nerve or selectively the axons or myelin causing axonal or demyelinating neuropathies, respectively. Lesions of the spinal cord or brain as seen for example following traumatic injury, ischemic stroke, cerebral hemorrhage, or multiple sclerotic plaques may lead to central neuropathic pain. Acupuncture inhibited pain transmission *via* the somatosensory system, and activated the descending pain control system.

lesion did not cause hyperalgesia to thermal or mechanical stimuli (Emery et al., 2011). HCN2 antagonist attenuated neuropathic hypersensitivity in neuropathic rats and inhibited spontaneous activity of C-nociceptors, but not Aβ fiber (Djouhri et al., 2018).

Electroacupuncture (EA) effectively reduced nociceptive sensitization in spared nerve injury (SNI) and spinal nerve ligation (SNL) by downregulating the expression ratio of TRPV1 in DRG (Fang et al., 2021). Administration of TRPV1 agonists reversed EA analgesia (Jiang et al., 2013; Du et al., 2021). Ca^{2+} imaging revealed that TRPV1 channel activity was increased in DRG neurons of paclitaxel-treated rats, whereas EA suppressed the increased TRPV1 channel activity. Pharmacological blockade of TRPV1 was similar to the analgesic effect of EA on pain allergy, while capsaicin reversed the effect of EA (Li et al., 2019b). EA might inhibit the activation of P2X3Rs in neuropathic pain and block primary afferent transmission mediated through P2X3Rs to alleviate mechanical and thermal nociceptive sensitization (Fei et al., 2020). Additionally, EA was more potent in reducing both mechanical allodynia and thermal hyperalgesia in combination with intrathecal A-317491 (a selective P2X3 and P2X2/3 receptor antagonist) (Wang et al., 2014). Therefore, EA and A-317491 might potentially have an additive effect in inhibiting the transmission of pain mediated by the P2X3 receptor. The protein levels of P2X4 and P2X7 in diabetes-induced neuropathy rats were significantly increased. 2 Hz EA improved the paw withdrawal latency and reduced the expression of P2X4 and P2X7 in DRG (Hu et al., 2022). EA attenuated $Na_v1.7$ and $Na_v1.8$ protein expression levels in the DRG during painful states (Yen et al., 2018). $Na_v1.3$ was

lacking in DRG neurons of normal adult rats, but was highly expressed in damaged sensory neurons (Waxman et al., 1994). EA diminished spinal cord injury (SCI)-induced upregulation of $Na_v1.3$ (Liu and Wu, 2017). EA also reduced mechanical allodynia and face-grooming in trigeminal neuropathic pain rats through downregulation of HCN expression in the gasserian ganglion (Yang et al., 2019). These results suggested that acupuncture blocks pain-related ion channels and increases pain thresholds (**Figure 5**).

The available evidence indicated that afferent nerve fibers and different receptors in the acupoints might played a key role in mediating the effects of acupuncture. Acupuncture inhibited neuronal activity caused by neuropathic pain, through reducing pain-related ion channels and receptors activation. At present, however, the specific pathway of changes in receptors and ion channels mediated by acupuncture stimulation points cannot be explained. More types of sham controls need to be employed to thoroughly evaluate the effects of acupoint specificity in future studies.

Acupuncture inhibits activation of protein kinases and glia in the spinal cord

The spinal cord neuronal activity caused by neuropathic pain is partially attributed to increased synaptic efficacy. Ionic and metabotropic glutamate receptors exhibit phosphorylation or translocation changes, resulting in increased excitatory postsynaptic potential (EPSP) frequency and amplitude (Kiyoyuki et al., 2015; Hildebrand et al., 2016). Multiple protein kinases have been implicated in regulating neuronal plasticity and pain sensitization following intense noxious stimuli or injuries. There is increasing evidence suggesting that serine/threonine kinases family especially Protein kinase A (PKA), Protein kinase C (PKC), mitogen-activated protein kinases (MAPKs) (Ma et al., 2021), is critical for the induction and maintenance of pain hypersensitivity after injuries (Ji et al., 2007). Extracellular signal regulated kinase (ERK) (Kondo and Shibuta, 2020), p38 mitogen-activated protein kinases (p38 MAPK) (Lin et al., 2014), and calmodulin-dependent protein kinase II (CaMKII) (Qian et al., 2019) are downstream to many kinases. These kinases are activated in primary sensory and dorsal horn neurons by nociceptive activity, contributing to the induction and maintenance of pain sensitization (Choi et al., 2019). Compared with the normal rat, more PKA-positive cells were observed in the spinal dorsal horn of SNL rat (Wu et al., 2021). PKC activation depolarized unmyelinated afferent neurons, which enhance currents in afferent neurons activated by nociceptive stimuli, and PKC inhibitors blocked sensitization of afferent neurons (Velázquez et al., 2007). p38 MAPK and ERK are present in spinal dorsal horn, and their inhibitors inhibit neuropathic pain (Inoue and Tsuda, 2018). The activation pattern of ERK in the spinal cord correlated with neuropathic pain behavior at different time points after SNL. Intrathecal injection of the non-competitive ERK inhibitor PD98059 attenuated SNL-induced mechanical nociceptive hypersensitivity (Kondo and Shibuta, 2020). Inhibition of spinal CaMKII expression has been shown to prevent thermal hyperalgesia and mechanical allodynia (Fang et al., 2002).

Electroacupuncture (EA) reduced the expression of p38 MAPK and inhibited pain transmission in rat spinal cord dorsal horn (Wei and Hsieh, 2020; Jin et al., 2021). PKA expression levels are elevated and involved in neuropathic pain by activating the p38 MAPK pathway to mediate apoptosis in spinal cord cells (Deng et al., 2020). EA exerted analgesic effects by decreasing the expression of PKA in SNL model rats (Wu et al., 2021). 2 Hz EA reduced the expression of P2X3 receptors by inhibiting the PKC pathway thus relieved pain (Zhou et al., 2018), CaMKII is crucially involved in synaptic plasticity and long-term potentiation (LTP) (Luo et al., 2014). It was found that EA reduced p-CaMKII levels in the spinal cord and was blocked by pretreatment with 5-hydroxytryptamine (5-HT) 1A receptor antagonists, suggesting that 5-HT1A receptors were involved in the inhibitory effect of EA on spinal p-CaMKII (Zhang Y. et al., 2018). These studies clearly showed that acupuncture blocked multiple protein kinases activation to reduce spinal cord neuronal activity in painful conditions and achieved pain relief.

Proliferation, shape change and activation of microglial populations in the spinal dorsal horn has been reported in several models of neuropathic pain (Ji et al., 2016). Diverse ensuing changes in the transcriptional and secretory profile of microglia have been linked to neuropathic pain, including release of inflammatory factor, ATP, chemokines, amongst others (Inoue and Tsuda, 2018). Moreover, astrocyte activation further promotes neuronal activity (Ji et al., 2016). It is known that p38 MAPK and ERK play important roles in the maintenance of neuropathic pain (Deng et al., 2020; Kondo and Shibuta, 2020). P-p38 MAPK and p-ERK upregulation are localized to activate microglia within the dorsal horn of the lumbar region after spinal cord injury. CX3C chemokine fractalkine (CX3CL1) released from damaged neurons activates CX3C-chemokine receptor 1 (CX3CR1) on microglial cells and leads to tumor necrosis factor α (TNF-α), and Interleukin-1β (IL-1β) secretion *via* p38 MAPKs/ERK. Released IL-1β and TNF-α acts on spinal dorsal horn neurons to enhance glutamate excitatory synaptic transmission and decreaseγ-aminobutyric acid (GABA)-mediated and glycine-mediated synaptic inhibition (Gim et al., 2011; Inoue and Tsuda, 2018). Furthermore, ERK in activated microglia mediates the release of prostaglandin E2 (PGE2), which binds to prostaglandin E receptor 2 (EP2) expressed in spinal cord neurons, inducing a change in their excitatory state and thus causing neuropathic pain (Zhao et al., 2007). Spinal brain-derived neurotrophic factor (BDNF) is a key neuromodulator of pain transmission, and P2X4R activates spinal microglia to induce p38 MAPK

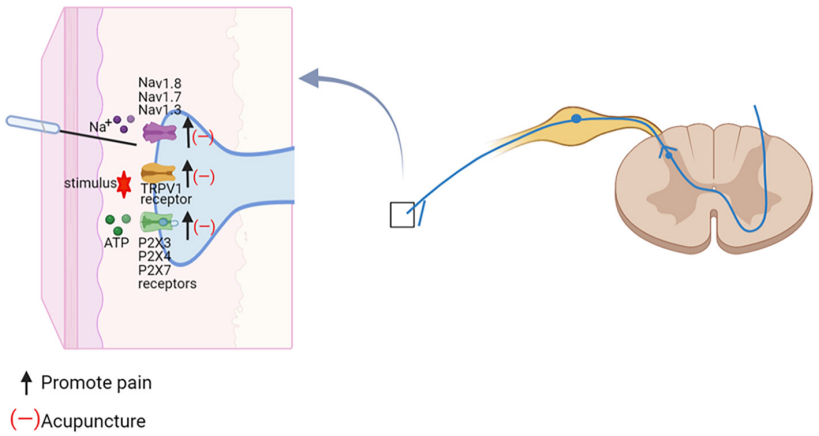

FIGURE 5
Acupuncture regulates ion-channel dysregulation in neuropathic pain. The effect of acupuncture on the spinal cord. Na+, sodium ion; Navs, Voltage-gated sodium channels; TRPV1, TRP vanolloid 1; ATP, adenosine triphosphate; P2X3, ATP-gated purinergic channels 3.

phosphorylation to release BDNF, which transmits noxious signals to layer I neurons, thereby contributing to the pathogenesis of pain (Cappoli et al., 2020).

Microglia and astrocytes, are important targets for acupuncture analgesic (**Figure 6**). EA reduced mechanical and thermal pain in rat models of neuropathic pain by preventing microglia, astrocyte activation (Liang et al., 2016). EA and intrathecal injection of the glial metabolism suppressant fluorocitrate might synergize against pain (Sun et al., 2006). Therefore, the inactivation of glial cells may be partly responsible for the acupuncture analgesic. In a SCI model, acupuncture applied to GB34 inhibited p38 MAPK and ERK phosphorylation in microglia of L4-5 spinal cord (Choi D. C. et al., 2012). EA down-regulated the neuronal chemokine CX3CL1, which acted on CX3CR1 in microglia, and prevented the p38 MAPKs/ERK signaling pathway, leading to reduce the release of inflammatory cytokines, resulting in pain relief (Li et al., 2019a). Acupuncture attenuated the ERK-dependent PGE2 releasing from activated microglia (Choi D. C. et al., 2012). EA prevented BDNF binding to spinal cord neuronal tyrosine kinase receptor B (Trk B) by decreasing microglia activation and BDNF expression, thereby reducing nociceptive hyperalgesia and neuropathic pain (Tu et al., 2018).

Adenosine is present at the extracellular space within the spinal cord dorsal horn and engaged in the processing of nociceptive sensory signals. Systemic or spinal administration of exogenous adenosine produces a potent analgesia against pathological pain. In rat spinal cord slices, adenosine increases postsynaptic inhibitory currents mediated by glycine receptors (GlyRs), and this synaptic potentiation is dependent on activation of adenosine A1 receptors (A1Rs) (Bai et al., 2017). Another study found that spinal A1R contributed to the inhibitory effects of EA on astrocyte activation as well as TNF-α upregulation (Zhang M. et al., 2018). The c-Jun N-terminal kinase (JNK), a major member of the MAPK family, has been shown to play a key role in intracellular signaling and contributes to central sensitization of chronic pain. Peripheral inflammation or nerve injury leads to JNK activation in spinal astrocytes. Activation of the JNK pathway lead to the production and release of several pro-inflammatory cytokines that play an important role as biological mediators in chronic pain (Wang et al., 2017).

It has been shown that EA first downregulated microglia activation (after 2 days of EA) and then astrocyte activation (after 1–2 weeks of EA treatment) (Wang et al., 2018). A1Rs expression in the L4–6 spinal segments were increased by EA, indicating that EA induced an increase in adenosine levels in the spinal cord, which in turn activated astrocyte A1Rs to produce an analgesic effect (Dai et al., 2020). In SCI rats, acupuncture inhibited JNK activation in astrocytes at the spinal cord L4-5 level. The level of p-c-Jun, a downstream molecule of JNK, was also decreased by acupuncture. In addition, the number of hypertrophic, activated astrocytes in the L4-5 dorsal horn I-II layers was significantly reduced in the acupuncture-treated group. It was suggested that the analgesic effect of acupuncture was mediated in part through inhibition of JNK activation in astrocytes after SCI (Lee J. Y. et al., 2013). Interleukin-10 (IL-10) is a powerful anti-inflammatory cytokine that improves inflammation and protects damaged nerve. It mainly distributes in the superficial spinal astrocytes. The anti-nociceptive effects of EA were blocked by the spinal IL-10 inhibitor, suggesting EA had a regulatory effect on IL-10 in spinal astrocytes (Dai et al., 2019).

The spinal cord is an important center for mediating the analgesic effects of acupuncture. EA down-regulated the neuronal chemokine CX3CL1 and prevented microglial p38 MAPK/ERK signaling pathway, leading to reduced release of TNF-α and IL-1β, BDNF, PGE2, and reducing the neuronal

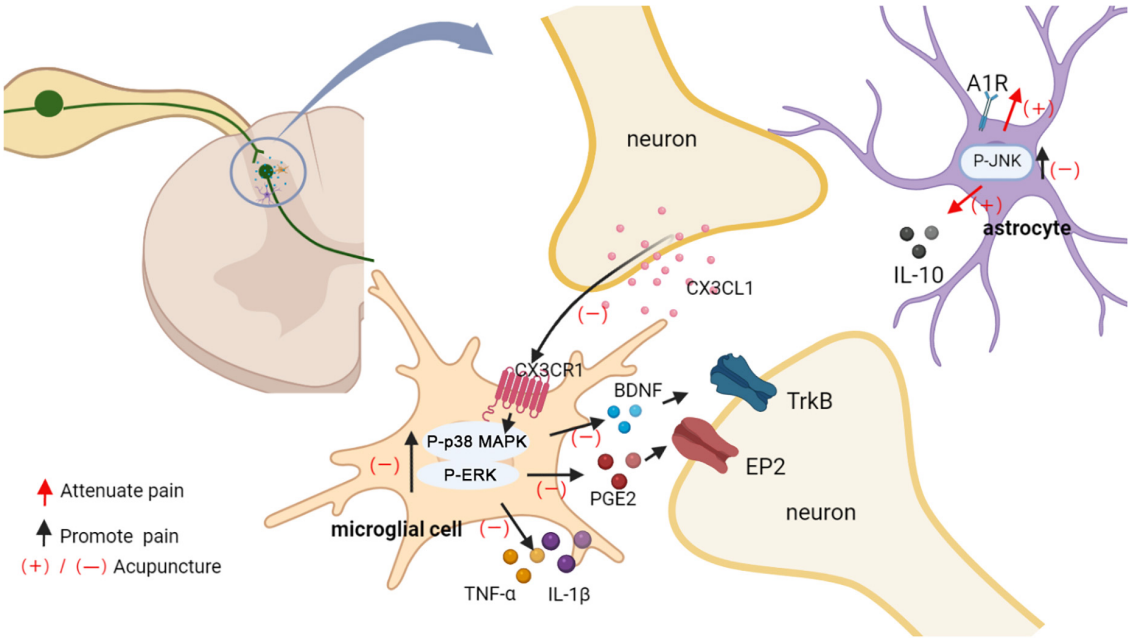

FIGURE 6
Acupuncture affects glial activation. EA down-regulated the neuronal chemokine CX3CL1 and prevented the p38 MAPK/ERK signaling pathway, leading to reduced release of TNF-α and IL-1β, BDNF, PGE2. Released IL-1β and TNF-α acts on spinal dorsal horn neurons to enhance the neuronal excitability and inflammatory response. PGE2 and BDNF bind to spinal cord neuronal EP2 and Trk B, respectively, inducing a change in their excitatory state and thus causing neuropathic pain. EA induced an increase in adenosine levels in the spinal cord, which in turn activated astrocyte A1Rs to produce an analgesic effect. Additionally, analgesic effect of acupuncture was mediated in part through inhibiting the activation of JNK and promoting the release of IL10 in astrocytes.

excitability and inflammatory response. The activation of microglial cells in the ipsilateral spinal cord dorsal horn increased within 1 day of nerve injury; however, astrocytes were activated later than microglial cells and were implicated in the maintenance of mechanical allodynia after spinal nerve injury. In the early and late stages of neuropathic pain, repeated EA therapy inhibited microglia and astrocyte activation, respectively (Liang et al., 2016). These results suggested that inhibition of spinal microglia activation was involved in the early stage of EA analgesia, while inhibition of astrocytes activation was involved in the maintenance of EA analgesia.

Acupuncture regulates descending pain control system

It is well-established that the descending pain control system of the midbrain and brainstem regulates the processing of nociceptive information in the spinal cord. The anterior cingulate cortex (ACC) promotes spinal excitatory synaptic transmission leading to nociceptive hyperalgesia. Descending corticospinal tract fibers originating from somatosensory cortex project not only to the spinal ventral horn, but also to the spinal dorsal horn and to the sensory synapse (Liu et al., 2018). Imaging studies in human volunteers showed that the rostral ACC likely mediated analgesia by activation of the periaqueductal gray (PAG) (Eippert et al., 2009). The PAG is known to be an important relay station receiving inputs from higher brain centers for descending modulation of pain through projections to the spinal cord *via* the rostroventral medulla (RVM) and the locus coeruleus (LC) (Alhadeff et al., 2018). In neuropathic pain, acupuncture plays a complex and critical role in the network of descending pathways projecting from brain structures to the spinal dorsal horn (**Figure 7**). The analgesic effect of descending pain control system relies on endogenous gamma-aminobutyric acid GABA, 5-HT, norepinephrine (NE), and endogenous opioids (Silva et al., 2013).

Gama aminobutyric acid (GABA) is an important inhibitory neurotransmitter. GABAergic projections of interneurons from the brainstem to the spinal cord control spinal cord nociceptive transmission (François et al., 2017). The PAG manages pain through downstream modulation of the spinal dorsal horn, and EA activation of PAG neurons involved the descending pain control system of GABAergic (Fusumada et al., 2007). In the neuropathic pain model induced by chronic constriction injury (CCI), EA up-regulated the level of GABA in the PAG (Huang C. P. et al., 2019). EA at Jiaji (EX-B2) acupoints improved neuropathic pain by increasing protein expression levels of GABA receptors in the spinal cord (Jiang et al., 2018). Using *in vivo* two-photon imaging in mice with chronic

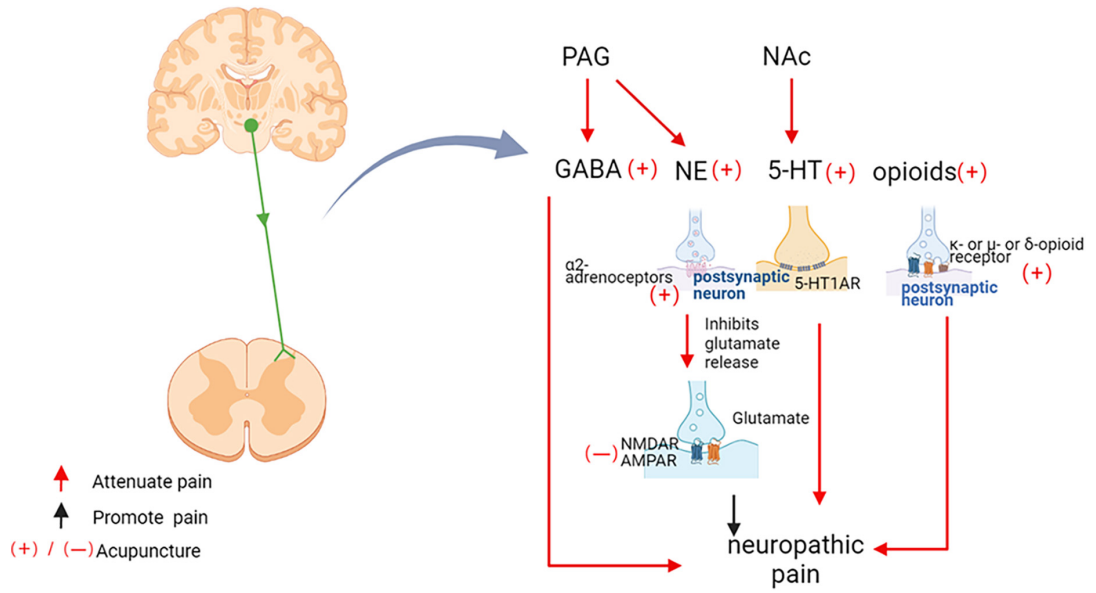

FIGURE 7
Acupuncture inhibits neuropathic pain through the downstream pathway. PAG, periaqueductal gray matter; nucleus accumbens, NAc; GABA, Gama aminobutyric acid; NE, Norepinephrine; 5-HT, 5-hydroxytryptamine; 5-HT1AR, 5-hydroxytryptamine 1A receptors; 5-HT3R, 5-hydroxytryptamine 3 receptors; NMDAR, *N*-methyl-D-aspartate receptors; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors.

systolic injury, it was found that EA therapy systematically modulates Ca^{2+} activity in primary somatosensory cortical neural circuits, including inhibition of excitatory pyramidal neurons, enhancement of GABA-ergic somatostatin positive interneurons, thereby mediating improved mechanical or thermal hypersensitivity (Wei et al., 2021).

5-HT and its receptors are key substances that regulate pain and play a crucial role in EA analgesia. 5-HT released from the nucleus accumbens (NAc) were increased in rats receiving acupuncture, which was observed 20 min after acupuncture treatment and persisted until 40 min after the end of acupuncture (Yoshimoto et al., 2006). Exogenous lateral ventricular 5-HT have analgesic effects that partially mimic the analgesic effects of EA (Chang et al., 2004). Current research indicated that the alleviation of cold nociceptive hypersensitivity by 2 Hz EA was mediated by spinal 5-HT1A and 5-HT3 receptors (Kim et al., 2005). In addition, it has been shown that lateral ventricular injections of 5-HT1A receptors antagonists blocked the analgesic effects induced by low-and high-frequency EA (Chang et al., 2004). Blockade of 5-HT1A receptors in the ventral tegmental area reversed morphine-/dextromethorphan-induced analgesia in pain model rats (Seddighfar et al., 2019). Spinal 5-HT2A receptors (5-HT2AR) mediate the downstream vulnerability of 5-HTergic axons through multiple mechanisms. Inhibition of spinal dorsal horn 5-HT2AR expression prevents mechanical nociceptive hypersensitivity of the face and associated changes in $PKC\gamma^{+}$ interneuron morphology (Alba-Delgado et al., 2018). However, there are no reports of neuropathic pain relief by acupuncture mediated by 5-HT2A, providing a direction for the next study.

Norepinephrine (NE) and α_2-adrenergic receptors are widely distributed in the brain and spinal cord, and activation of the NEergic descending pain control system is involved in the anti-nociceptive effects of EA (Silva et al., 2011). Acupuncture at Zusanli (ST 36) and Shangjuxu (ST 37) activated neurons in the PAG to exert anti-nociceptive effects, increased the release of NE from the PAG. The α_1-, α_2-, β-adrenoceptors were found to be located in the PAG, and noradrenalin and α_1-and α_2-agonists were found to activate the lateral and ventrolateral PAG neurons. These findings suggested that the modulation of pain by EA in PAG involved NE (Murotani et al., 2010). Intrathecal injection of drugs that increase spinal NE utilization promotes the long-term anti-nociceptive effects of EA (Silva et al., 2013). Intrathecal injection of the α_2–adrenergic antagonist yohimbine reduced EA-induced analgesia in a dose-dependent manner, suggesting that the analgesic effect of EA was dependent on the binding of norepinephrine to α_2 receptors (Koo et al., 2008).

Endogenous opioids were closely related to the analgesic effect of acupuncture. Acupuncture reduced the SNL-induced hypersensitivity response, which blocked by naloxone, a non-selective opioid receptor antagonist (Cidral-Filho et al., 2011), suggesting that the analgesic effect of acupuncture was dependent on the opioid system. Low-frequency and high-frequency EA activated different types of opioid receptors. Mu-or delta-opioid receptor antagonists blocked the 2 Hz EA anti-mechanical nociceptive hypersensitivity (Kim et al., 2004).

100 Hz EA leaded to the release of dynorphin, which binded to kappa-opioid receptors in the spinal cord and provides pain relief (Huang et al., 2008). However, 2/100 Hz EA activated both mu/delta and kappa opioid receptors, inducing a synergistic analgesic effect that was more effective than constant frequency stimulation (Han, 2003).

Ionic glutamate receptors include *n*-methyl-d-aspartate (NMDA) receptors and α-amino-3-hydroxy-5-methyl-4-isoxazolidinepropanoic acid (AMPA) receptors. Glutamate is the main excitatory neurotransmitter acting on ionotropic glutamate receptors to direct central sensitization (Groc and Choquet, 2006). EA inhibited phosphorylation of AMPA receptor (AMPAR) GluR2 subunit (Tang Y. et al., 2016). Low-frequency EA exerted analgesic effects by modulating the phosphorylation status of spinal NMDA receptor subunits NR1 and NR2B (Jung et al., 2010). EA improved SNI-induced pain behavior and decreased glutamate release in the spinal dorsal horn (Vidal-Torres et al., 2012). 5-HT1BR (Choi I. S. et al., 2012) and α_2-adrenoceptors (Li and Eisenach, 2001) activation reduced glutamate release from medullary dorsal horn neurons. However, in SNL-induced neuropathic pain rat model, 2 Hz EA activated the endogenous opioid system and induces NMDA receptor-dependent long-term depression (LTD) in the spinal dorsal horn (Xing et al., 2007). The different results may be explained by different acupoints and different parameters of EA.

After the initial injury healing, chronic pain will continue, which is related to brain remodeling (Kuner and Flor, 2016) and the treatment rely on post-therapeutic effect of EA. Brain processing of acupuncture stimuli in neuropathic pain patients or animals may underlie its beneficial effects. Previous study showed that neuropathic pain following brachial plexus avulsion injury (BPAI) induced metabolic connectivity changes significantly among sensorimotor-related areas and pain-related area in bilateral hemispheres (Huo R. et al., 2020). The decreased metabolic connectivity between ipsilateral dorsolateral thalamus and somatosensory cortex was related with BPAI-induced neuropathic pain. EA increased the thermal withdrawal latency of BPAI rat and improved the strength of connectivity among the above regions (Hou et al., 2020). Evidence suggested that neuropathic pain patients responded to acupuncture with more pronounced fMRI signal decrease in the amygdala and signal increase in the lateral hypothalamic area differently than healthy people. Acupuncture coordinated limbic response that included the hypothalamus and amygdala (Napadow et al., 2007). The hypothalamus is an important component of the central descending pain modulatory circuit from the cerebral cortex to the spinal cord (Ossipov et al., 2010), activation of hypothalamic neurons can inhibit the input of nociceptive signals to the pain centers in the cortex (Lin and Chen, 2008). CCI rat following EA intervention, there were 17 hypothalamic proteins identified with significant changes in the expression. EA attenuated pain may *via* regulation of expression of these proteins in the hypothalamus (Vogt, 2016). Although studies have indicated involvement of many brain structures in the modulation of acupuncture analgesia, hypothalamus might play a crucial role in this process. A study revealed that increased local synaptic activity in the ipsilateral somatosensory cortex and decreased in the contralateral somatosensory cortex after sciatic nerve injury. EA served as repeated sensory stimulation, might potentially induce increased synaptic activity in the corresponding cortices involving contralateral somatosensory cortex of sciatic nerve injury (Wu et al., 2018).

As can be seen, the descending pain modulation system, including the ACC, the PAG, the NAc, and the hypothalamus, plays an important role in EA analgesia. EA might reverse the maladaptive brain plasticity, promote the release of endogenous substances such as GABA, NE, 5-HT, and opioids. This could be an important mechanism underlying the post-therapeutic effect of EA, and it deserves further study.

Discussion

Acupuncture, which has a history of 2,000-year, is a useful adjunct therapy or an acceptable alternative treatment of pain (Wells et al., 2017). The research base for acupuncture is rapidly expanding. Somatosensory stimulation, including acupuncture, could thus act as an additional input to re-arrange the neural loop, nociceptive, and acupuncture signals was integrated at spinal and supraspinal levels (**Figure 4**). Acupuncture suppressed pain, probably due to effects on the afferent nerve. The gate control theory might play a role in acupuncture analgesia. Non-painful input by acupuncture closed the "gates" to painful input, which prevented pain sensations from traveling to the central nervous system (Lemmon, 2018). Modulation of sensory input occurs at the primary afferent neuron and spinal dorsal horn during an acupuncture treatment, which may depend on acupoints at the same spinal section as the pain site. We speculated, in this case, acupuncture inhibited neuronal activity caused by neuropathic pain, through reducing pain-related ion channels and protein kinases activation (**Figure 5**). In the spinal dorsal horn, EA down-regulated the neuronal chemokine CX3CL1 and prevented microglial p38 MAPK/ERK signaling pathway, leading to reduced release of TNF-α and IL-1β, BDNF, PGE2, and reducing the neuronal excitability and inflammatory response (**Figure 6**). Furthermore, functional magnetic resonance imaging studies have shown that acupuncture at specific acupoints modulated areas of the brain (Huang et al., 2021), and acupuncture activated descending pain control system. The distal acupoints such as Neiting (ST 44), Hegu 4 (LI 4), and Sanjian (LI 3) probably work in this way. Research has found that, in nerve injury model, contralateral but not ipsilateral acupuncture produced clear analgesia. This difference might be due to the damaged nerve blocks the conduction of acupuncture signals, while contralateral acupuncture inhibited pain through the central descending inhibitory, independent of the influence of

local damaged nerves (Zhang H. et al., 2018). GABA, NE, 5-HT, and endogenous opioids are involved centrally (**Figure 7**). Taken together, acupuncture, as a distinctive therapeutic modality to pain, produced physiologic changes in the brain, spinal cord, and at the periphery. Medication treatment was often associated with side effects, and many patients did not achieve adequate pain relief at tolerated doses (Finnerup et al., 2015). On the contrary, acupuncture is a relatively safe and well-tolerated treatment, with most patients experiencing no adverse effects at all (Pfister et al., 2010).

As a matter of fact, the optimal prescription of acupuncture treatment (acupuncture point, degree of stimulation, frequency of treatment, and a number of treatment sessions) for neuropathic pain is a controversial issue amongst acupuncture experts. EA and MA are two acupuncture manipulations commonly used in clinical practice. Our previous study found that EA and MA effectively improved pain symptoms in patients with osteoarthritis of the knee, but EA had a faster onset of action than MA (Xu et al., 2020). Current evidence does not yet support an efficacy difference between MA and EA in the treatment of neuropathic pain. MA involves inserting an acupuncture needle into an acupuncture point and then twisting it up and down with the hand. EA delivers an electric current to the acupoint through an inserted needle. In terms of "needling feeling," EA is often described as painful and numb, while MA is dominated by heaviness and distension in the deep tissue beneath the acupuncture point. MA activated all types of afferent fibers (Aβ, Aδ, and C) (Kagitani et al., 2010). The current intensity of EA was sufficient to excite Aβ-and some Aδ-fibers inducing analgesic effects (Zhao, 2008). Reason for this difference might be that MA and EA activated distinct ion-channels, but this inference had not been confirmed. In clinical practice, the intense intensity of EA was not suitable for patient analgesia, because the excitation of C fibers by synchronous strong electrical pulses would inevitably cause unbearable pain. The parameters of the EA could be precisely characterized. A study characterized the generation and transmission of electrical signals in Aβ-and some Aδ-fibers induced by acupuncture-like stimuli. EA in frequency-specific modes (2/15 Hz or 2/100 Hz) best mimicked MA (Huo R. et al., 2020). The efficacy of MA and EA also may be influenced by disease state, acupuncture duration, acupuncture parameters and acupoints. Therefore, clarifying the analgesic mechanisms of MA and EA and selecting the appropriate acupuncture modality are essential to improve clinical efficacy.

The frequency-dependent study for analgesia at high-and low-frequency highlighted the best operating parameters. Low-frequency (2 Hz) EA caused the release of neuropeptides such as encephalin and endorphin, which acedt on mu and/or delta opioid receptors to mediate analgesia; high-frequency (100 Hz) EA caused the release of dynorphin, which was mediated by kappa opioid receptors to mediate analgesia. Certain brain regions have been found to be associated with the release of various types of central opioid peptides (Zhang et al., 2003), but it was unclear how these brain regions were modulated by the 2 Hz and 100 Hz EA, respectively. The studies screened frequencies of EA, and the results indicated that in rats with neuropathic pain, 2 Hz EA induced a robust and longer lasting analgesic than 100 Hz EA (He et al., 2017; Xia et al., 2019). In type 2 diabetic neuropathic pain rat, EA at both 2, and 100 Hz down-regulated CGRP (Calcitonin gene related peptide) and P2X3 receptors overexpression in DRGs, but the analgesic effect of EA was stronger at 2 Hz (He et al., 2017). Another study showed that compared with 100 Hz EA, 2 Hz EA effectively regulated the expression level of genes in the arcuate nucleus region of the hypothalamus, especially those related to neurogenesis (Wang et al., 2012).

Acupoints have a characteristic that they become sensitive and even painful when exposed to pathological processes (Zhou and Benharash, 2014). The analysis of anatomical have revealed that acupoints have a number of elements such as a high density of nerve endings, A-and C-afferent fibers and vascular, which could perceive stimulation (Li et al., 2004). When stimulating acupoints, the local of acupoints may release biomolecules to exert the role of analgesia or neuromodulation. Acupuncture stimulates the somatic afferent nerves of the skin and muscles under the acupoints. Then, the somatic sensory information is carried to the spinal cord and cortex area of the brain that modulate spinal signal transmission and pain perception in the brain (Wang et al., 2008). Therefore, acupuncture analgesia was essentially a manifestation of integrative processes at different levels of the nervous system between afferent impulses from the pain regions and impulses from acupoints. The infiltration of procaine, a local anesthetic, into the deep tissues around the point of acupuncture entirely abolished the analgesic effect, suggesting that nerves was mediators of this response (Zhou and Benharash, 2014). The selection of acupoints may make the effect of acupuncture more targeted in different diseases and different pain sites. But there are no studies to explain in the treatment of pain why acupoints are effective and non-acupoints are not, or why this acupoint is effective and other acupoints are not. Therefore, the specificity of the acupoints should be studied further.

Acupuncture has been used to treat neuropathic pain caused by different diseases. In addition to nerve injury-induced peripheral neuropathic pain, a meta-analysis showed that benefit for acupuncture over control in the treatment of neuropathic pain caused by diabetes, human immunodeficiency virus (HIV), Bell's palsy, and carpal tunnel syndrome (Dimitrova et al., 2017). Here, it should be pointed out that diabetic neuropathy, a major complication of diabetes mellitus, refers to a collection of clinically diverse disorders affecting the nervous system. Despite most of diabetes peripheral neuropathy is characterized by hypoesthesia, it also may present with pain. Of all diabetic peripheral neuropathy patients, 20% develop neuropathic pain (Sloan et al., 2018). Acupuncture was

considered as a treatment option for diabetic neuropathic pain. Zusanli (ST 36), Feishu (BL 13), Pishu (BL 20), Sanyinjiao (SP 6), and Yinlingquan (SP 9) were the most widely used acupoints (Cho and Kim, 2021). And acupuncture could have a beneficial effect on neurological and motor function recovery (Fan et al., 2018). Previous research indicated that acupuncture also appeared to improve motor and sensory nerve conduction parameters, curing the disease from both sensory and functional aspects (Dimitrova et al., 2017). In spinal cord injury patients, acupuncture could be useful to improve pain and other complications if patients experience side effects or have no (or a weak) response to a conventional treatment (Heo et al., 2013). However, the analgesic effect of acupuncture on neuropathic pain induced by spinal cord injury has an obvious selectivity, which depends on the location and type of pain, as well as the type of injury (Siddall and Middleton, 2015). Acupuncture recipients with incomplete damage to central nervous system pathways that remained intact appeared to recover better than those with complete damage, and patients with musculoskeletal pain responded better to treatment compared with those with central pain. In addition, participants with moderate pain were more likely to achieve long-term pain relief than those suffering from severe pain (Mehta et al., 2013).

In clinical and preclinical models, some forms of neuropathic pain persist as a result of sympathetic nerve activity, and local sympathetic blockade or lesion is used to treat it (Xie et al., 2016, 2020). After peripheral nerve injury, sympathetic neurons sprout within DRG, sensitizing nociceptive neurons to adrenergic stimulation, although this remains controversial (Hoffman et al., 2018). A new study provided evidence that sympathetic sprouting in the DRG played a role in spontaneous pain in the SNI and related neuropathic pain models (Zheng et al., 2022). They concluded that norepinephrine released from sympathetic induced DRG neuronal clustering discharge, which correlated directly with spontaneous pain behavior caused by nerve injury. Research has shown that acupuncture reduced sympathetic nerve hyperactivity (Li et al., 2009). However, EA for neuropathic pain by modulating sympathetic nerves has not been studied.

Summary and future directions

In neuropathic pain conditions, acupuncture may improve pain through somatosensory system including both central and peripheral mechanism. In the periphery, acupuncture inhibited neuronal activity caused by neuropathic pain, through reducing pain-related ion channels and receptors activation (**Figure 5**). In the spinal dorsal horn, EA down-regulated the neuronal chemokine CX3CL1 and prevented microglial p38 MAPK/ERK signaling pathway, leading to reduced release of TNF-α and IL-1β, BDNF, PGE2, and reducing the neuronal excitability and inflammatory response (**Figure 6**). Furthermore, acupuncture activated descending pain control system (**Figure 7**). The cumulative evidence demonstrated that acupuncture provided an alternative or adjunctive therapy for neuropathic pain.

In traditional Chinese medicine, the choice of appropriate acupoints is the key to acupuncture treatment. Intensities, frequencies, and the course of treatment of acupuncture all affect analgesic effects. However, the differences between MA and EA, the effects of EA frequency on relevant brain regions and the possible systematic differences between acupoint and non-acupoint are currently unknown. Conducting these studies in the future will provide better evidence to guide clinical on acupuncture modalities, acupuncture parameters and acupoints for the treatment of neuropathic pain. In the light of addressing the above issues, future studies shall be conducted in a broader context. High-quality, multifaceted basic research that explores the mechanisms of acupuncture analgesia will provide more possibilities for pain management.

Author contributions

XM, H-PL, and C-ZL put forward the idea of performing the review. XM wrote the initial manuscript. H-PL and C-ZL revised and edited the manuscript. WC and X-WH draw the manuscript. N-NY, LW, and C-XT summarized the tables. All authors have approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Alba-Delgado, C., Mountadem, S., Mermet-Joret, N., Monconduit, L., Dallel, R., Artola, A., et al. (2018). 5-HT2A receptor-induced morphological reorganization of PKCγ-expressing interneurons gates inflammatory mechanical allodynia in rat. *J. Neurosci.* 38, 10489–10504. doi: 10.1523/JNEUROSCI.1294-18.2018

Alhadeff, A. L., Su, Z., Hernandez, E., Klima, M. L., Phillips, S. Z., Holland, R. A., et al. (2018). A neural circuit for the suppression of pain by a competing need state. *Cell* 173, 140–152.e15. doi: 10.1016/j.cell.2018.02.057

Apkarian, A. V., Bushnell, M. C., Treede, R.-D., and Zubieta, J.-K. (2005). Human brain mechanisms of pain perception and regulation in health and disease. *Eur. J. Pain.* 9, 463–484. doi: 10.1016/j.ejpain.2004.11.001

Bai, H.-H., Liu, J.-P., Yang, L., Zhao, J.-Y., Suo, Z.-W., Yang, X., et al. (2017). Adenosine A1 receptor potentiated glycinergic transmission in spinal cord dorsal horn of rats after peripheral inflammation. *Neuropharmacology* 126, 158–167. doi: 10.1016/j.neuropharm.2017.09.001

Bai, L., Qin, W., Tian, J., Liu, P., Li, L., Chen, P., et al. (2009). Time-varied characteristics of acupuncture effects in fMRI studies. *Hum. Brain Mapp.* 30, 3445–3460. doi: 10.1002/hbm.20769

Bannister, K., Sachau, J., Baron, R., and Dickenson, A. H. (2020). Neuropathic pain: Mechanism-based therapeutics. *Annu. Rev. Pharmacol. Toxicol.* 60, 257–274. doi: 10.1146/annurev-pharmtox-010818-021524

Barnes, P. M., Bloom, B., and Nahin, R. L. (2008). Complementary and alternative medicine use among adults and children: United States, 2007. *Natl. Health Stat. Rep.* 12, 1–23.

Basso, L., and Altier, C. (2017). Transient receptor potential channels in neuropathic pain. *Curr. Opin. Pharmacol.* 32, 9–15. doi: 10.1016/j.coph.2016.10.002

Becker, H., Stuifbergen, A. K., Schnyer, R. N., Morrison, J. D., and Henneghan, A. (2017). Integrating acupuncture within a wellness intervention for women with multiple sclerosis. *J. Holist. Nurs.* 35, 86–96. doi: 10.1177/0898010116644833

Bennett, D. L., Clark, A. J., Huang, J., Waxman, S. G., and Dib-Hajj, S. D. (2019). The role of voltage-gated sodium channels in pain signaling. *Physiol. Rev.* 99, 1079–1151. doi: 10.1152/physrev.00052.2017

Bouhassira, D. (2019). Neuropathic pain: Definition, assessment and epidemiology. *Revue Neurologique* 175, 16–25. doi: 10.1016/j.neurol.2018.09.016

Bouhassira, D., Lantéri-Minet, M., Attal, N., Laurent, B., and Touboul, C. (2008). Prevalence of chronic pain with neuropathic characteristics in the general population. *Pain* 136, 380–387. doi: 10.1016/j.pain.2007.08.013

Cao, X. (2002). Scientific bases of acupuncture analgesia. *Acupunct. Electro Therap. Res.* 27, 1–14. doi: 10.3727/036012902816026103

Cappoli, N., Tabolacci, E., Aceto, P., and Dello Russo, C. (2020). The emerging role of the BDNF-TrkB signaling pathway in the modulation of pain perception. *J. Neuroimmunol.* 349, 577406. doi: 10.1016/j.jneuroim.2020.577406

Chang, F.-C., Tsai, H.-Y., Yu, M.-C., Yi, P.-L., and Lin, J.-G. (2004). The central serotonergic system mediates the analgesic effect of electroacupuncture on ZUSANLI (ST36) acupoints. *J. Biomed. Sci.* 11, 179–185. doi: 10.1007/BF02256561

Cho, E., and Kim, W. (2021). Effect of acupuncture on diabetic neuropathy: A narrative review. *Int. J. Mol. Sci.* 22:8575. doi: 10.3390/ijms22168575

Choi, D. C., Lee, J. Y., Lim, E. J., Baik, H. H., Oh, T. H., and Yune, T. Y. (2012). Inhibition of ROS-induced p38MAPK and ERK activation in microglia by acupuncture relieves neuropathic pain after spinal cord injury in rats. *Exp. Neurol.* 236, 268–282. doi: 10.1016/j.expneurol.2012.05.014

Choi, I. S., Cho, J.-H., An, C.-H., Jung, J.-K., Hur, Y.-K., Choi, J.-K., et al. (2012). 5-HT(1B) receptors inhibit glutamate release from primary afferent terminals in rat medullary dorsal horn neurons. *Br. J. Pharmacol.* 167, 356–367. doi: 10.1111/j.1476-5381.2012.01964.x

Choi, S.-R., Beitz, A. J., and Lee, J.-H. (2019). Inhibition of cytochrome P450c17 reduces spinal astrocyte activation in a mouse model of neuropathic pain via regulation of p38 MAPK phosphorylation. *Biomed. Pharmacother.* 118:109299. doi: 10.1016/j.biopha.2019.109299

Choi, Y.-J., Lee, J.-E., Moon, W.-K., and Cho, S.-H. (2013). Does the effect of acupuncture depend on needling sensation and manipulation? *Complement. Ther. Med.* 21, 207–214. doi: 10.1016/j.ctim.2012.12.009

Cidral-Filho, F. J., da Silva, M. D., Moré, A. O. O., Córdova, M. M., Werner, M. F., and Santos, A. R. S. (2011). Manual acupuncture inhibits mechanical hypersensitivity induced by spinal nerve ligation in rats. *Neuroscience* 193, 370–376. doi: 10.1016/j.neuroscience.2011.07.076

Colleoni, M., and Sacerdote, P. (2010). Murine models of human neuropathic pain. *Biochim. Biophys. Acta* 1802, 924–933. doi: 10.1016/j.bbadis.2009.10.012

Comachio, J., Oliveira, C. C., Silva, I. F. R., Magalhães, M. O., and Marques, A. P. (2020). Effectiveness of manual and electrical acupuncture for chronic non-specific low back pain: A randomized controlled trial. *J. Acupunct. Meridian Stud.* 13, 87–93. doi: 10.1016/j.jams.2020.03.064

Cui, Y., Wang, F., Li, H., Zhang, X., Zhao, X., and Wang, D. (2021). Efficacy of acupuncture for herpes zoster: A systematic review and meta-analysis. *Complement. Med. Res.* 28, 463–472. doi: 10.1159/000515138

da Silva, J. R. T., da Silva, M. L., and Prado, W. A. (2013). Electroacupuncture at 2/100?hz activates antinociceptive spinal mechanisms different from those activated by electroacupuncture at 2 and 100?hz in responder rats. *Evid. Based Complement. Alternat. Med.* 2013:205316. doi: 10.1155/2013/205316

Dai, Q.-X., Huang, L.-P., Mo, Y.-C., Yu, L.-N., Du, W.-W., Zhang, A.-Q., et al. (2020). Role of spinal adenosine A1 receptors in the analgesic effect of electroacupuncture in a rat model of neuropathic pain. *J. Int. Med. Res.* 48:300060519883748. doi: 10.1177/0300060519883748

Dai, W.-J., Sun, J.-L., Li, C., Mao, W., Huang, Y.-K., Zhao, Z.-Q., et al. (2019). Involvement of interleukin-10 in analgesia of electroacupuncture on incision pain. *Evid. Based Complement. Alternat. Med.* 2019:8413576. doi: 10.1155/2019/8413576

Deng, Y., Yang, L., Xie, Q., Yang, F., Li, G., Zhang, G., et al. (2020). Protein kinase a is involved in neuropathic pain by activating the p38MAPK pathway to mediate spinal cord cell apoptosis. *Mediat. Inflamm.* 2020:6420425. doi: 10.1155/2020/6420425

Dib-Hajj, S. D., and Waxman, S. G. (2019). Sodium channels in human pain disorders: Genetics and pharmacogenomics. *Annu. Rev. Neurosci.* 42, 87–106. doi: 10.1146/annurev-neuro-070918-050144

Dimitrova, A., Murchison, C., and Oken, B. (2017). Acupuncture for the treatment of peripheral neuropathy: A systematic review and meta-analysis. *J. Alternat. Complement. Med.* 23, 164–179. doi: 10.1089/acm.2016.0155

Djouhri, L., Smith, T., Ahmeda, A., Alotaibi, M., and Weng, X. (2018). Hyperpolarization-activated cyclic nucleotide-gated channels contribute to spontaneous activity in L4 C-fiber nociceptors, but not Aβ-non-nociceptors, after axotomy of L5-spinal nerve in the rat in vivo. *Pain* 159, 1392–1402. doi: 10.1097/j.pain.0000000000001224

Du, J., Fang, J., Xiang, X., Yu, J., Le, X., Liang, Y., et al. (2021). Effects of low- and high-frequency electroacupuncture on protein expression and distribution of TRPV1 and P2X3 in rats with peripheral nerve injury. *Acupunct. Med.* 39, 478–490. doi: 10.1177/0964528420968845

Edwards, J. W., and Shaw, V. (2021). Acupuncture in the management of trigeminal neuralgia. *Acupunct. Med.* 39, 192–199. doi: 10.1177/0964528420924042

Eippert, F., Bingel, U., Schoell, E. D., Yacubian, J., Klinger, R., Lorenz, J., et al. (2009). Activation of the opioidergic descending pain control system underlies placebo analgesia. *Neuron* 63, 533–543. doi: 10.1016/j.neuron.2009.07.014

Emery, E. C., Young, G. T., Berrocoso, E. M., Chen, L., and McNaughton, P. A. (2011). HCN2 ion channels play a central role in inflammatory and neuropathic pain. *Science* 333, 1462–1466. doi: 10.1126/science.1206243

Fan, Q., Cavus, O., Xiong, L., and Xia, Y. (2018). Spinal cord injury: How could acupuncture help? *J. Acupunct. Merid. Stud.* 11, 124–132. doi: 10.1016/j.jams.2018.05.002

Fang, J., Du, J., Xiang, X., Shao, X., He, X., Jiang, Y., et al. (2021). SNI and CFA induce similar changes in TRPV1 and P2X3 expressions in the acute phase but not in the chronic phase of pain. *Exp. Brain Res.* 239, 983–995. doi: 10.1007/s00221-020-05988-4

Fang, L., Wu, J., Lin, Q., and Willis, W. D. (2002). Calcium-calmodulin-dependent protein kinase II contributes to spinal cord central sensitization. *J. Neurosci.* 22, 4196–4204.

Fei, X., He, X., Tai, Z., Wang, H., Qu, S., Chen, L., et al. (2020). Electroacupuncture alleviates diabetic neuropathic pain in rats by suppressing P2X3 receptor expression in dorsal root ganglia. *Purinergic Signal.* 16, 491–502. doi: 10.1007/s11302-020-09728-9

Finnerup, N. B., Attal, N., Haroutounian, S., McNicol, E., Baron, R., Dworkin, R. H., et al. (2015). Pharmacotherapy for neuropathic pain in adults: A systematic review and meta-analysis. *Lancet Neurol.* 14, 162–173. doi: 10.1016/S1474-4422(14)70251-0

Finnerup, N. B., Haroutounian, S., Kamerman, P., Baron, R., Bennett, D. L. H., Bouhassira, D., et al. (2016). Neuropathic pain: An updated grading system

for research and clinical practice. *Pain* 157, 1599–1606. doi: 10.1097/j.pain.0000000000000492

François, A., Low, S. A., Sypek, E. I., Christensen, A. J., Sotoudeh, C., Beier, K. T., et al. (2017). A brainstem-spinal cord inhibitory circuit for mechanical pain modulation by GABA and enkephalins. *Neuron* 93, 822–839.e6. doi: 10.1016/j.neuron.2017.01.008

Fusumada, K., Yokoyama, T., Miki, T., Wang, Z.-Y., Yang, W., Lee, N.-S., et al. (2007). C-Fos expression in the periaqueductal gray is induced by electroacupuncture in the rat, with possible reference to GABAergic neurons. *Okajimas Folia Anatomica Japonica* 84, 1–9. doi: 10.2535/ofaj.84.1

Gao, J., Zhao, C., Jiang, W., Zheng, B., and He, Y. (2019). Effect of acupuncture on cognitive function and quality of life in patients with idiopathic trigeminal neuralgia. *J. Nerv. Ment. Dis.* 207, 171–174. doi: 10.1097/NMD.0000000000000937

Gim, G.-T., Lee, J.-H., Park, E., Sung, Y.-H., Kim, C.-J., Hwang, W.-W., et al. (2011). Electroacupuncture attenuates mechanical and warm allodynia through suppression of spinal glial activation in a rat model of neuropathic pain. *Brain Res. Bull.* 86, 403–411. doi: 10.1016/j.brainresbull.2011.09.010

Goldman, N., Chen, M., Fujita, T., Xu, Q., Peng, W., Liu, W., et al. (2010). Adenosine A1 receptors mediate local anti-nociceptive effects of acupuncture. *Nat. Neurosci.* 13, 883–888. doi: 10.1038/nn.2562

Gomez-Ramirez, M., Hysaj, K., and Niebur, E. (2016). Neural mechanisms of selective attention in the somatosensory system. *J. Neurophysiol.* 116, 1218–1231. doi: 10.1152/jn.00637.2015

Groc, L., and Choquet, D. (2006). AMPA and NMDA glutamate receptor trafficking: Multiple roads for reaching and leaving the synapse. *Cell Tissue Res.* 326, 423–438. doi: 10.1007/s00441-006-0254-9

Han, J.-S. (2003). Acupuncture: Neuropeptide release produced by electrical stimulation of different frequencies. *Trends Neurosci.* 26, 17–22. doi: 10.1016/s0166-2236(02)00006-1

Han, J.-S. (2004). Acupuncture and endorphins. *Neurosci. Lett.* 361, 258–261. doi: 10.1016/j.neulet.2003.12.019

He, X.-F., Wei, J.-J., Shou, S.-Y., Fang, J.-Q., and Jiang, Y.-L. (2017). Effects of electroacupuncture at 2 and 100 Hz on rat type 2 diabetic neuropathic pain and hyperalgesia-related protein expression in the dorsal root ganglion. *J. Zhejiang Univ. Sci. B* 18, 239–248. doi: 10.1631/jzus.B1600247

Held, M., Karl, F., Vlckova, E., Rajdova, A., Escolano-Lozano, F., Stetter, C., et al. (2019). Sensory profiles and immune-related expression patterns of patients with and without neuropathic pain after peripheral nerve lesion. *Pain* 160, 2316–2327. doi: 10.1097/j.pain.0000000000001623

Heo, I., Shin, B.-C., Kim, Y.-D., Hwang, E.-H., Han, C. W., and Heo, K.-H. (2013). Acupuncture for spinal cord injury and its complications: A systematic review and meta-analysis of randomized controlled trials. *Evid. Based Complement. Alternat. Med.* 2013:364216. doi: 10.1155/2013/364216

Hildebrand, M. E., Xu, J., Dedek, A., Li, Y., Sengar, A. S., Beggs, S., et al. (2016). Potentiation of synaptic GluN2B NMDAR currents by fyn kinase is gated through BDNF-mediated disinhibition in spinal pain processing. *Cell Rep.* 17, 2753–2765. doi: 10.1016/j.celrep.2016.11.024

Hoffman, B. U., Baba, Y., Griffith, T. N., Mosharov, E. V., Woo, S.-H., Roybal, D. D., et al. (2018). Merkel cells activate sensory neural pathways through adrenergic synapses. *Neuron* 100, 1401–1413.e6. doi: 10.1016/j.neuron.2018.10.034

Hoffmann, T., Sauer, S. K., Horch, R. E., and Reeh, P. W. (2008). Sensory transduction in peripheral nerve axons elicits ectopic action potentials. *J. Neurosci.* 28, 6281–6284. doi: 10.1523/JNEUROSCI.1627-08.2008

Hou, A.-L., Zheng, M.-X., Hua, X.-Y., Huo, B.-B., Shen, J., and Xu, J.-G. (2020). Electroacupuncture-related metabolic brain connectivity in neuropathic pain due to brachial plexus avulsion injury in rats. *Front. Neural Circuits* 14:35. doi: 10.3389/fncir.2020.00035

Hu, H., Chen, L., Ma, R., Gao, H., and Fang, J. (2019). Acupuncture for primary trigeminal neuralgia: A systematic review and PRISMA-compliant meta-analysis. *Complement. Ther. Clin. Pract.* 34, 254–267. doi: 10.1016/j.ctcp.2018.12.013

Hu, Q.-Q., He, X.-F., Ma, Y.-Q., Ma, L.-Q., Qu, S.-Y., Wang, H.-Z., et al. (2022). Dorsal root ganglia P2X4 and P2X7 receptors contribute to diabetes-induced hyperalgesia and the downregulation of electroacupuncture on P2X4 and P2X7. *Purinergic Signal.* [Epub ahead of print]. doi: 10.1007/s11302-022-09844-8

Huang, C., Huang, Z.-Q., Hu, Z.-P., Jiang, S.-Z., Li, H.-T., Han, J.-S., et al. (2008). Electroacupuncture effects in a rat model of complete Freund's adjuvant-induced inflammatory pain: Antinociceptive effects enhanced and tolerance development accelerated. *Neurochem. Res.* 33, 2107–2111. doi: 10.1007/s11064-008-9721-x

Huang, C. P., Lin, Y.-W., Lee, D.-Y., and Hsieh, C.-L. (2019). Electroacupuncture relieves CCI-induced neuropathic pain involving excitatory and inhibitory neurotransmitters. *Evid. Based Complement. Alternat. Med.* 2019:6784735. doi: 10.1155/2019/6784735

Huang, L., Xu, G., He, J., Tian, H., Zhou, Z., Huang, F., et al. (2021). Bibliometric analysis of functional magnetic resonance imaging studies on acupuncture analgesia over the past 20 years. *J. Pain Res.* 14, 3773–3789. doi: 10.2147/JPR.S340961

Huang, Z., Liu, S., Zhou, J., Yao, Q., and Liu, Z. (2019). Efficacy and safety of acupuncture for chronic discogenic sciatica, a randomized controlled sham acupuncture trial. *Pain Med.* 20, 2303–2310. doi: 10.1093/pm/pnz167

Hui, K. K. S., Liu, J., Marina, O., Napadow, V., Haselgrove, C., Kwong, K. K., et al. (2005). The integrated response of the human cerebro-cerebellar and limbic systems to acupuncture stimulation at ST 36 as evidenced by fMRI. *Neuroimage* 27, 479–496. doi: 10.1016/j.neuroimage.2005.04.037

Huo, B.B., Zheng, M.-X., Hua, X.-Y., Shen, J., Wu, J.-J., and Xu, J.-G. (2020). Brain metabolism in rats with neuropathic pain induced by brachial plexus avulsion injury and treated via electroacupuncture. *J. Pain Res.* 13, 585–595. doi: 10.2147/JPR.S232030

Huo, R., Han, S.-P., Liu, F.-Y., Shou, X.-J., Liu, L.-Y., Song, T.-J., et al. (2020). Responses of primary afferent fibers to acupuncture-like peripheral stimulation at different frequencies: Characterization by single-unit recording in rats. *Neurosci. Bull.* 36, 907–918. doi: 10.1007/s12264-020-00509-3

Inoue, K., and Tsuda, M. (2018). Microglia in neuropathic pain: Cellular and molecular mechanisms and therapeutic potential. *Nat. Rev. Neurosci.* 19, 138–152. doi: 10.1038/nrn.2018.2

Iravani, S., Kazemi Motlagh, A. H., Emami Razavi, S. Z., Shahi, F., Wang, J., Hou, L., et al. (2020). Effectiveness of acupuncture treatment on chemotherapy-induced peripheral neuropathy: A pilot, randomized, assessor-blinded, controlled trial. *Pain Res. Manag.* 2020:2504674. doi: 10.1155/2020/2504674

Jang, J.-H., Song, E.-M., Do, Y.-H., Ahn, S., Oh, J.-Y., Hwang, T.-Y., et al. (2021). Acupuncture alleviates chronic pain and comorbid conditions in a mouse model of neuropathic pain: The involvement of DNA methylation in the prefrontal cortex. *Pain* 162, 514–530. doi: 10.1097/j.pain.0000000000002031

Jensen, T. S., Baron, R., Haanpää, M., Kalso, E., Loeser, J. D., Rice, A. S. C., et al. (2011). A new definition of neuropathic pain. *Pain* 152, 2204–2205. doi: 10.1016/j.pain.2011.06.017

Ji, R. R., Kawasaki, Y., Zhuang, Z. Y., Wen, Y. R., and Zhang, Y. Q. (2007). Protein kinases as potential targets for the treatment of pathological pain. *Handbook Exp. Pharmacol.* 177, 359–389. doi: 10.1007/978-3-540-33823-9_13

Ji, R.-R., Chamessian, A., and Zhang, Y.-Q. (2016). Pain regulation by non-neuronal cells and inflammation. *Science* 354, 572–577. doi: 10.1126/science.aaf8924

Jiang, M., Chen, X., Zhang, L., Liu, W., Yu, X., Wang, Z., et al. (2021). Electroacupuncture suppresses glucose metabolism and GLUT-3 expression in medial prefrontal cortical in rats with neuropathic pain. *Biol. Res.* 54:24. doi: 10.1186/s40659-021-00348-0

Jiang, S.-W., Lin, Y.-W., and Hsieh, C.-L. (2018). Electroacupuncture at Hua Tuo Jia Ji acupoints reduced neuropathic pain and increased GABAA receptors in rat spinal cord. *Evid. Based Complement. Alternat. Med.* 2018:8041820. doi: 10.1155/2018/8041820

Jiang, Y.-L., Yin, X.-H., Shen, Y.-F., He, X.-F., and Fang, J.-Q. (2013). Low frequency electroacupuncture alleviated spinal nerve ligation induced mechanical allodynia by inhibiting TRPV1 upregulation in ipsilateral undamaged dorsal root ganglia in rats. *Evid. Based Complement. Alternat. Med.* 2013:170910. doi: 10.1155/2013/170910

Jin, Y., Zhou, J., Xu, F., Ren, Z., Hu, J., Zhang, C., et al. (2021). Electroacupuncture alleviates the transition from acute to chronic pain through the p38 MAPK/TNF-α signalling pathway in the spinal dorsal horn. *Acupunct. Med.* 39, 708–715. doi: 10.1177/09645284211020766

Jung, T. G., Lee, J. H., Lee, I. S., and Choi, B. T. (2010). Involvement of intracellular calcium on the phosphorylation of spinal N-methyl-D-aspartate receptor following electroacupuncture stimulation in rats. *Acta Histochem.* 112, 127–132. doi: 10.1016/j.acthis.2008.09.009

Kagitani, F., Uchida, S., and Hotta, H. (2010). Afferent nerve fibers and acupuncture. *Autonom. Neurosci. Basic Clin.* 157, 2–8. doi: 10.1016/j.autneu.2010.03.004

Kelly, R. B., and Willis, J. (2019). Acupuncture for pain. *Am. Fam. Phys.* 100, 89–96.

Khan, A., Khan, S., and Kim, Y. S. (2019). Insight into pain modulation: Nociceptors sensitization and therapeutic targets. *Curr. Drug Targ.* 20, 775–788. doi: 10.2174/1389450120666190131114244

Kim, J. H., Min, B.-I., Na, H. S., and Park, D. S. (2004). Relieving effects of electroacupuncture on mechanical allodynia in neuropathic pain model of inferior caudal trunk injury in rat: Mediation by spinal opioid receptors. *Brain Res.* 998, 230–236. doi: 10.1016/j.brainres.2003.11.045

Kim, S. K., Park, J. H., Bae, S. J., Kim, J. H., Hwang, B. G., Min, B.-I., et al. (2005). Effects of electroacupuncture on cold allodynia in a rat model of neuropathic pain: Mediation by spinal adrenergic and serotonergic receptors. *Exp. Neurol.* 195, 430–436. doi: 10.1016/j.expneurol.2005.06.018

Kiyoyuki, Y., Taniguchi, W., Okubo, M., Yamanaka, H., Kobayashi, K., Nishio, N., et al. (2015). Leukotriene enhances NMDA-induced inward currents in dorsal horn neurons of the rat spinal cord after peripheral nerve injury. *Mol. Pain* 11:53. doi: 10.1186/s12990-015-0059-5

Kondo, M., and Shibuta, I. (2020). Extracellular signal-regulated kinases (ERK) 1 and 2 as a key molecule in pain research. *J. Oral Sci.* 62, 147–149. doi: 10.2334/josnusd.19-0470

Koo, S. T., Lim, K. S., Chung, K., Ju, H., and Chung, J. M. (2008). Electroacupuncture-induced analgesia in a rat model of ankle sprain pain is mediated by spinal alpha-adrenoceptors. *Pain* 135, 11–19. doi: 10.1016/j.pain.2007.04.034

Kuner, R., and Flor, H. (2016). Structural plasticity and reorganisation in chronic pain. *Nat. Rev. Neurosci.* 18, 20–30. doi: 10.1038/nrn.2016.162

Laedermann, C. J., Cachemaille, M., Kirschmann, G., Pertin, M., Gosselin, R.-D., Chang, I., et al. (2013). Dysregulation of voltage-gated sodium channels by ubiquitin ligase NEDD4-2 in neuropathic pain. *J. Clin. Invest.* 123, 3002–3013. doi: 10.1172/JCI68996

Laumet, G., Garriga, J., Chen, S.-R., Zhang, Y., Li, D.-P., Smith, T. M., et al. (2015). G9a is essential for epigenetic silencing of K(+) channel genes in acute-to-chronic pain transition. *Nat. Neurosci.* 18, 1746–1755. doi: 10.1038/nn.4165

Lee, J. Y., Choi, D. C., Oh, T. H., and Yune, T. Y. (2013). Analgesic effect of acupuncture is mediated via inhibition of JNK activation in astrocytes after spinal cord injury. *PLoS One* 8:e73948. doi: 10.1371/journal.pone.0073948

Lee, S., Kim, J.-H., Shin, K.-M., Kim, J.-E., Kim, T.-H., Kang, K.-W., et al. (2013). Electroacupuncture to treat painful diabetic neuropathy: Study protocol for a three-armed, randomized, controlled pilot trial. *Trials* 14:225. doi: 10.1186/1745-6215-14-225

Lee, S., Lee, C.-S., Moon, J. Y., Song, H.-G., Yoo, Y., Kim, J., et al. (2020). Electroacupuncture may improve burning and electric shock-like neuropathic pain: A prospective exploratory pilot study. *J. Alternat. Complement. Med.* 26, 1136–1143. doi: 10.1089/acm.2020.0307

Lemmon, R. (2018). Acupuncture for pain: 7 questions answered. *J. Fam. Pract.* 67, 224;226;228;230

Li, A.-H., Zhang, J.-M., and Xie, Y.-K. (2004). Human acupuncture points mapped in rats are associated with excitable muscle/skin-nerve complexes with enriched nerve endings. *Brain Res.* 1012, 154–159. doi: 10.1016/j.brainres.2004.04.009

Li, C., Yang, J., Sun, J., Xu, C., Zhu, Y., Lu, Q., et al. (2013). Brain responses to acupuncture are probably dependent on the brain functional status. *Evid. Based Complement. Alternat. Med.* 2013:175278. doi: 10.1155/2013/175278

Li, P., Tjen-A-Looi, S. C., Guo, Z.-L., Fu, L.-W., and Longhurst, J. C. (2009). Long-loop pathways in cardiovascular electroacupuncture responses. *J. Appl. Physiol.* 106, 620–630. doi: 10.1152/japplphysiol.91277.2008

Li, X., and Eisenach, J. C. (2001). Alpha2A-adrenoceptor stimulation reduces capsaicin-induced glutamate release from spinal cord synaptosomes. *J. Pharmacol. Exp. Therap.* 299, 939–944.

Li, Y., Yin, C., Li, X., Liu, B., Wang, J., Zheng, X., et al. (2019b). Electroacupuncture alleviates paclitaxel-induced peripheral neuropathic pain in rats via suppressing TLR4 signaling and TRPV1 upregulation in sensory neurons. *Int. J. Mol. Sci.* 20:E5917. doi: 10.3390/ijms20235917

Li, Y., Fang, Z., Gu, N., Bai, F., Ma, Y., Dong, H., et al. (2019a). Inhibition of chemokine CX3CL1 in spinal cord mediates the electroacupuncture-induced suppression of inflammatory pain. *J. Pain Res.* 12, 2663–2672. doi: 10.2147/JPR.S205987

Liang, Y., Qiu, Y., Du, J., Liu, J., Fang, J., Zhu, J., et al. (2016). Inhibition of spinal microglia and astrocytes contributes to the anti-allodynic effect of electroacupuncture in neuropathic pain induced by spinal nerve ligation. *Acupunct. Med.* 34, 40–47. doi: 10.1136/acupmed-2015-010773

Lin, J.-G., and Chen, W.-L. (2008). Acupuncture analgesia: A review of its mechanisms of actions. *Am. J. Chin. Med.* 36, 635–645. doi: 10.1142/S0192415X08006107

Lin, X., Wang, M., Zhang, J., and Xu, R. (2014). p38 MAPK: A potential target of chronic pain. *Curr. Med. Chem.* 21, 4405–4418. doi: 10.2174/09298673216614091514304O

Liu, J., and Wu, Y. (2017). Electro-acupuncture-modulated miR-214 prevents neuronal apoptosis by targeting Bax and inhibits sodium channel Nav1.3 expression in rats after spinal cord injury. *Biomed. Pharmacother.* 89, 1125–1135. doi: 10.1016/j.biopha.2017.02.077

Liu, S., Zhang, C. S., Cai, Y., Guo, X., Zhang, A. L., Xue, C. C., et al. (2019). Acupuncture for post-stroke shoulder-hand syndrome: A systematic review and meta-analysis. *Front. Neurol.* 10:433. doi: 10.3389/fneur.2019.00433

Liu, Y., Latremoliere, A., Li, X., Zhang, Z., Chen, M., Wang, X., et al. (2018). Touch and tactile neuropathic pain sensitivity are set by corticospinal projections. *Nature* 561, 547–550. doi: 10.1038/s41586-018-0515-2

Lovaglio, A. C., Socolovsky, M., Di Masi, G., and Bonilla, G. (2019). Treatment of neuropathic pain after peripheral nerve and brachial plexus traumatic injury. *Neurol. India* 67, S32–S37. doi: 10.4103/0028-3886.250699

Luo, C., Kuner, T., and Kuner, R. (2014). Synaptic plasticity in pathological pain. *Trends Neurosci.* 37, 343–355. doi: 10.1016/j.tins.2014.04.002

Ma, R., Liu, X., Clark, J., Williams, G. M., and Doi, S. A. (2015). The impact of acupuncture on neurological recovery in spinal cord injury: A systematic review and meta-analysis. *J. Neurotrauma* 32, 1943–1957. doi: 10.1089/neu.2014.3866

Ma, Y., Chen, J., Yu, D., Wei, B., Jin, H., Zeng, J., et al. (2021). CAMP-PKA signaling is involved in regulation of spinal HCN channels function in diabetic neuropathic pain. *Neurosci. Lett.* 750:135763. doi: 10.1016/j.neulet.2021.135763

Masoodifar, M., Hajihashemi, S., Pazhoohan, S., Nazemi, S., and Mojadadi, M.-S. (2021). Effect of the conditioned medium of mesenchymal stem cells on the expression levels of P2X4 and P2X7 purinergic receptors in the spinal cord of rats with neuropathic pain. *Purinergic Signal.* 17, 143–150. doi: 10.1007/s11302-020-09756-5

Mehta, S., Orenczuk, K., McIntyre, A., Willems, G., Wolfe, D. L., Hsieh, J. T. C., et al. (2013). Neuropathic pain post spinal cord injury part 1: Systematic review of physical and behavioral treatment. *Topics Spinal Cord Inj. Rehabil.* 19, 61–77. doi: 10.1310/sci1901-61

Mickle, A. D., Shepherd, A. J., and Mohapatra, D. P. (2015). Sensory TRP channels: The key transducers of nociception and pain. *Prog. Mol. Biol. Transl. Sci.* 131, 73–118. doi: 10.1016/bs.pmbts.2015.01.002

Millstine, D., Chen, C. Y., and Bauer, B. (2017). Complementary and integrative medicine in the management of headache. *BMJ (Clinical Research Ed.)* 357:j1805. doi: 10.1136/bmj.j1805

Murotani, T., Ishizuka, T., Nakazawa, H., Wang, X., Mori, K., Sasaki, K., et al. (2010). Possible involvement of histamine, dopamine, and noradrenalin in the periaqueductal gray in electroacupuncture pain relief. *Brain Res.* 1306, 62–68. doi: 10.1016/j.brainres.2009.09.117

Napadow, V., Kettner, N., Liu, J., Li, M., Kwong, K. K., Vangel, M., et al. (2007). Hypothalamus and amygdala response to acupuncture stimuli in Carpal Tunnel Syndrome. *Pain* 130, 254–266. doi: 10.1016/j.pain.2006.12.003

Nayak, S., Shiflett, S. C., Schoenberger, N. E., Agostinelli, S., Kirshblum, S., Averill, A., et al. (2001). Is acupuncture effective in treating chronic pain after spinal cord injury? *Arch. Phys. Med. Rehabil.* 82, 1578–1586. doi: 10.1053/apmr.2001.26624

Nilius, B., Owsianik, G., Voets, T., and Peters, J. A. (2007). Transient receptor potential cation channels in disease. *Physiol. Rev.* 87, 165–217. doi: 10.1152/physrev.00021.2006

Ossipov, M. H., Dussor, G. O., and Porreca, F. (2010). Central modulation of pain. *J. Clin. Invest.* 120, 3779–3787. doi: 10.1172/JCI43766

Pei, W., Zeng, J., Lu, L., Lin, G., and Ruan, J. (2019). Is acupuncture an effective postherpetic neuralgia treatment? A systematic review and meta-analysis. *J. Pain Res.* 12, 2155–2165. doi: 10.2147/JPR.S199950

Pfister, D. G., Cassileth, B. R., Deng, G. E., Yeung, K. S., Lee, J. S., Garrity, D., et al. (2010). Acupuncture for pain and dysfunction after neck dissection: Results of a randomized controlled trial. *J. Clin. Oncol.* 28, 2565–2570. doi: 10.1200/JCO.2009.26.9860

Qian, Y., Xia, T., Cui, Y., Chu, S., Ma, Z., and Gu, X. (2019). The role of CaMKII in neuropathic pain and fear memory in chronic constriction injury in rats. *Int. J. Neurosci.* 129, 146–154. doi: 10.1080/00207454.2018.1512986

Ruengwongroj, P., Muengtaweepongsa, S., Patumanond, J., and Phinyo, P. (2020). Effectiveness of press needle treatment and electroacupuncture in patients with postherpetic neuralgia: A matched propensity score analysis. *Complement. Ther. Clin. Pract.* 40:101202. doi: 10.1016/j.ctcp.2020.1020.1012021202

Scholz, J., Finnerup, N. B., Attal, N., Aziz, Q., Baron, R., Bennett, M. I., et al. (2019). The IASP classification of chronic pain for ICD-11: Chronic neuropathic pain. *Pain* 160, 53–59. doi: 10.1097/j.pain.0000000000001365

Seddighfar, M., Ghasemzadeh, Z., and Rezayof, A. (2019). The blockade of 5-HT1A receptors in the ventral tegmental area inhibited morphine/dextromethorphan-induced analgesia in pain rat models. *Brain Res.* 1715, 27–34. doi: 10.1016/j.brainres.2019.03.018

Sharif, B., Ase, A. R., Ribeiro-da-Silva, A., and Séguéla, P. (2020). Differential coding of itch and pain by a subpopulation of primary afferent neurons. *Neuron* 106, 940–951.e4. doi: 10.1016/j.neuron.2020.03.021

Siddall, P. J., and Middleton, J. W. (2015). Spinal cord injury-induced pain: Mechanisms and treatments. *Pain Manag.* 5, 493–507. doi: 10.2217/pmt.15.47

Silva, J. R. T., Silva, M. L., and Prado, W. A. (2011). Analgesia induced by 2- or 100-Hz electroacupuncture in the rat tail-flick test depends on the activation of different descending pain inhibitory mechanisms. *J. Pain* 12, 51–60. doi: 10.1016/j.jpain.2010.04.008

Silva, M. L., Silva, J. R. T., and Prado, W. A. (2013). Analgesia induced by 2- or 100-Hz electroacupuncture in the rat tail-flick test depends on the anterior pretectal nucleus. *Life Sci.* 93, 742–754. doi: 10.1016/j.lfs.2013.09.012

Sloan, G., Shillo, P., Selvarajah, D., Wu, J., Wilkinson, I. D., Tracey, I., et al. (2018). A new look at painful diabetic neuropathy. *Diabetes Res. Clin. Pract.* 144, 177–191. doi: 10.1016/j.diabres.2018.08.020

Sollie, M., Pind, R., Madsen, C. B., and Sørensen, J. A. (2022). Acupuncture (superficial dry-needling) as a treatment for chronic postherpetic neuralgia—a randomized clinical trial. *Br. J. Pain* 16, 96–108. doi: 10.1177/20494637211023075

Song, S., Xu, Y., Liu, J., Jia, Y., Lin, X., Liu, Y., et al. (2021). Strong twirling-rotating manual acupuncture with 4r/s Is superior to 2r/s in relieving pain by activating C-fibers in rat models of CFA-induced pain. *Evid. Based Complement. Alternat. Med.* 2021:5528780. doi: 10.1155/2021/5528780

Spaeth, R. B., Camhi, S., Hashmi, J. A., Vangel, M., Wasan, A. D., Edwards, R. R., et al. (2013). A longitudinal study of the reliability of acupuncture deqi sensations in knee osteoarthritis. *Evid. Based Complement. Alternat. Med.* 2013:204259. doi: 10.1155/2013/204259

Su, Y.-S., Yang, Z.-K., Xin, J.-J., He, W., Shi, H., Wang, X.-Y., et al. (2014). Somatosensory nerve fibers mediated generation of de-qi in manual acupuncture and local moxibustion-like stimuli-modulated gastric motility in rats. *Evid. Based Complement. Alternat. Med.* 2014:673239. doi: 10.1155/2014/673239

Sun, R.-Q., Wang, H.-C., and Wang, Y. (2002). [Effect of electroacupuncture with different frequencies on neuropathic pain in a rat model]. *Zhongguo Ying Yong Sheng Li Xue Za* 18, 128–131.

Sun, S., Chen, W.-L., Wang, P.-F., Zhao, Z.-Q., and Zhang, Y.-Q. (2006). Disruption of glial function enhances electroacupuncture analgesia in arthritic rats. *Exp. Neurol.* 198, 294–302. doi: 10.1016/j.expneurol.2005.11.011

Tang, Q., Jiang, Q., Sooranna, S. R., Lin, S., Feng, Y., Zhang, Q., et al. (2016). Effects of electroacupuncture on pain threshold of laboring rats and the expression of norepinephrine transporter and α2 adrenergic receptor in the central nervous system. *Evid. Based Complement. Alternat. Med.* 2016:9068257. doi: 10.1155/2016/9068257

Tang, Y., Yin, H.-Y., Rubini, P., and Illes, P. (2016). Acupuncture-induced analgesia: A neurobiological basis in purinergic signaling. *Neuroscientist* 22, 563–578. doi: 10.1177/1073858416654453

Torrance, N., Smith, B. H., Bennett, M. I., and Lee, A. J. (2006). The epidemiology of chronic pain of predominantly neuropathic origin. Results from a general population survey. *J. Pain* 7, 281–289. doi: 10.1016/j.jpain.2005.11.008

Tu, W.-Z., Li, S.-S., Jiang, X., Qian, X.-R., Yang, G.-H., Gu, P.-P., et al. (2018). Effect of electro-acupuncture on the BDNF-TrkB pathway in the spinal cord of CCI rats. *Int. J. Mol. Med.* 41, 3307–3315. doi: 10.3892/ijmm.2018.3563

Velázquez, K. T., Mohammad, H., and Sweitzer, S. M. (2007). Protein kinase C in pain: Involvement of multiple isoforms. *Pharmacol. Res.* 55, 578–589. doi: 10.1016/j.phrs.2007.04.006

Vidal-Torres, A., Carceller, A., Zamanillo, D., Merlos, M., Vela, J. M., and Fernández-Pastor, B. (2012). Evaluation of formalin-induced pain behavior and glutamate release in the spinal dorsal horn using in vivo microdialysis in conscious rats. *J. Pharmacol. Sci.* 120, 129–132. doi: 10.1254/jphs.12105sc

Vogt, B. A. (2016). Midcingulate cortex: Structure, connections, homologies, functions and diseases. *J. Chem. Neuroanat.* 74, 28–46. doi: 10.1016/j.jchemneu.2016.01.010

Vranken, J. H. (2012). Elucidation of pathophysiology and treatment of neuropathic pain. *Cent. Nerv. Syst. Agents Med. Chem.* 12, 304–314. doi: 10.2174/187152412803760645

Wang, J.-Y., Gao, Y.-H., Qiao, L.-N., Zhang, J.-L., Duan-Mu, C.-L., Yan, Y.-X., et al. (2018). Repeated electroacupuncture treatment attenuated hyperalgesia through suppression of spinal glial activation in chronic neuropathic pain rats. *BMC Complement. Alternat. Med.* 18:74. doi: 10.1186/s12906-018-2134-8

Wang, K., Zhang, R., He, F., Lin, L.-B., Xiang, X.-H., Ping, X.-J., et al. (2012). Electroacupuncture frequency-related transcriptional response in rat arcuate nucleus revealed region-distinctive changes in response to low- and high-frequency electroacupuncture. *J. Neurosci. Res.* 90, 1464–1473. doi: 10.1002/jnr.23028

Wang, L., Gao, Z., Niu, X., Yuan, M., Li, Y., Wang, F., et al. (2020). Acupuncture for diabetic neuropathic pain: A protocol for systematic review and meta analysis. *Medicine* 99:e23244. doi: 10.1097/MD.0000000000023244

Wang, S.-M., Kain, Z. N., and White, P. (2008). Acupuncture analgesia: I. The scientific basis. *Anesth. Analg.* 106, 602–610. doi: 10.1213/01.ane.0000277493.42335.7b

Wang, W.-S., Tu, W.-Z., Cheng, R.-D., He, R., Ruan, L.-H., Zhang, L., et al. (2014). Electroacupuncture and A-317491 depress the transmission of pain on primary afferent mediated by the P2X3 receptor in rats with chronic neuropathic pain states. *J. Neurosci. Res.* 92, 1703–1713. doi: 10.1002/jnr.23451

Wang, Y.-R., Xu, H., Tao, M., Xu, L.-H., and Fu, X.-C. (2017). Ligustilide relieves complete Freund's adjuvant-induced mechanical hyperalgesia through inhibiting the activation of spinal c-Jun N-terminal Kinase/c-Jun pathway in rats. *Pharmacogn. Mag.* 13, 634–638. doi: 10.4103/pm.pm_546_16

Waxman, S. G., Kocsis, J. D., and Black, J. A. (1994). Type III sodium channel mRNA is expressed in embryonic but not adult spinal sensory neurons, and is reexpressed following axotomy. *J. Neurophysiol.* 72, 466–470. doi: 10.1152/jn.1994.72.1.466

Wei, J.-A., Hu, X., Zhang, B., Liu, L., Chen, K., So, K.-F., et al. (2021). Electroacupuncture activates inhibitory neural circuits in the somatosensory cortex to relieve neuropathic pain. *iScience* 24:102066. doi: 10.1016/j.isci.2021.102066

Wei, T.-H., and Hsieh, C.-L. (2020). Effect of acupuncture on the p38 signaling pathway in several nervous system diseases: A systematic review. *Int. J. Mol. Sci.* 21:E4693. doi: 10.3390/ijms21134693

Wells, R. E., Baute, V., and Wahbeh, H. (2017). Complementary and integrative medicine for neurologic conditions. *Med. Clin. North Am.* 101, 881–893. doi: 10.1016/j.mcna.2017.04.006

Wu, J.-J., Lu, Y.-C., Hua, X.-Y., Ma, S.-J., Shan, C.-L., and Xu, J.-G. (2018). Cortical remodeling after electroacupuncture therapy in peripheral nerve repairing model. *Brain Res.* 1690, 61–73. doi: 10.1016/j.brainres.2018.04.009

Wu, Q., Chen, J., Yue, J., Ying, X., Zhou, Y., Chen, X., et al. (2021). Electroacupuncture improves neuronal plasticity through the A2AR/cAMP/PKA signaling pathway in SNL rats. *Neurochem. Int.* 145:104983. doi: 10.1016/j.neuint.2021.104983

Xia, Y.-Y., Xue, M., Wang, Y., Huang, Z.-H., and Huang, C. (2019). Electroacupuncture alleviates spared nerve injury-induced neuropathic pain and modulates HMGB1/NF-κB signaling pathway in the spinal cord. *J. Pain Res.* 12, 2851–2863. doi: 10.2147/JPR.S220201

Xie, W., Chen, S., Strong, J. A., Li, A.-L., Lewkowich, I. P., and Zhang, J.-M. (2016). Localized sympathectomy reduces mechanical hypersensitivity by restoring normal immune homeostasis in rat models of inflammatory pain. *J. Neurosci.* 36, 8712–8725. doi: 10.1523/JNEUROSCI.4118-15.2016

Xie, W., Strong, J. A., and Zhang, J.-M. (2020). Localized sympathectomy reduces peripheral nerve regeneration and pain behaviors in 2 rat neuropathic pain models. *Pain* 161, 1925–1936. doi: 10.1097/j.pain.0000000000001887

Xing, G.-G., Liu, F.-Y., Qu, X.-X., Han, J.-S., and Wan, Y. (2007). Long-term synaptic plasticity in the spinal dorsal horn and its modulation by electroacupuncture in rats with neuropathic pain. *Exp. Neurol.* 208, 323–332. doi: 10.1016/j.expneurol.2007.09.004

Xu, H., Yang, Y., Deng, Q.-W., Zhang, B.-B., Ruan, J.-W., Jin, H., et al. (2021). Governor vessel electro-acupuncture promotes the intrinsic growth ability of spinal neurons through activating calcitonin gene-related peptide/α-calcium/calmodulin-dependent protein kinase/neurotrophin-3 pathway after spinal cord injury. *J. Neurotrauma* 38, 734–745. doi: 10.1089/neu.2020.7155

Xu, S., Yu, L., Luo, X., Wang, M., Chen, G., Zhang, Q., et al. (2020). Manual acupuncture versus sham acupuncture and usual care for prophylaxis of episodic migraine without aura: Multicentre, randomised clinical trial. *BMJ* 368:m697. doi: 10.1136/bmj.m697

Yang, L., Ding, W., You, Z., Yang, J., Shen, S., Doheny, J. T., et al. (2019). Alleviation of trigeminal neuropathic pain by electroacupuncture: The role of hyperpolarization-activated cyclic nucleotide-gated channel protein expression in the Gasserian ganglion. *Acupunct. Med.* 37, 192–198. doi: 10.1177/0964528419841614

Yen, L.-T., Hsu, Y.-C., Lin, J.-G., Hsieh, C.-L., and Lin, Y.-W. (2018). Role of ASIC3, Nav1.7 and Nav1.8 in electroacupuncture-induced analgesia in a mouse model of fibromyalgia pain. *Acupunct. Med.* 36, 110–116. doi: 10.1136/acupmed-2016-011244

Yoshimoto, K., Fukuda, F., Hori, M., Kato, B., Kato, H., Hattori, H., et al. (2006). Acupuncture stimulates the release of serotonin, but not dopamine, in the rat nucleus accumbens. *Tohoku J. Exp. Med.* 208, 321–326. doi: 10.1620/tjem.208.321

Yu, B., Li, M., Huang, H., Ma, S., Huang, K., Zhong, Z., et al. (2021). Acupuncture treatment of diabetic peripheral neuropathy: An overview of systematic reviews. *J. Clin. Pharm. Therap.* 46, 585–598. doi: 10.1111/jcpt.13351

Yu, S.-W., Lin, S.-H., Tsai, C.-C., Chaudhuri, K. R., Huang, Y.-C., Chen, Y.-S., et al. (2019). Acupuncture effect and mechanism for treating pain in patients with Parkinson's disease. *Front. Neurol.* 10:1114. doi: 10.3389/fneur.2019.01114

Yun, J.-M., Lee, S.-H., Cho, J.-H., Kim, K.-W., and Ha, I.-H. (2020). The effects of acupuncture on occipital neuralgia: A systematic review and meta-analysis. *BMC Complement. Med. Ther.* 20:171. doi: 10.1186/s12906-020-02955-y

Zhang, H., Sun, J., Xin, X., Huo, Z., and Li, D. (2018). Contralateral electroacupuncture relieves chronic neuropathic pain in rats with spared nerve injury. *Med. Sci. Monit.* 24, 2970–2974. doi: 10.12659/MSM.909741

Zhang, M., Dai, Q., Liang, D., Li, D., Chen, S., Chen, S., et al. (2018). Involvement of adenosine A1 receptor in electroacupuncture-mediated inhibition of astrocyte activation during neuropathic pain. *Arquivos Neuro Psiquiatria* 76, 736–742. doi: 10.1590/0004-282X20180128

Zhang, N., Wang, L.-Q., Li, J.-L., Su, X.-T., Yu, F.-T., Shi, G.-X., et al. (2021). The management of sciatica by acupuncture: An expert consensus using the improved delphi survey. *J. Pain Res.* 14, 13–22. doi: 10.2147/JPR.S280404

Zhang, W.-T., Jin, Z., Cui, G.-H., Zhang, K.-L., Zhang, L., Zeng, Y.-W., et al. (2003). Relations between brain network activation and analgesic effect induced by low vs. high frequency electrical acupoint stimulation in different subjects: A functional magnetic resonance imaging study. *Brain Res.* 982, 168–178. doi: 10.1016/s0006-8993(03)02983-4

Zhang, Y., Li, A., Xin, J., Ren, K., Berman, B. M., Lao, L., et al. (2018). Electroacupuncture alleviates chemotherapy-induced pain through inhibiting phosphorylation of spinal CaMKII in rats. *Eur. J. Pain* 22, 679–690. doi: 10.1002/ejp.1132

Zhao, L., Li, D., Zheng, H., Chang, X., Cui, J., Wang, R., et al. (2019). Acupuncture as adjunctive therapy for chronic stable angina: A randomized clinical trial. *JAMA Intern. Med.* 179, 1388–1397. doi: 10.1001/jamainternmed.2019.2407

Zhao, P., Waxman, S. G., and Hains, B. C. (2007). Extracellular signal-regulated kinase-regulated microglia-neuron signaling by prostaglandin E2 contributes to pain after spinal cord injury. *J. Neurosci.* 27, 2357–2368. doi: 10.1523/JNEUROSCI.0138-07.2007

Zhao, Z.-Q. (2008). Neural mechanism underlying acupuncture analgesia. *Prog. Neurobiol.* 85, 355–375. doi: 10.1016/j.pneurobio.2008.05.004

Zheng, Q., Xie, W., Lückemeyer, D. D., Lay, M., Wang, X.-W., Dong, X., et al. (2022). Synchronized cluster firing, a distinct form of sensory neuron activation, drives spontaneous pain. *Neuron* 110, 209–220.e6. doi: 10.1016/j.neuron.2021.10.019

Zhi, W. I., Ingram, E., Li, S. Q., Chen, P., Piulson, L., and Bao, T. (2018). Acupuncture for bortezomib-induced peripheral neuropathy: Not just for pain. *Integr. Cancer Ther.* 17, 1079–1086. doi: 10.1177/1534735418788667

Zhou, W., and Benharash, P. (2014). Effects and mechanisms of acupuncture based on the principle of meridians. *J. Acupunct. Merid. Stud.* 7, 190–193. doi: 10.1016/j.jams.2014.02.007

Zhou, Y.-F., Ying, X.-M., He, X.-F., Shou, S.-Y., Wei, J.-J., Tai, Z.-X., et al. (2018). Suppressing PKC-dependent membrane P2X3 receptor upregulation in dorsal root ganglia mediated electroacupuncture analgesia in rat painful diabetic neuropathy. *Purinergic Signal.* 14, 359–369. doi: 10.1007/s11302-018-9617-4

Zou, J., Dong, X., Wang, K., Shi, J., and Sun, N. (2021). Electroacupuncture inhibits autophagy of neuron cells in postherpetic neuralgia by increasing the expression of mir-223-3p. *BioMed Res. Int.* 2021:6637693. doi: 10.1155/2021/6637693

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Proteomic and metabolomic profiling of acupuncture for migraine reveals a correlative link via energy metabolism

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Migraine is a neurovascular disease with a high disability rate. Acupuncture treatment has emerged as a safe and viable alternative prophylactic therapy that can effectively alleviate the duration and frequency of migraine attacks. However, the therapeutic mechanisms underlying the effects of acupuncture are yet to be systematically elucidated. In this study, we enrolled female patients with migraine without aura (n = 20) and healthy controls (n = 10). Patients received acupuncture treatment on DU20, DU24, bilateral GB13, GB8, and GB20, applied three times per week over the course of 4 weeks for 12 sessions in total. Blood samples were collected from the median cubital vein before and after acupuncture treatment. Proteomic and metabolomic profiling was performed using liquid chromatography-mass spectrometry to determine the characteristics of differentially expressed molecules and expression of their corresponding biological pathways as well as to elucidate the pathogenesis of migraine and the biological effects underlying the treatment of migraine with acupuncture. Proteomic and metabolomic profiling of plasma samples from patients with migraine without aura before and after acupuncture treatment revealed enrichment of immune-related pathway functions and the arginine synthesis pathway. Joint pathway analyses revealed significant enrichment of the pentose phosphate and glycolysis/gluconeogenesis pathways in patients with migraine. The glycolysis/gluconeogenesis and riboflavin metabolism pathways were significantly enriched after acupuncture treatment. The expression levels of various key proteins and metabolites, including α-D-glucose, flavin adenine dinucleotide, biliverdin reductase B, and L-glutamate, were significantly differentially expressed before and after acupuncture treatment in patients with migraine without aura. Treatment of migraine with acupuncture was associated with significant changes in key molecules and

pathways, indicative of physiological changes in the trigeminovascular system, glutamate neurotoxicity, and other migraine-related physiological changes. Overall, our comprehensive analysis using proteomic and metabolomic profiling demonstrates that energy metabolism may serve as a key correlative link in the occurrence of migraine and the therapeutic effects of acupuncture treatment. Our findings may facilitate the identification of diagnostic and therapeutic modalities in the ongoing search for effective treatments for migraine attacks.

KEYWORDS

migraine, proteomics, metabolomics, acupuncture, energy metabolism pathways

Introduction

Migraine is a painful chronic neurological disorder characterized by recurrent moderate or severe headaches that typically manifest unilaterally in a pulsating manner (Ashina et al., 2021b). Migraines are partly due to genetic predisposition (Gormley et al., 2016) but can also be aggravated by environmental triggers, leading to nausea, vomiting, and hypersensitivity to light and sound (Headache Classification Committee of the International Headache Society (IHS), 2018). Migraines adversely impact the lives of patients, afflicting approximately 1 billion people regardless of culture and socioeconomic status (GBD 2016 Disease and Injury Incidence and Prevalence Collaborators, 2017; Ashina, 2020). Indeed, migraine is currently the second most burdensome neurological disease worldwide (GBD 2016 Neurology Collaborators, 2019). Migraines impose a heavy economic burden due to both direct and indirect costs. Direct costs include costs incurred by specialist visits, medications, and diagnostic tests, which vary among countries according to different healthcare systems and administration policies. Indirect costs include costs due to lost jobs and reduced productivity, and the economic impact is evident in stressful environments (Agosti, 2018). A large European study reported that 17.7% of men and 28.0% of women lost more than 10 days of activity over a 3-month period due to migraine (Steiner et al., 2014).

Current medications used for migraine treatment include non-steroidal anti-inflammatory drugs, triptans, ergot-type preparations, and calcitonin gene-related peptide (CGRP) receptor antagonists (Ashina et al., 2021a). However, the clinical application of migraine medications is limited due to side effects such as cardiovascular and gastrointestinal issues (Katsarava et al., 2018), as well as their short-term therapeutic effects, which can lead to medication overuse and dissatisfaction with available treatments (Krymchantowski, 2004). Reports suggest that excessive use of antimigraine therapies or analgesics can increase the frequency of headaches (Tfelt-Hansen et al., 2012), while the use of different therapeutic drugs can lead to toxic reactions in the liver, heart, brain, and other organs (Lionetto et al., 2013). Accordingly, there is a critical unmet need to reconsider treatment approaches for the relief and prevention of migraines. As a complementary alternative therapy without severe adverse effects, acupuncture has been reported to exert long-term effects for alleviating migraine recurrence, significantly decreasing the occurrence of days with migraine and migraine attacks, and for reducing pain intensity (Zhao et al., 2017; Xu et al., 2020). A retrospective analysis revealed that acupuncture treatment is cost-effective for individuals with migraine, as the medical costs of patients with migraine and hospitalization of patients with severe migraine can reduce with the use of acupuncture add-on therapy (Tsai et al., 2020). However, the exact pathophysiology of migraine and physiological changes underlying acupuncture treatment of migraine are not fully understood. Previous reports have demonstrated that trigeminovascular system activation, immune effects, and oxidative stress may underscore migraine pathophysiology. Extant research suggests that the onset, progression, and termination of migraine may be associated with neuronal and trigeminovascular pain pathways, and trigeminovascular system activation has been linked to metabolic factors (Goadsby et al., 2017; Charles, 2018). In a rat model of migraine, dysregulation of the mitochondrial dynamic regulatory network was observed in trigeminal neurons, accompanied by suppression of mitochondrial

Abbreviations: CSD, cortical spreading depression; MS, mass spectrometry; EDTA, ethylenediaminetetraacetic acid; DIA, data-independent acquisition; AGC, automatic gain control; MeOH, methanol; ACN, acetonitrile; QC, quality control; DEPs, differentially expressed proteins; DEMs, differentially expressed metabolites; OPLS-DA, orthogonal partial least squares discriminant analysis; SVM, support vector machine; TIC, total ion chromatograms; PKM, pyruvate kinase; G6PD, glucose-6-phosphate dehydrogenase; HK1, hexokinase 1; FAD, flavin adenine dinucleotide; ENO1, enolase 1; BLVRB, biliverdin reductase B; NO, nitric oxide; NOS, nitric oxide synthase; CGRP, calcitonin gene-related peptide; BBB, blood–brain barrier; ANLS, astrocyte-to-neuron lactate shuttle.

biogenesis and a dynamic shift toward mitochondrial fission (Dong et al., 2017). Mitochondria also interact with the immune system. For instance, the cortical spreading depression (CSD)-induced neuroinflammatory signaling pathway may be associated with activation of the mitochondrial stress-induced mtDNA-triggered NLRP3 inflammasome (Kursun et al., 2021). Insufficient energy in the brain and high levels of oxidative stress are pathophysiological characteristics of patients with migraine (Gross et al., 2019). These findings support the notion that mitochondrial dysfunction is a key factor in migraine pathophysiology.

Given the multifactorial nature of migraines, integrated analysis combining proteomics and metabolomics is crucial for assessing migraine pathophysiology (Lionetto et al., 2013). Proteomics, as a supplement to other "omics" technologies, enables the identification and quantification of overall proteins in biological systems. Technological advances have led to substantial improvements in the quantitative accuracy of mass spectrometry (MS)-based quantitative proteomics, which supports the quantification of more proteins (Liu et al., 2014; Chi et al., 2018). Accordingly, quantitative proteomics holds promising prospects for development and application in the future, including in the discovery of biomarkers and clinical applications. This technology is currently applied in multiple fields and is used for detecting multiple diagnostic biomarkers, revealing pathogenicity mechanisms of disease, screening vaccine candidates for manufacturing, regulating expression patterns of different signals, and interpreting different functional pathways (Suhre et al., 2021). Moreover, metabolomic analyses afford novel perspectives for understanding an organism's overall health status by evaluating key metabolic changes in living systems. This method reflects changes in genetic modifications, physiological stimuli (e.g., diet and environmental factors), and the gut microbiome to expound on the identification of biomarkers and alterations in biochemical pathways. As such, this method has the potential to promote the development of better strategies for disease prevention and treatment (Kaddurah-Daouk et al., 2008; Wishart, 2019).

To the best of our knowledge, although several studies have performed an "omics" analysis of patients with migraine (Zielman et al., 2016), there is yet to be a study examining the systemic proteomic and metabolomic effects of acupuncture treatment in patients with migraine. In this regard, deeper understanding of the underlying physiological changes will assist in the development of effective treatment strategies. Therefore, the objectives of our study were to elucidate the biological mechanisms of acupuncture treatment in patients with migraine and to identify the differentially expressed molecules and corresponding enriched pathways using proteomic and untargeted metabolic profiling. We hypothesized that several metabolic factors would be associated with acupuncture treatment of migraine. Ultimately, our study aimed to provide more comprehensive and detailed understanding of the association between the molecular status of proteins and metabolites following acupuncture for migraine.

Materials and methods

Patients and samples

All patients in our study were enrolled at the Outpatient Department of Acupuncture and Moxibustion, Beijing Hospital of Traditional Chinese Medicine. The study protocol was approved by the Research Ethical Committee of the Beijing Hospital of Traditional Chinese Medicine (ref: 2016BL-081-02). All individuals provided written informed consent for the recruitment for scientific purposes before enrollment. This study was conducted in accordance with the latest version of the Declaration of Helsinki.

Patients with migraine without aura (n = 20) who fulfilled the criteria of the International Classification of Headache Disorders (3rd edition, beta version) (Headache Classification Committee of the International Headache Society, 2013) and healthy controls (n = 10) were recruited. All the enrolled patients were diagnosed by an experienced neurologist. Patients and controls were matched based on age (20–40 years) and sex (female) to ensure that the groups were as homogenous as possible. Other inclusion criteria included the presence of 2–8 migraine attacks during the 4-week baseline phase, patients with migraine with an initial onset before 50 years of age, and a history of migraine lasting more than 1 year.

The exclusion criteria included other types of primary (e.g., tension-type headache, cluster headache, or chronic migraine) and secondary headaches, history of severe systemic diseases such as immune and nervous system diseases, cardio-metabolic disorders, acute infectious disease, hematopathy, allergies, clinically diagnosed psychiatric disorders, pregnancy, breastfeeding, non-compliance with the baseline headache diary, and the use of any type of prophylactic acupuncture or medication within 3 months before the baseline phase. Subsequently, all patients were instructed to avoid any other analgesics or initiating any other interventions.

Venous blood samples were collected from the median cubital vein in patients with migraine before and after acupuncture treatment (24 h) and in healthy control participants. All samples were drawn using ethylenediaminetetraacetic acid (EDTA)-containing plasma tubes (Shandong Aosaite Medical Devices Co., Ltd., Shandong, China) between 7:30 and 8:30 am. To achieve blinded measurement, the samples were coded. Plasma samples were centrifuged at 1,500 × g for 20 min at 4°C and immediately stored at −80°C until the analysis of proteins and metabolites using high-performance liquid chromatography-tandem mass spectrometry (HPLC–MS/MS) was performed. During the 48 h before sampling, participants were instructed to avoid taking analgesics or antimigraine medications, and there were no restrictions on dietary intake.

Study interventions

The treatments were administered by two licensed acupuncturists with more than 20 years of clinical experience. The acupuncturists received specific training regarding the study purpose, treatment strategies, and quality control (QC). Each patient received 12 acupuncture treatment sessions of 30-min duration, three times per week over 4 weeks. **Supplementary Table 1** presents information about the location of acupuncture points and depth of needle insertion.

The acupuncturists performed manual acupuncture at eight acupuncture points, including DU20, DU24, bilateral GB13, bilateral GB8, and bilateral GB20. The therapy involved the use of sterile acupuncture needles (ANDE Needles, Guizhou ANDE Medical Equipment, Co., Ltd., Guizhou, China) with a diameter of 0.25 mm and length of 25–40 mm. After sterilization, acupuncture needles were inserted into acupuncture points and applied with manual manipulation to elicit the "Deqi" sensation. In cases of severe pain [visual analog score (VAS) > 8], ibuprofen (300 mg/capsule; maximal tolerated dose 1,200 mg/day) was used as a rescue medication.

Assessment of migraine

Detailed headache diaries regarding the duration of migraine diagnosis, number of migraine days, and headache intensity (VAS score) within the 4-week baseline phase and 4-week treatment phase (before and after acupuncture treatment) were obtained from migraineurs. The number of migraine days and average headache intensity were calculated every 4 weeks over an 8-week period. Each patient completed a baseline headache diary for screening and was subsequently interviewed by an experienced neurologist before enrollment.

All patients completed a standardized questionnaire encompassing demographics, headache characteristics, the six-item Headache Impact Test (HIT-6) (Yang et al., 2011), Migraine-Specific Quality-of-Life Questionnaire (MSQ) (Cole et al., 2007), Beck Depression Inventory-II (BDI-II) (Geisser et al., 1997), Beck Anxiety Inventory (BAI) (Leyfer et al., 2006), Montreal Cognitive Assessment (MoCA) (Freitas et al., 2013), and Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989) at baseline and at the end of the 4-week treatment phase. **Tables 1**, **2** present the relevant demographic and clinical characteristics of the participants.

Protein extraction and enzymatic solution

Plasma samples were diluted with 8 M urea containing 1% sodium dodecyl sulfate (containing a protease inhibitor) and lysed on ice, and vortex oscillations were performed every 10 min. After centrifugation at 12,000 × *g* for 20 min at 4°C, soluble protein lysates were collected and identified using the bicinchoninic acid protein assay. Then, 100 μg of protein from each sample was transferred into a fresh Eppendorf tube. After addition of 2 μL of 0.5 M tris(2-carboxyethyl)phosphine at 37°C, 4 μL of 1 M iodoacetamide was added, and the mixture was protected from light at room temperature for 40 min. After adding five volumes of prechilled acetone at −20°C, precipitation was conducted overnight. The precipitates were washed with 90% acetone solution and centrifuged at 12,000 × *g* for 20 min at 4°C. After thorough drying at room temperature, the proteins were dissolved in 100 μL of 100 mM tetraethylammonium bromide. Sequence-grade modified trypsin (Promega, Madison, WI, USA) was then added at a mass ratio of 1:50 enzyme/protein overnight at 37°C.

Liquid chromatography with tandem mass spectrometry analysis for proteomics

Desalination was performed using C18 ZipTip (Millipore, Burlington, MA, USA). A Pierce quantitative colorimetric peptide assay (Thermo Fisher Scientific, Waltham, MA, USA) was used for quantification followed by lyophilization. Subsequently, 30 μL of 0.1% formic acid aqueous solution (solvent A) was added to each sample. The whole system comprised an Orbitrap Exploris 480 mass spectrometer (Thermo Fisher Scientific) coupled to an EASY-nanoLC 1200 system (Thermo Fisher Scientific). Samples (3 μL) (Acclaim PepMap C18, 75 μm × 25 cm) were separated with a 130-min gradient. The column temperature was set at 40°C, with a constant flow rate of 250 nL/min. A mass spectrometer with electrospray at an inlet voltage of 2 kV was used. Using 0.1% formic acid aqueous solution (mobile phase A) and 0.1% formic acid acetonitrile (ACN) solution (mobile phase B), a 130 min separation gradient (0 min, 4% B; 10 min, 6% B; 120 min, 50% B; 121 min, 95% B; 130 min, 95% B) was established. The mass spectrometer was operated in data-independent acquisition (DIA) mode, and one full scan was followed by eight windows. The following MS parameters were used: (1) MS: scan range (m/z) = 350–1,200, resolution = 120,000, automatic gain control (AGC) target = 300%, maximum injection time = 50 ms, and charge states = 2–6; (2) HCD-MS/MS: resolution = 30,000, isolation window = 2, AGC target = 200%, maximum injection time = 50 ms, collision energy = 25, 30, 35; (3) variable isolation windows were used for DIA, and each window overlapped by 1 m/z. The DIA MS data were processed using DIA-NN (v.1.7.12) (Demichev et al., 2020), and the "Generate Spectrum Library" option was set. The workflow of DIA-NN commenced with a peptide-centric approach, and the collection of precursor ions supported automatic generation from a protein sequence database (library-free mode) (Demichev et al., 2020). The FASTA file used a human proteome database containing Swiss-Prot sequences (downloaded from the UniProt database on March 21, 2020; containing 20,365 proteins). A false discovery rate of <0.01 was set.

TABLE 1 Demographics and clinical characteristics of healthy controls and migraine patients.

Demographics	Healthy controls (n = 10)	Migraine (n = 20)	P-value[a]
Age–year			
Mean (SD)[b]	32.60 (3.95)	32.00 (4.31)	0.7151
Median (IQR)	33.00 (29.00–35.00)	32.00 (30.00–35.25)	
Range	23.00–39.00	26.00–39.00	
Sex–no. (%)			
Female[c]	10 (100.00)	20 (100.00)	1.00
Education–no. (%)			
Above/below bachelor level[c]	7 (70.00)/3 (30.00)	16 (80.00)/4 (20.00)	0.5416
Marriage–no. (%)			
Married/single[c]	9 (90.00)/1 (10.00)	17 (85.00)/3 (15.00)	0.7041
Duration of migraine diagnosis at baseline–year, mean (SD)	NA	13.70 (7.55)	NA
Accompanying symptoms–no. (%)			
Nausea or vomiting	NA	17 (85.00)	NA
Photophobia or phonophobia	NA	15 (75.00)	NA
Use of acute pain medication–no. (%)	NA	7 (35.00)	NA

[a]All tests were two-sided. Statistical significance was set at $P < 0.05$.
[b]Analyzed using independent samples t-test.
[c]Analyzed using Chi-square test. SD, standard deviation; IQR, interquartile range; no. (%), number; NA, not applicable.

TABLE 2 Clinical characteristics of 20 migraine patients before and after treatment.

Clinical characteristics	Baseline	After treatment	MD (95% CI)	P-value[a]
Days with migraine per 4 weeks, mean (SD)[b,c]	6.10 (5.55)	1.52 (1.66)	4.58 (2.43, 6.73)	0.0003
Mean VAS score, mean (SD)[b]	8.10 (1.62)	4.90 (1.65)	3.20 (1.98, 4.42)	<0.0001
HIT-6, mean (SD)[b]	66.20 (5.02)	57.30 (7.36)	8.90 (5.76, 12.04)	<0.0001
MSQ, mean (SD)[b]				
Role restrictive subscale	55.43 (13.45)	77.14 (15.73)	−42.00 (−59.30, −24.69)	0.0001
Role preventive subscale	71.25 (13.56)	85.75 (15.07)	−14.50 (−21.70, −7.30)	0.0005
Emotional subscale	67.67 (22.51)	84.67 (11.87)	−17.00 (−26.58, −7.43)	0.0015
BDI-15 mean (SD)[b]	9.40 (4.99)	4.85 (4.17)	4.55 (2.28, 6.82)	0.0005
BAI, mean (SD)[b]	9.75 (7.49)	4.75 (3.97)	5.00 (2.09, 7.91)	0.0015
MoCA, mean (SD)[b]	27.60 (2.09)	28.15 (1.84)	−0.55 (−1.25, 0.15)	0.1183
PSQI, mean (SD)[b]	5.00 (2.32)	4.50 (2.06)	0.50 (−0.69, 1.69)	0.3905

[a]All tests were two-sided. Statistical significance was set at $P < 0.05$.
[b]Analyzed using paired t-test.
[c]Number of days with migraine was defined as the duration of migraine attacks. SD, standard deviation; MD, mean difference; CI, confidence interval; VAS, visual analog scale; HIT-6, six-item Headache Impact Test; MSQ, Migraine-Specific Quality of Life Questionnaire; BDI-II, Beck Depression Inventory-II; BAI, Beck Anxiety Inventory; MoCA, Montreal Cognitive Assessment; PSQI, Pittsburgh Sleep Quality Index.

Metabolite extraction

For untargeted metabolomic analysis, the stored samples were thawed at 4°C. Then, 400 μL of methanol (MeOH) and 400 μL of ACN were added to a 100 μL plasma sample and centrifuged at 13,000 rpm for 15 min at 4°C. The supernatant was collected and evaporated to dry with a vacuum concentrator. The dry extract was resuspended in a 1:1 ACN:H_2O solution (100 μL centrifuged at 13,000 rpm for 15 min at 4°C). The supernatant was collected and stored at −80°C.

Liquid chromatography with tandem mass spectrometry analysis for metabolomics

For untargeted metabolomic analysis in both positive and negative ion mode, the whole system comprised an HPLC–MS/MS on a Triple TOF 6600plus mass spectrometer (AB SCIEX, Foster City, CA, USA) coupled to an Agilent 1290 LC system (Agilent, Palo Alto, CA, USA), while the ACQUITY UPLC BEH Amide column (100 mm × 2.1 mm, 1.7 μm, Waters) was used for LC separation. For positive

and negative ion modes, the ion spray voltage was set to 5,000 V (ESI+) and −4,000 V (ESI−), respectively. The MS scanning range was collected between 60 and 1,200 m/z. The curtain, heating, and atomization gas flow was set at 35, 60, and 60 psi, respectively. The injection volume was set at 5 μL, and the gradient separation was 12 min. The flow rate of the column was 500 μL/min, and the column temperature was set to 25°C. Mobile phase A was water (containing 25 mM ammonium acetate and 25 mM ammonia), and mobile phase B was pure ACN. Gradient settings were set at 95% of the initial conditions of mobile phase B for 0.5 min, then the linear gradient shifted from 95 to 65% of mobile phase B in 6.5 min, and then from 65 to 40% in 1 min; 40% of the elasticity of mobile phase B was maintained for 1 min. Subsequently, a 0.1-min gradient was used to return to the initial condition, which lasted for another 2.9 min. In the instrumental analysis, one QC sample was inserted for every seven analytical samples. For untargeted metabolomics analysis, the original data collected by MS were converted using ProteoWizard (version 3.0.6150). The converted file was further processed using XCMS (version 1.46.0) for peak identification and retention time alignment to obtain the peak list. The main parameters were as follows: minimum peak width, 5 s; maximum peak width, 30 s; ppm deviation, 25 ppm; and signal-to-noise threshold (snthresh), 3.

Statistical analysis

Continuous data are summarized as mean [standard deviation (SD)] and median [interquartile range (IQR)] values, and categorical data are described as numbers (%). The Shapiro–Wilk test was performed to assess normality. Comparisons of two groups (before vs. after treatment/healthy control vs. migraine) were conducted using an independent samples/paired *t*-test or Mann–Whitney *U*-test. The relationship between categorical variables was evaluated using the chi-squared test and Fisher's exact test. Bonferroni adjustment was applied to control the family wise error rate in these comparisons in consideration of the main hypothesis of the study. In the analysis of demographics and clinical characteristics, Statistica 20.0 software (TIBCO Software Inc, Palo Alto, CA, USA) was used, and the statistical significance level was set at $P < 0.05$.

Missing values were processed using the "Wu kong" platform (Wang et al., 2020). Protein data rows with a missing value ratio of >80% in the quantitative results were deleted. The Seq-KNN method was used to fill in missing values. For quantitative proteins, deviation between samples was reduced using the median normalization method. The *P*-value of the protein was calculated using an unpaired two-sided Welch's *t*-test, and the fold change was calculated according to the ratio of the two groups. The data were filtered according to a *P*-value of <0.05, and fold change of >1.2 or $<1.2^{-1}$ in volcano plots. The gene names of differentially expressed proteins (DEPs) were drawn using the UniProtKB/Swiss-Prot public database. clusterProfiler (v.3.18.0) in R studio (v.1.2.5033) was used to enrich the Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (Yu et al., 2012).

MetDNA[1] was used for metabolite identification (Shen et al., 2019). Statistical analyses were performed using the StatTarget package (Luan et al., 2018). The QC-base random forest signal correction algorithm (QC-RFSC) was used to perform signal correction based on QC. We generated a preliminary filter according to a *P*-value of <0.05 and fold change of >1.2 or $<1.2^{-1}$ in volcano plots. Subsequently, the data in the positive and negative ion modes were merged, and multivariable statistical analysis was conducted using SIMCA-P software version 14.1 (Umetrics, Umea, Sweden). An orthogonal partial least squares discriminant analysis (OPLS-DA) model was constructed, and the model was verified by a substitution test of 200 iterations. Differentially expressed metabolites (DEMs) were screened by variable importance in projection of >1, *P*-value of <0.05, and fold change of >1.2 or $<1.2^{-1}$. Metabolic and joint pathway analyses were performed using MetaboAnalyst 5.0[2] (Pang et al., 2021). Sample normalization by median was selected. Pareto scaling and logarithmic normalization were selected for data scaling and transformation, respectively.

SIMCA-P software was used to construct an OPLS-DA model for multivariate correlation analysis. Data were scaled to unit variance and subjected to linear transformation. After data processing, regression analysis was performed between quantified plasma molecules (proteins and metabolites) and clinical variables of pain intensity (VAS) in patients with migraine who received acupuncture treatment. VAS was used as a single y-variable, and x-variables were molecular quantitative values. In S-plot plots, the abscissa and ordinate coordinates represented the co-correlation and correlation coefficients of the principal component and molecule, respectively. To verify the results obtained using the above method, Spearman correlation analysis was performed, and the results were illustrated as a heatmap.

Machine learning methods provide requisite data parsing, integration and analysis, and binary output capability characteristics, which enables the construction of multiple classification models using combinations of biomarkers. In recent years, machine learning has demonstrated broad application prospects for disease diagnosis, health management, and other medical and health fields. In this study, machine learning analysis was performed using MetaboAnalyst 5.0

1 http://metdna.zhulab.cn/
2 https://www.metaboanalyst.ca/

(see text footnote 2). The classification was performed using linear support vector machines (SVMs), a supervised machine learning algorithm that efficiently separates datasets by constructing a hyperplane in an N-dimensional space. The mass spectrum intensity values were used as the input of the machine learning model to construct receiver operating characteristic curve analysis and calculate the area under the curve (AUC) to characterize the relationship between specificity and sensitivity.

Results

Demographic features and clinical characteristics

We recruited 20 patients with migraine without aura and 10 age- and sex-matched healthy controls to ensure the groups were as homogenous as possible (**Table 1**). To evaluate the impact of acupuncture on proteomics and metabolomics in patients with migraine without aura, we compared paired samples obtained before and after treatment (n = 20). The headache characteristics and comorbidities of the migraineurs before and after acupuncture treatment are presented in **Table 2**. **Table 2** provides mean scores on the BDI-II (mean score 9.40 is indicative of normal, BDI-II score, 0–10), the BAI (mean score 9.75 is indicative of normal, BAI score, 0–14), the MoCA (mean score 27.60 is indicative of normal, MoCA score, 26–30) and the PSQI (mean score 5.00 is indicative of normal, PSQI score, 0–5) at baseline. Co-morbid depression, anxiety, cognitive impairment and sleep disturbance were not found in these migraine patients. Significant changes were observed in the number of migraine days, VAS score, HIT-6 scale, and MSQ scale after acupuncture treatment, which are consistent with the results of our previous studies (Wang et al., 2011; Zhao et al., 2017; Xu et al., 2020; **Table 2**). Specifically, acupuncture resulted in significantly greater reduction in the number of migraine days after the treatment phase, with a mean difference of 4.58 (95% confidence interval 2.43–6.73; $P = 0.0003$) days compared with that at the baseline phase. A significant decrease in VAS score was observed from baseline to the end of acupuncture treatment (8.10 ± 1.62 vs. 4.90 ± 1.65; $P < 0.0001$). At baseline, the mean HIT-6 score was 66.20, suggesting that headache had a severe impact. The decrease in mean HIT-6 score from baseline was significant at week 4 ($P < 0.0001$), resulting in a mean HIT-6 score at week 4 that was below the severe impact threshold. All subscales of the MSQ scale improved significantly by week 4 relative to baseline. Moreover, in patients with paired samples before and after acupuncture treatment, total scores on the BDI-II and BAI scales were significantly lower at week 4 than at baseline. However, we did not observe any significant differences in the changes in MoCA and PSQI scores from baseline to week 4.

Proteomic and metabolomic profiling of plasma from patients with migraine

We collected 50 plasma samples in total, including 20 blood samples from the same group of migraine patients without aura before and after acupuncture treatment, 20 plasma samples from patients before acupuncture treatment (M group), and 20 plasma samples from patients after acupuncture treatment (A group). In addition, 10 plasma samples of healthy control group (H group) were included. To characterize changes in protein and metabolite expression, we used DIA proteomics and UPLC-MS/MS untargeted metabolomics to analyze the protein and metabolite profiles in patients with migraine after acupuncture treatment (**Figure 1**). In total, 1,354 proteins (**Supplementary Table 2**) and 2,828 metabolites (1,389 in negative ion mode and 1,439 in positive ion mode) (**Supplementary Tables 3, 4**) were identified and quantified. For untargeted metabolomics, total ion chromatograms (TIC) of QC samples under positive and negative ion modes (**Supplementary Figure 1**) revealed good overlap between the retention time and response intensity of each chromatographic peak. Furthermore, the violin plot of the results of median normalization for proteomic and metabolomic

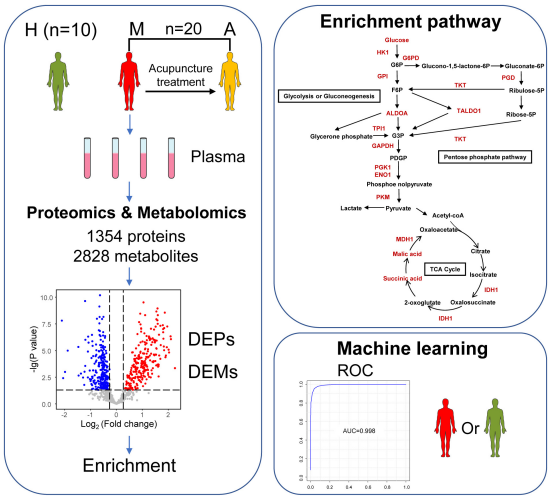

FIGURE 1
Study design and workflow. Overview patterns of blood sample collection from migraine without aura patients, including M group (migraine patients before acupuncture treatment) (n = 20), A group (migraine patients after acupuncture treatment) (n = 20), and H group (healthy controls) (n = 10). A total of 1,354 proteins and 2,828 metabolites were identified by proteomics and metabolomics. Differentially expressed biomarkers (DEPs and DEMs) are involved in pathways including a variety of immune responses and changes in energy metabolism. This may help us to understand the pathogenesis of migraine and the potential biological effects of acupuncture in the treatment of migraine. Furthermore, this may allow the identification of potential biomarker combinations for the classification of migraine without aura patients and healthy controls by using a machine learning strategy.

data demonstrated that the biases between samples decreased (Supplementary Figure 2). Collectively, these results highlight the stability and reproducibility of the data.

Differentially expressed protein and differentially expressed metabolite expression and functional enrichment between patients with migraine and healthy controls

Differentially expressed proteins were screened according to the criteria of fold change and *P*-value (Supplementary Figure 3). The volcano plots and OPLS-DA model were further used to screen DEMs according to the combination of *P*-value of <0.05, fold change of >1.2 or $<1.2^{-1}$ (Supplementary Figure 3), and variable importance in projection value of >1. The permutation test with 200 iterations (Supplementary Figure 4) and high R2X, R2Y, and Q2 confirmed the good quality of each supervised model.

We compared the proteomic changes in patients with migraine without aura and identified 526 DEPs that centered on the involvement of the humoral immune response and complement and coagulation cascades (Figures 2A,B) between the M and H groups. In addition, we identified 114 DEMs (59 in negative ion mode and 55 in positive ion mode) in the comparison of the M and H groups. The related metabolic

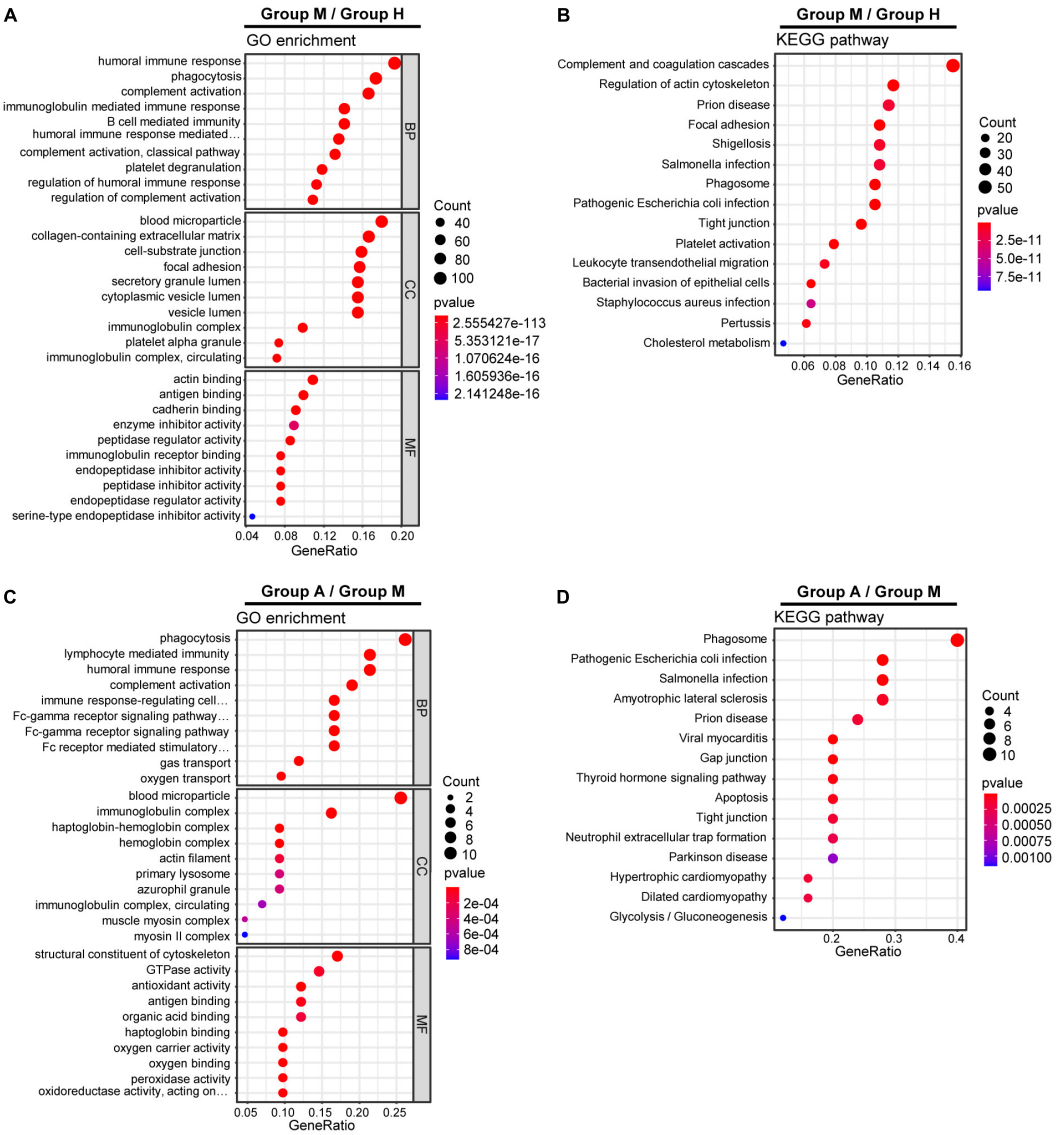

FIGURE 2
Gene Ontology (GO) terms and KEGG pathways enrichment analysis of DEPs. GO enrichment analysis **(A)** and KEGG pathway analysis **(B)** of DEPs in M group and H group. GO enrichment analysis **(C)** and KEGG pathway analysis **(D)** of DEPs in M group and A group.

pathways (Supplementary Table 5) predominantly involved arginine biosynthesis (Figure 3A). Joint pathway analysis (Supplementary Table 6) revealed significant enrichment of the pentose phosphate and glycolysis/gluconeogenesis pathways (Supplementary Figure 5A).

Our analysis also revealed three energy metabolism-related enzymes, including pyruvate kinase (PKM), glucose-6-phosphate dehydrogenase (G6PD), and hexokinase 1 (HK1) (Figure 4). In addition to these proteins, we identified key dysregulated metabolites, including L-arginine, ADP, and L-noradrenaline. These changes in key molecules, especially α-D-glucose and ADP, were indicative of energy metabolism disorders and oxidative stress in patients with migraine.

Differentially expressed protein and differentially expressed metabolite expression and functional enrichment after acupuncture treatment for patients with migraine

We identified 29 DEPs between groups A and M. GO and KEGG pathway enrichment analyses revealed that DEPs were highly enriched in immune response and complement activation (Figures 2C,D). Further, we identified 69 DEMs (45 in negative ion mode and 24 in positive ion mode) in the comparison of groups A and M. DEMs were uploaded to MetaboAnalyst, and the pathway impact value and *P*-value were calculated to evaluate the importance of the affected metabolomic pathways. Among these altered pathways (Supplementary Table 7), arginine biosynthesis, arginine metabolism, and proline metabolism were notable pathways of interest (Figure 3B). To integrate the DEPs and DEMs in the pathway, we conducted a joint pathway analysis (Supplementary Table 8). We observed that the DEPs and DEMs were significantly enriched in riboflavin metabolism, arginine biosynthesis, and glycolysis/gluconeogenesis pathways (Supplementary Figure 5B).

Figure 5 summarizes the key dysregulated molecules, including alpha-D-glucose, flavin adenine dinucleotide (FAD), L-glutamate, biliverdin reductase B (BLVRB), citrulline, and enolase 1 (ENO1). We evaluated the correlation between these key dysregulated molecules and pain intensity based on VAS. We observed multivariable correlations of these key molecules with pain intensity in the OPLS-DA model. Among the key molecules, alpha-D-glucose, citrulline, BLVRB, FAD, and L-noradrenaline exhibited a significant correlation with pain intensity, and VAS was positively associated with BLVRB and FAD and negatively related with alpha-D-glucose, citrulline, and L-noradrenaline (Supplementary Figure 6).

Identification of patients with migraine using machine learning

To further explore potential diagnostic biomarkers to distinguish migraine cases from controls, we adopted a machine learning approach using SVMs based on DEPs

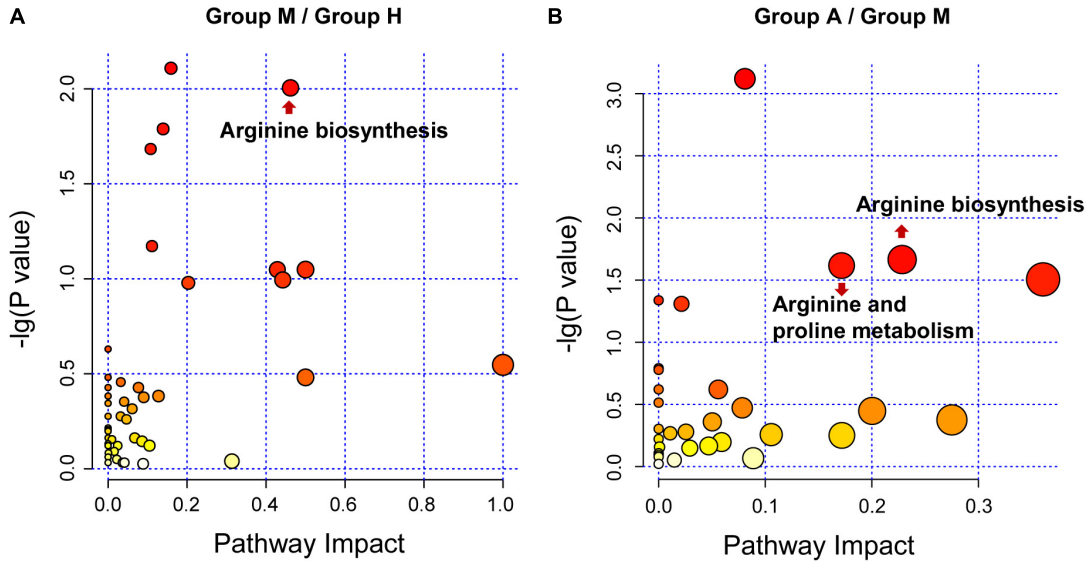

FIGURE 3
Pathway analysis for DEMs. **(A)** The metabolic pathway analysis between H group and M group. **(B)** The metabolic pathway analysis between M group and A group. The size and color of each bubble is based on the pathway impact value and *P*-value, respectively.

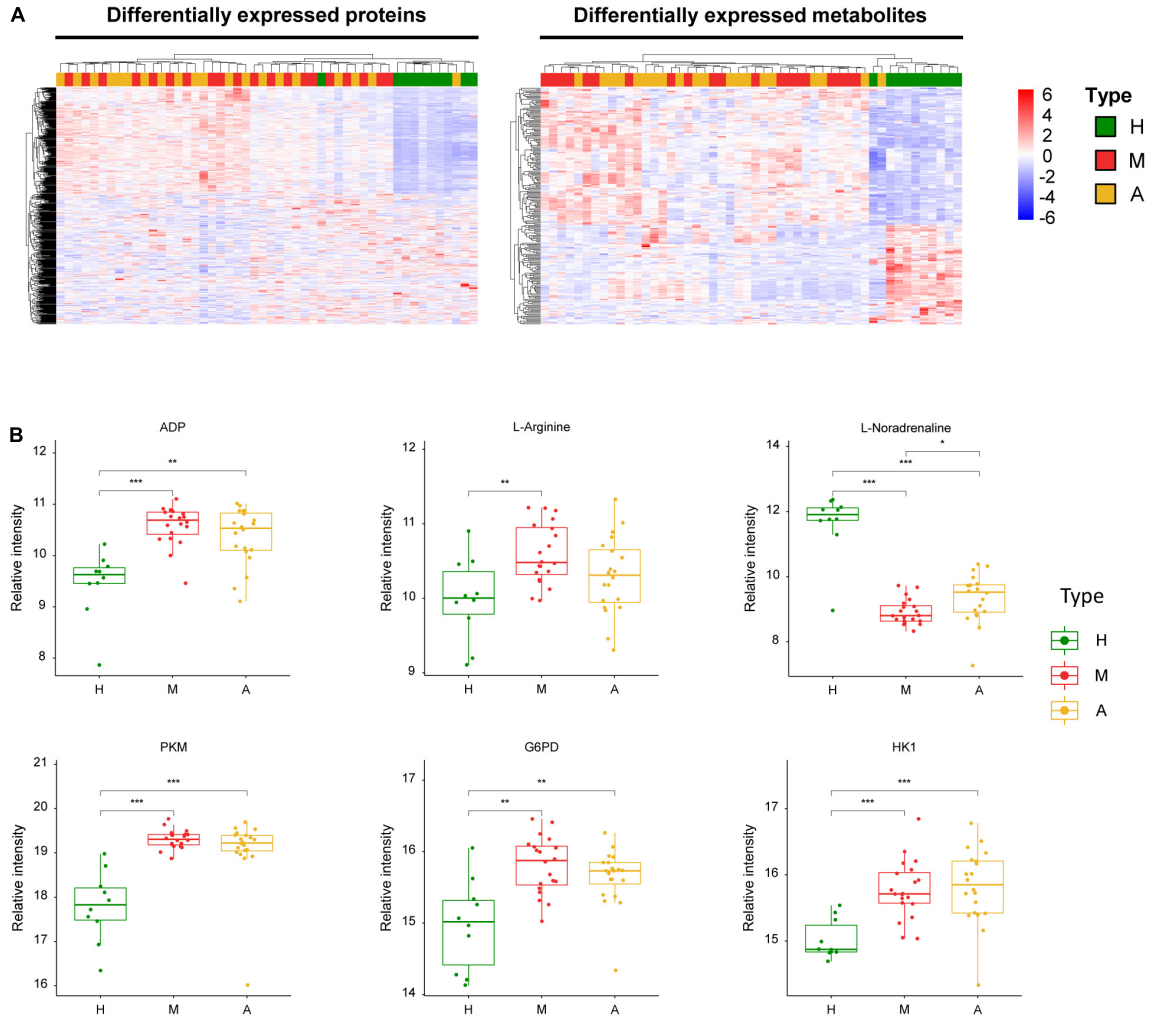

FIGURE 4
Dysregulated proteins and metabolites in H group and M group. **(A)** Heatmap of DEPs and DEMs between H group and M group. **(B)** The expression level change of the key proteins and metabolites with significant difference between H group and M group. Asterisks indicate statistical significance based on unpaired two-sided Welch's *t*-test. Significance levels: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

and DEMs screened from proteomic and metabolomic data. The model achieved a high AUC for classification based only on the first 25 variables, providing sufficient estimates of the AUC results of the first 5–100 biomarkers (**Supplementary Figure 7**). The 25 key biomarkers, including 16 proteins and 9 metabolites, were ranked by average importance (**Figure 6A**). The model achieved an AUC of 0.998 (**Figure 6B**) in our dataset. Receiver operating characteristic curve analysis was performed to quantify the diagnostic performance of each of the 25 previously highlighted individual plasma metabolites. Notably, FAD was a highly predictive metabolite identified by OPLS-DA in the dataset and exhibited the highest average importance in the construction of the SVM model.

Discussion

Energy metabolism

Migraines may be interpreted as an adaptive and conservative behavioral response to an imbalance between the supply of and demand for brain energy. In recent years, advanced high-throughput techniques have increasingly been applied to different aspects of brain metabolism in migraineurs, and the metabolic mechanisms underlying migraine pathophysiology have partially been elucidated in animal studies (Gross et al., 2019). The recovery of brain energy and reduction of oxidative stress are thought to be facilitated by the onset of the migraine itself (Borkum, 2018, 2021). Moreover, growing clinical evidence has implicated an

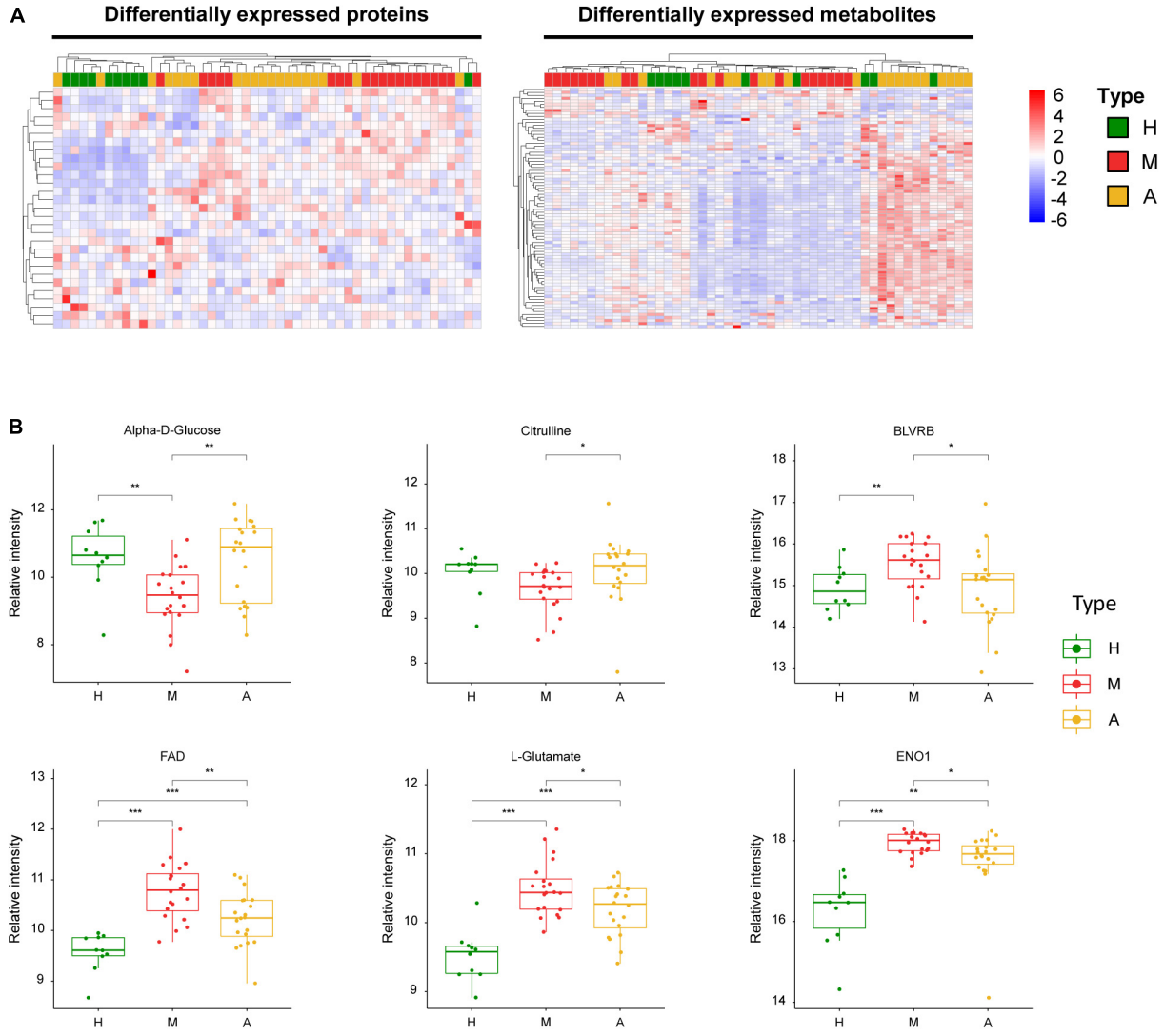

FIGURE 5
Dysregulated proteins and metabolites between M group and A group. **(A)** Heatmap of DEPs and DEMs between M group and A group. **(B)** The expression level change of the key proteins and metabolites with significant difference between M group and A group. Asterisks indicate statistical significance based on unpaired two-sided Welch's *t*-test. Significance levels: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

insufficient amount of brain energy or limited antioxidant capacity in migraine attacks (Aytaç et al., 2014). The application of ^{31}P-magnetic resonance spectroscopy (^{31}P-MRS) has revealed that mitochondrial oxidative phosphorylation in the brains of migraineurs is impaired during both ictal (Welch et al., 1989) and interictal periods (Montagna et al., 1994; Kim et al., 2010; Reyngoudt et al., 2012). Numerous reports based on ^{31}P-MRS and morphological, biochemical, and genetic studies (Stuart and Griffiths, 2012) have indicated that an energy supply imbalance (i.e., insufficient energy generation coupled with increased consumption) occurs in patients with migraines. These findings suggest that when the energy demand exceeds a threshold level, the metabolic prerequisites for a migraine may be attained, which triggers the occurrence of migraines. Riboflavin and coenzyme Q10 are key components in the regulation of the mitochondrial respiratory chain and are recommended as effective prophylactic treatments for migraines (Holland et al., 2012; Pringsheim et al., 2012). These notable findings suggest that improved energy metabolism may reduce migraine susceptibility. In this study, we compared patients with migraine and healthy individuals, as well as patients with migraine before and after acupuncture treatments. Our results indicated that acupuncture treatment for migraine without aura reversed the levels of key molecules and stimulated systematic changes in immune-, arginine-, and energy metabolism-related pathways based on proteomic and metabolomic data. Further, we established a correlation between pain intensity and key molecular changes in patients with migraine. Collectively, our

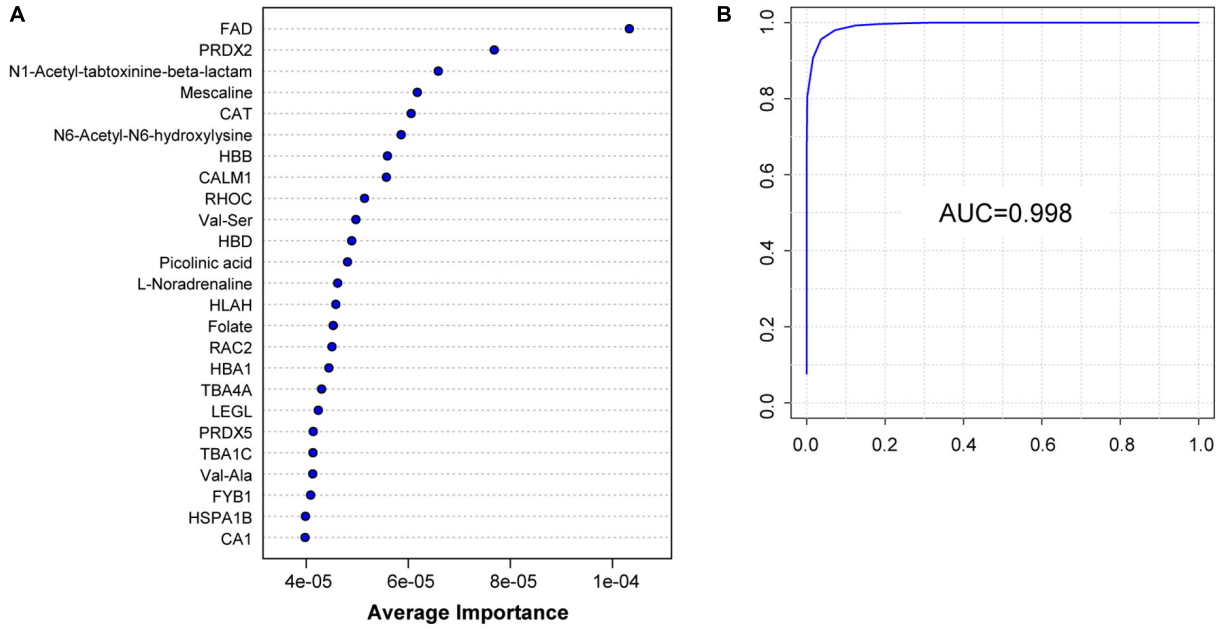

FIGURE 6
Identification of H group and M group by machine learning of proteomic and metabolomic features. **(A)** Top 25 characteristic molecules prioritized by SVM model by the average importance. **(B)** Receiver operating characteristic (ROC) of the SVM model can classify H group and M group perfectly; AUC: 0.998.

analyses of associated pathways and corresponding molecular changes related to energy metabolism provide evidence for the influence of energy metabolism on migraines.

We detected three energy metabolism-related enzymes (PKM, G6PD, and HK1) among the DEPs in the healthy controls and patients with migraine (**Figure 4B**). These enzymes are primarily involved in pentose phosphate and glycolysis/gluconeogenesis pathways (**Supplementary Figure 5A**). Via the catalysis of PKM in the glycolytic pathway reaction, a phosphate group is transferred from phosphoenolpyruvate to ADP, resulting in the production of ATP (Wang et al., 2018). The rate-limiting enzyme G6PD generates cytosolic NADPH in the oxidative pentose phosphate pathway for use in biosynthesis and oxidative defense (Zhong et al., 2021). HK1 phosphorylates glucose by consuming extracellular ATP. As the major contributors to brain energy metabolism (glucose metabolism), these enzymes that are implicated in ATP synthesis and glucose catabolism play a crucial role in migraine pathophysiology (Gross et al., 2019); therefore, the importance of these enzymes in energy metabolism-related pathways cannot be ignored. Several medications for acute migraine, such as corticosteroids, induce gluconeogenesis and are effective for treating refractory migraines and status migrainosus (Woldeamanuel et al., 2015). In hypoglycemia, cortisol, adrenaline, and noradrenaline protect cells by inhibiting insulin activity, inducing protein catabolism, and enhancing gluconeogenesis and glycogenolysis. Glycolysis and pentose phosphate pathways involved in the biological effects of changes in patients with migraine and healthy individuals are key clues to changes in energy metabolism, in which the response of these three key proteins perform to be instrumental and can serve as key evidence for changes in energy metabolism.

We also observed lower levels of alpha-D-glucose (**Figure 5B**) and higher levels of ADP (**Figure 4B**) in patients with migraine compared than in healthy controls. The aforementioned impairment in oxidative phosphorylation manifests as decreased organic phosphate levels and phosphorylation potential, higher ADP levels, and glucose hypometabolism (Welch et al., 1989; Kim et al., 2010), all of which lead to energy metabolism dysregulation, which is consistent with our data. There is also evidence of impaired oxidative phosphorylation in the brains of migraineurs during the ictal (Welch et al., 1989) and interictal periods (Montagna et al., 1994; Kim et al., 2010; Reyngoudt et al., 2012).

Previous studies have suggested that patients with migraines have insufficient metabolic reserves to satisfy the cerebral high energy demands (Lisicki et al., 2018). This causes these individuals to be susceptible to perturbations in cortical homeostasis. Therefore, it is conceivable that acupuncture treatment, in addition to riboflavin, may be beneficial for migraineurs with impaired energy metabolism, since acupuncture may provide an alternative source of fuel for the brain. Further clinical and animal studies are needed to

validate the effects of acupuncture on energy metabolism in patients with migraine. Acupuncture may increase antioxidant levels, decrease oxidative stress, and downregulate neuronal reactivity, thereby reducing the energy demands of the brain, increasing mitochondrial biogenesis, and restoring brain energy homeostasis. Our results suggest that energy metabolism regulation underscores the effects of acupuncture in patients with migraine, which is consistent with the proteomic and metabolomic findings in the healthy controls and patients with migraine.

The comparison of patients with migraine before and after acupuncture treatments revealed several key molecules, including L-arginine (**Figure 4B**) and alpha-D-glucose (**Figure 5B**). However, no significant differences were observed in the levels of these molecules between the acupuncture and healthy groups ($P > 0.05$). Conversely, we observed that the levels of several key molecules, including alpha-D-glucose, FAD, L-glutamate, citrulline, ENO1, and BLVRB, were significantly altered after acupuncture treatment compared with pre-treatment values (**Figure 5B**) ($P < 0.05$). ENO1, which is a metabolic enzyme involved in pyruvate synthesis, is a key enzyme in glycolysis. As a plasminogen receptor, ENO1 activates plasmin and degrades the extracellular matrix. Based on current characterization of the biochemical and immunological features of ENO1, it may affect the induction of strong cellular immune and specific humoral responses.

Our joint pathway analysis revealed that changes occurred in riboflavin metabolism and glycolysis pathways after acupuncture compared with pre-treatment values. In accordance with the evidence-based American Academy of Neurology guidelines (Holland et al., 2012), riboflavin is recommended as a Level B medication for migraines in adults, and its effectiveness for migraine prevention has been reported in many clinical studies (Schoenen et al., 1998; Boehnke et al., 2004; Thompson and Saluja, 2017). The properties of riboflavin are also reflected in its neuroprotective functions, which include improvements in neuroinflammation, glutamate excitotoxicity, mitochondrial dysfunction, and oxidative stress. In various neurological diseases, such as migraines, multiple sclerosis, and Parkinson's disease, the neuroprotective effects of riboflavin may play a key role in multiple pathways (e.g., iron metabolism, mitochondrial function, myelin formation, and antioxidation) that may be impaired at the physiological level. Riboflavin is involved in the oxidized glutathione recycling process and the metabolism of fats, proteins, and carbohydrates. Further, it plays a crucial role in the mitochondrial electron transport chain (Bütün et al., 2015; Marashly and Bohlega, 2017), however, a point that cannot be ignored is the excessive use of riboflavin may induce compensatory downregulation of riboflavin dependent enzymes. These changes in biological effects before and after acupuncture treatment can undoubtedly be used as evidence of changes in energy metabolism. These changes themselves may be crisscrossing and complex, but when we relate them to the corresponding molecular changes and consider the correlative intrinsic interrelationships, the role of changes in energy metabolism emerges.

Biliverdin reductase B family members are general flavin reductases that play a key role in the maintenance of cellular redox. BLVRB is thought to be capable of dictating cell fate alone (Duff et al., 2020). The BLVRB class of enzymes catalyzes the NADPH-dependent reduction of multiple flavin substrates and has emerged as a critical player in cellular redox regulation (Redzic et al., 2021). In the current study, FAD was identified as the molecule with the highest average importance in the SVM classification model that distinguished patients with migraine from healthy controls. FAD expression is upregulated in migraine patients, which may be due to the presence of reductive block in hypoxia and inflammatory signaling. Meanwhile, the level of FAD decreased after acupuncture treatment. These findings suggest alterations in the electron transport chain, which could impact ATP synthesis and energy metabolism. FAD is an essential coenzyme form of riboflavin, and plasma FAD levels have been reported to be consistent in healthy individuals who were administered low doses of riboflavin (Hustad et al., 2002). In humans, mitochondria also produce FAD, which plays a key role in mitochondrial energy metabolism (Mosegaard et al., 2020). These results highlight potential energy metabolism disorders and imbalance in energy regulation in patients with migraine.

Arginine metabolism

Arginine is an essential amino acid that regulates blood flow by modulating vascular endothelial cells. An increase in serum L-arginine level during the migraine interictal period has been reported (Reyhani et al., 2017). Clinical evidence suggests that nitric oxide (NO) also plays a key role in migraines. NO is synthesized from arginine via endothelial nitric oxide synthase (NOS). Studies focusing on substances that release NO and trigger migraines have provided evidence for the importance of NO in migraine pathogenesis (Demartini et al., 2019). In patients with migraines, endothelial dysfunction and oxidative stress have been linked to L-arginine/NO system dysfunction (Erdélyi-Bótor et al., 2017). The present metabolomics analysis identified changes in the arginine synthesis pathway in patients with migraine compared with those in healthy controls (**Figure 3A**), as well as in patients with migraine before and after acupuncture treatment (**Figure 3B**). Our data also revealed the presence of arginine biosynthesis dysregulation, including dysregulation of L-arginine, L-glutamate, citrulline, and L-aspartate.

The glutamate excitotoxicity hypothesis suggests that excessive glutamate damages neurons by causing dysfunction and degeneration (Lau and Tymianski, 2010). Glutamate is a critical neurotransmitter in the pathophysiology of migraine

headaches and central sensitization due to its excitatory action on nociceptive neurons in the trigeminovascular system (Hoffmann and Charles, 2018). Our data revealed increased L-glutamate levels in patients with migraines (**Figure 5B**). Elevated glutamate levels in blood samples have been reported in migraineurs during both ictal and interictal periods (Ferrari et al., 1990; Martínez et al., 1993; Campos et al., 2013). Notably, in the present study, we observed a decrease in glutamate levels after acupuncture treatment. Effective preventive treatments for migraine that have distinct mechanisms of action have been reported to significantly lower plasma glutamate levels (Ferrari et al., 2009).

Citrulline, which is a precursor of NO synthesis, was downregulated in migraine patients (**Figure 5B**). This could be at least partly because migraine attacks were caused by enhanced NO production via the NOS-induced conversion of arginine to citrulline and NO, resulting in the high-output NO synthesis pathway (Gruber et al., 2010). NO may participate in the mechanisms underlying migraines by triggering neurogenic inflammation and activation of fibers conveying nociceptive inputs to the trigeminal ganglion. Citrulline has been reported to remain at low levels in other types of primary headaches, which is consistent with our data (D'Andrea et al., 2019). While citrulline was upregulated after acupuncture treatment, rodent studies in which citrulline attenuated the propagation velocity of KCl-induced CSD support the notion that increased citrulline levels alleviate migraines (Kurauchi et al., 2017).

High NO levels induce CGRP expression and activate the trigeminovascular system. In this regard, NO may modulate mitochondrial function via various mechanisms (Ghasemi et al., 2018). NO-mediated suppression of the mitochondrial electron transport chain may constitute an effect of NO on mitochondrial activity (Bolanos et al., 1994). Additionally, alterations in platelet activity may play a critical role in migraine pathophysiology through mechanisms that involve the NO pathway (Paolucci et al., 2021). Consequently, the presence of NO in platelets has been proposed as a promising tool for studying changes in NO during migraines.

Molecular insights into the pathogenesis of migraines: Link between altered bioenergetics and trigeminovascular activation

Factors that trigger migraine may be attributable to dysfunction in brain energy metabolism, hormones, and environmental factors. As a multifactorial disorder, individual differences exist in the primary pathophysiology of migraines. Increasing evidence indicates that migraines may constitute a disorder of brain energetics. The pathophysiology of migraines was the major focus of this study, and we identified the role of energy metabolism and mitochondrial function in migraine pathogenesis.

Extensive evidence suggests that individuals with migraines exhibit abnormalities in energy metabolism in the brain. An increase in sensory stimulation-induced brain reactivity was observed in patients with migraine in almost every sensory modality (de Tommaso et al., 2014), indicating that migraineurs have an imbalance in energy supply demand in the brain, characterized by an increase in demand and decrease in supply. Increased brain energy deficits and/or oxidative stress are also linked to a lower threshold for sensory pain. ATP-sensitive potassium channels, which are widely expressed in the trigeminovascular system, are involved in the relationship between trigeminovascular activation and metabolic stress and are influenced by the intracellular ATP/ADP ratio as well as by cAMP and cGMP pathways (Al-Karagholi et al., 2017). In a study examining the effects of insulin, glucagon, and leptin in a rat model of migraine, changes in the transmission of trigeminal nociceptive inputs were identified, which provides insight into the dysregulated glucose state associated with migraines (Martins-Oliveira et al., 2017). As mentioned above, the nature of blood glucose disorders in migraines is complex and should form the focus of future research efforts.

Collectively, these findings indicate that oxidative stress and energy metabolism play key roles in triggering or exacerbating migraines. Indeed, abnormalities in multiple metabolic pathways in migraines are well established. Disruption of brain energy homeostasis in the trigeminovascular system is also noteworthy, as it may contribute to the pathological changes underpinning migraines. A common pathway for the activation of trigeminal nociceptors may be related to its limbic connections, primarily via the activation of brainstem chemosensitive neurons or direct stimulation of the release of sensory neuropeptides (e.g., pituitary adenylyl cyclase-activating polypeptide and CGRP) by activated meningeal afferent fibers. Conversely, chemosensitive neurons in the diencephalic region and brainstem (Lisicki et al., 2018) may be capable of sensing metabolic changes and inducing the sensitization of the trigeminovascular system. Factors associated with enhanced oxidative stress may also act as contributors to migraine pain (Benemei et al., 2014; Kozai et al., 2014).

Glycogen reserves in the human brain are limited for various reasons, including the presence of the blood–brain barrier (BBB), which excludes the entry of large, energy-dense molecules. In addition, the brain has high energy requirements and is highly dependent on circulating energy sources. The insulin-independent glucose transporter type 1 plays a key role in the transport of glucose in astrocytes, oligodendrocytes, and endothelial cells in the BBB. Notably, the physiological increase in the levels of brain lactate induced by stimulation and the theory of the astrocyte-to-neuron lactate shuttle (ANLS) provide a highly plausible explanation in this regard (Magistretti and Pellerin, 1999).

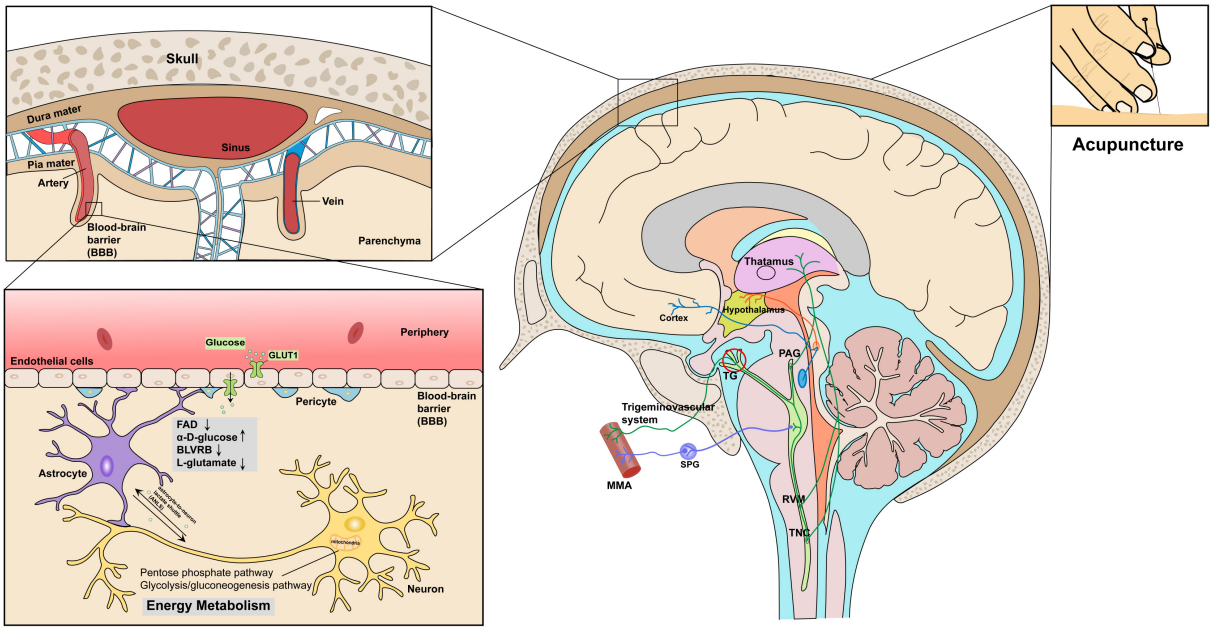

FIGURE 7
Key proteins and metabolites profiling in migraine patients after acupuncture treatment could reveal the correlative link between acupuncture and energy metabolism in the context of trigeminovascular system. Proteomics and metabolomics profiles indicated that acupuncture treatments may decrease oxidative stress levels (FAD, BLVRB, L-glutamate) while increasing glucose availability (α-D-glucose), thereby aiding in restoring energy homeostasis.

Glucose is the only carbon source of energy for metabolism in the brain and is transported into brain cells across the BBB. As such, brain tissue is highly sensitive to hypoglycemia. The ANLS theory suggests that astrocytes convert blood glucose into lactate during neuronal activation and play a pivotal role in neuronal energy metabolism, including in retinal ganglion cells (Mori et al., 2020). These results and evidence of diminished migraine energy reserves serve as the basis for our understanding of the role of glucose fluctuations and metabolic abnormalities in migraine pathophysiology.

In addition to their effects on brain excitability and reactivity, most preventive migraine therapies have the potential to improve metabolic function (Yang et al., 2014; Gross et al., 2019). Our data shed light on changes in proteomics and metabolomics profiles of the plasma of patients with migraine after acupuncture treatment. Our data reveal a correlative link between acupuncture treatment of migraine and energy metabolism (**Figure 7**). We hypothesize that acupuncture treatments may decrease oxidative stress levels while increasing glucose availability in the brain, thereby facilitating the restoration of energy homeostasis (**Figure 7**). In addition, increased mitochondrial biogenesis, reduced excitatory synaptic transmission in pain and inflammatory pathways, and increased antioxidant capacity are possible additional effects of acupuncture on migraine pathophysiology and treatment (Qu et al., 2020; Zhao et al., 2020). Nevertheless, more research is warranted to confirm these potential effects and clinical outcomes.

Limitations and prospects

Despite the need for a larger sample size, the findings described in this study indicate the existence of several promising biomarker candidates. Our results suggest that acupuncture treatments may be beneficial for patients with migraines by enhancing brain energetics; however, further research (e.g., via MRS and 18F-fluorodeoxyglucose PET) on brain metabolism before and after acupuncture treatment is warranted to draw definite conclusions. Moreover, the impact of placebo acupuncture on proteomic/metabolomic profiles should be evaluated in future studies, as this would enhance the power of the correlations identified herein. Given that sampling from the median cubital vein is more convenient for patients with migraine, we collected plasma samples before and after acupuncture treatment. In this regard, migraine-generating tissues (e.g., meningeal, trigeminal nerve, or CSF) should also be collected to confirm the correlative link between biological differences in disease-specific proteomic/metabolomic profiles and acupuncture treatment for migraine.

Future investigations should harness combined evaluation of brain and energy metabolism with sensory information processing to elucidate the imbalance between brain activity and energy metabolism in patients with migraines. Similarly, studies investigating the role of specific alterations in energy metabolism for other migraine subgroups, such as chronic migraines and migraines with aura, are necessary. Moreover, additional clinical data are required to confirm whether acupuncture treatments that ameliorate dysregulation of energy metabolism result in changes in brain energy usability and sensory information processing.

Conclusion

Our study provides a comprehensive proteomic and metabolomic analysis of plasma samples from patients with migraines before and after acupuncture treatments and a group of healthy control participants. We analyzed key protein and metabolite changes to characterize the underlying molecular and physiological effects and the potential of acupuncture to treat migraines. Our results indicate that energy metabolism pathways may provide a key link in the association between the development of migraines and the molecular changes underscoring acupuncture treatments. Significant changes in key molecules such as FAD, L-noradrenaline, α-D-glucose, BLVRB, and L-glutamate and their corresponding pathways after acupuncture treatment suggest that physiological changes may involve glutamate neurotoxicity, alongside alterations in the trigeminovascular system and other migraine-related biological effects. Migraine triggers are generally thought to be related to energy imbalances, oxidative stress, and various forms of metabolic dysregulation, including glucose metabolism disorders. Indeed, our conclusions are consistent with these hypotheses. Our data offer an overview of molecular changes in the blood that are induced by activation of the trigeminovascular pain pathway. This information may catalyze future investigations on diagnostic and therapeutic modalities in the ongoing effort to identify effective treatments for migraine attacks.

Data availability statement

The mass spectrometry data were deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=PXD028441) via the iProX partner repository (Ma et al., 2019) with the dataset identifier PXD028441. The access link in iProX is: https://www.iprox.cn/page/project.html?id=IPX0003451000.

Ethics statement

The studies involving human participants were reviewed and approved by the Research Ethical Committee of Beijing Hospital of Traditional Chinese Medicine. The patients/participants provided their written informed consent to participate in this study.

Author contributions

CL and BL conceived and designed the study, revised the manuscript, and provided key intellectual content. LL, DL, and YZ were responsible for sample collection. WL and LL performed the statistical analyses. LL, WL, and PG wrote the manuscript and prepared the figures and tables. TL, PG, XL, YG, MT, and HH contributed to a thorough revision of the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2022.1013328/full#supplementary-material

References

Agosti, R. (2018). Migraine Burden of Disease: From the Patient's Experience to a Socio-Economic View. *Headache* 58(Suppl. 1), 17–32. doi: 10.1111/head.13301

Al-Karagholi, M. A., Hansen, J. M., Severinsen, J., Jansen-Olesen, I., and Ashina, M. (2017). The KATP channel in migraine pathophysiology: a novel therapeutic target for migraine. *J. Headache Pain* 18:90. doi: 10.1186/s10194-017-0800-8

Ashina, M. (2020). Migraine. *N. Engl. J. Med.* 383, 1866–1876. doi: 10.1056/NEJMra1915327

Ashina, M., Buse, D. C., Ashina, H., Pozo-Rosich, P., Peres, M. F. P., Lee, M. J., et al. (2021a). Migraine: integrated approaches to clinical management and emerging treatments. *Lancet* 397, 1505–1518. doi: 10.1016/s0140-6736(20)32342-4

Ashina, M., Terwindt, G. M., Al-Karagholi, M. A., de Boer, I., Lee, M. J., Hay, D. L., et al. (2021b). Migraine: disease characterisation, biomarkers, and precision medicine. *Lancet* 397, 1496–1504. doi: 10.1016/S0140-6736(20)32162-0

Aytaç, B., Coşkun, Ö, Alioğlu, B., Durak, Z. E., Büber, S., Tapçi, E., et al. (2014). Decreased antioxidant status in migraine patients with brain white matter hyperintensities. *Neurol. Sci.* 35, 1925–1929. doi: 10.1007/s10072-014-1864-8

Benemei, S., Fusi, C., Trevisan, G., and Geppetti, P. (2014). The TRPA1 channel in migraine mechanism and treatment. *Br. J. Pharmacol.* 171, 2552–2567. doi: 10.1111/bph.12512

Boehnke, C., Reuter, U., Flach, U., Schuh-Hofer, S., Einhäupl, K. M., and Arnold, G. (2004). High-dose riboflavin treatment is efficacious in migraine prophylaxis: an open study in a tertiary care centre. *Eur. J. Neurol.* 11, 475–477. doi: 10.1111/j.1468-1331.2004.00813.x

Bolanos, J. P., Peuchen, S., Heales, S. J., Land, J. M., and Clark, J. B. (1994). Nitric oxide-mediated inhibition of the mitochondrial respiratory chain in cultured astrocytes. *J. Neurochem.* 63, 910–916. doi: 10.1046/j.1471-4159.1994.63030910.x

Borkum, J. M. (2018). The Migraine Attack as a Homeostatic, Neuroprotective Response to Brain Oxidative Stress: Preliminary Evidence for a Theory. *Headache* 58, 118–135. doi: 10.1111/head.13214

Borkum, J. M. (2021). Brain energy deficit as a source of oxidative stress in migraine: A molecular basis for migraine susceptibility. *Neurochem. Res.* 46, 1913–1932. doi: 10.1007/s11064-021-03335-9

Bütün, A., Nazıroğlu, M., Demirci, S., Çelik, Ö, and Uğuz, A. C. (2015). Riboflavin and vitamin E increase brain calcium and antioxidants, and microsomal calcium-ATP-ase values in rat headache models induced by glyceryl trinitrate. *J. Membr. Biol.* 248, 205–213. doi: 10.1007/s00232-014-9758-5

Buysse, D. J., Reynolds, C. F. III, Monk, T. H., Berman, S. R., and Kupfer, D. J. (1989). The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res.* 28, 193–213. doi: 10.1016/0165-1781(89)90047-4

Campos, F., Sobrino, T., Pérez-Mato, M., Rodríguez-Osorio, X., Leira, R., Blanco, M., et al. (2013). Glutamate oxaloacetate transaminase: a new key in the dysregulation of glutamate in migraine patients. *Cephalalgia* 33, 1148–1154. doi: 10.1177/0333102413487444

Charles, A. (2018). The pathophysiology of migraine: implications for clinical management. *Lancet Neurol.* 17, 174–182. doi: 10.1016/s1474-4422(17)30435-0

Chi, H., Liu, C., Yang, H., Zeng, W. F., Wu, L., Zhou, W. J., et al. (2018). Comprehensive identification of peptides in tandem mass spectra using an efficient open search engine. *Nat. Biotechnol.* [Online ahead of print] doi: 10.1038/nbt.4236

Cole, J. C., Lin, P., and Rupnow, M. F. (2007). Validation of the Migraine-Specific Quality of Life Questionnaire version 2.1 (MSQ v. 2.1) for patients undergoing prophylactic migraine treatment. *Qual. Life Res.* 16, 1231–1237. doi: 10.1007/s11136-007-9217-1

D'Andrea, G., Gucciardi, A., Perini, F., and Leon, A. (2019). Pathogenesis of Cluster Headache: From Episodic to Chronic Form, the Role of Neurotransmitters and Neuromodulators. *Headache* 59, 1665–1670. doi: 10.1111/head.13673

de Tommaso, M., Ambrosini, A., Brighina, F., Coppola, G., Perrotta, A., Pierelli, F., et al. (2014). Altered processing of sensory stimuli in patients with migraine. *Nat. Rev. Neurol.* 10, 144–155. doi: 10.1038/nrneurol.2014.14

Demartini, C., Greco, R., Zanaboni, A. M., Sances, G., De Icco, R., Borsook, D., et al. (2019). Nitroglycerin as a comparative experimental model of migraine pain: From animal to human and back. *Prog. Neurobiol.* 177, 15–32. doi: 10.1016/j.pneurobio.2019.02.002

Demichev, V., Messner, C. B., Vernardis, S. I., Lilley, K. S., and Ralser, M. (2020). DIA-NN: neural networks and interference correction enable deep proteome coverage in high throughput. *Nat. Methods* 17, 41–44. doi: 10.1038/s41592-019-0638-x

Dong, X., Guan, X., Chen, K., Jin, S., Wang, C., Yan, L., et al. (2017). Abnormal mitochondrial dynamics and impaired mitochondrial biogenesis in trigeminal ganglion neurons in a rat model of migraine. *Neurosci. Lett.* 636, 127–133. doi: 10.1016/j.neulet.2016.10.054

Duff, M. R., Redzic, J. S., Ryan, L. P., Paukovich, N., Zhao, R., Nix, J. C., et al. (2020). Structure, dynamics and function of the evolutionarily changing biliverdin reductase B family. *J. Biochem,* 168, 191–202. doi: 10.1093/jb/mvaa039

Erdélyi-Bótor, S., Komáromy, H., Kamson, D. O., Kovács, N., Perlaki, G., Orsi, G., et al. (2017). Serum L-arginine and dimethylarginine levels in migraine patients with brain white matter lesions. *Cephalalgia* 37, 571–580. doi: 10.1177/0333102416651454

Ferrari, A., Spaccapelo, L., Pinetti, D., Tacchi, R., and Bertolini, A. (2009). Effective prophylactic treatments of migraine lower plasma glutamate levels. *Cephalalgia* 29, 423–429. doi: 10.1111/j.1468-2982.2008.01749.x

Ferrari, M. D., Odink, J., Bos, K. D., Malessy, M. J., and Bruyn, G. W. (1990). Neuroexcitatory plasma amino acids are elevated in migraine. *Neurology* 40, 1582–1586. doi: 10.1212/wnl.40.10.1582

Freitas, S., Simões, M. R., Alves, L., and Santana, I. (2013). Montreal cognitive assessment: validation study for mild cognitive impairment and Alzheimer disease. *Alzheimer Dis. Assoc. Disord.* 27, 37–43. doi: 10.1097/WAD.0b013e3182420bfe

GBD 2016 Disease and Injury Incidence and Prevalence Collaborators (2017). Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet* 390, 1211–1259. doi: 10.1016/S0140-6736(17)32154-2

GBD 2016 Neurology Collaborators (2019). Global, regional, and national burden of neurological disorders, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* 18, 459–480. doi: 10.1016/S1474-4422(18)30499-X

Geisser, M. E., Roth, R. S., and Robinson, M. E. (1997). Assessing depression among persons with chronic pain using the Center for Epidemiological Studies-Depression Scale and the Beck Depression Inventory: a comparative analysis. *Clin. J. Pain* 13, 163–170. doi: 10.1097/00002508-199706000-00011

Ghasemi, M., Mayasi, Y., Hannoun, A., Eslami, S. M., and Carandang, R. (2018). Nitric Oxide and Mitochondrial Function in Neurological Diseases. *Neuroscience* 376, 48–71. doi: 10.1016/j.neuroscience.2018.02.017

Goadsby, P. J., Holland, P. R., Martins-Oliveira, M., Hoffmann, J., Schankin, C., and Akerman, S. (2017). Pathophysiology of migraine: A disorder of sensory processing. *Physiol. Rev.* 97, 553–622. doi: 10.1152/physrev.00034.2015

Gormley, P., Anttila, V., Winsvold, B. S., Palta, P., Esko, T., Pers, T. H., et al. (2016). Meta-analysis of 375,000 individuals identifies 38 susceptibility loci for migraine. *Nat. Genet.* 48, 856–866. doi: 10.1038/ng.3598

Gross, E. C., Lisicki, M., Fischer, D., Sándor, P. S., and Schoenen, J. (2019). The metabolic face of migraine - from pathophysiology to treatment. *Nat. Rev. Neurol.* 15, 627–643. doi: 10.1038/s41582-019-0255-4

Gruber, H. J., Bernecker, C., Lechner, A., Weiss, S., Wallner-Blazek, M., Meinitzer, A., et al. (2010). Increased nitric oxide stress is associated with migraine. *Cephalalgia* 30, 486–492. doi: 10.1111/j.1468-2982.2009.01964.x

Headache Classification Committee of the International Headache Society (2013). The International Classification of Headache Disorders, 3rd edition (beta version). *Cephalalgia* 33, 629–808. doi: 10.1177/0333102413485658

Headache Classification Committee of the International Headache Society (IHS) (2018). The International Classification of Headache Disorders, 3rd edition. *Cephalalgia* 38, 1–211. doi: 10.1177/0333102417738202

Hoffmann, J., and Charles, A. (2018). Glutamate and Its Receptors as Therapeutic Targets for Migraine. *Neurotherapeutics* 15, 361–370. doi: 10.1007/s13311-018-0616-5

Holland, S., Silberstein, S. D., Freitag, F., Dodick, D. W., Argoff, C., Ashman, E., et al. (2012). Evidence-based guideline update: NSAIDs and other complementary treatments for episodic migraine prevention in adults: report of the Quality Standards Subcommittee of the American Academy of Neurology and the American Headache Society. *Neurology* 78, 1346–1353. doi: 10.1212/WNL.0b013e3182535d0c

Hustad, S., McKinley, M. C., McNulty, H., Schneede, J., Strain, J. J., Scott, J. M., et al. (2002). Riboflavin, flavin mononucleotide, and flavin adenine dinucleotide in human plasma and erythrocytes at baseline and after low-dose riboflavin supplementation. *Clin. Chem.* 48, 1571–1577.

Kaddurah-Daouk, R., Kristal, B. S., and Weinshilboum, R. M. (2008). Metabolomics: a global biochemical approach to drug response and disease. *Annu. Rev. Pharmacol. Toxicol.* 48, 653–683. doi: 10.1146/annurev.pharmtox.48.113006.094715

Katsarava, Z., Mania, M., Lampl, C., Herberhold, J., and Steiner, T. J. (2018). Poor medical care for people with migraine in Europe - evidence from the Eurolight study. *J. Headache Pain* 19:10. doi: 10.1186/s10194-018-0839-1

Kim, J. H., Kim, S., Suh, S. I., Koh, S. B., Park, K. W., and Oh, K. (2010). Interictal metabolic changes in episodic migraine: a voxel-based FDG-PET study. *Cephalalgia* 30, 53–61. doi: 10.1111/j.1468-2982.2009.01890.x

Kozai, D., Ogawa, N., and Mori, Y. (2014). Redox regulation of transient receptor potential channels. *Antioxid. Redox. Signal.* 21, 971–986. doi: 10.1089/ars.2013.5616

Krymchantowski, A. V. (2004). Acute treatment of migraine. Breaking the paradigm of monotherapy. *BMC Neurol.* 4:4. doi: 10.1186/1471-2377-4-4

Kurauchi, Y., Mokudai, K., Mori, A., Sakamoto, K., Nakahara, T., Morita, M., et al. (2017). l-Citrulline ameliorates cerebral blood flow during cortical spreading depression in rats: Involvement of nitric oxide- and prostanoids-mediated pathway. *J. Pharmacol. Sci.* 133, 146–155. doi: 10.1016/j.jphs.2017.02.004

Kursun, O., Yemisci, M., van den Maagdenberg, A., and Karatas, H. (2021). Migraine and neuroinflammation: the inflammasome perspective. *J. Headache Pain* 22:55. doi: 10.1186/s10194-021-01271-1

Lau, A., and Tymianski, M. (2010). Glutamate receptors, neurotoxicity and neurodegeneration. *Pflugers Arch.* 460, 525–542. doi: 10.1007/s00424-010-0809-1

Leyfer, O. T., Ruberg, J. L., and Woodruff-Borden, J. (2006). Examination of the utility of the Beck Anxiety Inventory and its factors as a screener for anxiety disorders. *J. Anxiety Disord.* 20, 444–458. doi: 10.1016/j.janxdis.2005.05.004

Lionetto, L., Gentile, G., Bellei, E., Capi, M., Sabato, D., Marsibilio, F., et al. (2013). The omics in migraine. *J. Headache Pain* 14:55. doi: 10.1186/1129-2377-14-55

Lisicki, M., D'Ostilio, K., Coppola, G., Scholtes, F., Maertens de Noordhout, A., Parisi, V., et al. (2018). Evidence of an increased neuronal activation-to-resting glucose uptake ratio in the visual cortex of migraine patients: a study comparing (18)FDG-PET and visual evoked potentials. *J. Headache Pain* 19:49. doi: 10.1186/s10194-018-0877-8

Liu, C., Song, C. Q., Yuan, Z. F., Fu, Y., Chi, H., Wang, L. H., et al. (2014). pQuant improves quantitation by keeping out interfering signals and evaluating the accuracy of calculated ratios. *Anal. Chem.* 86, 5286–5294. doi: 10.1021/ac404246w

Luan, H., Ji, F., Chen, Y., and Cai, Z. (2018). statTarget: A streamlined tool for signal drift correction and interpretations of quantitative mass spectrometry-based omics data. *Anal. Chim. Acta* 1036, 66–72. doi: 10.1016/j.aca.2018.08.002

Ma, J., Chen, T., Wu, S., Yang, C., Bai, M., Shu, K., et al. (2019). iProX: an integrated proteome resource. *Nucleic Acids Res.* 47, D1211–D1217. doi: 10.1093/nar/gky869

Magistretti, P. J., and Pellerin, L. (1999). Cellular mechanisms of brain energy metabolism and their relevance to functional brain imaging. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 354, 1155–1163. doi: 10.1098/rstb.1999.0471

Marashly, E. T., and Bohlega, S. A. (2017). Riboflavin has neuroprotective potential: Focus on Parkinson's disease and migraine. *Front. Neurol.* 8:333. doi: 10.3389/fneur.2017.00333

Martínez, F., Castillo, J., Rodríguez, J. R., Leira, R., and Noya, M. (1993). Neuroexcitatory amino acid levels in plasma and cerebrospinal fluid during migraine attacks. *Cephalalgia* 13, 89–93. doi: 10.1046/j.1468-2982.1993.1302089.x

Martins-Oliveira, M., Akerman, S., Holland, P. R., Hoffmann, J. R., Tavares, I., and Goadsby, P. J. (2017). Neuroendocrine signaling modulates specific neural networks relevant to migraine. *Neurobiol. Dis.* 101, 16–26. doi: 10.1016/j.nbd.2017.01.005

Montagna, P., Cortelli, P., Monari, L., Pierangeli, G., Parchi, P., Lodi, R., et al. (1994). 31P-magnetic resonance spectroscopy in migraine without aura. *Neurology* 44, 666–669. doi: 10.1212/wnl.44.4.666

Mori, S., Kurimoto, T., Miki, A., Maeda, H., Kusuhara, S., and Nakamura, M. (2020). Aqp9 Gene Deletion Enhances Retinal Ganglion Cell (RGC) Death and Dysfunction Induced by Optic Nerve Crush: Evidence that Aquaporin 9 Acts as an Astrocyte-to-Neuron Lactate Shuttle in Concert with Monocarboxylate Transporters To Support RGC Function and Survival. *Mol. Neurobiol.* 57, 4530–4548. doi: 10.1007/s12035-020-02030-0

Mosegaard, S., Dipace, G., Bross, P., Carlsen, J., Gregersen, N., and Olsen, R. K. J. (2020). Riboflavin Deficiency-Implications for General Human Health and Inborn Errors of Metabolism. *Int. J. Mol. Sci.* 21:3847. doi: 10.3390/ijms21113847

Pang, Z., Chong, J., Zhou, G., de Lima Morais, D. A., Chang, L., Barrette, M., et al. (2021). MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. *Nucleic Acids Res.* 49, W388–W396. doi: 10.1093/nar/gkab382

Paolucci, M., Altamura, C., and Vernieri, F. (2021). The role of endothelial dysfunction in the pathophysiology and cerebrovascular effects of migraine: A narrative review. *J. Clin. Neurol.* 17, 164–175. doi: 10.3988/jcn.2021.17.2.164

Pringsheim, T., Davenport, W., Mackie, G., Worthington, I., Aube, M., Christie, S. N., et al. (2012). Canadian Headache Society guideline for migraine prophylaxis. *Can. J. Neurol. Sci.* 39(2 Suppl. 2), S1–S59.

Qu, Z., Liu, L., Yang, Y., Zhao, L., Xu, X., Li, Z., et al. (2020). Electro-acupuncture inhibits C-fiber-evoked WDR neuronal activity of the trigeminocervical complex: Neurophysiological hypothesis of a complementary therapy for acute migraine modeled rats. *Brain Res.* 1730:146670. doi: 10.1016/j.brainres.2020.146670

Redzic, J. S., Duff, M. R., Blue, A., Pitts, T. M., Agarwal, P., and Eisenmesser, E. Z. (2021). Modulating Enzyme Function via Dynamic Allostery within Biliverdin Reductase B. *Front. Mol. Biosci.* 8:691208. doi: 10.3389/fmolb.2021.691208

Reyhani, A., Celik, Y., Karadag, H., Gunduz, O., Asil, T., and Sut, N. (2017). High asymmetric dimethylarginine, symmetric dimethylarginine and L-arginine levels in migraine patients. *Neurol. Sci.* 38, 1287–1291. doi: 10.1007/s10072-017-2970-1

Reyngoudt, H., Achten, E., and Paemeleire, K. (2012). Magnetic resonance spectroscopy in migraine: what have we learned so far? *Cephalalgia* 32, 845–859. doi: 10.1177/0333102412452048

Schoenen, J., Jacquy, J., and Lenaerts, M. (1998). Effectiveness of high-dose riboflavin in migraine prophylaxis. A randomized controlled trial. *Neurology* 50, 466–470. doi: 10.1212/wnl.50.2.466

Shen, X., Wang, R., Xiong, X., Yin, Y., Cai, Y., Ma, Z., et al. (2019). Metabolic reaction network-based recursive metabolite annotation for untargeted metabolomics. *Nat. Commun.* 10:1516. doi: 10.1038/s41467-019-09550-x

Steiner, T. J., Stovner, L. J., Katsarava, Z., Lainez, J. M., Lampl, C., Lanteri-Minet, M., et al. (2014). The impact of headache in Europe: principal results of the Eurolight project. *J. Headache Pain* 15:31. doi: 10.1186/1129-2377-15-31

Stuart, S., and Griffiths, L. R. (2012). A possible role for mitochondrial dysfunction in migraine. *Mol. Genet. Genom.* 287, 837–844. doi: 10.1007/s00438-012-0723-7

Suhre, K., McCarthy, M. I., and Schwenk, J. M. (2021). Genetics meets proteomics: perspectives for large population-based studies. *Nat. Rev. Genet.* 22, 19–37. doi: 10.1038/s41576-020-0268-2

Tfelt-Hansen, P., Pascual, J., Ramadan, N., Dahlof, C., D'Amico, D., Diener, H. C., et al. (2012). Guidelines for controlled trials of drugs in migraine: third edition. A guide for investigators. *Cephalalgia* 32, 6–38. doi: 10.1177/0333102411417901

Thompson, D. F., and Saluja, H. S. (2017). Prophylaxis of migraine headaches with riboflavin: A systematic review. *J. Clin. Pharm. Ther.* 42, 394–403. doi: 10.1111/jcpt.12548

Tsai, S. T., Tseng, C. H., Lin, M. C., Liao, H. Y., Teoh, B. K., San, S., et al. (2020). Acupuncture reduced the medical expenditure in migraine patients: Real-world data of a 10-year national cohort study. *Medicine* 99:e21345. doi: 10.1097/MD.0000000000021345

Wang, B., Liu, S., Fan, B., Xu, X., Chen, Y., Lu, R., et al. (2018). PKM2 is involved in neuropathic pain by regulating ERK and STAT3 activation in rat spinal cord. *J. Headache Pain* 19:7. doi: 10.1186/s10194-018-0836-4

Wang, L. P., Zhang, X. Z., Guo, J., Liu, H. L., Zhang, Y., Liu, C. Z., et al. (2011). Efficacy of acupuncture for migraine prophylaxis: a single-blinded, double-dummy, randomized controlled trial. *Pain* 152, 1864–1871. doi: 10.1016/j.pain.2011.04.006

Wang, S., Li, W., Hu, L., Cheng, J., Yang, H., and Liu, Y. (2020). NAguideR: performing and prioritizing missing value imputations for consistent bottom-up proteomic analyses. *Nucleic Acids Res.* 48:e83. doi: 10.1093/nar/gkaa498

Welch, K. M., Levine, S. R., D'Andrea, G., Schultz, L. R., and Helpern, J. A. (1989). Preliminary observations on brain energy metabolism in migraine studied by in vivo phosphorus 31 NMR spectroscopy. *Neurology* 39, 538–541. doi: 10.1212/wnl.39.4.538

Wishart, D. S. (2019). Metabolomics for investigating physiological and pathophysiological processes. *Physiol. Rev.* 99, 1819–1875. doi: 10.1152/physrev.00035.2018

Woldeamanuel, Y. W., Rapoport, A. M., and Cowan, R. P. (2015). The place of corticosteroids in migraine attack management: A 65-year systematic review with pooled analysis and critical appraisal. *Cephalalgia* 35, 996–1024. doi: 10.1177/0333102414566200

Xu, S., Yu, L., Luo, X., Wang, M., Chen, G., Zhang, Q., et al. (2020). Manual acupuncture versus sham acupuncture and usual care for prophylaxis of episodic migraine without aura: multicentre, randomised clinical trial. *BMJ* 368:m697. doi: 10.1136/bmj.m697

Yang, M., Rendas-Baum, R., Varon, S. F., and Kosinski, M. (2011). Validation of the Headache Impact Test (HIT-6^{TM}) across episodic and chronic migraine. *Cephalalgia* 31, 357–367. doi: 10.1177/0333102410379890

Yang, M., Yang, J., Zeng, F., Liu, P., Lai, Z., Deng, S., et al. (2014). Electroacupuncture stimulation at sub-specific acupoint and non-acupoint induced distinct brain glucose metabolism change in migraineurs: a PET-CT study. *J. Transl. Med.* 12:351. doi: 10.1186/s12967-014-0351-6

Yu, G., Wang, L. G., Han, Y., and He, Q. Y. (2012). clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS* 16, 284–287. doi: 10.1089/omi.2011.0118

Zhao, L., Chen, J., Li, Y., Sun, X., Chang, X., Zheng, H., et al. (2017). The long-term effect of acupuncture for migraine prophylaxis: A randomized clinical trial. *JAMA Intern. Med.* 177, 508–515. doi: 10.1001/jamainternmed.2016.9378

Zhao, L., Liu, L., Xu, X., Qu, Z., Zhu, Y., Li, Z., et al. (2020). Electroacupuncture Inhibits Hyperalgesia by Alleviating Inflammatory Factors in a Rat Model of Migraine. *J. Pain Res.* 13, 75–86. doi: 10.2147/jpr.S225431

Zhong, B., Jiang, D., Hong, Y., Li, L., Qiu, L., Yang, R., et al. (2021). Glucose-6-phosphate dehydrogenase neutralizes stresses by supporting reductive glutamine metabolism and AMPK activation. *Signal. Transduct. Target Ther.* 6:46. doi: 10.1038/s41392-020-00399-x

Zielman, R., Postma, R., Verhoeven, A., Bakels, F., van Oosterhout, W. P., Meissner, A., et al. (2016). Metabolomic changes in CSF of migraine patients measured with (1)H-NMR spectroscopy. *Mol. Biosyst.* 12, 3674–3682. doi: 10.1039/c6mb00424e

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Effectiveness of acupuncture combined with rehabilitation training vs. rehabilitation training alone for post-stroke shoulder pain: A systematic review and meta-analysis of randomized controlled trials

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Background: Post-stroke shoulder pain (PSSP) is characterized by shoulder pain on the hemiplegic side, which can limit physical activity in patients with stroke. Acupuncture combined with rehabilitation training (AR) has been widely used in PSSP, but the evidence of its effectiveness is still unclear.

Objective: The study aimed to evaluate the effect and safety of AR vs. rehabilitation training (RT) alone on PSSP.

Methods: We searched PubMed, the Cochrane Library, the Chinese Biological Medicine Database (CBM), the Chinese Scientific Journal Database (VIP), China National Knowledge Infrastructure (CNKI), and the WAN FANG database for relevant studies from their inception to February 2022. Only randomized controlled trials (RCTs) comparing the effect of AR with RT alone on PSSP were considered. The primary outcome was shoulder pain. Secondary outcomes included upper limb motor function, activities of daily living (ADL), shoulder range of motion (ROM), and adverse events (AEs). Subgroup analysis and sensitivity analysis were also conducted. Quality assessment was implemented based on Cochrane risk of bias (ROB) criteria, which consist of seven items. When more than four items in a study were judged as low ROB, the overall quality of this study was considered low risk.

Results: A total of 40 studies were included in the qualitative analysis, and 35 (87.5%) studies with 2,554 patients were included in the meta-analysis.

Of the 40 studies, 14 (35.0%) were of moderate-to-high quality. The meta-analysis results showed that AR is better than RT alone in reducing shoulder pain (MD −1.32, 95% CI −1.58 to −1.07), improving upper limb motor function (MD 6.81, 95% CI 4.95–8.67), ADL (MD 11.17, 95% CI 9.44–12.91), and shoulder ROM (internal rotation: MD 10.48, 95% CI 8.14–12.83; backward extension: MD 7.82, 95% CI 6.00–9.64; anteflexion: MD 12.88, 95% CI 5.47–20.29; external rotation: MD 11.40, 95% CI 6.17–16.64; abduction: MD 16.96, 95% CI 8.61–25.31) without obvious AEs.

Conclusion: AR may be better than RT alone for the improvement of shoulder pain, upper limb motor function, ADL, and shoulder ROM, without obvious AEs in patients with PSSP. However, considering the clinical and statistical heterogeneity, our findings need to be interpreted with caution. More rigorous RCTs in this area should be conducted in the future.

Systematic review registration: [www.crd.york.ac.uk], identifier [CRD42022326763].

KEYWORDS

post-stroke shoulder pain, acupuncture, rehabilitation training, alternative and complementary medicine, meta-analysis

Introduction

Post-stroke shoulder pain (PSSP) is a common complication of stroke, characterized by shoulder pain on the hemiplegic side (1–3). PSSP occurs more frequently in the chronic phase after stroke than in the acute phase and usually occurs 2–3 months after stroke (4). Recently, a review including 3,496 patients with stroke demonstrated that the relatively conservative estimate of the total annual incidence of PSSP fluctuates at 0.30 (1). The prevalence may be higher among those in rehabilitation because they tend to have more risk factors (5). To date, due to the etiology of shoulder pain being complex and multifactorial, the exact pathogenesis of PSSP still remains controversial (6, 7). Altered peripheral and central nervous activities, such as spasticity, severe arm paralysis, central post-stroke pain, complex regional pain syndrome, and central hypersensitivity, are considered to be related to PSSP, as well as musculoskeletal disorders, such as supraspinatus tendon pathology, frozen shoulder, and impingement syndrome (5, 8–11). Furthermore, the aforementioned causes may contribute to the development of PSSP individually or in combination. The negative effects of PSSP, such as limited physical activity, depressive states, and sleep disturbances, significantly deteriorate patients' quality of life (12–15), predispose patients to withdraw from rehabilitation programs, and prolong hospitalization (1, 16), which impose a great burden on both patients and society.

Rehabilitation training (RT) and symptomatic treatment (physical therapy, occupational therapy, transcutaneous electrical nerve stimulation, peripheral nerve stimulation, robotic-assisted shoulder rehabilitation, good limb position, and ROM exercises for the affected shoulder) are the main intervention methods of PSSP (7, 17–20). However, shortcomings exist in the single rehabilitation treatment, including the short duration of efficacy, the limited scope of medication indications, potential adverse drug reactions, and most importantly, limited analgesic efficacy (21–23). For example, neuromuscular electrical stimulation has preventive and therapeutic effects on subluxation, but not pain relief (24, 25). Pharmacological therapy such as corticosteroid injections may not be applied to some type of PSSP owing to its potential side effects (26).

In recent years, acupuncture has become an increasingly popular technique and is used worldwide for the management of pain, headache, musculoskeletal diseases, and other health problems (27). Previous studies demonstrated that acupuncture is effective and safe for PSSP, especially in reducing pain intensity (27–30). It has been incorporated into some guidelines as adjuvant therapy for PSSP (31). Acupuncture combined with rehabilitation (AR) is more effective than rehabilitation training (RT) alone in relieving shoulder pain, improving upper limb movement, and increasing joint range of motion (ROM) in patients with PSSP (17, 32–34). The combined therapy can also shorten the treatment duration, increase blood flow, and reduce edema with fewer adverse reactions (32, 35–37). In 2015, a review including 13 randomized controlled trials (RCTs) demonstrated that AR may be better than RT alone in reducing pain and improving upper limb motor function and activities of daily living (ADL) (38). The number of studies included in

this meta-analysis was limited, the quality of studies included was mostly low, and the reliability of its conclusions needs to be improved. In recent years, a large number of RCTs focusing on AR in the treatment of PSSP have been published. We conducted this systematic review (SR) and meta-analysis (MA) to comprehensively update the existing evidence to clarify the role of AR in PSSP. The results of this study may provide evidence for the rehabilitation management of PSSP.

Methods

We conducted this SR and MA according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). Only RCTs compared the efficacy of AR with RT alone on PSSP and published in Chinese or English were considered eligible for our MA. A total of six databases, such as PubMed and the Cochrane Library, were searched systematically from their inception to February 2022 for eligible studies. The protocol was registered in the International Prospective Register of Systematic Reviews (No. CRD42022326763). In this review, no ethical approval or patient consent was required because all data analyses were from previously published studies.

Inclusion and exclusion criteria

Types of studies

We included all RCTs comparing the effectiveness of AR with RT alone for PSSP. The studies should be published in Chinese or English. We contacted the authors by email if there was any incomplete information, and studies were excluded if data remained incomplete. The studies would still be excluded if (1) the study compared the effect of different RT techniques, different acupuncture parameters, or different types of acupuncture; and (2) the study type is crossover trials, case reports, animal experiments, abstracts, thesis, or review articles.

Population

We included studies in which participants were diagnosed with ischemic or hemorrhagic stroke by recognized criteria or brain imaging technology (e.g., brain CT, MRI, and DSA). Studies were included if the participants in the study had hemiplegic shoulder pain following a stroke, and shoulder pain was assessed by using a visual analog scale (VAS), or other recognized instruments [e.g., McGi pain questionnaire (MPQ) (39), numeric rating scale (NRS) (40), and faces pain scale (FPS) (41)]. The inclusion criteria included participants > 18 years, regardless of gender, race, clinical setting, or the time since stroke. We excluded studies that recruited participants with the shoulder-hand syndrome (i.e., complex regional pain syndrome) or shoulder dislocation. We also excluded studies that included participants who suffered from shoulder periarthritis, shoulder trauma, and other shoulder diseases before the stroke.

Intervention and comparison

We included studies assessing the effectiveness of AR vs. RT alone on PSSP. The types of acupuncture included auricular acupuncture, abdominal acupuncture (AA), balancing acupuncture (BAA), body acupuncture (BA), carpus–ankle acupuncture (CAA), electroacupuncture (EA), fire acupuncture (FA), relaxing needling at meridian-muscle nodes (RNN), scalp acupuncture (SA), traditional acupuncture (TA), and warm acupuncture (WA), but not moxibustion. The RT mainly included good limb position, Bobath, Brunnstrom, Rood, and shoulder joint ROM exercise. The participants in the AR group received AR, while the participants in the RT group, received RT alone. The RT regimens and base medicines in the two groups must be similar.

Outcomes

Primary outcome

The primary outcome was shoulder pain assessed by VAS, NRS, MPQ, or FPS at the end of treatment. The VAS score changes between 0 and 10 points, the NRS uses the numbers 0–10 to indicate pain intensity, the MPQ evaluates pain *via* 11 questions, and the FPS uses different facial expressions to show six levels of pain. These scales indicate that the higher the score, the more severe the pain.

Secondary outcome

The secondary outcomes were the motor function of the upper limb, ADL, shoulder ROM, and adverse events (AEs). The assessment time of the outcomes was at the end of treatment. The upper limb motor function was assessed by the Fugl-Meyer scale for the upper limb (FMA-U), ADL evaluated by the Barthel Index (BI) or the modified Barthel Index (MBI), and shoulder ROM measured by the protractor. The FMA-U scale contains 33 items, with a full score of 66 points. The higher the FMA-U score, the better the upper limb function (42). The BI or MBI includes 10 items (e.g., eating, personal hygiene, bathing, toileting, dressing, anal control, bladder control, bed and chair transfer, level walking, and stairs), with a full score of 100 points. The lower the score, the more serious the ADL (43, 44). The potential AEs related to RT or AR may include local subcutaneous ecchymosis, nausea, dizziness, infection, and palpitation.

Data sources and searches

We searched six electronic databases from their inception to February 2022: PubMed, the Cochrane Library, the Chinese Biological Medicine Database (CBM), the Chinese Scientific Journal Database (VIP), China National Knowledge Infrastructure (CNKI), and the WAN FANG database. We performed a systematic search using Medical Subject Headings, titles, keywords, and free words related to acupuncture, RT, and PSSP. The detailed electronic search strategies for all databases are provided in **Supplementary Appendix 1**. We manually searched additional studies by screening the reference lists of the included articles and the relevant reviews.

Data collection and analysis

Selection of studies

Initially, all articles were imported into EndNote (version X9) for automatic deduplication. After removing duplicates, two authors (XW and CT) independently reviewed the titles and abstracts of all articles to determine whether these articles met the inclusion and exclusion criteria. Full-text articles that potentially met the eligibility criteria were retrieved. Then, the same two authors independently read these full-text articles to identify eligible studies. When multiple studies described the same trial, we included only the earliest published study. During the study selection process, any disagreements could be resolved by discussion or by consulting with a third author (JZ) as necessary.

Data extraction and management

Microsoft Excel was used by two authors (XY and PZ) independently to extract predefined data from the studies included. Where a study considered multiple intervention groups, data were extracted only for AR and RT groups. These authors conducted data extraction in duplicate and then checked the accuracy of these data. During the data extraction process, any disagreements could be resolved by discussion or by consulting with a third author (JZ), as necessary. The predefined data included (1) basic characteristics of the study, such as author name, publication year, sample size, gender, age, time since stroke, and type of stroke; (2) detailed information about interventions, such as types of acupuncture, selected acupoints, duration of needle retention, and frequency and duration of treatment; and (3) outcome measures, such as VAS, FMA-U, BI/MBI, shoulder ROM, and AEs.

Quality assessment

The risk of bias (ROB) assessment tool in the *Cochrane Handbook for Systematic Reviews of Interventions* (45) was used by two authors (CT and XY) independently to assess the methodological quality of the studies included. This tool included seven criteria: randomization method, allocation concealment, blinding of the participant and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other bias. Each of the criteria is classified as "low risk," "high risk," or "unclear risk." Where a study had more than four low risks in the seven criteria, we considered the overall quality of this study as moderate-to-high quality. Any disagreements could be resolved by discussion or by consulting with a third author (JZ), as necessary.

Measures of treatment effect

We used the post-treatment mean and standard deviation of the two groups to obtain the pooled effect size. When outcomes were evaluated by using the same scale, we used weighted mean differences (WMDs) with 95% confidence intervals (CIs) to describe continuous variables; otherwise, we used standardized mean differences (SMDs) with 95% CIs.

Unit of analysis issues

We managed and analyzed the data of non-standard design studies in accordance with the guidelines recommended in the Cochrane Handbook for Systematic Reviews of Interventions (version 5.1.0) (45).

Dealing with missing data

We contacted the corresponding authors or relevant authors of the studies included by email to obtain relevant data and information missing from the study. When the authors of these studies had provided data on at least one outcome among shoulder pain, upper limb motor function, and ADL before and after the intervention, we included those studies in our meta-analysis; otherwise, only a qualitative synthesis of those was performed.

Assessment of heterogeneity

We tested the clinical heterogeneity of studies included *via* analysis of the basic characteristics of participants (e.g., gender, age, type of stroke, and time since stroke), protocols of intervention (e.g., types of acupuncture, duration of needle retention, and frequency and duration of treatment), outcome measures, and trial design (e.g., randomization method, allocation concealment, and double-blinding).

We tested the statistical heterogeneity of the studies included using the Cochrane I-squared statistic. The I-squared statistic quantified the percentage of heterogeneity in the outcome measures. When I-squared was more than 25, 50, and 75%, the heterogeneity between studies was considered low, moderate, or high, respectively.

Assessment of reporting biases

We tested the publication biases by using visual funnel plots and Egger's test when the number of studies included was beyond 10.

Data synthesis

We conducted statistical analyses using RevMan version 5.3 (Nordic Cochrane Centre, the Cochrane Collaboration 2014) and RStudio Desktop 1.4.1717.[1] Shoulder pain was the primary outcome of this meta-analysis. Given the small number of studies included and moderate-to-high heterogeneity, we conducted a meta-analysis for the primary outcome using a random effects model. For secondary outcomes (e.g., FMA-U, MBI, and ROM), we used a random effects model to pool data when the statistic heterogeneity was significant; otherwise, we used a fixed effects model to conduct the meta-analysis. Furthermore, we performed a narrative summary for AEs. For all meta-analyses, we considered two-tailed *P*-values less than 0.05 as statistically significant.

Subgroup analysis

Considering the clinical heterogeneity of studies included, we performed subgroup analyses based on different types of acupuncture as follows: EA plus RT vs. RT alone, BAA plus RT vs. RT alone, and TA plus RT vs. RT alone.

Sensitivity analysis

We conducted a sensitivity analysis for the primary outcome by removing each study individually to test the robustness of the meta-analysis results.

Results

Study selection

In total, 2,090 electronic publications were identified from the selected databases. After removing duplicates (n = 614), we further excluded 1,307 articles by screening titles and abstracts, and then 169 articles were retained with full text. Of the 169 articles, 127 were removed for the following reasons: unrelated to PSSP (n = 11), not RCTs (n = 14), irrelevant intervention or comparison (n = 72), thesis (n = 24), separate articles for the same trial (n = 2), not published in Chinese or English (n = 2), and missing data (n = 2). Finally, 40 studies (46–85) were included in the qualitative analysis, and 35 studies with 2,554 patients were included in the meta-analysis due to five studies (54, 58, 60, 65, 77) without appropriate outcomes data. The selection flow of studies is described in **Figure 1**.

1 https://www.rstudio.com/products/rstudio/download/

Characteristics of eligible studies

With sample sizes ranging from 26 to 164, the publication years of studies included were distributed between 2002 and 2022. Except for 11 studies without reporting how long after stroke PSSP occurred, the remaining studies reported PSSP occurred in the acute and chronic phases of stroke, ranging from days to months. Overall, 20 studies covered stroke patients with infarction or hemorrhage, two studies (60, 84) were limited to cerebral infarction, and 18 studies had no further information on the type of stroke. Of the 40 studies included, 10 compared EA plus RT vs. RT alone, nine compared TA plus RT with RT alone, two compared AA plus RT vs. RT alone, three compared BAA plus RT with RT alone, four compared CAA plus RT vs. RT alone, two compared WA plus RT with RT alone, and four compared SA and BA plus RT vs. RT alone. The remaining studies compared the effectiveness of TA and BAA plus RT vs. RT alone, EA and BA plus RT vs. RT alone, SA plus RT vs. RT alone, WA and EA plus RT vs. RT alone, FA plus RT vs. RT alone, and RNN plus RT vs. RT alone, respectively. The highest needle retention time was 30 min, except for the fire acupuncture, which only needed 30 s. The treatment duration ranged from 5 to 60 sessions with the days ranging from 7 days to 4 months. Of the 40 eligible studies, 31 assessed shoulder pain by using VAS, one by NRS, one by FPS, and one by MPQ. A total of 29 studies assessed the upper limb motor function *via* the FMA-U, 12 studies used the MBI or BI to evaluate ADL, and five studies evaluated the shoulder ROM by the protractor. The characteristics of the included studies are given in **Supplementary Table 1**.

The quality of studies included

Of the 40 eligible studies, 14 (35.0%) were of moderate-to-high quality based on the ROB criteria. In terms of selection bias, 17 articles (42.5%) reported on the randomization process using a random number table approach were judged as low risk, two were considered high risk due to inappropriate randomization procedures, and 21 were considered an unclear risk due to lack of reporting. As for allocation concealment, only two (5%) articles were classified as low risk because correct allocation concealment processes using opaque envelopes were reported. Others were judged as an unclear risk due to the absence of the random number allocation process. For performance bias, patients could not be blinded because there were no sham or placebo controls, so we focused on whether the articles had blinding of acupuncture practitioners. Of all, only one RCT (2.5%) was considered low risk because its acupuncturists were blinded to the grouping. In terms of the blinding of outcome assessment, only three articles (7.5%) were judged as low risk and their outcomes were assessed by those who did not participate in acupuncture practice and unknown to the

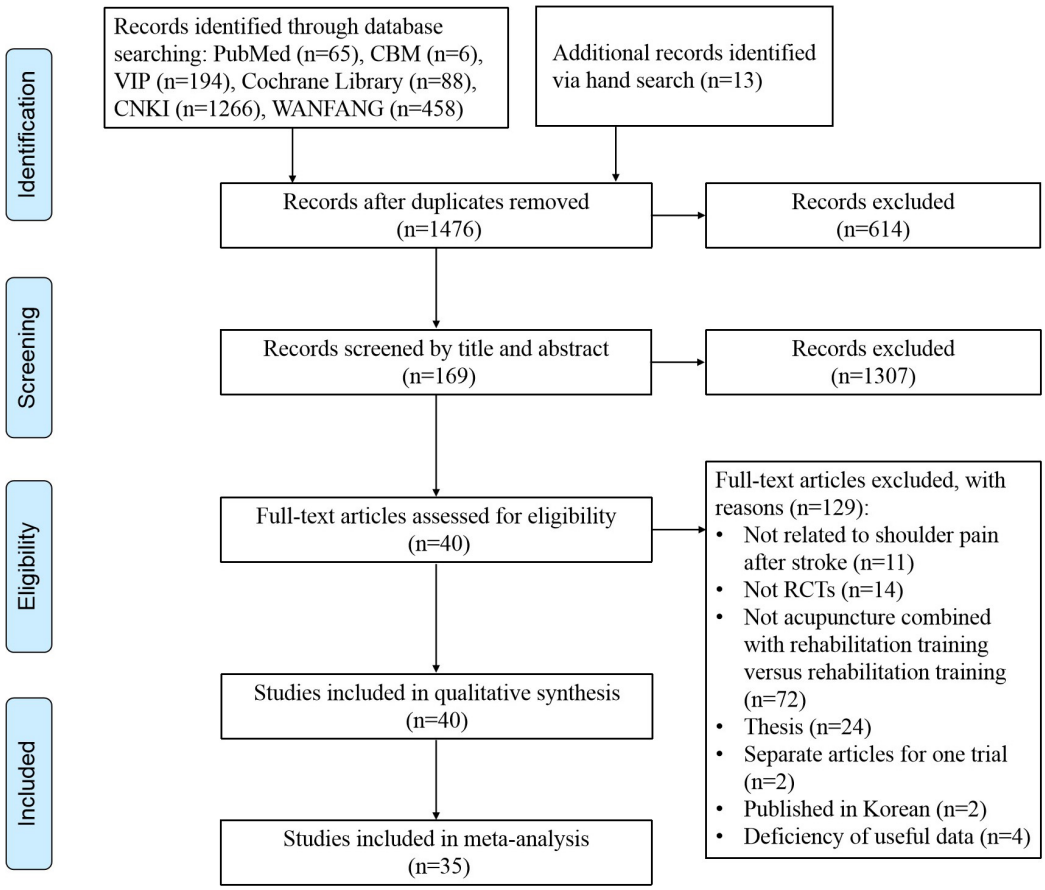


FIGURE 1
Study flow diagram.

grouping. Two studies (5%) reported cases of dropouts but did not specify the reasons for dropout, and 38 studies (95%), which reported all outcome data, were considered as low ROB due to attrition bias. In total, seven studies (17.5%) were judged as high risk of reporting bias because one or more outcomes of interest to this SR were poorly or not reported in these studies, which prevented them from being included in the meta-analysis. Other biases were not found in all the studies. The risk of bias assessments of studies included is shown in Figure 2.

Meta-analysis

Primary outcome

Shoulder pain

A total of 31 studies with 2,290 patients compared the effectiveness of AR vs. RT alone on shoulder pain assessed by using VAS. We used the random effects model to pool the data due to the high statistical heterogeneity ($I^2 = 93\%$, $P < 0.01$). The results of pooling data showed that AR is superior to RT alone in reducing shoulder pain in patients with PSSP (MD −1.32, 95% CI: −1.58 to −1.07, $Z = -10.10$, $P < 0.01$) (Figure 3). Only one study with 88 patients used the NRS to evaluate shoulder pain, and there was a significant difference between AR and RT alone (2.83 ± 2.24 vs. 3.95 ± 2.31, $P < 0.05$); one study with 60 patients used the FPS to assess the improvement of shoulder pain between AR and RT alone, and the difference between AR vs. RT alone was significant (1.23 ± 0.77 vs. 5.30 ± 1.44, $P < 0.05$); and one study including 25 patients used the MPQ to evaluate shoulder pain, and AR was better than RT alone in reducing shoulder pain (10.54 ± 3.01 vs. 17.25 ± 2.77, $P < 0.05$).

Secondary outcome

Motor function of upper limb

A total of 29 studies including 2,033 patients compared the effectiveness of AR vs. RT alone on upper limb motor function assessed by using the FMA-U. Because of significantly statistical heterogeneity ($I^2 = 97.3\%\%$, $P < 0.01$), we selected the random effects model to pool the data. The results of the meta-analysis showed that AR is better than RT alone in improving the upper

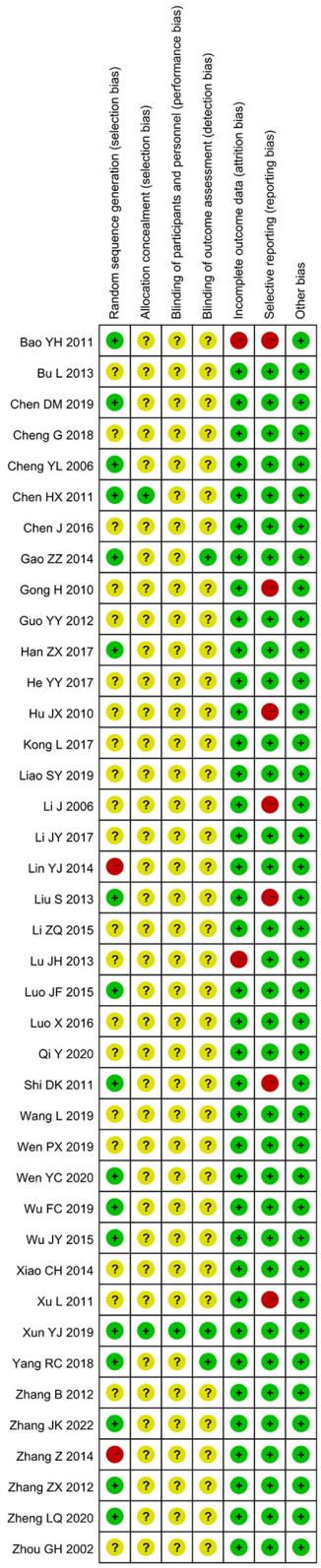


FIGURE 2
Risk of bias assessments of included studies.

limb motor function (MD 6.81, 95% CI: 4.95–8.67, $Z = 7.18$, $P < 0.01$) (Figure 4).

Activities of daily living

In total, 12 studies including 906 patients compared the effectiveness of AR vs. RT alone on ADL assessed by using the MBI or BI. We used the fixed effects model to pool the data because of the low statistical heterogeneity ($I^2 = 35.4\%$, $P = 0.11$). The results of pooling data showed that the effectiveness of AR on ADL is better than that of RT alone (MD 11.17, 95% CI: 9.44–12.91, $Z = 12.61$, $P < 0.01$) (Figure 5).

Shoulder range of motion

Only two studies including 110 patients compared the effectiveness of AR vs. RT alone on ROM of shoulder internal rotation and backward extension, respectively. The effects on ROM of shoulder internal rotation and backward extension were analyzed by a fixed effects model due to low to moderate statistical heterogeneity ($I^2 = 0.0\%$, $P = 0.42$; $I^2 = 62.4\%$, $P = 0.10$; respectively). AR for the improvement of ROM of shoulder internal rotation and backward extension was better than RT alone, respectively (MD 10.48, 95% CI: 8.14–12.83, $Z = 8.76$, $P < 0.01$; MD 7.82, 95% CI: 6.00–9.64, $Z = 8.44$, $P < 0.01$; respectively) (Figure 6).

Only five studies including 392 patients compared the effectiveness of AR vs. RT alone on ROM of shoulder anteflexion, external rotation, and abduction, respectively. The effects on ROM of shoulder anteflexion, external rotation, and abduction was analyzed using a random effects model, owing to significant heterogeneity ($I^2 = 95.0\%$, $P < 0.01$; $I^2 = 96.0\%$, $P < 0.01$; $I^2 = 98.0\%$, $P < 0.01$; respectively). The effect of AR on ROM of shoulder anteflexion, external rotation, and abduction, respectively, was better than that of RT alone (MD 12.88, 95% CI: 5.47–20.29, $Z = 3.41$, $P < 0.01$; MD 11.40, 95% CI: 6.17–16.64, $Z = 4.27$, $P < 0.01$; MD 16.96, 95% CI: 8.61–25.31, $Z = 3.98$, $P < 0.01$; respectively) (Figure 7).

Adverse events

Only one study reported no AEs related to AR or RT alone, and the other studies did not mention AEs.

Subgroup analysis

Electroacupuncture plus rehabilitation training vs. rehabilitation training alone

A total of nine studies including 527 patients compared the effectiveness of EA plus RT vs. RT alone on shoulder pain assessed by VAS. A random effects model was used to pool the data due to significant heterogeneity ($I^2 = 91\%$, $P < 0.01$). The results of the meta-analysis showed that EA plus RT was better than that RT alone in reducing shoulder pain (MD −0.76, 95% CI −1.08 to −0.44, $Z = 4.68$, $P < 0.01$) (Figure 8).

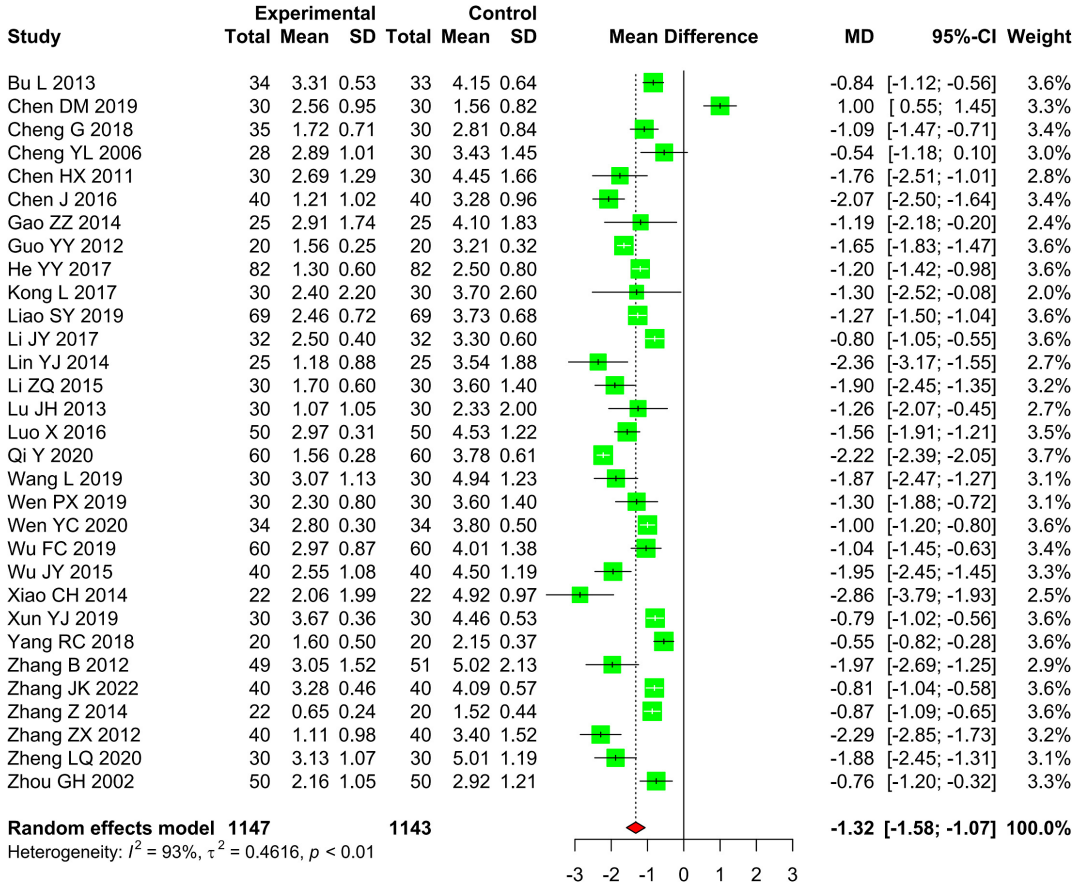


FIGURE 3
Forest plot and meta-analysis of VAS.

Traditional acupuncture plus rehabilitation training vs. rehabilitation training

Overall, six studies including 430 patients used VAS to assess shoulder pain. We used a random effects model to conduct meta-analysis because of significant heterogeneity (I^2 = 80%, P < 0.01). There was a significant difference in reducing shoulder pain between TA plus RT and RT alone (MD −1.25, 95% CI −1.65 to −0.85, Z = 6.15, P < 0.01) (Figure 8).

Balancing acupuncture plus rehabilitation training vs. rehabilitation training alone

A total of three studies with 220 patients compared the effectiveness of BAA plus RT vs. RT alone on shoulder pain assessed by VAS. Due to a significant heterogeneity (I^2 = 70%, P = 0.04), we used a random effects model to pool the data. BAA plus RT was better than RT alone in reducing shoulder pain (MD −1.90, 95% CI −2.44 to −1.35, Z = 6.83, P < 0.01) (Figure 8).

Sensitivity analysis

The stability of the pool data of the primary outcome was tested by removing studies one by one, and the result also supports that the effectiveness of AR in reducing shoulder pain was better than that of RT alone (P < 0.01) (Table 1).

Publication bias

All included studies were roughly well distributed on both sides of the funnel based on VAS and the MBI. Meanwhile, Egger's test based on VAS (t = −0.27, P = 0.79) and the MBI (t = 0.44, P = 0.67) also did not find obvious publication bias. However, some studies based on the FMA-U did not distribute inside 95% CIs, and Egger's test (t = 4.30, P < 0.01) demonstrated obvious publication bias (Figure 9).

Discussion

Our systematic review contained 40 RCTs focusing on the effectiveness of AR vs. RT alone for the improvement of symptoms and function in PSSP. Of the 40 studies included, 14 (35.0%) were of moderate-to-high quality based on the ROB criteria. The meta-analysis results of 35 RCTs with 2554 patients demonstrated that AR is better

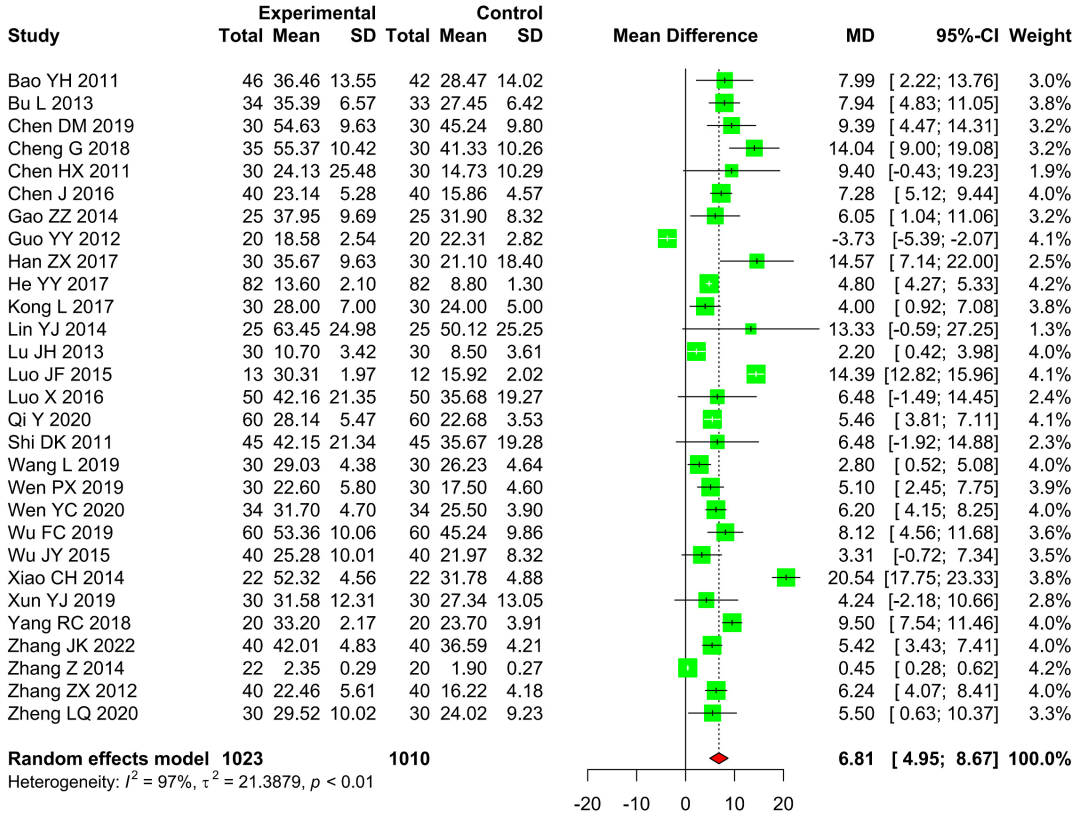


FIGURE 4
Forest plot and meta-analysis of FMA-U.

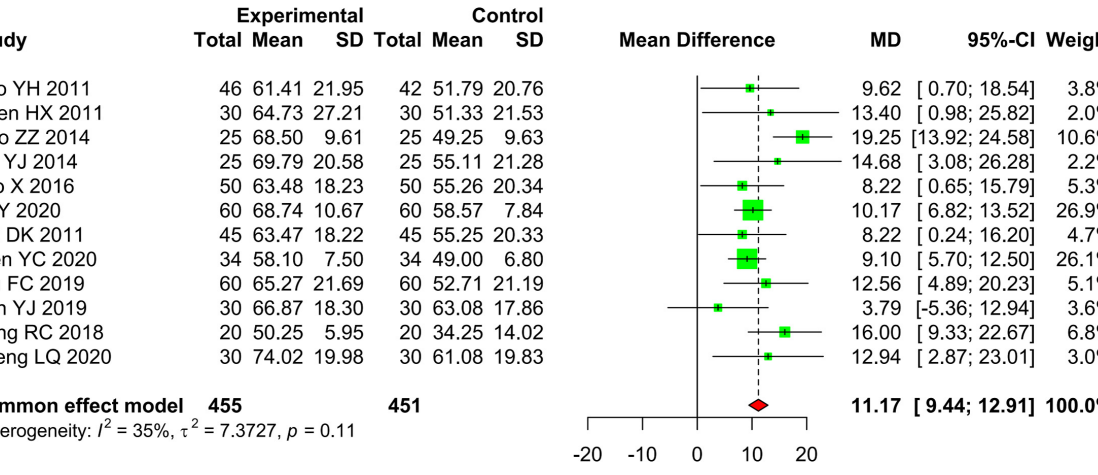


FIGURE 5
Forest plot and meta-analysis of MBI.

than RT alone in reducing shoulder pain and improving upper limb motor function, ADL, and shoulder ROM, without obvious AEs. Nevertheless, these conclusions must be interpreted with caution on account of substantial heterogeneity between studies.

In this meta-analysis, we found AR was superior to RT alone in the improvement of shoulder pain, motor function of the upper limb, ADL, and shoulder ROM of patients with PSSP. The robustness of the meta-analysis results was also confirmed by sensitivity analysis. In addition, a subgroup analysis of the

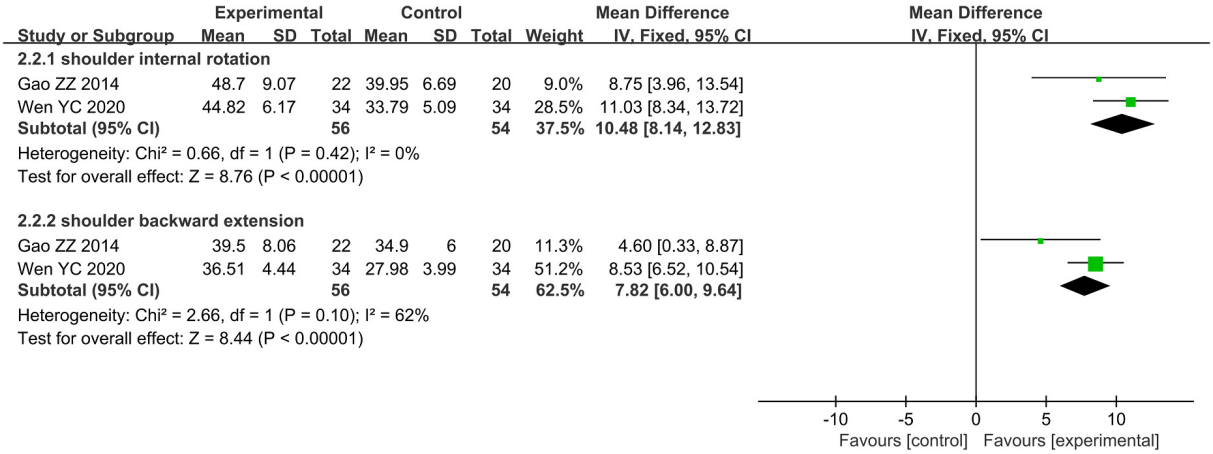


FIGURE 6
Forest plot and meta-analysis of ROM of internal rotation and backward extension.

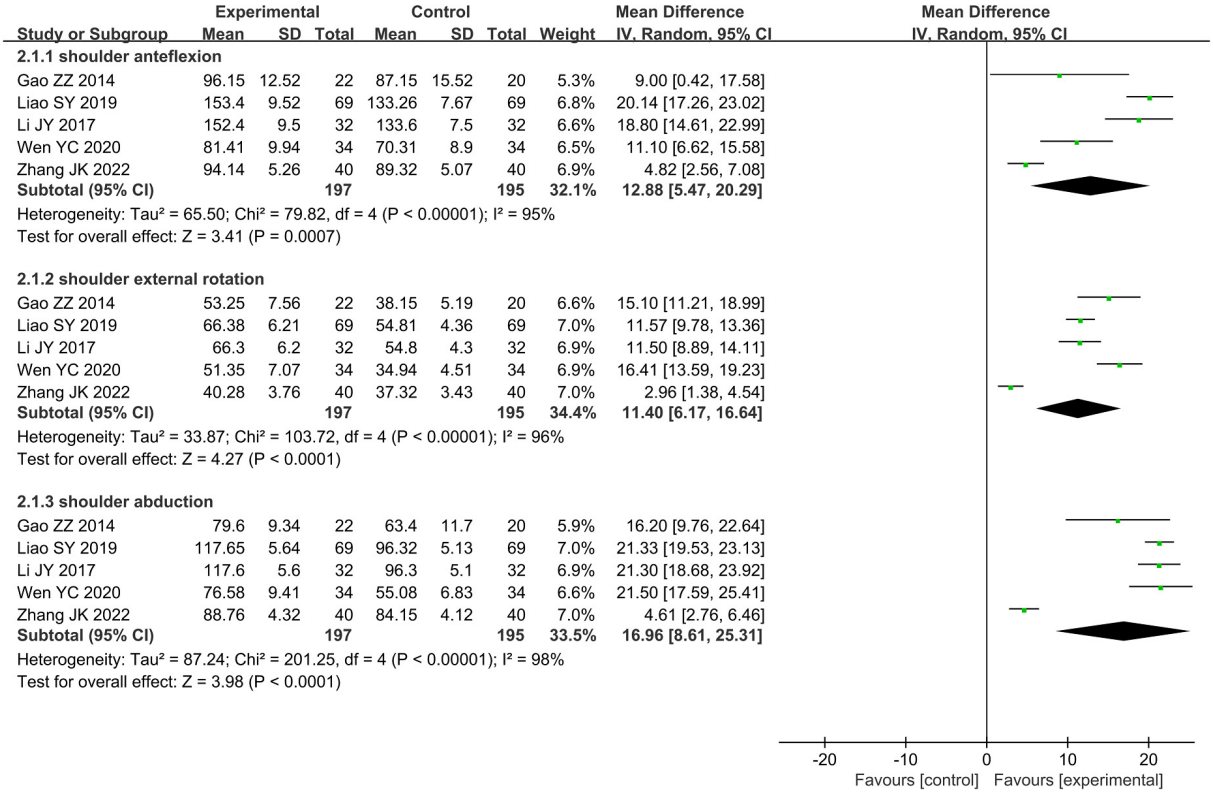


FIGURE 7
Forest plot and meta-analysis of ROM of anteflexion, external rotation, and abduction.

efficacy of acupuncture on analgesia was conducted based on the different acupuncture types. Efficacy data of the same type of acupuncture from a single study were excluded for a more reliable result. The pooled results revealed that the EA, or TA, or BAA plus RT was better than that of RT alone on shoulder pain in patients with PSSP. Beyond that, when RT was combined with BAA, patients experienced a greater reduction in shoulder pain than combined with TA or EA, as well as with relatively low heterogeneity. The BAA seems to be a more promising solution for PSSP among the varied acupuncture. This may be a helpful finding for future research on acupuncture for PSSP. Overall, the heterogeneous sources

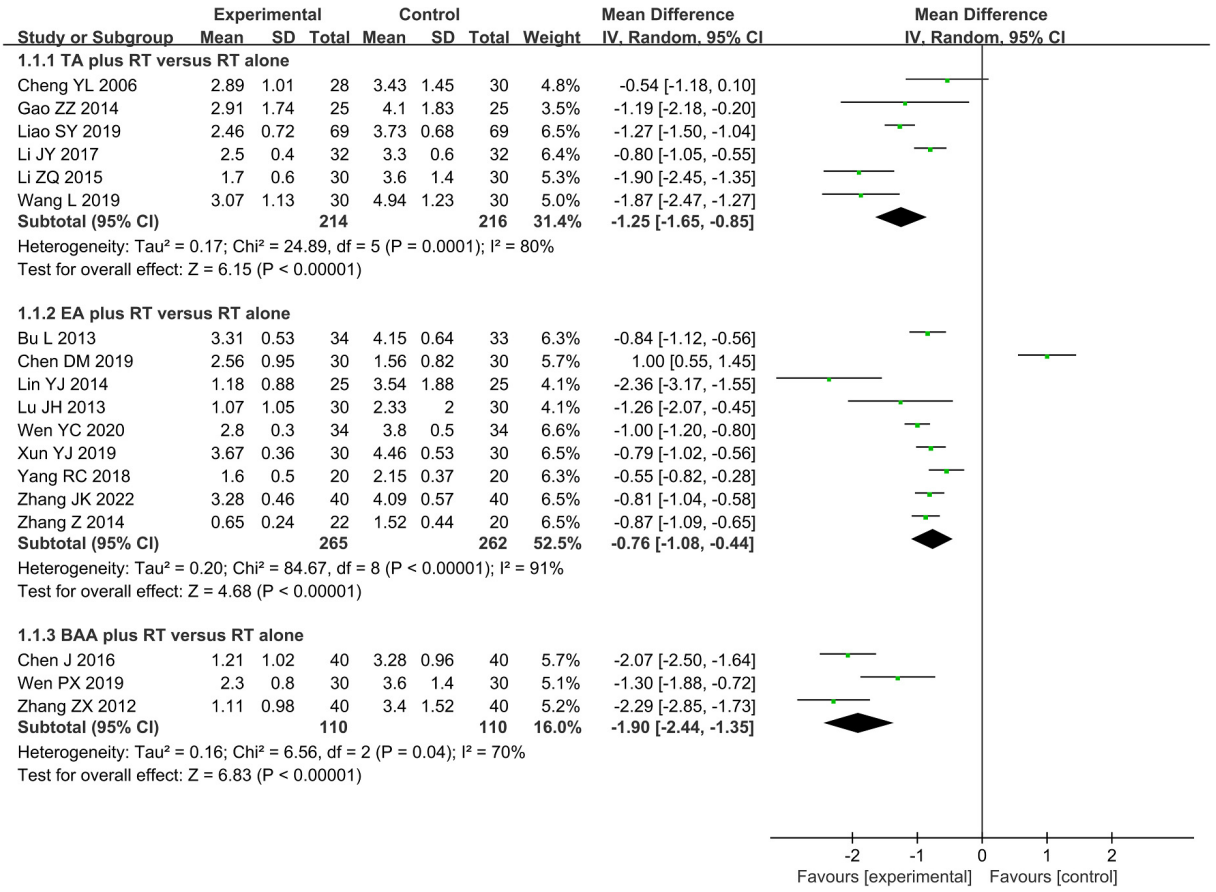


FIGURE 8
Forest plot and subgroup meta-analysis of VAS (BAA, balancing acupuncture; EA, electroacupuncture; RT, rehabilitation training; TA, traditional acupuncture; VAS, visual analog scale).

of studies included mainly were diversified acupuncture regimens, such as the selected acupoints, manipulation of acupuncture, needle retention duration, and frequency and session of acupuncture. For instance, among RCTs using TA as intervention, one study (52) reported that the acupuncturist performed neutral supplementation and the draining method after stabbing the needles, while another study (53) reported that the acupuncturist conducted the lifting—thrusting supplementation and draining method to achieve Deqi [i.e., a feeling of soreness, numbness, distension, heaviness, or the electric shock sensation (86)]. Only 35% of the included articles were rated as moderate-to-high quality, which reflects the high possibility of methodological heterogeneity in the included literature. As we can see, neither randomization nor blinding was performed for the majority of RCTs included.

From the perspective of traditional Chinese medicine, pain is caused by the blockage of the meridians and collaterals, and acupuncture uses metal needles to penetrate specific acupoints in the body to regulate the Qi in the meridians and achieve its analgesic effect by unblocking the meridians and collaterals (87–89). From the perspective of modern medicine, acupuncture analgesia is a comprehensive effect that is considered to be achieved by the transmission of impulse signals generated by acupoints to the nervous system, thereby adjusting the generation and release of neurotransmitters (88, 90, 91). Some neurotransmitters related to pain regulation pathways, such as opioid peptides, γ-aminobutyric acid (GABA), 5-hydroxytryptamine (5-HT), glutamate, and norepinephrine (92–95), have been found to be involved in acupuncture analgesia. The most well-recognized mechanism therein of acupuncture analgesia is the endogenous opioid mechanism (88, 90). However, the exact physiological mechanism of acupuncture analgesia is still unclear.

Compared with previous studies, three reviews are similar to ours, published in 2018 (27), 2015 (46), and 2012 (96). The review published in 2018 specified a wider range of shoulder pain for eligible patients, including shoulder-hand syndrome. Also, the review took routine stroke care as the comparative intervention, which may make the conclusion less

TABLE 1 Results of sensitivity analysis based on VAS.

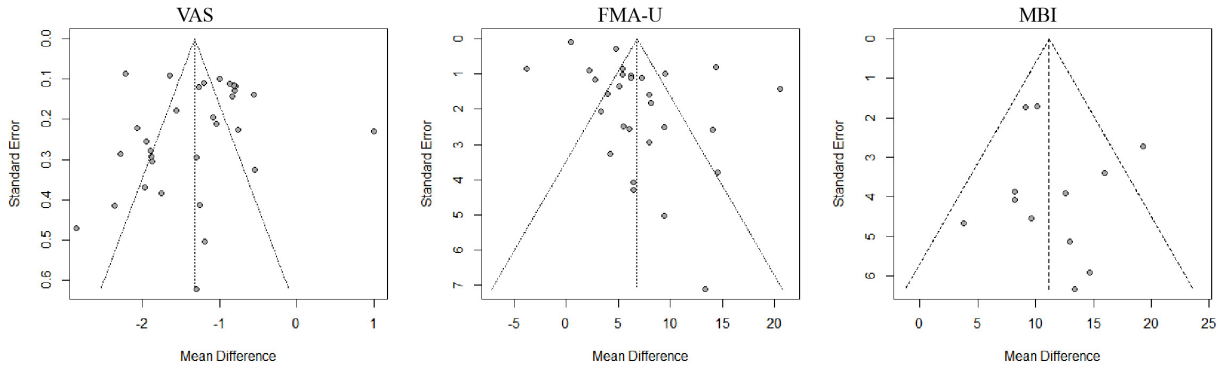
	MD	95%-CI	*P*-value	tau^2	tau	I^2
Omitting Bu L 2013	−1.3412	[−1.6052; −1.0771]	<0.0001	0.4728	0.6876	93.0%
Omitting Chen DM 2019	−1.3888	[−1.5989; −1.1786]	<0.0001	0.2790	0.5282	91.1%
Omitting Cheng G 2018	−1.3320	[−1.5976; −1.0665]	<0.0001	0.4795	0.6925	93.1%
Omitting Cheng YL 2006	−1.3471	[−1.6076; −1.0867]	<0.0001	0.4613	0.6792	93.1%
Omitting Chen HX 2011	−1.3107	[−1.5735; −1.0478]	<0.0001	0.4719	0.6869	93.1%
Omitting Chen J 2016	−1.2966	[−1.5566; −1.0366]	<0.0001	0.4575	0.6764	92.9%
Omitting Gao ZZ 2014	−1.3268	[−1.5899; −1.0637]	<0.0001	0.4753	0.6894	93.1%
Omitting Guo YY 2012	−1.3113	[−1.5764; −1.0461]	<0.0001	0.4768	0.6905	92.8%
Omitting He YY 2017	−1.3285	[−1.5948; −1.0621]	<0.0001	0.4818	0.6942	93.1%
Omitting Kong L 2017	−1.3239	[−1.5860; −1.0617]	<0.0001	0.4733	0.6880	93.1%
Omitting Liao SY 2019	−1.3259	[−1.5923; −1.0594]	<0.0001	0.4822	0.6944	93.1%
Omitting Li JY 2017	−1.3427	[−1.6064; −1.0791]	<0.0001	0.4711	0.6863	92.9%
Omitting Lin YJ 2014	−1.2934	[−1.5503; −1.0365]	<0.0001	0.4488	0.6699	93.0%
Omitting Li ZQ 2015	−1.3041	[−1.5663; −1.0420]	<0.0001	0.4671	0.6835	93.0%
Omitting Lu JH 2013	−1.3254	[−1.5894; −1.0613]	<0.0001	0.4773	0.6908	93.1%
Omitting Luo X 2016	−1.3152	[−1.5806; −1.0498]	<0.0001	0.4787	0.6919	93.1%
Omitting Qi Y 2020	−1.2875	[−1.5442; −1.0309]	<0.0001	0.4425	0.6652	89.6%
Omitting Wang L 2019	−1.3057	[−1.5681; −1.0433]	<0.0001	0.4685	0.6845	93.1%
Omitting Wen PX 2019	−1.3245	[−1.5897; −1.0593]	<0.0001	0.4796	0.6925	93.1%
Omitting Wen YC 2020	−1.3358	[−1.6014; −1.0703]	<0.0001	0.4783	0.6916	93.0%
Omitting Wu FC 2019	−1.3336	[−1.5988; −1.0684]	<0.0001	0.4784	0.6917	93.1%
Omitting Wu JY 2015	−1.3018	[−1.5635; −1.0402]	<0.0001	0.4646	0.6816	93.0%
Omitting Xiao CH 2014	−1.2819	[−1.5324; −1.0314]	<0.0001	0.4249	0.6519	92.9%
Omitting Xun YJ 2019	−1.3432	[−1.6068; −1.0796]	<0.0001	0.4706	0.6860	92.9%
Omitting Yang RC 2018	−1.3512	[−1.6114; −1.0909]	<0.0001	0.4573	0.6763	92.7%
Omitting Zhang B 2012	−1.3039	[−1.5651; −1.0426]	<0.0001	0.4652	0.6820	93.1%
Omitting Zhang JK 2022	−1.3425	[−1.6063; −1.0787]	<0.0001	0.4715	0.6866	92.9%
Omitting Zhang Z 2014	−1.3404	[−1.6049; −1.0760]	<0.0001	0.4740	0.6885	92.9%
Omitting Zhang ZX 2012	−1.2905	[−1.5472; −1.0337]	<0.0001	0.4457	0.6676	92.9%
Omitting Zheng LQ 2020	−1.3051	[−1.5674; −1.0428]	<0.0001	0.4680	0.6841	93.1%
Omitting Zhou GH 2002	−1.3427	[−1.6057; −1.0797]	<0.0001	0.4697	0.6853	93.1%
Pooled estimate	−1.3228	[−1.5794; −1.0662]	<0.0001	0.4616	0.6794	92.90%

MD, mean difference; CI, confidence interval. Details on meta-analytical method: Inverse variance method; Restricted maximum-likelihood estimator for tau^2.

specific. In addition, only narrative summaries, rather than meta-analyses of data, were performed in the review, which made the conclusions lack the support of objective data. The reviews published in 2015 and 2012 have different inclusion criteria for eligible studies, such as the study type, control group, outcomes measurements, and quality assessment. The quality of the literature included in the aforementioned reviews was generally low, and there was a high risk of selective bias and measurement bias. Moreover, these three reviews have been published for a long time, and there may be hysteresis in their conclusions. In addition, as shown in **Supplementary Table 1**, some evidence of RCTs for AR on PSSP has emerged since 2018. Therefore, a comprehensive update of the available evidence is necessary to clarify the role of AR in PSSP.

There were some advantages in this review. We performed a comprehensive literature search using a combination of machine and manual methods after consulting professional library searchers. Meanwhile, we also rigorously conducted this SR and MA in accordance with PRISMA and the guideline of Cochrane Collaboration. In addition, we performed subgroup analysis and sensitivity analysis on the included studies, further investigated the effects of different types of acupuncture therapy, and explored the possible sources of heterogeneity among the studies. This can help our conclusions be more reliable and helpful to the actual condition.

There are some limitations to our study. First, we did not limit the intervention to a specific type of RT, which may lead to the amplification of the meta-analysis results. Second, we restricted the published languages of studies



Linear regression test of funnel plot asymmetry

	bias	se.bias	intercept	se.intercept	*t*	*P*-value	tau^2
VAS	**-0.39**	**1.45**	**-1.19**	**0.24**	**-0.27**	**0.79**	**14.53**
FMA-U	**4.35**	**1.01**	**0.46**	**0.43**	**4.30**	**< 0.01**	**22.86**
MBI	**0.38**	**0.88**	**10.10**	**2.69**	**0.44**	**0.67**	**1.67**

FIGURE 9
Funnel plots illustrating meta-analysis of VAS, FMA-U, and MBI. (FMA-U, Fugl-Meyer Assessment Scale for upper extremity; MBI, modified Barthel Index; VAS, visual analog scale).

to Chinese or English, but only one eligible study was published in English, and all studies included were conducted in the Chinese population, which may lead to linguistic and regional biases that are difficult to eliminate. Third, due to the lack of clear descriptions of randomization, blinding, and allocation concealment in the protocols of most studies included, we cannot judge whether the authors performed these steps, which may affect the accuracy of our findings. Finally, there was a significantly statistical heterogeneity in this meta-analysis, which may increase the uncertainty of our results.

Conclusion

In this review, we found AR is better than RT alone for the improvement of shoulder pain, upper limb motor function, ADL, and shoulder ROM, without obvious AEs in patients with PSSP. However, considering the clinical and statistical heterogeneity, our findings need to be interpreted with caution. In the future, more rigorous and standardized trials on AR for PSSP should be conducted.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author/s.

Author contributions

JZ, JL, and LL were responsible for the conception and design of this systematic review. JZ and XW drafted the manuscript. JL and LL revised the manuscript. JZ and LL designed the search strategies. JZ and PZ conducted the electronic search. XW, RC, and YD manually screened the reference lists of the included studies and all relevant reviews. XY and PZ extracted the data. CT and XY independently assessed the risk of bias. JZ, XW, and LL analyzed and interpreted the data. HC and JZ arbitrated any disagreements during the process of systematic review. All authors approved the submitted version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.947285/full#supplementary-material

References

1. Zhang Q, Chen D, Shen Y, Bian M, Wang P, Li J. Incidence and prevalence of poststroke shoulder pain among different regions of the world: a systematic review and meta-analysis. *Front Neurol.* (2021) 12:724281. doi: 10.3389/fneur.2021.724281

2. Coskun Benlidayi I, Basaran S. Hemiplegic shoulder pain: a common clinical consequence of stroke. *Pract Neurol.* (2014) 14:88–91. doi: 10.1136/practneurol-2013-000606

3. Qiu H, Li J, Zhou T, Wang H, Li J. Electrical stimulation in the treatment of hemiplegic shoulder pain: a meta-analysis of randomized controlled trials. *Am J Phys Med Rehabil.* (2019) 98:280–6. doi: 10.1097/PHM.0000000000001067

4. Adey-Wakeling Z, Arima H, Crotty M, Leyden J, Kleinig T, Anderson CS, et al. Incidence and associations of hemiplegic shoulder pain poststroke: prospective population-based study. *Arch Phys Med Rehabil.* (2015) 96:241–7. doi: 10.1016/j.apmr.2014.09.007

5. Wilson RD, Chae J. Hemiplegic shoulder pain. *Phys Med Rehabil Clin N Am.* (2015) 26:641–55.

6. Hou Y, Wang Y, Sun X, Lou Y, Yu Y, Zhang T. Effectiveness of suprascapular nerve block in the treatment of hemiplegic shoulder pain: a systematic review and meta-analysis. *Front Neurol.* (2021) 12:723664. doi: 10.3389/fneur.2021.723664

7. Kumar P, Turton A, Cramp M, Smith M, McCabe C. Management of hemiplegic shoulder pain: a UK-wide online survey of physiotherapy and occupational therapy practice. *Physiother Res Int.* (2021) 26:e1874. doi: 10.1002/pri.1874

8. Kim YH, Jung SJ, Yang EJ, Paik NJ. Clinical and sonographic risk factors for hemiplegic shoulder pain: a longitudinal observational study. *J Rehabil Med.* (2014) 46:81–7. doi: 10.2340/16501977-1238

9. Kibler WB, Uhl TL, Maddux JW, Brooks PV, Zeller B, McMullen J. Qualitative clinical evaluation of scapular dysfunction: a reliability study. *J Shoulder Elbow Surg.* (2002) 11:550–6. doi: 10.1067/mse.2002.126766

10. Kalichman L, Ratmansky M. Underlying pathology and associated factors of hemiplegic shoulder pain. *Am J Phys Med Rehabil.* (2011) 90:768–80. doi: 10.1097/PHM.0b013e318214e976

11. Soo Hoo J, Paul T, Chae J, Wilson RD. Central hypersensitivity in chronic hemiplegic shoulder pain. *Am J Phys Med Rehabil.* (2013) 92:1–13. doi: 10.1097/PHM.0b013e31827df862

12. Anwer S, Alghadir A. Incidence, prevalence, and risk factors of hemiplegic shoulder pain: a systematic review. *Int J Environ Res Public Health.* (2020) 17:4962. doi: 10.3390/ijerph17144962

13. Chae J, Mascarenhas D, Yu DT, Kirsteins A, Elovic EP, Flanagan SR, et al. Poststroke shoulder pain: its relationship to motor impairment, activity limitation, and quality of life. *Arch Phys Med Rehabil.* (2007) 88:298–301. doi: 10.1016/j.apmr.2006.12.007

14. Adey-Wakeling Z, Liu E, Crotty M, Leyden J, Kleinig T, Anderson CS, et al. Hemiplegic shoulder pain reduces quality of life after acute stroke: a prospective population-based study. *Am J Phys Med Rehabil.* (2016) 95:758–63. doi: 10.1097/PHM.0000000000000496

15. Kumar P. Hemiplegic shoulder pain in people with stroke: present and the future. *Pain Manag.* (2019) 9:107–10. doi: 10.2217/pmt-2018-0075

16. Pan R, Zhou M, Cai H, Guo Y, Zhan L, Li M, et al. A randomized controlled trial of a modified wheelchair arm-support to reduce shoulder pain in stroke patients. *Clin Rehabil.* (2018) 32:37–47. doi: 10.1177/0269215517714830

17. de Sire A, Moggio L, Demeco A, Fortunato F, Spanò R, Aiello V, et al. Efficacy of rehabilitative techniques in reducing hemiplegic shoulder pain in stroke: systematic review and meta-analysis. *Ann Phys Rehabil Med.* (2021) 65:101602. doi: 10.1016/j.rehab.2021.101602

18. Kim MS, Kim SH, Noh SE, Bang HJ, Lee KM. Robotic-assisted shoulder rehabilitation therapy effectively improved poststroke hemiplegic shoulder pain: a randomized controlled trial. *Arch Phys Med Rehabil.* (2019) 100:1015–22. doi: 10.1016/j.apmr.2019.02.003

19. Wilson RD, Knutson JS, Bennett ME, Chae J. The effect of peripheral nerve stimulation on shoulder biomechanics: a randomized controlled trial in comparison to physical therapy. *Am J Phys Med Rehabil.* (2017) 96:191–8. doi: 10.1097/PHM.0000000000000677

20. Doucet BM, Lam A, Griffin L. Neuromuscular electrical stimulation for skeletal muscle function. *Yale J Biol Med.* (2012) 85:201–15.

21. Yajima H, Takayama M, Nobe R, Schlaeger JM, Takakura N. Acupuncture for post-stroke shoulder pain: a case report. *Acupunct Med.* (2020) 38:446–8. doi: 10.1177/0964528420920292

22. Benlidayi IC, Basaran S. Hemiplegic shoulder pain: a common clinical consequence of stroke. *Pract. Neurol.* (2014) 14:88–91.

23. Chiu YH, Chang KV, Wu WT, Hsu PC, Özçakar L. Comparative effectiveness of injection therapies for hemiplegic shoulder pain in stroke: a systematic review and network meta-analysis. *Pharmaceuticals (Basel).* (2021) 14:788. doi: 10.3390/ph14080788

24. Koyuncu E, Nakipoğlu-Yüzer GF, Doğan A, Ozgirgin N. The effectiveness of functional electrical stimulation for the treatment of shoulder subluxation and shoulder pain in hemiplegic patients: a randomized controlled trial. *Disabil Rehabil.* (2010) 32:560–6. doi: 10.3109/09638280903183811

25. Harvey R, Roth E, Yu D, Celnik P. Stroke syndromes. 4th ed. In: Braddom RL editor. *Braddom's Physical Medicine and Rehabilitation*. (Philadelphia, PA: Saunders) (2011). p. 1200–1.

26. Lakse E, Gunduz B, Erhan B, Celik EC. The effect of local injections in hemiplegic shoulder pain: a prospective, randomized, controlled study. *Am J Phys Med Rehabil.* (2009) 88:805–11.

27. Chau JPC, Lo SHS, Yu X, Choi KC, Lau AYL, Wu JCY, et al. Effects of acupuncture on the recovery outcomes of stroke survivors with shoulder pain: a systematic review. *Front Neurol.* (2018) 9:30. doi: 10.3389/fneur.2018.00030

28. Liu T, Wen X, Kuang W, Wang X, Jiang Y, Qiu X, et al. Therapeutic effect of Fu's subcutaneous needling for hemiplegic shoulder pain among stroke survivors: study protocol for a randomized controlled trial. *Medicine (Baltimore).* (2019) 98:e15507. doi: 10.1097/MD.0000000000015507

29. Zheng J, Wu Q, Wang L, Guo T. A clinical study on acupuncture in combination with routine rehabilitation therapy for early pain recovery of post-stroke shoulder-hand syndrome. *Exp Ther Med.* (2018) 15:2049–53. doi: 10.3892/etm.2017.5659

30. Peng L, Zhang C, Zhou L, Zuo HX, He XK, Niu YM. Traditional manual acupuncture combined with rehabilitation therapy for shoulder hand syndrome after stroke within the Chinese healthcare system: a systematic review and meta-analysis. *Clin Rehabil.* (2018) 32:429–39. doi: 10.1177/0269215517729528

31. Stroke Foundation,. *(Australian) Clinical Guidelines for Stroke Management*. (2017). Available online at: https://app.magicapp.org/#/guideline/WE8wOn/section/jXNMen

32. Zhan J, Ai Y, Zhan L, Pan R, Wang Y, Dong C, et al. Effect of abdominal acupuncture combined with routine rehabilitation training on shoulder-hand syndrome after stroke: a randomized controlled trial. *Integr Med Res.* (2022) 11:100805. doi: 10.1016/j.imr.2021.100805

33. Mendigutía-Gómez A, Quintana-García MT, Martín-Sevilla M, de Lorenzo-Barrientos D, Rodríguez-Jiménez J, Fernández-de-Las-Peñas C, et al. Post-needling soreness and trigger point dry needling for hemiplegic shoulder pain following stroke. *Acupunct Med.* (2020) 38:150–7. doi: 10.1177/0964528419882941

34. Eslamian F, Farhoudi M, Jahanjoo F, Sadeghi-Hokmabadi E, Darabi P. Electrical interferential current stimulation versus electrical acupuncture in management of hemiplegic shoulder pain and disability following ischemic stroke-a randomized clinical trial. *Arch Physiother.* (2020) 10:2. doi: 10.1186/s40945-019-0071-6 (accessed September 12, 2022).

35. Sun Y, Wang Y, Wang W. Effect of acupuncture plus rehabilitation training on shoulder-hand syndrome due to ischemic stroke. *J Acupunct Tuina Sci.* (2012) 10:109–13. doi: 10.1007/s11726-012-0583-z

36. Melzack R, Stillwell DM, Fox EJ. Trigger points andacupuncture points for pain: correlations and implications. *Pain.* (1977) 3:3–23. doi: 10.1016/0304-3959(77)90032-

37. Liu S, Zhang CS, Cai Y, Guo X, Zhang AL, Xue CC, et al. Acupuncture for post-stroke shoulder-hand syndrome: a systematic review and meta-analysis. *Front Neurol.* (2019) 10:433. doi: 10.3389/fneur.2019.00433

38. Lin Y, Li Z, Fu J, Liu X. The systematic evaluation of acupuncture combined with rehabilitation training For the treatment of post-strokeshoulder pain. *J Nurs Train.* (2015) 30:1004–09. doi: 10.16821/j.cnki.hsjx.2015.11.011

39. Melzack R. The short-form McGill pain questionnaire. *Pain*. (1987) 30:191–7. doi: 10.1016/0304-3959(87)91074-8

40. Williamson A, Hoggart B. Pain: a review of three commonly used pain rating scales. *J Clin Nurs.* (2005) 14:798–804. doi: 10.1111/j.1365-2702.2005.01121.x

41. Taylor LJ, Herr K. Pain intensity assessment: a comparison of selected pain intensity scales for use in cognitively intact and cognitively impaired African American older adults. *Pain Manag Nurs.* (2003) 4:87–95. doi: 10.1016/s1524-9042(02)54210-7

42. Gladstone DJ, Danells CJ, Black SE. The fugl-meyer assessment of motor recovery after stroke: a critical review of its measurement properties. *Neurorehabil Neural Repair.* (2002) 16:232–40. doi: 10.1177/154596802401105171

43. Li KC, Tang D, Liu XY, Xu YW. Review of the application of Barthel index and ModifiedBarthel index in Mainland China. *Chin J Rehabil Med.* (2009) 24:737–40.

44. Ohura T, Hase K, Nakajima Y, Nakayama T. Validity and reliability of a performance evaluation tool based on the modified Barthel index for stroke patients. *BMC Med Res Methodol.* (2017) 17:131. doi: 10.1186/s12874-017-0409-2

45. Higgins JPT, Green S editors. *Cochrane Handbook for Systematic Reviews of Interventions.* Version 5.1.0. London: The Cochrane Collaboration (2011).

46. Bao YH, Wang YW, Chu JM, Zhu GX, Wang CM, Hou HM. Effect of electroacupuncture combined with rehabilitation in treating patients with hemiplegic shoulder pain. *Chin Arch Tradit Chin Med.* (2011) 29: 2536–9. doi: 10.13193/j.archtcm.2011.11.162.baoyh.038

47. Bu, L, Xu HQ, Tan WJ, Di RK. Effects of electroacupuncture combined with scapular control training on shoulder pain and upper limbs function in hemiplegia patients. *Glob Tradit Chin Med.* (2013) 6:246–7.

48. Chen DM, Jin YF, He QC, Zhao XC, Pu J. Clinical observation of electroacupuncture combined with rehabilitation training for shoulder pain after stroke. *Med World.* (2019) 15:114.

49. Chen HX, He MF, Xie RM. Clinical observation on the combination of abdominal acupuncture and rehabilitation in treating omalgia after stroke. *J Nanjing Univ Tradit Chin Med.* (2011) 27:333–5. doi: 10.3969/j.issn.1000-5005.2011.04.009

50. Chen J. Clinical effect observation of acupuncture combined with kinesitherapy for hemiplegia shoulder pain after stroke. *Womens Health Res.* (2016) 9:79–81.

51. Cheng G. The Effect of Rehabilitation Training Combined With Acupuncture on Shoulder Pain After Stroke. *China Contin Med Educ.* (2018) 10:149–51. doi: 10.3969/j.issn.1674-9308.2018.12.081

52. Cheng YL, Li SH. Jieqi Tongjing acupuncture combined with exercise rehabilitation for post-stroke shoulder pain. *J Clin Acupunct Moxibust.* (2006) 22:39–40. doi: 10.3969/j.issn.1005-0779.2006.05.028

53. Gao ZZ, Xu DM, Li YC, Guo HY. Observation of curative effect of acupuncture combined with shoulder control training on shoulder pain after stroke. *Chin J Rehabil Med.* (2014) 29:370–2. doi: 10.3969/j.issn.1001-1242.2014.04.018

54. Gong H, Tang Q. Clinical observation of acupuncture combined with rehabilitation for hemiplegia shoulder pain after stroke. *J Clin Acupunct Moxibust.* (2010) 26:10–1. doi: 10.3969/j.issn.1005-0779.2010.01.004

55. Guo YY, Chen JP. Clinical study of warm needle for shoulder pain after stroke. *Chin J Rehabil Med.* (2012) 27:275–7. doi: 10.3969/j.issn.1001-1242.2012.03.021

56. Han ZX, Qi LL, Zhou YX, Zhang H, Xu WJ, Wang HL, et al. Clinical observation of relaxing needling at meridian-muscle nodes plus PNF method in improving upper-limb motor function and quality of life in hemiplegic shoulder pain. *Shanghai J Acupunct Moxibust.* (2017) 36:1420–4. doi: 10.13460/j.issn.1005-0957.2017.12.1420

57. He YY. Observation on curative effect of scalp acupuncture and body acupuncture in combined with rehabilitation exercise in the treatment of hemiplegic shoulder pain after stroke. *China Health Care Nutr.* (2017) 27:37–8. doi: 10.3969/j.issn.1004-7484.2017.12.047

58. Hu JX, Xue LW. Acupuncture Combined with Rehabilitation Training for shoulder pain after stroke in 40 cases. *Shaanxi J Tradit Chin Med.* (2010) 31:1392–3. doi: 10.3969/j.issn.1000-7369.2010.10.082

59. Kong L, Li YF, Si SL. Effects of abdominal acupuncture combined with rehabilitation training on shoulder pain after stroke. *J Pract Med Tech.* (2017) 24:1235–7. doi: 10.19522/j.cnki.1671-5098.2017.11.036

60. Li J, Wang Y, Liu B. Effect of acupuncture combine with rehabilitation technique on shoulder pain after stroke. *Chin J Trauma Disabil Med.* (2006) 14:37–8. doi: 10.3969/j.issn.1673-6567.2006.01.022

61. Li JY, Yu LW, Wu XQ, Yi D, Ma YS, Wu XF, et al. Clinical effect of acupuncture-exercise therapy combined with joint mobilization on the post-stroke shoulder pain. *Nurs Integr Tradit Chin Western Med.* (2017) 3:64–7. doi: 10.11997/nitcwm.2017012017

62. Li ZQ, Li CR, Zhao JH, Fang XY. Brief evaluation of shoulder acupoint acupuncture combined with kinesitherapy in the treatment of shoulder pain after stroke. *China Pract Med.* (2015) 10:5–7. doi: 10.14163/j.cnki.11-5547/r.2015.20.003

63. Liao SY, Xie QF. Clinical observation of joint mobilization combined with functional acupuncture in treating hemiplegic shoulder pain. *Chin J Clin Ration Drug Use.* (2019) 12:149–50. /r.2019.07.086 doi: 10.15887/j.cnki.13-1389

64. Lin YJ, Xu LY. Effect of acupuncture combined with rehabilitation training on shoulder pain after stroke. *Asia Pac Tradit Med.* (2014) 10:64–5.

65. Liu S, Shi ZY. Observation on the therapeutic effect of scalp acupuncture and body acupuncture in combination with rehabilitation exercise for hemiplegia and shoulder pain after stroke. *World J Acupunct Moxibust.* (2013) 23:21–6.

66. Lu JH, Lu J, Zhang LX, Liu KJ, Wang T, Chen XM, et al. Clinical observation of electroacupuncture combined with rehabilitation manipulation for post-stroke shoulder pain in 30 cases. *Jiangsu J Tradit Chin Med.* (2013) 45:60–1. doi: 10.3969/j.issn.1672-397X.2013.01.038

67. Luo JF, Jia L, Ni CM. Effect of acupuncture combined with proprioceptive neuromuscular promotion technique for shoulder pain after stroke. *Chin J Rehabil Med.* (2015) 30:1284–5. doi: 10.3969/j.issn.1001-1242.2015.12.019

68. Luo X, Liu LQ, Sun XY. Clinical research on wrist – ankle acupuncture combined with rehabilitation therapy for shoulder pain after stroke. *Chin J Trauma Disabil Med.* (2016) 24:58–9. doi: 10.13214/j.cnki.cjotadm.2016.15.044

69. Qi Y, Sun Y. Effect of proprioceptive neuromuscular stimulation combined with scalp-body needling on motor function and pain score in patients with shoulder pain after stroke. *J North Sichuan Med Coll.* (2020) 35:679–82. doi: 10.3969/j.issn.1005-3697.2020.04.31

70. Shi DK, Tang XS. Carpus-ankle acupuncture combined with physical therapy for patients with post-stroke shoulder pain: a randomized controlled trial. *J Chengdu Univ Tradit Chin Med.* (2011) 34:33–5. doi: 10.13593/j.cnki.51-1501/r.2011.01.012

71. Wang L, Wang EL. Clinical observation of acupuncture combined with rehabilitation training for shoulder pain after stroke. *Yunnan J Tradit Chin Med Mater Med.* (2019) 40:57–8. doi: 10.3969/j.issn.1007-2349.2019.08.022

72. Wen PX, Chen JB, Zheng JL. Effect of acupuncture combined with kinesitherapy for hemiplegia shoulder pain after stroke. *Shenzhen J Integr Tradit Chin Western Med.* (2019) 29:42–3. doi: 10.16458/j.cnki.1007-0893.2019.11.020

73. Wen YC, Li N, Wu XR. The clinical study on warm needling and electroacupuncture combined with exercise and TENS treating patients of hemiplegia after stroke with shoulder pain. *Int J Tradit Chin Med.* (2020) 42:226–30. doi: 10.3760/cma.j.cn115398-20190525-00171

74. Wu FC, Chen PMY, Chen ZB. Clinical observation of scalp acupuncture combined with exercise rehabilitation training for post-stroke shoulder pain. *Hebei J Tradit Chin Med.* (2019) 41:532–5. doi: 10.3969/j.issn.1002-2619.2019.04.011

75. Wu JY, Ye BY, Xue XH, Huang SE, Lin ZC, Hong JC. Observations on the efficacy of wrist-ankle acupuncture plus continuous exercise therapy for post-stroke shoulder pain. *Shanghai J Acupunct Moxibust.* (2015) 34:409–11. doi: 10.13460/j.issn.1005-0957.2015.05.0409

76. Xiao CH, Li M. Effect of warm acupuncture combined with rehabilitation therapy for shoulder pain after stroke in 22 cases. *Hebei J Tradit Chin Med.* (2014) 36:1672–3. doi: 10.3969/j.issn.1002-2619.2014.11.036

77. Xu L, Jiang Y, Cao XF, Zhang XY, Si WN. Treatment of hemiplegic shoulder pain with wrist-ankle acupuncture combined with rehabilitation exercises. *Chin J Gen Pract.* (2011) 09:1865–6.

78. Xun YJ, Yan QW, Liu PJ, Zhan Y, Lei DB, Zhai HY. Effects of electroacupuncture on hemiplegic shoulder pain: a single-blind randomized controlled trial. *Chin J Integr Tradit Western Med.* (2019) 39:1345–8. doi: 10.7661/j.cjim.20190828.309

79. Yang RC, Yang JH, Mao ZF. Effect of electroacupuncture with rehabilitation training on the recovery of upper limb motor function in patients with shoulder pain after stroke. *Chin J Tradit Med Sci Technol.* (2018) 25:239–41.

80. Zhang B. Effect of acupuncture for hemiplegia shoulder pain. *Chin J Clin Ration Drug Use.* (2012) 5:83–83. doi: 10.3969/j.issn.1674-3296.2012.15.062

81. Zhang JK, Yin RX, Fan JZ. Therapeutic effect of electroacupuncture combined with new Bobath technology on shoulder pain after stroke. *Hainan Med J.* (2022) 33:39–42. doi: 10.3969/j.issn.1003-6350.2022.01.011

82. Zhang Z. Analysis of therapeutic effect of acupuncture combined with modern rehabilitation treatment of shoulder pain after stroke. *Med Inf.* (2014) 27:272. doi: 10.3969/j.issn.1006-1959.2014.25.316

83. Zhang ZX, Zhang Y, Yu TY, Gao HY. The effects of acupuncture on Jiantong point combined with exercise for post-stroke shoulder pain patients. *Shandong Med J.* (2012) 52:82–3. doi: 10.3969/j.issn.1002-266X.2012.27.032

84. Zheng LQ, Qi J, He YF, Sun CM, Zhang ZQ. Effect of filiform fire-needling at the tendon joint point for post-stroke shoulder pain in 30 cases. *J Extern Ther Tradit Chin Med.* (2020) 29:64–5. doi: 10.3969/j.issn.1006-978X.2020.03.034

85. Zhou GH. Observation of curative effect of acupuncture combined with rehabilitation training on hemiplegia patients with shoulder pain. *Chin J Rehabil Theory Pract.* (2002) 8:244–5. doi: 10.3969/j.issn.1006-9771.2002.04.025

86. World Health Organization. *WHO International Standard Terminologies on Traditional Medicine in the Western Pacific Region*. Geneva: World Health Organization (2007).

87. Vickers AJ. Can acupuncture have specific effects on health? A systematic review of acupuncture antiemesis trials. *J R Soc Med.* (1996) 89:303–11.

88. Qiao L, Guo M, Qian J, Xu B, Gu C, Yang Y. Research advances on acupuncture analgesia. *Am J Chin Med.* (2020) 48:245–58. doi: 10.1142/S0192415X20500135

89. Kaptchuk TJ. Acupuncture: theory, efficacy, and practice. *Ann Intern Med.* (2002) 136:374–83. doi: 10.7326/0003-4819-136-5-200203050-00010

90. Zhang M, Shi L, Deng S, Sang B, Chen J, Zhuo B, et al. Effective oriental magic for analgesia: acupuncture. *Evid Based Complement Alternat Med.* (2022) 2022:1451342. doi: 10.1155/2022/1451342

91. Chen T, Zhang WW, Chu YX, Wang YQ. Acupuncture for pain management: molecular mechanisms of action. *Am J Chin Med.* (2020) 48:793–811. doi: 10.1142/S0192415X20500408

92. Chen S, Wang S, Rong P, Wang J, Qiao L, Feng X, et al. Acupuncture for visceral pain: neural substrates and potential mechanisms. *Evid Based Complement Alternat Med.* (2014) 2014:609594. doi: 10.1155/2014/609594

93. Lyu Z, Guo Y, Gong Y, Fan W, Dou B, Li N, et al. The role of neuroglial crosstalk and synaptic plasticity-mediated central sensitization in acupuncture analgesia. *Neural Plast.* (2021) 2021:8881557. doi: 10.1155/2021/8881557

94. Dai QX, Geng WJ, Zhuang XX, Wang HF, Mo YC, Xin H, et al. Electroacupuncture-induced neuroprotection against focal cerebral ischemia in the rat is mediated by adenosine A1 receptors. *Neural Regen Res.* (2017) 12:228–34. doi: 10.4103/1673-5374.200806

95. Cui L, Ding Y, Zeng J, Feng Y, Li M, Ding M. Spinal glutamate transporters are involved in the development of electroacupuncture tolerance. *Int J Mol Sci.* (2016) 17:357. doi: 10.3390/ijms17030357

96. Nie W, Liu Z, Zhao H. Systematic review of acupuncture for treatment of post-stroke shoulder pain. *Chin J Inf Tradit Chin Med.* (2012) 19:25–8.

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Electroacupuncture relieves hyperalgesia by regulating neuronal–glial interaction and glutamate transporters of spinal dorsal horns in rats with acute incisional neck pain

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Objective: Glial cells are involved in the analgesic effect of electroacupuncture (EA) in rats with chronic neurological pain. The objective of this study was to observe the role of neuronal–glial interaction and glutamate (Glu) transporters in EA-induced acute neck pain relief in rats.

Materials and methods: Male rats were placed into the following five groups: control, model, EA Futu (LI18), EA Hegu (LI4)-Neiguan (PC6), and EA Zusanli (ST36)-Yanglingquan (GB34). The incisional neck pain model was established by making a longitudinal incision along the midline of the neck. The thermal pain threshold (TPT) was measured using a radiation heat detector. The immunoactivities of glial fibrillary acidic protein (GFAP), ionized calcium-binding adapter molecule 1 (Iba-1), neurokinin-1 receptor (NK-1R), Glu aspartate transporter (GLAST), and Glu transporter-1 (GLT-1) in the dorsal horns (DHs) of the cervico-spinal cord (C2–C5) were detected using immunofluorescence histochemistry. The expression levels of GFAP, Iba-1, GLAST, and GLT-1 mRNAs were determined using quantitative real-time polymerase chain reaction (PCR).

Results: The TPT and levels of mRNAs expression and immunoactivity of GLT-1 and GLAST were significantly decreased, and those of Iba-1 and GFAP were significantly increased in the model group than those of the control group ($P < 0.05$). The activated microgliacytes were gathered around the NK-1R positive neurons, and co-expression of NK-1R and astrocytes was observed in the model group. EA LI18 significantly increased the TPT and expression of GLAST and GLT-1 mRNAs ($P < 0.05$) and notably decreased the number of Iba-1 positive cells and Iba-l mRNA expression ($P < 0.05$), whereas GLAST and GLT-1 antagonists inhibited the analgesic effect of EA LI18. However, these effects, except for the downregulation of Iba-1 mRNA, were not observed

in the EA ST36-GB34 group. Fewer NK-1R-positive neurons were visible in the spinal DHs in the EA LI18 group, and the co-expression of NK-1R and astrocytes was also lower than that in the three EA groups.

Conclusion: Electroacupuncture of LI18 had an analgesic effect in rats with neck incisions, which may be related to its functions in suppressing the neuronal–glial cell interaction through NK-1R and upregulating the expression of GLAST and GLT-1 in the spinal DHs.

KEYWORDS

glial cells, electroacupuncture, incisional neck pain, glutamate transporter-1, glutamate–aspartate transporter

Introduction

Postoperative incisional pain is a common complication of surgery that may seriously affect patient quality of life. Within 4 days after thyroidectomy or parathyroid and endoscopic surgery, many patients complain of severe neck pain and anterior chest discomfort (Wattier et al., 2016; Kim et al., 2018). Moreover, in some cases, chronic pain may occur due to a topical inflammatory reaction, central hypersensitivity, changes in patient psychological or pathophysiological factors, or improper treatment of acute pain (Lin, 2014; Lou et al., 2017). Block (2016) conducted a comprehensive analysis of previous studies and concluded that surgery-induced inflammation at the injury site further caused low-grade inflammation in the central nervous system (CNS), leading to an imbalance of neuronal–glial interaction and communication. Thus, enhanced excitability of the excitatory neurons and increased pain-signal transmission developed, inducing a transition from acute incisional pain to persistent postsurgical pain. During this process, the neuronal–glial interaction plays an important role in pain maintenance (Gwak et al., 2017; Zhang et al., 2017).

As excitatory neurotransmitters, glutamate (Glu) and substance P (SP) play a critical role in pain-signal transmission and maintenance. In the spinal dorsal horns (DHs), they are released from the peripheral endings of small diameter afferent fibers upon noxious stimulation (Kingery et al., 2002; Jin et al., 2009; Clarke et al., 2011), whereas, neurokinin-1 receptors (NK-1Rs) and Glu receptors (GluR) are expressed on spinal DH neurons (Ma et al., 2015; Wang et al., 2019). The NK-1Rs are also expressed in non-neuronal cells in the CNS, including microglia and astrocytes, and these glial cells have important immune functions (Härtel et al., 2009; Johnson et al., 2016; Burmeister et al., 2017; Jiang et al., 2020). The combination of SP and NK-1R functions in promoting inflammatory immune responses by the activated microglia and astrocytes (Johnson et al., 2016), which may be involved in acute incisional pain that progresses to chronic pain. The Glu aspartate transporter (GLAST) and Glu transporter-1 (GLT-1) are two major transporters expressed in astrocytes of the spinal cord that absorb extracellular Glu to maintain normal extracellular levels and protect neurons from neurotoxicity. Inhibition of GLAST expression in the spinal cord reduces excitatory synaptic activity and spontaneous responses after nociceptive stimulation of the paw (Niederberger et al., 2006). In the spinal DHs, GLT-1 and GLAST are densely expressed in laminae I and II, and their expression levels in the activated astrocytes (neither microglia nor neurons) were evidently decreased at both 7 and 14 days after partial sciatic nerve ligation (Xin et al., 2009). These results indicate that NK-1R, GLAST, and GLT-1 are all involved in the nociceptive input processing in DHs of the spinal cord.

Our previous research showed that EA of LI18 (close to the neck incision) and LI4-PC6 (located at the neighboring nerve segments of the neck incision) suppressed incisional neck pain or neck inflammatory pain and inhibited the immunoactivity of SP and NK-1R, as well as protein expression of the NR2B subunit of *N*-methyl-*D*-aspartate (NMDA) receptors in cervical spinal cord DHs in rats with inflammatory (Gao et al., 2009) and incisional neck pain (Qiao et al., 2010). Repeated EA treatment attenuated hyperalgesia by inhibiting spinal glial activation in rats with chronic neuropathic pain (Wang et al., 2018). Thus, EA may reduce pain by modulating SP/NK-1R signaling and GLAST activities, which have a close relationship with glial cells. However, it remains unclear whether the neuron–glial interaction *via* SP/NK-1R signaling and GLAST in the cervical spinal DHs are involved in EA-induced relief of acute incisional neck pain. Therefore, the present study was designed to investigate the role of spinal neuronal–glial cross-talk by regulating NK-1R and astrocytic GLAST in incisional pain induction and EA analgesia.

Abbreviations: EA, electroacupuncture; GLT-1, glutamate transporter-1; GLAST, glutamate–aspartate transporter; TPT, thermal pain threshold; Iba-1, ionized calcium-binding adapter molecule 1; GFAP, glial fibrillary acidic protein; CNS, central nervous system; NK-1R, neurokinin-1 receptor; GluR, glutamate receptors; SP, substance P; Glu, glutamate; LSD, least significant difference; NMDA, *N*-methyl-*D*-aspartate; EAAC1, excitatory amino acid carrier 1.

Materials and methods

Animals and grouping

All experimental protocols and animal care were approved by the Institutional Animal Welfare and Use Committee of the Institute of Acupuncture and Moxibustion of the China Academy of Chinese Medical Sciences (Approval No. 2013021801) and were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23, revised 1985). A total of 65 male Sprague-Dawley (SD) rats (200–220 g in weight) were obtained from the Experimental Animal Center of the China Academy of Medical Sciences [license number: SCXK (Jing, 2014-0013)] and housed under standard laboratory conditions at 22 ± 2°C with a 12:12-h light–dark cycle. The rats were given food and water freely and acclimatized to the laboratory conditions for 7 days prior to the experiment. Sixty-five rats were randomly assigned to the following five groups (n = 13 in each group): normal control, model (incisional neck pain), EA Futu (LI18), EA Hegu (LI4)-Neiguan (PC6), and EA Zusanli (ST36)-Yanglingquan (GB34) by using a random number table. To validate the effects of GLAST and GLT-1 in EA analgesia, another 30 male SD rats were randomized into the following three groups: vehicle + EA, glutamate aspartate transporter (GLIST) antagonist + EA, and GLT-1 antagonist + EA; these rats received intrathecal injection (i.t.) of the related vehicle or antagonists.

Incisional neck pain model establishment

Rat neck hair was removed with an appropriate amount of hair removal cream (barium sulfide) 1 day before the incision operation. To establish the incisional neck pain model, the rats were anesthetized with isoflurane (1–2% in oxygen), delivered by a nose cone in a tabletop animal anesthesia ventilator system (VME Matrix, Midmark, Dayton, OH, USA). A 1.5 cm longitudinal incision was made along the midline of the neck, and a pair of forceps was used to separate and manipulate the bilateral sternohyoideus muscles in the region of the thyroid gland repeatedly for approximately 30 min. Next, the incision was sutured in layers with surgical silk (gauge 4.0) at intervals of approximately 0.5 cm. After the operation, the rats were placed back into the cages for recovery.

Electroacupuncture intervention

According to the acupoint chart for rats in the book "*Experimental Acupuncture*" (Li, 2007), and the positions of acupoints in the human body, LI18 is located on the lateral part of the neck between the anterior and posterior margins of the sternocleidomastoid muscle on the horizontal level of the 4th cervical vertebra. LI4 is located between the first and second metacarpal bones of the forelimb. PC6 is on the ventral side of the forelimb, approximately 3 mm from the wrist, between the ulna and the radius. ST36 is on the lateral side of the knee joint of the hind limb, 5 mm under the capitulum fibulae. GB34 is approximately 4 mm superior-lateral to ST36. Bilateral acupoints were selected for this study (Figure 1A).

Under the same anesthesia, isoflurane (1–2% in oxygen), the filiform needles were inserted into the acupoints mentioned above. EA treatment was administered using a HANS Apparatus (Hans-200A, Jisheng Medical Technology, Co., Ltd., Nanjing, China) immediately after the neck surgery was completed, and 20 h after the incision, with the following parameters: 1 mA, alternative frequency of 2/100 Hz, and duration of

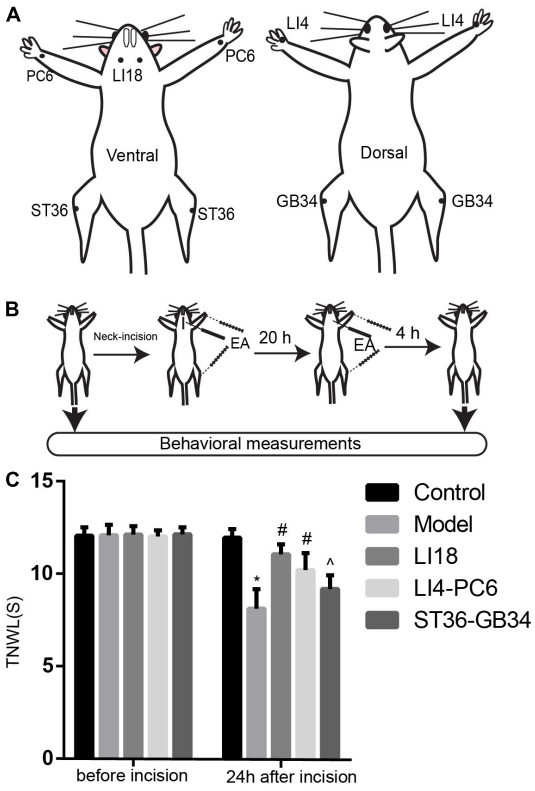

FIGURE 1
Electroacupuncture (EA) increases thermal pain threshold (TPT, thermal neck withdrawal latency, TNWL) in rats with incisional neck pain. **(A)** The location of LI18, LI4, PC6, ST36, and GB34 in rats. **(B)** Schematic diagram of experimental procedures of EA treatment and behavioral measurements. **(C)** Effect of EA at Futu (LI18), Hegu (LI14)-Neiguan (PC6), and Zusanli (ST36)–Yanglingquan (GB34) on TPT in rats with incisional neck pain (mean ± SD, n = 13/group). The TPT was significantly increased in both EA LI18 and EA PC6-LI4 groups but not in the EA ST36-GB34 group 24 h after incision. *$P < 0.01$, vs. the control group; #$P < 0.05$, vs. the model group; ^$P < 0.05$, vs. the EA LI18 group.

TABLE 1 Primer sequences.

Primers	Sequences	Length (bp)
Iba-1	Forward, 5′-AGCGAATGCTGGAGAAACTTG-3′ Reverse, 5′-AGTTGGCTTCTGGTGTTCTTTG-3′	194
GFAP	Forward, 5′-GCTAATGACTATCGCCGCCAACTG-3′ Reverse, 5′-CCTCCTGGTAACTCGCCGACTCC-3′	136
GLAST	Forward, 5′-GCCATCATGAGATTGGTAGCGGT-3′ Reverse, 5′-GGAAGTAGAGGAGAGGCAGGACGA-3′	187
Glt-1	Forward, 5′-GAACTTCGGTCAATGTAGTGGGCG-3′ Reverse, 5′-TGGACTGCGTCTTGGTCATTTCG-3′	132
GAPDH	Forward, 5′-TTCCTACCCCCAATGTATCCG-3′ Reverse, 5′-CCACCCTGTTGCTGTAGCCATA-3′	270

30 min. The rats in the normal control and model groups were administered the same anesthesia without acupuncture needle insertion and EA stimulation.

Measurement of thermal pain threshold

The thermal pain threshold (TPT, i.e., thermal neck withdrawal latency, TNWL) of the neck incision region was measured before and 24 h after neck incision using a tail-flick unit (37360, UGO Basile, Gemonio, VA, Italy) while the rat was fully awake (**Figure 1B**). The heat intensity was set to 50 units, with a cutoff time of 30 s to avoid tissue damage. The TPT was measured as described in our previous reports (Gao et al., 2009; Qiao et al., 2010). During the incisional neck pain measurement, the rat was held in place with the neck incision region over the mounted window of the radiant heat source of the tail-flick unit. The TPT was recorded automatically when the rat swiftly moved its neck away from the heat source. The measurement was repeated three times for each rat, with an interval of approximately 5 min between every two measurements, and the average value was used. The researcher who analyzed the TPT data was blinded to animal grouping and did not participate in EA interventions.

Surgery for intrathecal injection

The rats used for intrathecal injection (i.t.) under light anesthesia (isoflurane) were placed in a stereotaxic apparatus. After exposing the dura mater of the lumbar (L5–L6) spinal cord, a polyethylene (PE10) catheter [outside diameter (OD) 0.61 mm, internal diameter (ID) 0.28 mm, Smiths Medical, ICU Medical Inc., Minneapolis, MN, USA] prefilled with sterilized 0.9% NaCl solution was inserted into the subdural space and moved rostrally about 8 cm to the spinal subarachnoid space of the cervical vertebrae C2–C5 (Chen et al., 2012). The local muscles and skin were sutured in layers with 3-0 silk stitches, the catheter was fixed and buried in the muscle layers and sealed with a cautery pen, and approximately 2–3 cm of the catheter end was left exposed. The rats were allowed to recover for 7 days before beginning the next experimental procedure. After completing the experiment, the location of the catheter was verified by injecting lidocaine, and only rats who developed a brief forelimb paresis after lidocaine injection were used.

Intrathecal administration of glutamate aspartate transporter and glutamate transporter-1 antagonists

One week after catheter implantation, the rats were placed under light anesthesia (isoflurane) and received one of the following treatments by i.t.: 100 μg/10 μl DL-threo-β-benzyloxyaspartate (DL-TBOA, a competitive, non-transportable blocker of excitatory amino acid transporters such as GLAST), 100 μg/10 μl DHK (a GLT-1 specific antagonist), or 10 μl vehicle (0.9% saline, n = 10/group) through the catheter by using a micro-osmotic pump (0.5 μl/h), once daily for 5 days. Any residual reagent solution or vehicle was flushed from the catheter with subsequent delivery of 10 μl of saline, with the outer end sealed by heat every time. This treatment was followed by the neck incision procedure. Thermal hyperalgesia of the neck incision area was measured before incision, at 4 and 24 h after incision.

Quantitative real-time polymerase chain reaction

Under deep anesthesia with pentobarbital sodium (35 mg/kg, i.p.), the C2–C5 segments of the cervical dorsal spinal cord (semi-section) were quickly collected on ice 24 h after neck incision and stored in liquid nitrogen. The total RNA of spinal cord tissue was extracted using the TRIzol method. The reverse transcription of cDNA was performed using the Prime ScriptTM Reagent Kit (Takara Bio, Shiga, Japan). Gene expression levels were measured using a fluorescence quantitative real-time PCR system (ABI7500, Applied Biosystems, Waltham, MA, USA), with the primer sequences summarized in **Table 1**. Each reaction mixture consisted of 2 μl cDNA, 10 μl REAL SYBR Mixture (2×), 0.8 μl (10 μmol/μl) of both forward and reverse primers, and 7.2 μl PCR-grade water, equating to a final volume of 20 μl. PCR was performed under the following conditions: 95°C, 30 s; 40 PCR cycles (95°C, 5 s, 60°C, 40 s); followed by 95°C for 10 s, 60°C for 60 s, and 95°C for 15 s. The data were analyzed by $2^{-\Delta\Delta Ct}$.

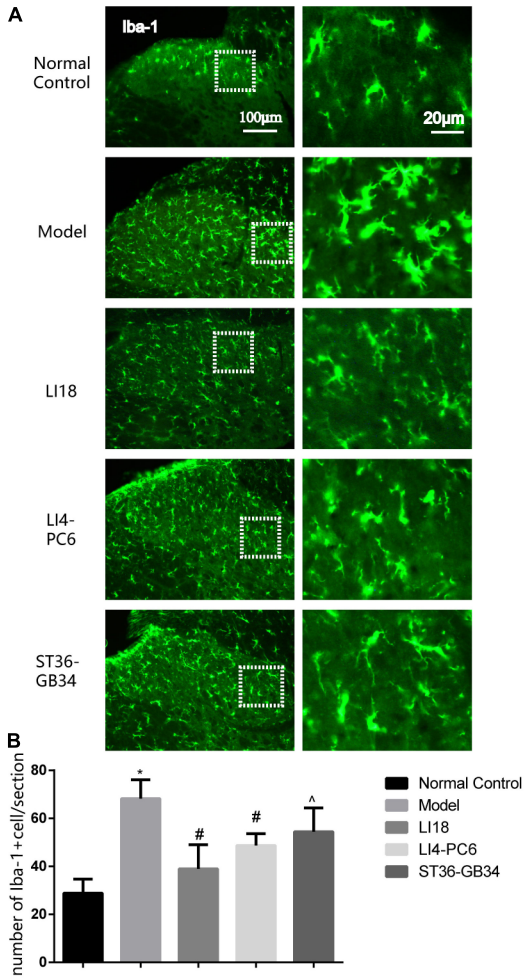

FIGURE 2
Comparison of the effects of electroacupuncture (EA) on activities of microglia in the dorsal horns (DHs) of cervical (C2–C5) spinal cord at 24 h after neck incision in different groups (mean ± SD, n = 5/group). **(A)** Representative fluorescence micrographs of immunofluorescent staining of ionized calcium-binding adapter molecule 1 (Iba-1) for microgliacytes in the spinal DHs in different groups. The magnified microglial cells shown in the right column images were chosen from the dashed squares of the left column images in the five groups. The scale bar in the five images on the left column is 100 μm, and 20 μm in the five images on the right column. **(B)** Bar graphs showing the numbers of microglia in the DHs of spinal cord at 24 h after incision in different groups. One-way ANOVA revealed that the number of Iba-1 immunoreaction (IR) positive cells was significantly increased in the model group (vs. the control group) and considerably decreased in both EA LI18 and EA PC6-LI4 groups but not in the EA ST36-GB34 group (vs. the model group). *$P < 0.01$, vs. the control group, #$P < 0.05$, vs. the model group, ^$P < 0.05$, vs. the EA LI18 group.

Immunofluorescence labeling

Under deep anesthesia with pentobarbital sodium (35 mg/kg, i.p.), the rats were initially perfused with normal saline followed by a 4% paraformaldehyde solution. The cervical spinal cord (C2–C5, semi-section) was removed and dehydrated with 30% sucrose solution and then sliced into 40 μm thick sections with a freezing microtome (Thermo Fisher Scientific, Bremen, Germany). NK-1 and glial fibrillary acidic protein (GFAP)/ionized calcium-binding adapter molecule 1 (Iba-1), or GFAP and GLT-1/GLAST double immunofluorescence analyses were performed. Free-floating tissue sections were incubated in the following primary antibodies: rabbit anti-NK-1 (1:1,000, AB5060, Sigma-Aldrich, Burlington, MA, USA) and mouse anti-GFAP (1:1,000, 3670S, Cell Signaling Technology, Danvers, MA, USA)/goat anti-Iba-1 (1:500, ab5076, Abcam, Waltham, MA, USA), or mouse anti-GFAP and rabbit anti-GLAST (1:200, ab416, Abcam, Waltham, MA, USA)/rabbit anti-GLT-1 (1:200, ab41621, Abcam, Waltham, MA, USA) overnight at 4°C. Next, the sections were incubated with fluorescent secondary antibodies: Alexa Fluor 594-conjugated donkey anti-rabbit antibodies (1:500, A32754, Life Technologies, Waltham, MA, USA) and Alexa Fluor 488-conjugated donkey anti-mouse antibodies (1:500, A21202, Life Technologies, Waltham, MA, USA)/Alexa Fluor 488-conjugated donkey anti-goat antibodies (1:500, A11055, Life Technologies, Waltham, MA, USA), or Alexa Fluor 488-conjugated donkey anti-mouse antibodies (1:500, A21202, Life Technologies, Waltham, MA, USA), and Alexa Fluor 594-conjugated donkey anti-rabbit antibodies (1:500, A32754, Life Technologies, Waltham, MA, USA) for 2 h at room temperature. The 435/455 Blue Fluorescent Nissl (1:1,000, N21479, Invitrogen, Waltham, MA, USA) was also used for the identification of cellular nuclei. The images of three sections (within the superficial DHs) of each rat were captured with a fluorescent microscope (E600, Eclipse, Nikon,Tokyo, Japan) or a laser scanning confocal microscope (FV1200, Olympus, Tokyo, Japan) equipped with a digital camera (DP70, Olympus, Tokyo, Japan). Under the microscope, five areas in the same section of DHs of the spinal cord in each tissue section were randomly selected for counting the number of Iba-1 positive cells or for measuring the intensity of GFAP/GLAST/GLT-1 immunofluorescence with the Nikon Imaging Software (NIS) elements (Nikon, Tokyo, Japan). The mean number or the mean intensity of each section in the five areas was calculated. Next, the mean number or mean intensity of three slices from the same rat was taken as the positive cell count or fluorescence intensity of that rat. Control immunostaining was obtained by substituting the primary antibody with normal serum.

Statistical analyses

All data were expressed as the mean ± standard deviation (mean ± SD) and were analyzed with IBM SPSS software (IBM Corp., Armonk, NY, USA). Repeated measures ANOVA was used to analyze the TPT data, and one-way ANOVA was used

to analyze the remaining data from the present study. A *post-hoc* test for least significant difference (LSD) was performed to compare differences between the two groups. Statistical significance was set at $P < 0.05$.

Results

Effect of electroacupuncture on thermal pain

Before the neck incision, we found no significant differences in the TPT levels among the five groups ($P > 0.05$, Figure 1C). After neck incision, the TPTs were significantly decreased in the model group when compared with the control group at 24 h after neck incision ($P < 0.05$). Also, when compared with the model group, the TPTs were observably increased 24 h after neck incision in both the EA LI18 and EA LI14-PC6 groups ($P < 0.05$, Figure 1C), but not in the EA ST36-GB34 group ($P > 0.05$, Figure 1C). The TPT of the EA LI18 group was markedly higher than that of the EA ST36-GB34 24 h after neck incision ($P < 0.05$, Figure 1C).

Effect of electroacupuncture on activities of microgliacytes and astrocytes

Under the microscope, 24 h after neck incision, the microgliacytes labeled by Iba-1 exhibited small cell bodies and thin processes in the superficial layers of the spinal DHs in the control group, and those in the model group exhibited an ameboid shape with enlarged cell bodies and thick and short processes (Figure 2A). When compared with the control group, the number of Iba-1 positive cells in the model group was observably increased 24 h after neck incision ($P < 0.05$, Figure 2B), suggesting microgliacyte activation after neck incision. The microgliacytes in the EA LI18 group exhibited a similar state as those in the control group. When compared with the model group, the number of Iba-1 positive cells at 24 h was significantly decreased in both the EA LI18 and EA LI4-PC6 groups ($P < 0.05$), whereas, the number in the EA ST36-GB34 was slightly decreased ($P > 0.05$). The number of Iba-1 positive cells was markedly lower in the EA LI18 group than that in the EA ST36-GB34 group 24 h after neck incision ($P < 0.05$, Figure 2B).

In the normal control group, a few GFAP-labeled astrocytes were evenly distributed in the DHs of the cervical spinal cord and exhibited thin dendrites. The astrocytes in the model group exhibited an activated state marked by hypertrophied cell bodies and thicker processes, with a significantly increased mean intensity of GFAP than that in the control group ($P < 0.05$, Figure 3A). The average intensity of GFAP in the EA LI18 group (but not in the EA LI4-PC6 and ST36-GB34 groups, Figure 3B) was significantly lower than that in the model group 24 h after incision ($P < 0.05$), suggesting a suppression of the astrocytic activity only in the EA LI18 group after EA.

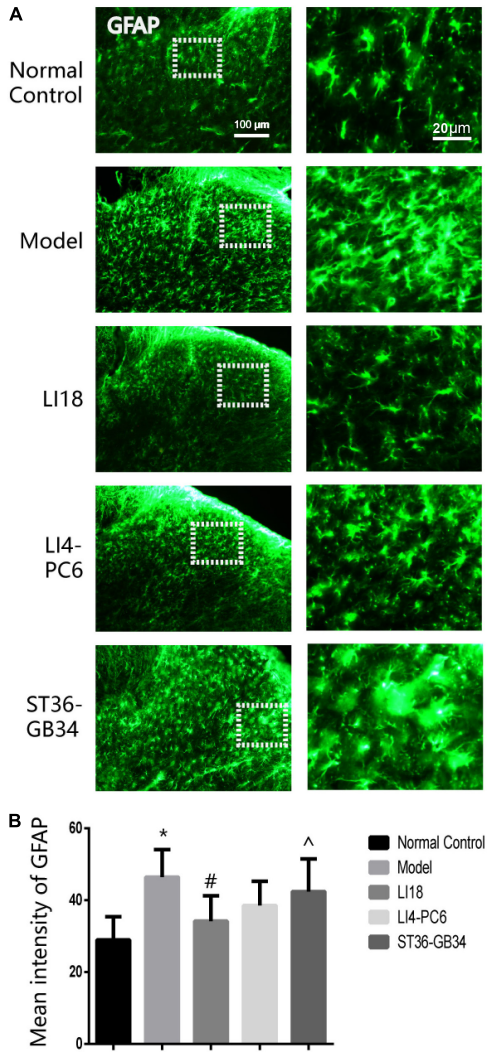

FIGURE 3
Effect of electroacupuncture (EA) on activities of astrocytes in dorsal horns (DHs) of cervical spinal cord at 24 h after neck incision in different groups. **(A)** Representative fluorescence micrographs of immunofluorescence staining of GFAP for astrocytes in the cervical (C2–C5) spinal DHs in the five groups. The magnified astrocytes shown in the right column images were chosen from the dashed squares of the right column images in the five groups. The scale bar in the five images on the left column is 100 μm, and 20 μm in the five images on the right column. **(B)** Bar graphs showing the mean intensity of glial fibrillary acidic protein (GFAP) immunoreaction (IR) in the DHs (mean ± SD, n = 5/group). One-way ANOVA analysis revealed that the mean intensity of GFAP IR was significantly increased in the model group (vs. the control group), and considerably decreased in the EA LI18 but not in the EA PC6-LI4 and EA ST36-GB34 groups (vs. the model group). *$P < 0.01$, vs. the control group, #$P < 0.05$, vs. the model group, ^$P < 0.05$, vs. the EA LI18 group.

Effect of electroacupuncture on the expression of ionized calcium-binding adapter molecule 1 and glial fibrillary acidic protein mRNAs

The expression levels of Iba-1 mRNA and GFAP mRNA in the cervical dorsal spinal cord in the model group were markedly higher than those in the normal control group 24 h after incision ($P < 0.05$, Figures 4A,B). When compared with the model group, the expression levels of Iba-1 mRNA and GFAP mRNA in the EA LI18 group, and those of Iba-1 mRNA in the EA LI14-PC6 and EA ST36-GB34 groups, were significantly decreased ($P < 0.05$, Figures 4A,B) 24 h after incision. Meanwhile, no remarkable differences were found among the three EA groups in the expression of Iba-1 mRNA, and between the EA LI18 and EA LI14-PC6 groups in the expression of GFAP mRNA. Also, no significant differences in the expression levels of GFAP mRNA were found between the EA ST36-GB34 group and the model group ($P > 0.05$, Figure 4B).

Electroacupuncture suppresses the cross-talk between neurokinin-1 receptor and glial cells

To analyze the effect of EA on the interaction between NK-1R and glial cells in the cervical spinal cord, we examined the co-labeled state of NK-1R and Iba-1 or GFAP in the DHs by using an immunofluorescence dual-labeling technique. Confocal microscopic observation results indicated that the NK-1Rs expression was mainly on neurons but not on microgliacytes.

In the normal control group, NK-1R expression was found in the superficial layers of the DHs of the spinal cord (lamina I) where both C and Aδ fibers terminate, and the Iba-1-labeled microglial cells remained in a resting state. NK-1R was clearly expressed on the neuronal bodies and axons (in the lamina I), which extended to the laminae II and III (Figure 5) in the model group 24 h after neck incision. Simultaneously, it was found that some activated microgliacytes gathered around the neurons expressing NK-1R in the spinal cord DHs. Following EA intervention, it was difficult to detect NK-1R-labeled neurons in the spinal cord DHs in the EA LI18 group, whereas NK-1R was expressed in neurons in the superficial layer of the spinal cord DHs in the EA LI4-PC6 and EA ST36-GB34 groups.

Immunofluorescence triple-labeling showed that few NK-1R- and GFAP-positive astrocytes were found in the superficial layers of spinal cord DHs in the normal control group rats (Figure 6), and many GFAP-labeled astrocytes were activated in laminae II–III 24 h after incision in the model group (Figure 6). This co-expression of NK-1R and GFAP was not found in the EA LI18 and EA LI4-PC6 groups, probably due to an EA-induced inhibitory effect on NK-1-positive astrocytes (Figure 6). Moreover, a markedly lower level of NK-1R-positive astrocytes was observed in the EA ST36-GB34 group.

Effect of electroacupuncture on the expression of glutamate aspartate transporter and glutamate transporter-1

Immunofluorescence staining analysis showed that GLAST immunoreaction-positive product was found in the superficial layer of spinal cord DHs (lamina I) where C/Aδ fibers terminate and transmit nociceptive information (Figure 7A). When compared with the normal control group, the immunofluorescence intensity of GLAST and expression of GLAST mRNA were markedly decreased in the model group 24 h after neck incision ($P < 0.05$, Figures 7B,C). Moreover, when compared with the model group, the expression of GLAST mRNA in the EA LI18 group and the immunofluorescence intensity of GLAST in both the EA LI18 and EA LI4-PC6 groups were significantly increased ($P < 0.05$, Figures 7B,C), but this increase was not observed in the EA ST36-GB34 group ($P > 0.05$). When compared with the normal control group, the mean immunofluorescence intensity of GLT-1 and the expression of GLT-1 mRNA were significantly decreased in the model group ($P < 0.05$, Figures 8A–C). In contrast to the model group, the expression of GLT-1 immunofluorescence intensity and GLT-1 mRNA was significantly increased in both the EA LI18 and EA LI4-PC6 groups ($P < 0.05$, Figures 8B,C), but not in the EA ST36-GB34 group ($P > 0.05$). The effect of the EA LI18 group in upregulating the immunofluorescence intensity levels of GLT-1 and GLAST of spinal cord DHs was significantly superior to that of the EA ST36-GB34 group ($P < 0.05$, Figures 7B, 8B).

Glutamate aspartate transporter and glutamate transporter-1 antagonists weaken the analgesic effect of electroacupuncture

To confirm the involvement of GLAST and GLT-1 in the analgesic effect of EA, we employed i.t. of antagonists of GLAST (TBOA) and GLT-1 (DHK) in rats with incisional neck pain (Figure 9A). The outcomes showed that following EA intervention of bilateral LI18, the TPT levels were significantly lower at 4 and 24 h after modeling in the i.t. TBOA + EA and DHK + EA groups than those in the i.t. saline + EA group ($P < 0.05$, Figures 9B,C). A marked weakened analgesic effect of EA was found after the administration of both GLAST and GLT-1 antagonists, suggesting that both GLAST and GLT-1 in cervical spinal cord DHs contribute to the analgesic effect of EA of LI18 in rats with neck incisions.

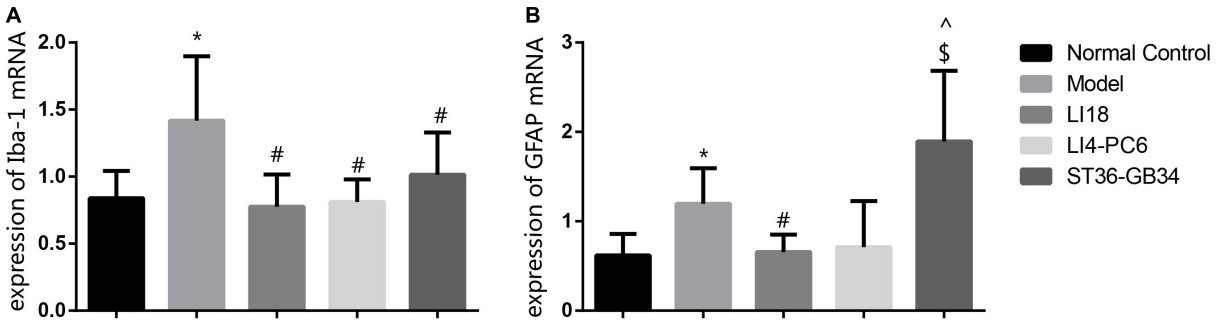

FIGURE 4
Effect of electroacupuncture (EA) intervention on ionized calcium-binding adapter molecule 1 (Iba-1) mRNA **(A)** and glial fibrillary acidic protein (GFAP) mRNA **(B)** of dorsal spinal cord (C2–C5) at 24 h after neck incision (mean ± SD, n = 8/group). The expression levels of Iba-1 mRNA and GFAP mRNA were significantly upregulated in the model group (vs. the control group), that of Iba-1 mRNA was obviously downregulated in the three EA groups (vs. the model group), and that of GFAP mRNA was obviously downregulated in the EA LI18 group but not in the EA PC6-LI4 and EA ST36-GB34 groups (vs. the model group). *$P < 0.01$, vs. the control group, #$P < 0.05$, vs. the model group, ^$P < 0.05$, vs. the EA LI18 group, \$$P < 0.05$, vs. the EA LI14-PC6 group.

Discussion

Our previous studies revealed that EA of LI18 and PC6-LI4 induced pain relief in rats with neck incision at 4 h after modeling (Qiao et al., 2010); however, the effect was not observed 24 h after neck incision when EA treatment was given twice. In the present study, an analgesic effect was observed after EA treatment was given twice in EA LI18 and EA LI4-PC6 groups (one immediately after the incision and another 20 h later) and was higher than that in EA ST36-GB34. These results were similar to the outcomes of Jia et al. (2016) in which EA of ST36 and Kunlun (BL 60) effectively reduced both mechanical and thermal hyperalgesia in rats with pelmatic incisional pain. The difference in the analgesic effect among the three acupoint groups is probably due to different segmental innervations. LI18 is close to the neck incision (in the same nerve segment, thus having the best EA effect), PC6 and LI4 are in the neighboring nerve segment, and ST38 and GB34 are quite distant from the neck incision, exhibiting a better and a poorer effect, respectively. Our previous electrophysiological research (Liu et al., 1993) showed that in normal rabbits, the spontaneous discharges of DH neurons in the thoracic spinal cord (T2–T3) were activated by EA stimulation of PC6 (the same nerve segment as T2–T3) in 35.48% (11/31) units and activated by EA of ST36 (the nerve segment distant from T2–T3) in only 7.14% (2/28) units. Moreover, acupuncture analgesia includes local analgesia that is achieved by activating A-type nerve fibers (innocuous intensity, triggering segmental nerve inhibition) and systemic analgesia which is obtained by activating Aδ and C nerve afferents (noxious stimulation). The latter may recruit the diffuse noxious inhibitory controls system (Xu et al., 2003; Zhu, 2015) of the brain stem. Thus, the stimulation strength is critical in achieving regional or systemic analgesic effects. These findings explain why in the present study, the analgesic effect of EA LI18 at 1 mA (lower strength) was significantly superior to that of EA ST36-GB34.

As the largest number of cells in the CNS, gliacytes play an important role in both physiological and pathological processes. Over the past several decades, a growing body of evidence indicated that activation of microglia and astrocytes was involved in pain induction and maintenance (Scholz and Woolf, 2007; Chen et al., 2018). The microgliacytes are essential for synaptic plasticity and chronic pain in the spinal cord DHs (Xu et al., 2014), and the activated astrocytes are involved in the pathogenesis of hypersensitivity in a chronic post-ischemia pain model (Fu et al., 2006). The findings of the present study showed that along with the appearance of incisional neck pain, the number of Iba-1-labeled microgliacytes was apparently increased and the GFAP-labeled astrocytes also exhibited an activation state, marked by hypertrophied cell bodies with thicker processes 24 h after neck incision. In addition, a notable upregulation of Iba-1 mRNA and GFAP mRNA was observed in the cervical spinal cord DHs 24 h after neck incision.

It is reported that even in the very early postoperative period after plantar incision, the peripheral sensory afferent neurons exhibited an increased spontaneous and stimulus-evoked activity that mediated hyperalgesia and allodynia (Xu and Brennan, 2009). Administration of SP produced mechanical allodynia in a dose-dependent fashion in mice, and administration of the NK-1R antagonist LY303870 attenuated the allodynia (Peyman et al., 2009). The SP is produced at high levels within the CNS, and NK-1R is abundantly expressed on neurons and is also present in glial cells including microglia and astrocytes (Johnson et al., 2016). C-fiber afferent SP/NK-1R signaling supports spinal microglial and astrocytic activation after noxious stimulation (Li et al., 2015). Thus, SP/NK-1 signaling plays an important role in nociceptive sensitization by facilitating the interaction of neuron–glial cells, and the relationship of NK-1R and glial cells during the occurrence of

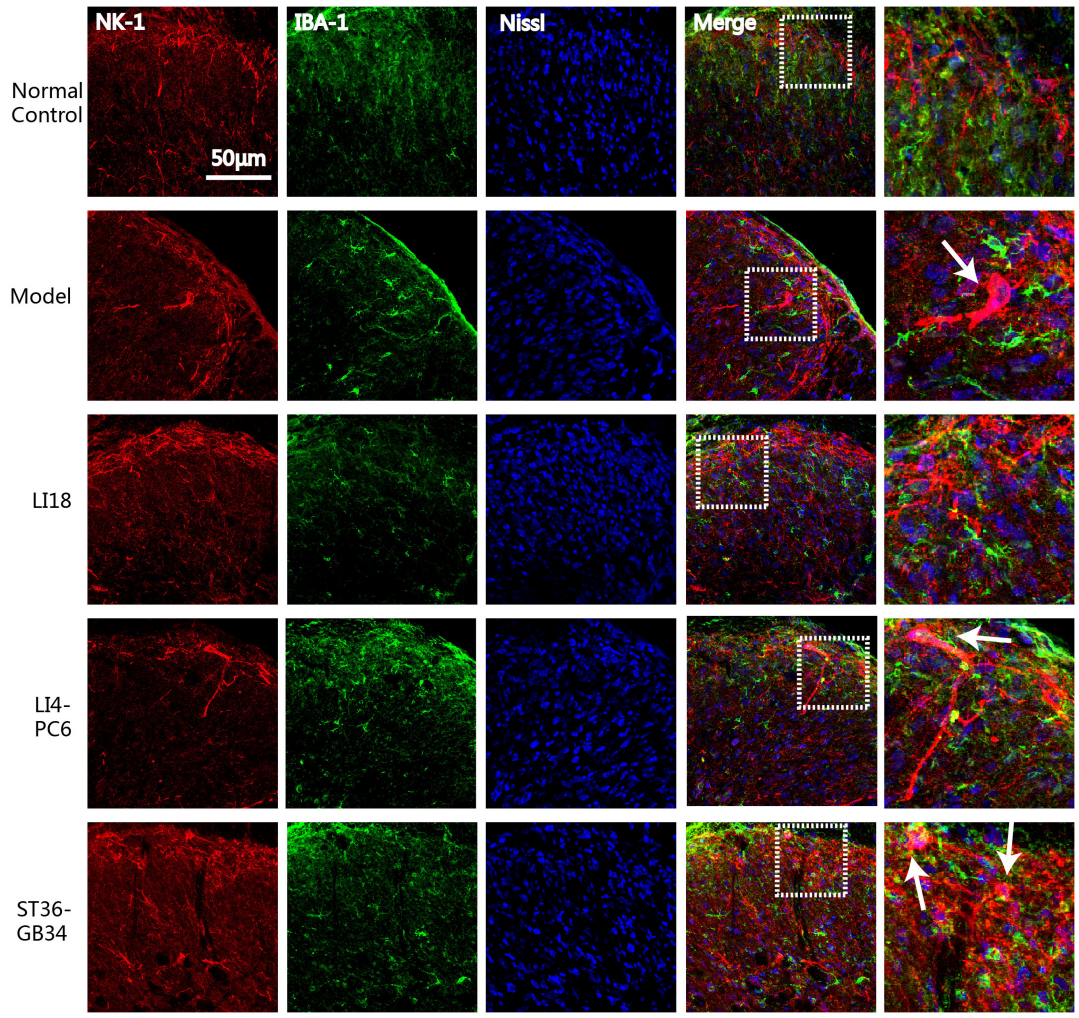

FIGURE 5
Confocal micrographs showing the expression of neurokinin-1 receptor (NK-1R) (red) and ionized calcium-binding adapter molecule 1 (Iba-1) (green) in the superficial dorsal horns (DHs) of the cervical spinal cord in different groups at 24 h after neck incision. Nissl staining (blue) shows the nucleus of cells. The scale bar is 50 μm. The white arrows indicate the NK-1 immunoreaction (IR) (red) positive neurons which are gathered around by the activated microgliacytes (green) in the DHs of cervical spinal cord.

neck incision acute pain was observed by confocal imaging. After the neck incision, the increased NK-1R was expressed on the neuronal bodies in lamina I, and the activated microgliacytes gathered around the NK-1R-positive neurons. Co-expression of NK-1R and GFAP was observed in astrocytes in laminae II–III, which was deeper than those predominantly expressed in the NK-1R area. Therefore, NK-1R may be involved in the induction of hyperalgesia after neck incision through a glial-cell-mediated inflammatory response. Inhibiting a spinal glial cell activation and inflammation reaction may be one of the effective ways to mitigate pain. Our previous study showed that repeated EA suppressed the activated glial cells induced by chronic neuropathological pain (Wang et al., 2018), but the effect of EA on glial cells was unknown in rats with incisional pain. When EA intervention was applied twice in the present study, along with the occurrence of pain relief, the Iba-1 IR positive cells and expression of Iba-1 mRNA in both EA LI18 and EA PC6-LI4 groups, and the expression of GFAP mRNA in the EA LI18 group were significantly reduced. This indicate that suppression of activities of both microgliacytes and astrocytes may also contribute to the analgesic effect of EA in rats with acute pain. NK-1R is expressed by neurons and glial cells of the CNS. Our study demonstrated that in LI18 and LI4-PC6, EA stimulation-induced downregulation of SP and NK-1R immunoactivity levels in the dorsal cervico-spinal cord may have contributed to their effects in relieving neck incision pain (Qiao et al., 2010), but the relationship of the SP/NK-1R signal and the glial cells was not clear after EA treatment. The findings of the present study demonstrated that following EA intervention, almost no NK-1R-positive neurons were visible in

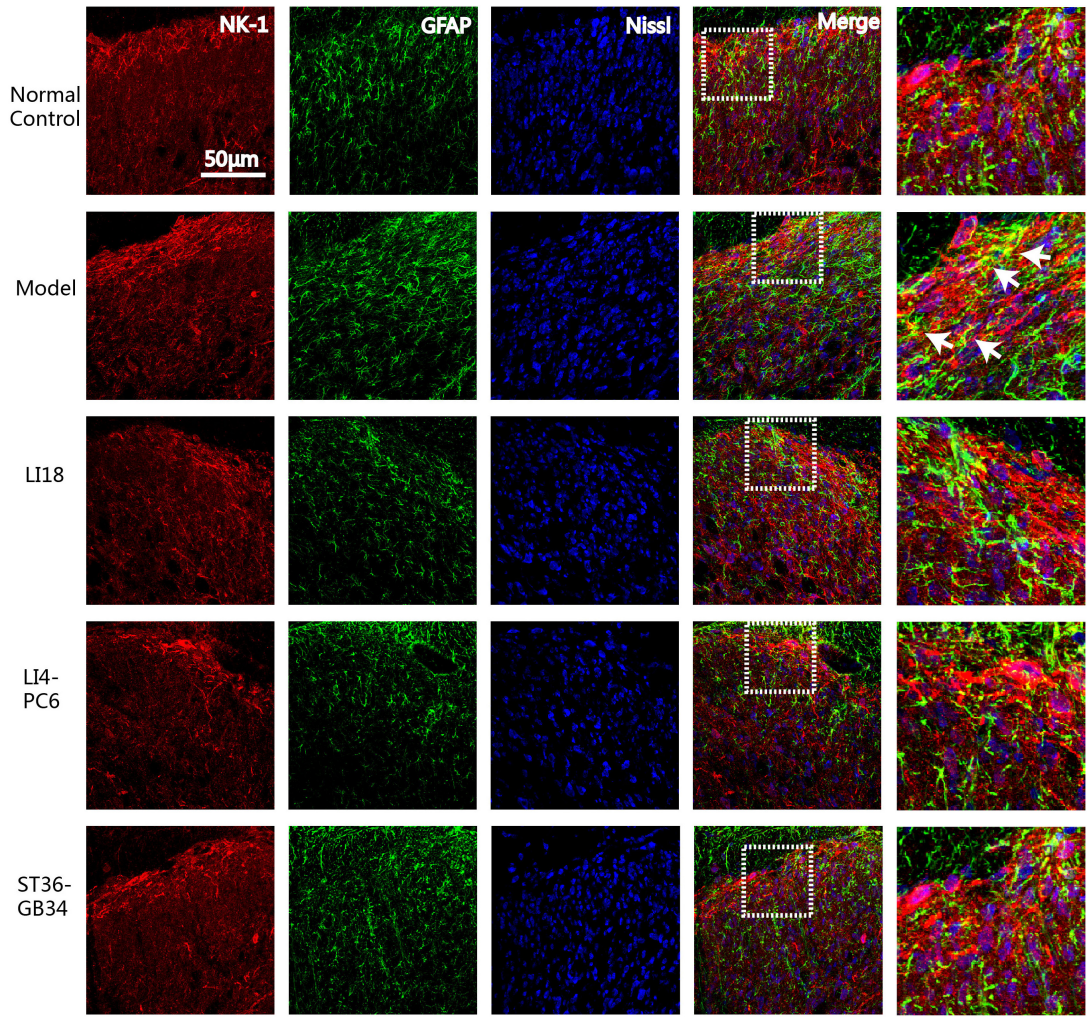

FIGURE 6
Confocal micrographs showing the co-expression (yellow) of NK-1 (red) and glial fibrillary acidic protein (GFAP) (green) in dorsal horns (DHs) of the cervical spinal cord in different groups at 24 h after neck incision. The scale bar is 50 μm. The white arrows indicate the NK-1R positive astrocytes. The expression of NK-1 immunoreaction (IR) and GFAP IR and co-expression were clearly seen in the superficial layer of spinal DHs (laminae II–III) in the model group and reduced in the three EA groups.

the spinal cord DHs in the EA LI18 group, and the number of NK-1R-positive astrocytes was also fewer to be found in DHs of the 3 EA groups, suggesting that EA regulates the neuron–glial cell interaction through NK-1R to achieve an analgesic effect. These findings have not previously been demonstrated in similar incisional pain animal models.

Glutamate is a major excitatory neurotransmitter in the central neuronal circuits, including the spinal cord DHs, and plays an important role in the nociceptive sensory transmission from injured tissue peripherals to the DHs of the spinal cord (Neugebauer, 2007). When Glu is released from the presynaptic nerve endings, it acts on Glu receptors of the post-synaptic membrane, such as NMDA, a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and metabotropic Glu receptors. Activation of these receptors causes the spinal cord neurons to become more sensitive to peripheral inputs, leading to central sensitization. Excessive extracellular Glu induces an overload of Ca^{2+} influx that may result in excitotoxicity and neuronal death (Kung et al., 2013). The clearance of neurotoxic concentrations of Glu is completed by a high-affinity Glu uptake system made up of the following five types of Na^{+}-dependent high-affinity Glu transporters: GLT-1, GLAST, excitatory amino acid carrier 1 (EAAC1), excitatory amino acid transporter 4 (EAAT4), and EAAT5. Among them, GLT-1 and GLAST are expressed mainly on glial cells and differentially expressed on sensory neurons and post-synaptic spinal interneurons (Gadea and Lopez-Colome, 2001). In addition to the major role in extracellular Glu removal, Glu transporters also have more sophisticated functions in the modulation of neurotransmissions, such as modifying the time

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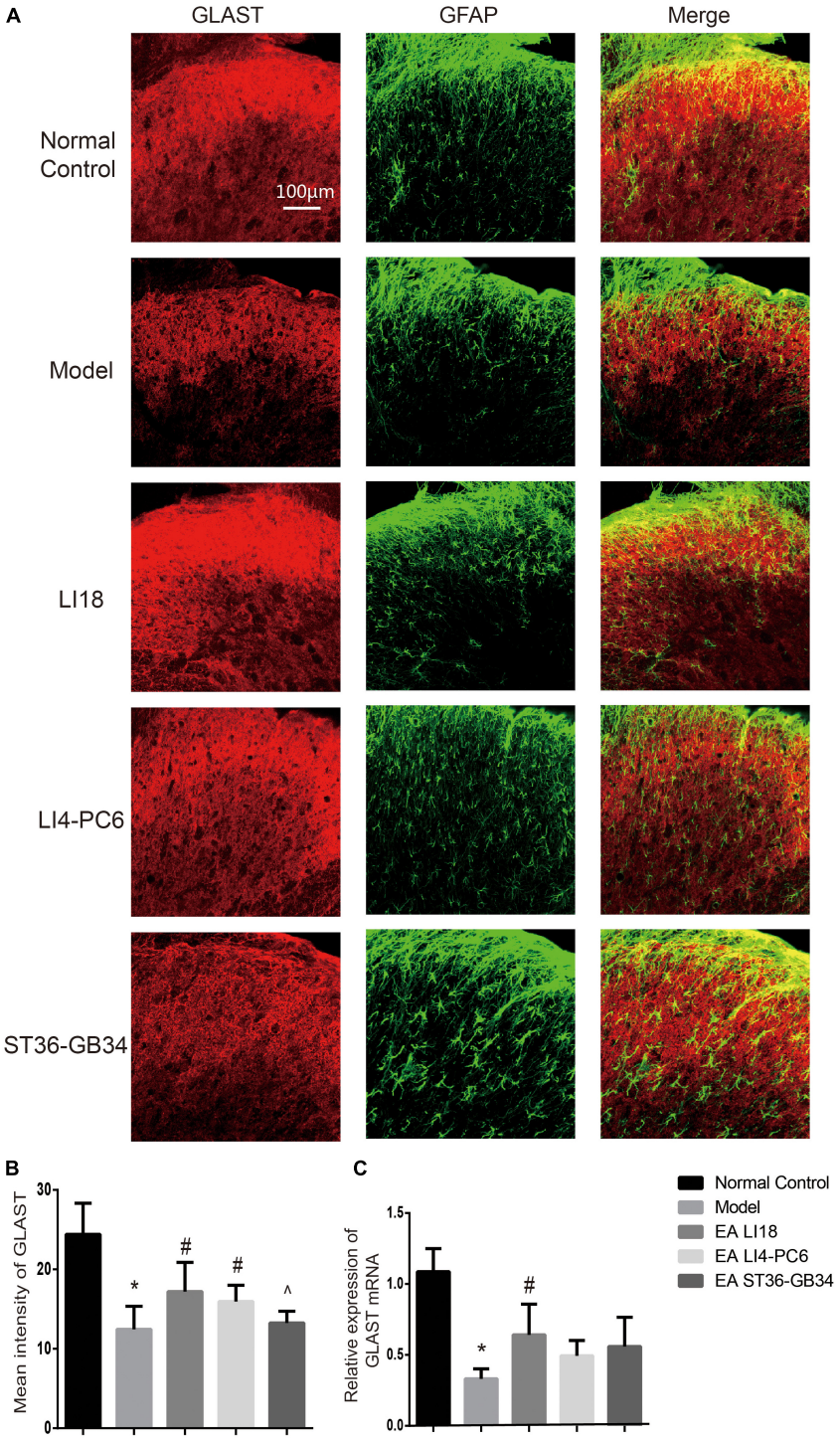

FIGURE 7

Comparison of expression of glu aspartate transporter (GLAST) in different groups (mean ± SD, n = 6/group). **(A)** Fluorescence micrographs of immunohistochemistry staining showing the co-expression (yellow) of GLAST (red) and glial fibrillary acidic protein (GFAP) (green) in the cervical spinal cord dorsal horns (DHs) at 24 h after neck incision. The scale bar is 100 μm. **(B)** Bar graphs showing the mean fluorescence intensity of GLAST in the five groups. The expression of GLAST protein was reduced in the model group relevant to the control group. After electroacupuncture (EA), the expression level of GLAST protein was significantly upregulated in both EA LI18 and EA PC6-LI4 groups but not in the EA ST36-GB32 group. **(C)** Bar graphs showing the relative expression of GLAST mRNA in the five groups. The expression of GLAST mRNA was significantly downregulated in the model group (vs. the control group) and significantly increased in EA LI18 group, rather than in the EA PC6-LI4 and EA ST36-GB34 groups (vs. the model group). *$P < 0.01$, vs. the control group, #$P < 0.05$, vs. the model group, $\hat{}P < 0.05$, vs. the EA LI18 group.

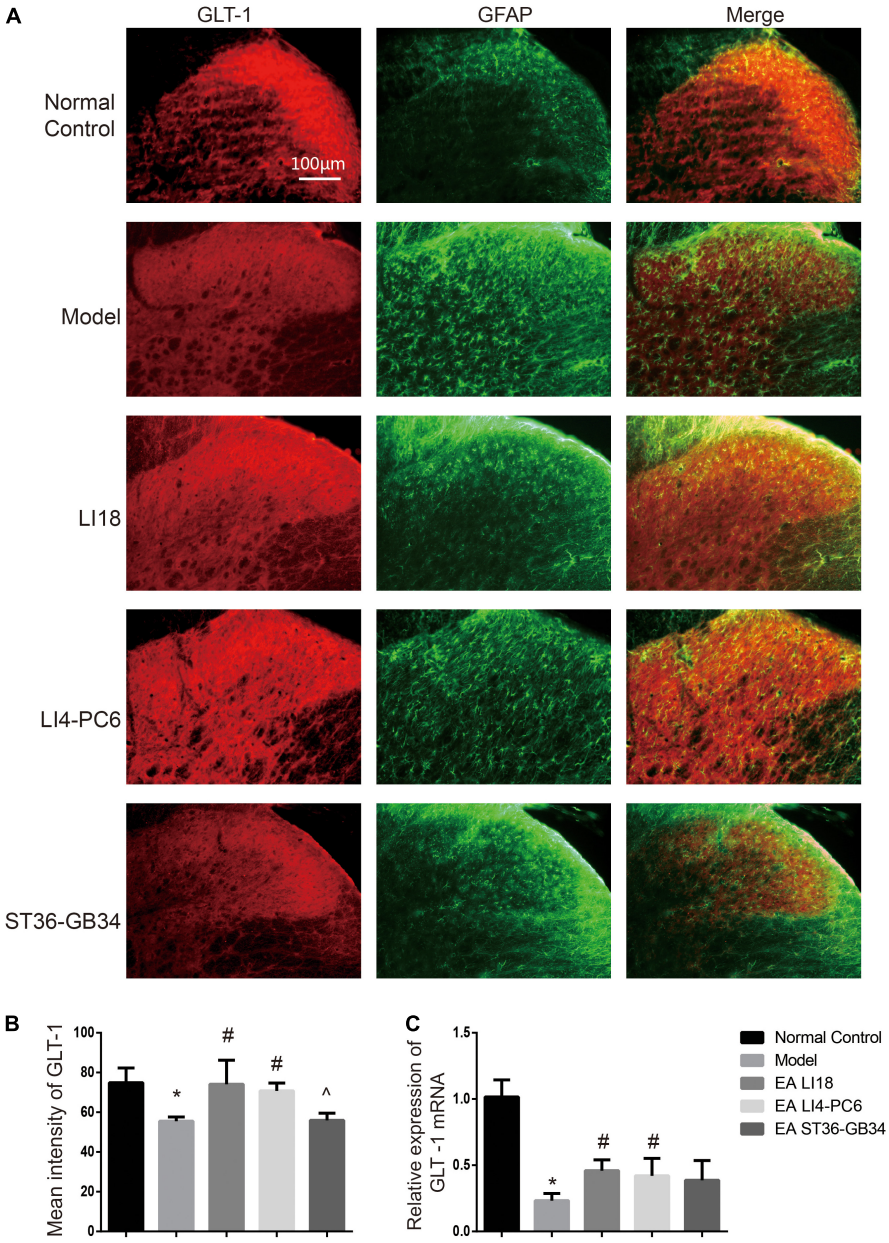

FIGURE 8
Comparison of expression of glu transporter-1 (GLT-1) in different groups (mean ± SD, n = 6/group). **(A)** Fluorescence micrographs of immunohistochemistry staining showing the co-expression (yellow) of GLT-1 (red) and glial fibrillary acidic protein (GFAP) (green) in different groups in the cervical spinal dorsal horns (DHs) 24 h after neck incision. GLT-1 was densely expressed by astrocytes in the normal control group, relatively denser in the EA LI18 and EA LI4-PC6 groups, and lower in both model and EA ST36-GB34 group. The scale bar is 100 μm. **(B)** Bar graphs showing the mean fluorescence intensity of GLT-1 in the five groups. GLT-1 expression was reduced in the model group relevant to the control group. After electroacupuncture (EA), the expression levels of GLT-1 were significantly upregulated in both EA LI18 and EA PC6-LI4 groups but not in the EA ST36-GB32 group (vs. the model group). **(C)** Bar graphs showing the relative expression of GLT-1 mRNA in the five groups. The expression of GLT-1 mRNA was observably decreased in the model group compared to the control group and significantly increased in both EA LI18 and EA PC6-LI4 groups (rather than in the EA ST36-GB34 group) in contrast to the model group. *$P < 0.01$, vs. the control group, #$P < 0.05$, vs. the model group, $^{\wedge}P < 0.05$, vs. the EA LI18 group.

course of synaptic events and the extent and pattern of activation and desensitization of receptors extending outside the synaptic cleft and at the neighboring synapses (Danbolt, 2001). The outcomes of the present study showed that after neck incision, the protein and mRNA levels of GLAST and GLT-1 were downregulated accordingly in the model group and significantly upregulated in both EA LI18 and LI4-PC6 groups (rather than in the EA ST36-GB34 group), along with the occurrence and relief

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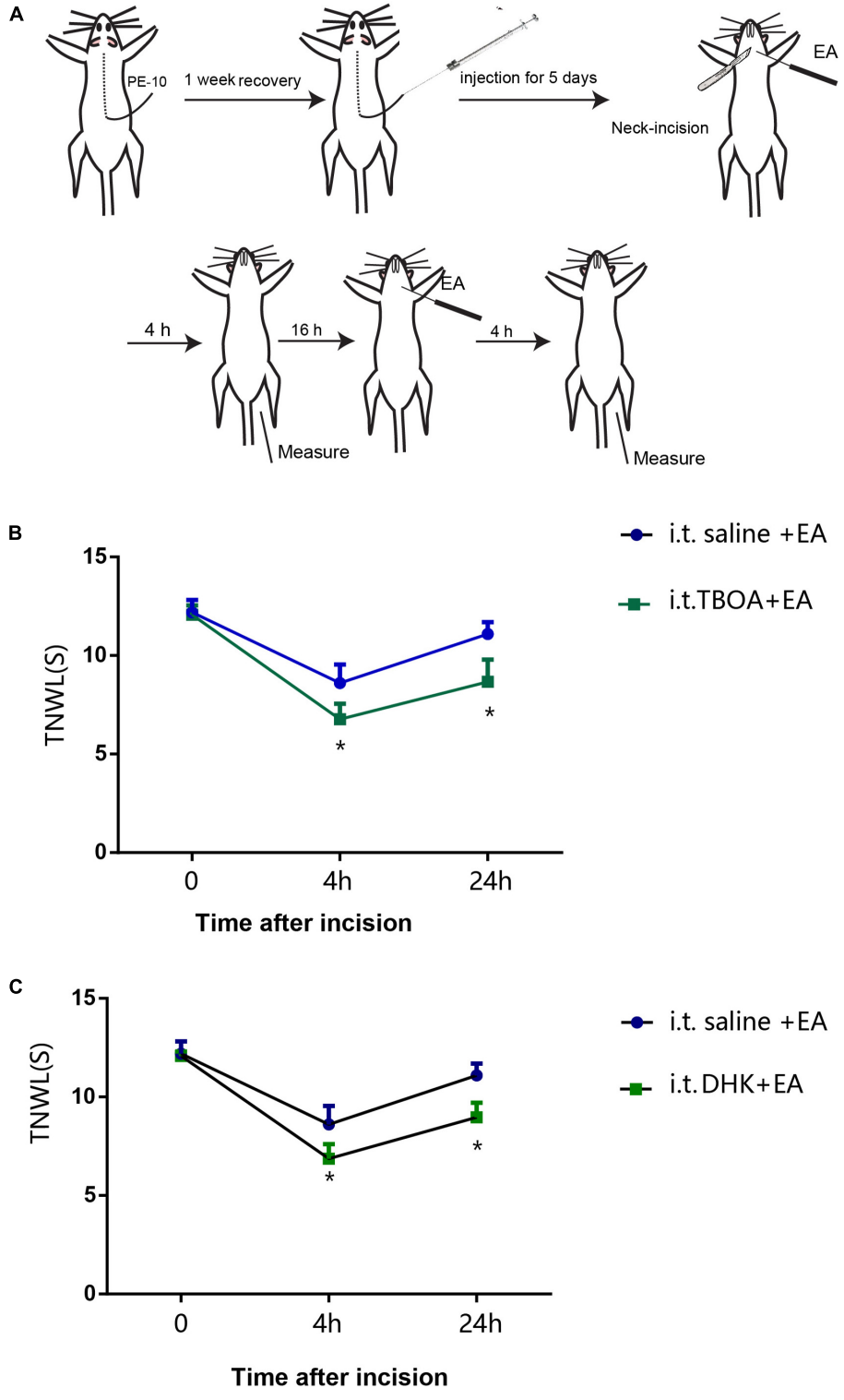

FIGURE 9
Intrathecal injection of glu aspartate transporter (GLAST) antagonist (DL-threo-beta-Benzyloxyaspartate, DL-TBOA) and glu transporter-1 (GLT-1) antagonist (dihydrokainate, DHK) diminishes the analgesic effect of EA LI18. **(A)** Schematic diagram showing the experimental procedures of i.t., electroacupuncture (EA) intervention of LI18, and behavioral measurements. **(B,C)** Significant reductions in the thermal pain threshold (TPT, thermal neck withdrawal latency, TNWL) at different time point after i.t. of GLAST antagonist TBOA **(B)**, and GLT-1 antagonist DHK **(C)**, separately (mean ± SD, $N = 10$ per group). i.t.: intrathecal injection; $^{*}P < 0.05$, vs. the i.t.-saline + EA group.

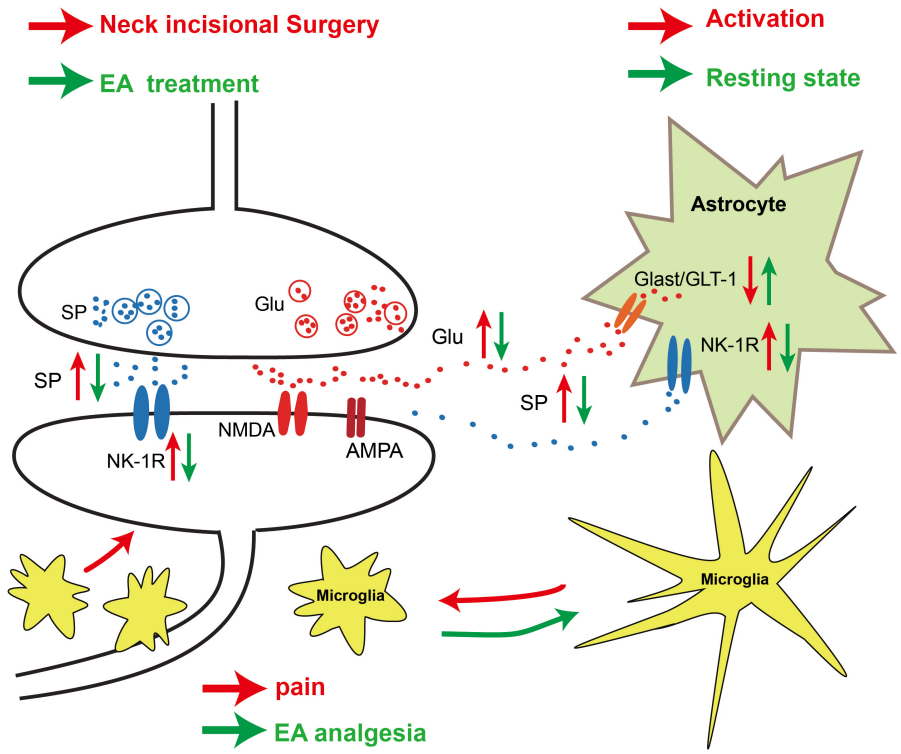

FIGURE 10
Schematic diagram showing the neuronal–glial mechanism of electroacupuncture (EA) analgesia in DHs of the cervical spinal cord in rats with incisional neck pain. Following neck incision, more substance P (SP) and glutamate (Glu) were released from the presynaptic terminals of the activated primary afferent nerve fibers in the superficial layers, and more neurokinin-1 receptor (NK-1R) was expressed on the post-synaptic membrane of the neurons and astrocytes. The expression levels of glu aspartate transporter (GLAST)/glu transporter-1 (GLT-1) on astrocytes were decreased, and more extracellular Glu acted on *N*-methyl-*D*-aspartate (NMDA)/amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) of the post-synaptic neurons after neck incision. Those led to an activation of NK-1R-positive neurons and glial fibrillary acidic protein (GFAP)-labeled astrocytes and ionized calcium-binding adapter molecule 1 (Iba-1)-labeled microgliacytes. The activated microgliacytes gathered around the NK-1R-positive neurons. These activated neurons and glial cells may contribute to the formation of hyperalgesia. EA stimulation upregulated the expression of GLAST/GLT-1 on astrocytes and downregulated the expression of NK-1R on neurons and astrocytes, to reduce the extracellular Glu level and the transmission of injury information to the post-synaptic neurons. The microgliacytes and astrocytes returned to the resting state, and the aggregation of microgliacytes around the NK-1R-positive neurons was also alleviated after EA treatment. These changes between glial cells and neurons may be one of the mechanisms of EA analgesia.

of the incisional neck pain. GLAST and GLT-1 antagonists also weakened the analgesic effect of EA LI18. Immunofluorescence dual labeling revealed that both GLAST and GLT-1 were expressed in astrocytes of the spinal cord DHs. As in rats with incisional pain, EA treatment also significantly upregulated GLAST and GLT-1 in rats with neuropathic pain, and i.t. of glutamate transport (GT) inhibitors attenuated the EA-induced analgesic effect (Zeng et al., 2016). Cui et al. (2016) revealed that the expression levels of spinal GTs increased on days 2 and 4 and gradually decreased as the time of EA intervention increased. Our previous study showed that EA significantly downregulated the expression levels of mGluR5 mRNA and the NMDAR 2B subunit mRNA and protein in rats with neck pain after neck incision (Lin et al., 2012), or subcutaneous injection of formalin at the neck (Gao et al., 2009). Therefore, EA alleviates incisional acute pain possibly by decreasing the expression of mGluR5 and NMDAR 2B on neurons and increasing the expression of GLAST and GLT-1 to reduce the neurotoxicity of Glu.

This study had several limitations. First, SP/NK-1R and GLAST/GLT-1 are only part of the interaction mechanisms of EA on neurons and glial cells. Fewer results were obtained on interactions between the microgliacytes and astrocytes and between the neurons and microgliacytes. Second, the techniques used in the present study were relatively limited, reducing the depth of the research. Thus, further studies should be conducted to elucidate the underlying mechanisms of EA analgesia in the same incision pain model.

In conclusion, our results demonstrate that EA of LI18 and LI4-PC6 can relieve thermal hyperalgesia probably by modulating neuronal–glial interaction in two ways, that is, by reducing SP/NK-1R signaling (nociceptive signal transmission) and glial cell activities, and by upregulating GLAST and GLT-1 to increase the intake of Glu in astrocytes in DHs of the cervical

spinal cord (Figure 10). These results may provide experimental evidence for the clinical complementary application of EA of LI18 and LI4-PC6 in surgery of the thyroid gland region.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was reviewed and approved by the Institutional Animal Welfare and Use Committee of the Institute of Acupuncture and Moxibustion of China Academy of Chinese Medical Sciences.

Author contributions

J-LL, P-JR, and J-YW designed the experiments. J-LL and J-YW prepared the manuscript and figures. J-YW, J-LingZ, S-PC, YC, and YZ performed the experiments. Y-HG and J-LiangZ analyzed the data. All authors reviewed and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Block, L. (2016). Glial dysfunction and persistent neuropathic postsurgical pain. *Scand. J. Pain* 10, 74–81. doi: 10.1016/j.sjpain.2015.10.002

Burmeister, A. R., Johnson, M. B., Vinita, S., Chauhan, V. S., Moerdyk-Schauwecker, M. J., Young, A. D., et al. (2017). Human microglia and astrocytes constitutively express the neurokinin-1 receptor and functionally respond to substance P. *J. Neuroinflammation* 14:245. doi: 10.1186/s12974-017-1012-5

Chen, G., Zhang, Y. Q., Qadri, Y. J., Serhan, C., and Ji, R. R. (2018). Microglia in pain: Detrimental and protective roles in pathogenesis and resolution of pain. *Neuron* 100, 1292–1311. doi: 10.1016/j.neuron.2018.11.009

Chen, L., Jiang, M., and Pei, L. (2012). Comparison of three methods of drug delivery in the rat lumbar spinal subarachnoid space. *Anat. Rec.* 295, 1212–1220. doi: 10.1002/ar.22506

Clarke, J. N., Anderson, R. L., Haberberger, R. V., and Gibbins, I. L. (2011). Non-peptidergic small diameter primary afferents expressing VGluT2 project to lamina I of mouse spinal dorsal horn. *Mol. Pain* 7:95. doi: 10.1186/1744-8069-7-95

Cui, L. Y., Ding, Y., Zeng, J., Feng, Y., Li, M., and Ding, M. M. (2016). Spinal glutamate transporters are involved in the development of electroacupuncture tolerance. *Int. J. Mol. Sci.* 17:357. doi: 10.3390/ijms17030357

Danbolt, N. C. (2001). Glutamate uptake. *Prog. Neurobiol.* 65, 1–105. doi: 10.1016/S0301-0082(00)00067-8

Fu, D., Guo, Q. L., Ai, Y. H., Cai, H. W., Yan, J. Q., and Dai, R. P. (2006). Glial activation and segmental upregulation of interleukin-1beta (IL-1beta) in the rat spinal cord after surgical incision. *Neurochem. Res.* 31, 333–340. doi: 10.1007/s11064-005-9032-4

Gadea, A., and Lopez-Colome, A. M. (2001). Glial transporters for glutamate, glycine and GABA I. Glutamate transporters. *J. Neurosci. Res.* 63, 453–460. doi: 10.1002/jnr.1039

Gao, Y. H., Chen, S. P., Wang, J. Y., Qiao, L. N., Xu, Q. L., and Liu, J. L. (2009). Effects of electroacupuncture at different acupoints on the pain behavior and NMDA receptor 2 B subunit mRNA and protein expression and phosphorylation level in the cervical spinal cord in rats with thyroid regional pain. *Zhen Ci Yan Jiu* 34, 376–382.

Gwak, Y. S., Hulsebosch, C. E., and Leem, J. W. (2017). Neuronal-glial interactions maintain chronic neuropathic pain after spinal cord injury. *Neural Plast.* 2017:2480689. doi: 10.1155/2017/2480689

Härtel, K., Schnell, C., and Swen, H. (2009). Astrocytic calcium signals induced by neuromodulators via functional metabotropic receptors in the ventral respiratory group of neonatal mice. *Glia* 57, 815–827. doi: 10.1002/glia.20808

Jia, W. H., Chi, W. Y., Lou, C., and Wang, G. Z. (2016). Effect of Electroacupuncture intervention on β-endorphin levels of hypothalamus and

spinal cord tissues in rats with pelmatic incisional pain. *Zhen Ci Yan Jiu* 41, 225–229.

Jiang, W. F., Wang, X. Y., Wang, W., Hua, F., Zhang, Z. S., Zhang, Z. H., et al. (2020). Inhibition of NK1R attenuates LPS-induced microglial inflammation and consequent death of PC12 cells. *Brain Res. Bull.* 162, 115–124. doi: 10.1016/j.brainresbull.2020.05.015

Jin, Y. H., Yamaki, F., Takemura, M., Koike, Y., Furuyama, A., and Yonehara, N. (2009). Capsaicin-induced glutamate release is implicated in nociceptive processing through activation of ionotropic glutamate receptors and group I metabotropic glutamate receptor in primary afferent fibers. *J. Pharmacol. Sci.* 109, 233–241. doi: 10.1254/jphs.08262FP

Johnson, M. B., Young, A. D., and Marriott, I. (2016). The Therapeutic Potential of Targeting Substance P/NK-1R Interactions in Inflammatory CNS Disorders. *Front. Cell. Neurosci.* 10:296. doi: 10.3389/fncel.2016.00296

Kim, B. G., Moon, J. Y., Choi, J. Y., Park, I. S., Oh, A. Y., Jeon, Y. T., et al. (2018). The Effect of intraoperative Nefopam administration on acute postoperative pain and chronic discomfort after robotic or endoscopic assisted thyroidectomy: A randomized clinical trial. *World J. Surg. Actions* 42, 2094–2101. doi: 10.1007/s00268-017-4421-9

Kingery, W. S., Agashe, G. S., Guo, T. Z., Davies, M. F., Clark, J. D., and Maze, M. (2002). Capsaicin sensitive afferents mediate the development of heat hyperalgesia and hindpaw edema after sciatic section in rats. *Neurosci. Lett.* 318, 39–43. doi: 10.1016/S0304-3940(01)02464-8

Kung, L. H., Gong, K., Adedoyin, M., Ng, J., Bhargava, A., Ohara, P. T., et al. (2013). Evidence for glutamate as a neuroglial transmitter within sensory ganglia. *PLoS One* 8:e68312. doi: 10.1371/journal.pone.0068312

Li, W. W., Guo, T. Z., Shi, X., Sun, Y., Wei, T., and Clark, D. J. (2015). Substance P spinal signaling induces glial activation and nociceptive sensitization after fracture. *Neuroscience* 310, 73–90. doi: 10.1016/j.neuroscience.2015.09.036

Li, Z. R. (2007). *Experimental Acupuncture*. Beijing: Chinese Press of Traditional Chinese Medicine, 273–274.

Lin, D., Kan, Y., Qiao, L. N., Wang, J. Y., Chen, S. P., Han, Y. J., et al. (2012). Effects of electroacupuncture at "Futu"(LI18), etc. on pain threshold and cervico-spinal mGlu receptor 5/cAmp/CREB signaling in rats with neck incision pain. *Zhen Ci Yan Jiu* 37, 191–196.

Lin, Y. (2014). *Clinical study of chronic postsurgical pain after thyroid surgery*. Guangzhou: Guangzhou Medical University.

Liu, J. L., Han, Z. J., Chen, S. P., and Cao, Q. S. (1993). Influence electroacupuncture on electrical activity of neurons in the dorsal horns of thoracic spinal cord. *Zhen Ci Yan Jiu* 18, 267–270.

Lou, I., Chennell, T. B., Schaefer, S. C., Chen, H., Sippel, R. S., Balentine, C., et al. (2017). Optimizing outpatient pain management after thyroid and parathyroid surgery: A two-institution experience. *Ann. Surg. Oncol.* 24, 1951–1957. doi: 10.1245/s10434-017-5781-y

Ma, Y., Zhou, C., Li, G., Tian, Y., Liu, J., Yan, L., et al. (2015). Effects on Spatial Cognition and Nociceptive Behavior Following Peripheral Nerve Injury in Rats with Lesion of the Striatal Marginal Division Induced by Kainic Acid. *Neurochem. Res.* 40, 2357–2364. doi: 10.1007/s11064-015-1727-6

Neugebauer, V. (2007). Glutamate receptor ligands. *Handb. Exp. Pharmacol.* 177, 217–249. doi: 10.1007/978-3-540-33823-9_8

Niederberger, E., Schmidtko, A., Coste, O., Marian, C., Ehnert, C., and Geisslinger, G. (2006). The glutamate transporter GLAST is involved in spinal nociceptive processing. *Biochem. Biophys. Res. Commun.* 346, 393–399. doi: 10.1016/j.bbrc.2006.05.163

Peyman, S., Shi, X. Y., Guo, T. Z., Qiao, Y., Yeomans, D., Kingery, W., et al. (2009). Role of substance P signaling in enhanced nociceptive sensitization and local cytokine production after incision. *Pain* 145, 341–349. doi: 10.1016/j.pain.2009.06.037

Qiao, L. N., Wang, J. Y., Chen, S. P., Gao, Y. H., Yang, Y. S., and Liu, J. L. (2010). Effect of electroacupuncture at "Futu"(LI18) on the immunoactivity of Substance P, 5-HT 1AR, etc. of the cervical spinal dorsal horn in rats with neck incision pain. *Zhen Ci Yan Jiu* 35, 91–98.

Scholz, J., and Woolf, C. J. (2007). The neuropathic pain triad: Neurons, immune cells and glia. *Nat. Neurosci.* 10, 1361–1368. doi: 10.1038/nn1992

Wang, J. Y., Gao, Y. H., Qiao, L. N., Zhang, J. L., Duan-Mu, C. L., Yan, Y. X., et al. (2018). Repeated electroacupuncture treatment attenuated hyperalgesia through suppression of spinal glial activation in chronic neuropathic pain rats. *BMC Complement. Altern. Med.* 18:74. doi: 10.1186/s12906-018-2134-8

Wang, Z. L., Hao, L. Y., Zhao, M. L., Xia, X. C., Bai, X. G., Zhang, J. F., et al. (2019). Correlation of behavioral performance with expressions of substance P and neurokinin-1 receptor in the L5-S2 spinal cord of chronic prostatitis rats. *Zhonghua Nan Ke Xue* 25, 489–495.

Wattier, J. M., Caiazzo, R., Andrieu, G., Kipins, E., Pattou, F., and Lebuffe, G. (2016). Chronic post-thyroidectomy pain: Incidence, typology, and risk factors. *Anaesth. Crit. Care Pain Med. Actions* 35, 197–201. doi: 10.1016/j.accpm.2015.10.006

Xin, W. J., Weng, H. R., and Dougherty, P. M. (2009). Plasticity in expression of the glutamate transporters GLT-1 and GLAST in spinal dorsal horn glial cells following partial sciatic nerve ligation. *Mol. Pain* 5:15. doi: 10.1186/1744-8069-5-15

Xu, B., Guan, X. H., Yu, J. X., Lv, J., Zhang, H. X., Fu, Q. C., et al. (2014). Activation of spinal phosphatidylinositol 3-kinase/protein kinase B mediates pain behavior induced by plantar incision in mice. *Exp. Neurol.* 255, 71–82. doi: 10.1016/j.expneurol.2014.02.019

Xu, J., and Brennan, T. J. (2009). Comparison of skin incision vs. skin plus deep tissue incision on ongoing pain and spontaneous activity in dorsal horn neurons. *Pain* 144, 329–339. doi: 10.1016/j.pain.2009.05.019

Xu, W. D., Zhu, B., Rong, P. J., Ben, H., Gao, X. Y., and Li, Y. Q. (2003). The pain-relieving effects induced by electroacupuncture with different intensities at homotopic and heterotopic acupoints in humans. *Am. J. Chin. Med.* 31, 791–802. doi: 10.1142/S0192415X03001478

Zeng, J., Cui, L. Y., Feng, Y., and Ding, M. M. (2016). Electroacupuncture relieves neuropathic pain via upregulation of glutamate transporters in the spinal cord of rats. *Neurosci. Lett.* 620, 38–42. doi: 10.1016/j.neulet.2016.03.041

Zhang, Z. J., Jiang, B. C., and Gao, Y. J. (2017). Chemokines in neuron-glial cell interaction and pathogenesis of neuropathic pain. *Cell. Mol. Life Sci.* 74, 3275–3291. doi: 10.1007/s00018-017-2513-1

Zhu, B. (2015). Section 7. Relationship between acupuncture analgesia and acupoint and nerve segmental innervation in chapter 13 of *Systemic Acupuncture Medicine-Renewing Body Surface Medicine* People's Medical Publishing House, 294–297.

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Transcutaneous electrical acupoint stimulation combined with electroacupuncture promotes rapid recovery after abdominal surgery: Study protocol for a randomized controlled trial

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Introduction: The most frequent complications after abdominal surgery include a decrease or loss of appetite, abdominal distension, abdominal pain caused by reduced gastrointestinal motility, anal arrest with intestinal distension and defecation, and nausea and vomiting due to anesthetic and opioid analgesic administration. These complications severely affect postoperative recovery, prolong hospital stay, and increase the financial burden. The objective of this study is to investigate the efficacy and safety of three acupoint stimulation modalities (electroacupuncture [EA], transcutaneous electrical acupoint stimulation [TEAS], and transcutaneous acupoint electrical stimulation combined with EA [TEAS+EA]), and two EA instrument waveforms (continuous wave and dilatational wave) for rapid recovery after abdominal surgery.

Methods and analysis: A total of 560 patients will be recruited and randomly allocated to receive one of the following seven interventions: continuous wave EA, continuous wave TEAS, continuous wave TEAS + EA, dilatational wave EA, dilatational wave TEAS, dilatational wave TEAS + EA, and a control. For this study, continuous waves at 2 Hz, and dilatational waves at 2/50 Hz would be selected. The points to be stimulated by EA are the bilateral Neiguan (PC6), Hegu (LI6), Zusanli (ST36), Shangjuxu (ST37), and Xiajuxu (ST39), and TEAS would stimulate the bilateral Liangmen (ST21) and Daheng (SP15). The control group will neither receive EA nor TEAS. All patients will undergo an enhanced recovery plan after surgery and be provided with standardized perioperative management. Treatment will start on the first postoperative day and be administered once daily in the morning until the patient regains spontaneous bowel movements and can tolerate oral intake of solid food. The primary outcome is a composite of time to first defecation and time to tolerance of a solid diet. Secondary outcomes include time to first exhaustion; time of first defecation; time of tolerance of a solid diet; time to the first

ambulation; length of hospital stay from surgery to discharge; visual analog scale score for postoperative daily pain, nausea, and vomiting; incidence of postoperative complications; and treatment acceptability.

Discussion: This study will compare the efficacy and safety of three acupoint stimulation methods and two EA instrument waveforms for rapid recovery after abdominal surgery.

Trial Registration: Chinese Clinical Trial Registry (http://www.chictr.org.cn), ChiCTR2100043883.

KEYWORDS

transcutaneous electrical acupoint stimulation, electroacupuncture, rapid recovery, abdominal surgery, randomized controlled trial

Introduction

Postoperative complications of abdominal surgery include gastrointestinal dysfunction, pain, nausea, and vomiting (1, 2). These complications adversely affect the patient's quality of life, prolong the length of hospital stay, increase hospitalization costs, and the risk of needing a second operation (3). Therefore, reducing postoperative complications and promoting rapid recovery after surgery have been widely studied (4).

An Increasing amount of clinical evidence supports the use of acupuncture for enhanced recovery after surgery (ERAS) (5–7). Nevertheless, there is no unified standard for the use of specific acupuncture methods, acupoint selection, and electroacupuncture (EA) instrument parameters (8, 9). The majority of previous studies evaluating the use of acupuncture during postoperative rehabilitation after abdominal surgery have utilized distal limb acupoints (10, 11). This may be related to the surgical wound after abdominal surgery and changes in the structure and state of the abdominal organs which affect the acupuncture procedure and its safety. However, emerging evidence in recent years has indicated that acupuncture applied to select abdominal or limb acupoints can lessen the degree of abdominal pain and distension (12, 13). The use of abdominal meridian points may be particularly effective in decreasing abdominal pain (14, 15). This study is based on many early clinical practices and on the application of EA at meridian points in the distal extremities where efficacy was compared with the addition of a safer transcutaneous electrical acupoint stimulation (TEAS) of the abdominal acupoints.

Stimulation parameters are critical factors for the effectiveness of EA, as different stimulation parameters will bring about different therapeutic effects (16, 17). The use of a continuous wave in EA results in muscle contraction, exciting sensation, and motor nerve. The continuous wave is the most commonly used waveform to strengthen the effect of acupuncture in the clinic. In contrast, dilatational waves can increase metabolism, promote blood circulation, and improve muscle weakness and other functions. Dilatational waves are frequently used to facilitate postoperative rehabilitation (18). To date, no studies have compared the effectiveness of these two waveforms in promoting rapid rehabilitation after abdominal surgery (19).

Therefore, the primary purpose of this randomized controlled study is to compare the efficacy and safety of three acupoint stimulation methods and two EA instrument waveforms in facilitating rapid recovery after abdominal surgery, with the overarching aim of informing the development of an objective and unified standard for acupuncture use to facilitate rapid postoperative recovery.

Methods and analysis

Study design

This is a prospective, single-center, parallel, single-blind, randomized controlled trial. Effectiveness and safety will be compared among three acupoint stimulation methods (EA, TEAS, TEAS+EA) and two EA instrument waveforms (continuous waves, dilatational waves) to determine their suitability for promoting them in postoperative rehabilitation. This study will be conducted in accordance with the principles of the Declaration of Helsinki. The "Standard Protocol Items: Recommendations for Interventional Trials" checklist is provided in Supplementary Material 1.

Abbreviations: cEA, continuous wave electroacupuncture; cTEAS, continuous wave transcutaneous electrical acupoint stimulation; dEA, dilatational wave electroacupuncture; dTEAS, dilatational wave transcutaneous electrical acupoint stimulation; EA, electroacupuncture; ERAS, enhanced recovery after surgery; TEAS, transcutaneous electrical acupoint stimulation; VAS, visual analog scale.

Recruitment

This trial will be conducted from 1 April 2021 to 31 March 2023, at West China Hospital of Sichuan University. A total of 560 patients who have undergone abdominal surgery (i.e., hepatobiliary, gastrointestinal, renal, or bladder tumor resection) and meet the study inclusion and exclusion criteria will be enrolled in this study. All patients will be requested to provide written informed consent prior to study participation. Figure 1 presents the study flow chart.

Inclusion criteria

Patients will be included if they (1) are aged 18–70 years old, male or female; (2) have undergone resection of hepatobiliary, gastrointestinal, renal, or bladder tumors under general anesthesia; and (3) participate voluntarily and provide written informed consent.

Exclusion criteria

The exclusion criteria would comprise the following: (1) the surgical incision was made through the abdominal acupoints selected for this study; (2) local skin infection is evident at the selected acupoints; (3) patients are unable to understand or cooperate with the assessments (e.g., visual analog scale [VAS]); (4) metal allergy or severe fear of acupuncture, TEAS, or EA; (5) uncontrolled diabetes, severe coagulopathy, or cardiac, central nervous system, or psychiatric disorders; (6) pacemaker; and (7) concurrent enrolment in other research trials.

Withdrawal criteria

Patients will be withdrawn from the study if they (1) experience serious adverse events, (2) have a serious complication or illness during the study that requires urgent action; or (3) withdraw informed consent.

Randomization and blinding

This study has a single-blind design. Patients will be unaware of their group allocation, which will only be known to the lead investigator and acupuncture physician. The randomization sequence and allocation ratio (1:1:1:1:1:1) will be generated using the statistical software SPSS 26.0. Group allocation will be concealed using opaque envelopes, which will be distributed sequentially after patient enrolment.

Intervention

Each group will receive an Enhanced Recovery After Surgery (ERAS)-standardized perioperative management (20). The acupoints used in the treatment group are referenced from the China National Standard Nomenclature and Location of Meridian Points (GB 12346-2021) (21). The selected acupoints and their locations are shown in Figures 2–4. The Hwato SDZ-V (Suzhou Medical Supplies Factory) EA apparatus will be used, and the current intensity will be adjusted to the tolerance level of the patient. Each treatment will last for 30 min and the initial session will commence on the first postoperative day. Treatment sessions will be provided once daily in the morning until the patient regains spontaneous flatus and can tolerate oral intake of solid food. All acupuncture maneuvers will be performed independently by the same acupuncturist, who has at least 5 years of work experience. The acupuncturist will not be replaced at any point during the study.

Continuous wave EA (cEA) group

EA will be used to stimulate the bilateral Neiguan (PC6), Hegu (LI6), Zusanli (ST36), Shangjuxu (ST37), and Xiajuxu (ST39). The current frequency will be a continuous wave at 2 Hz.

Continuous wave TEAS (cTEAS) group

TEAS will be used to stimulate the bilateral Liangmen (ST21) and Daheng (SP15). The current frequency will be a continuous wave at 2 Hz.

cTEAS + EA group

EA will be used to stimulate the bilateral Neiguan (PC6), Hegu (LI6), Zusanli (ST36), Shangjuxu (ST37), and Xiajuxu (ST39). TEAS will be used to stimulate the bilateral Liangmen (ST21) and Daheng (SP15). The current frequency will be a continuous wave at 2 Hz.

Dilatational wave EA (dEA) group

EA will be used to stimulate the bilateral Neiguan (PC6), Hegu (LI6), Zusanli (ST36), Shangjuxu (ST37), and Xiajuxu (ST39). The current frequency will be a dilatational wave at 2/50 Hz.

Dilatational wave TEAS (dTEAS) group

TEAS will be used to stimulate the bilateral Liangmen (ST21) and Daheng (SP15). The current frequency will be a dilatational wave at 2/50 Hz.

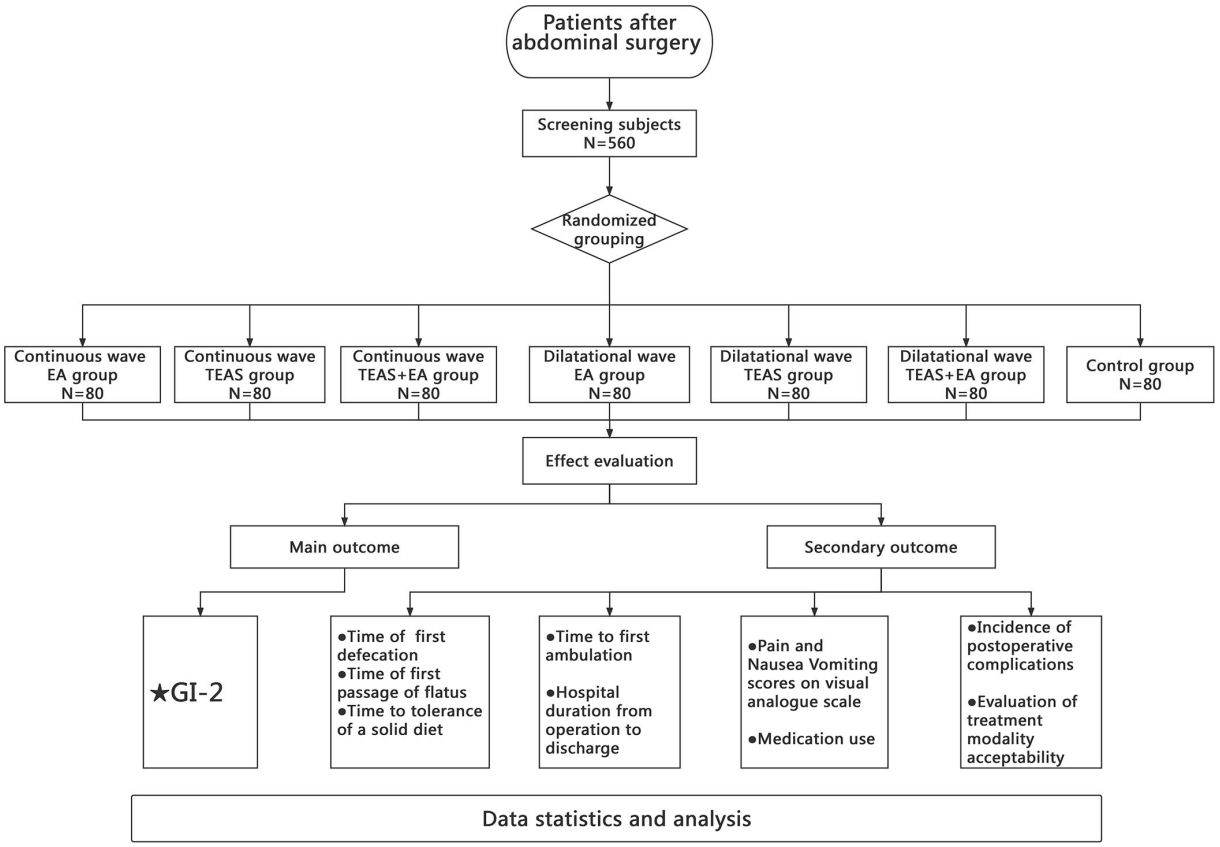

FIGURE 1
Flowchart of the study protocol.

dTEAS + EA group

EA will be used to stimulate the bilateral Neiguan (PC6), Hegu (LI6), Zusanli (ST36), Shangjuxu (ST37), and Xiajuxu (ST39). TEAS will be used to stimulate the bilateral Liangmen (ST21) and Daheng (SP15). The current frequency will be a dilatational wave at 2/50 Hz.

Control group

Only ERAS-standardized perioperative management, without TEAS or EA, will be performed in the control group.

All acupoints will be routinely disinfected. The acupoints in the distal limb will be punctured straight through the skin to a depth of 25–30 mm using disposable stainless steel needles (0.25 × 40 mm, Suzhou Jiajian, Jiangsu, China). The needle will then be twisted slightly to achieve the de qi sensation, and the EA apparatus will be connected with a set of electrodes in the ipsilateral Neiguan (PC6), Hegu (LI4), Zusanli (ST36), and Xiajuxu (ST39). The abdominal acupoints will be stimulated with a self-adhesive electrode sheet with electrical conductivity, and the ipsilateral Liangmen (ST21) will be connected with a set of electrodes at Daheng (SP15).

Outcome measures

Primary outcome

The primary outcome is Gastrointestinal-2, which is a composite outcome of time to first defecation and time to tolerance of a solid diet (22).

Secondary outcomes

Secondary outcomes comprise the following: (1) time of first spontaneous exhaustion after operation; (2) time of first spontaneous defecation after operation; (3) time of first tolerance to oral intake of solid food after operation; (4) time to first ambulation after surgery; (5) VAS scores for postoperative daily pain and nausea and vomiting; (6) postoperative daily incidence of nausea and vomiting; (7) length of hospital stay, from surgery to discharge; (8) incidence of postoperative complications; and (9) acceptability of acupuncture therapy on a 5-point Likert scale (very acceptable, moderately acceptable, somewhat acceptable, moderately unacceptable, and totally unacceptable).

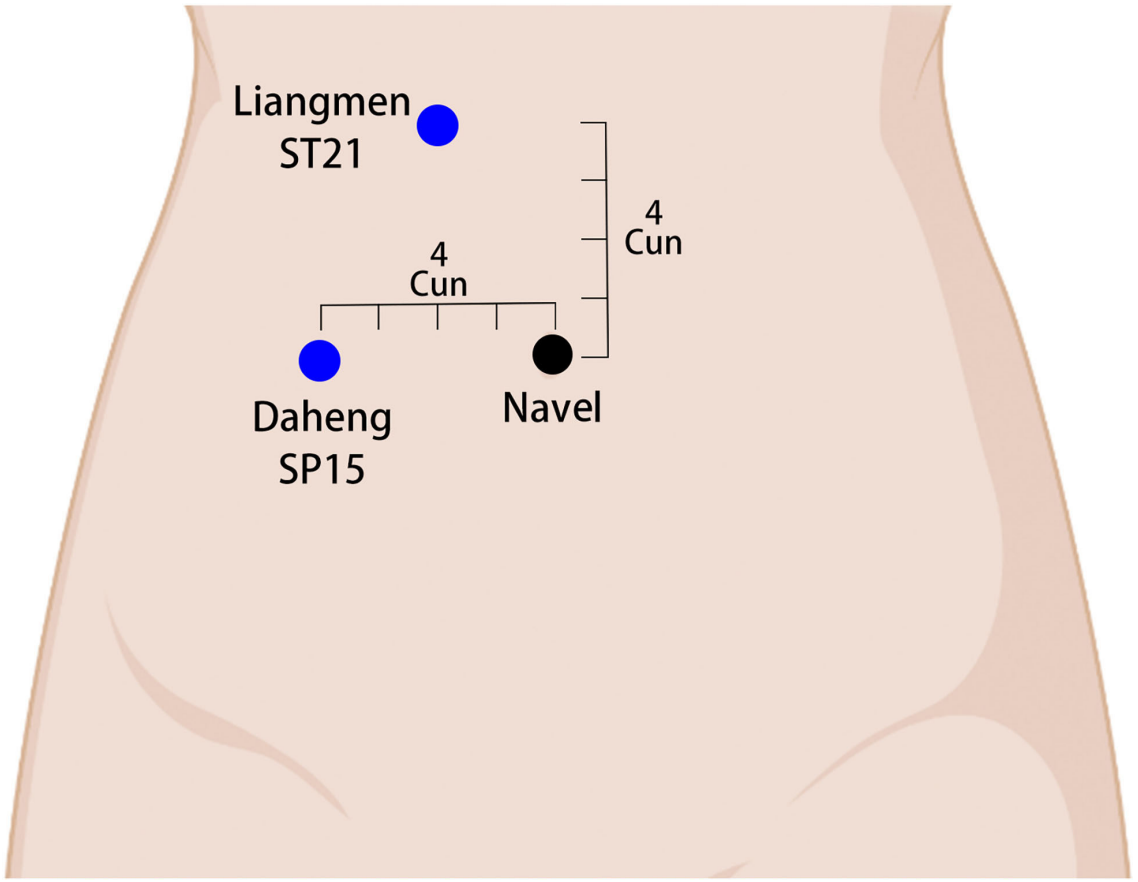

FIGURE 2
Localization of abdomen acupoints and electrode connection. ST21 (Liangmen) in the upper abdomen, 4 cun above the navel and 2 cun from the anterior midline. SP15 (Daheng) in the ventral part, 4 cun from the navel. A set of electrodes is attached at the blue dot (transcutaneous electrical acupoint stimulation).

Safety evaluation

EA-related safety evaluation during treatment includes the documentation of broken needles, fainting due to needles, intolerable pinprick pain, local hematoma, infection, abscess, and other incidences of discomfort after pinprick. Adverse events will be recorded by the acupuncture physician in a standardized form.

Sample size

The determination of the sample size was based on the following pre-experimental results for the primary outcome measure, Gastrointestinal-2: cEA, 84.8 ± 36.1 h; cTEAS, 86.8 ± 40.1 h; cTEAS+EA, 70.3 ± 39.3 h; dEA, 78.3 ± 42.2 h; dTEAS, 81.2 ± 44.2 h; dTEAS+EA, 64.1 ± 36.3 h; and control, 110.8 ± 42.3 h. PASS 15 software was used to determine the sample size of 560 patients (80 patients in each group; $\alpha = 0.05$ [two-sided], $\beta = 0.1$ [90% power], with an assumed 20% dropout rate).

Statistical analysis

The data will be analyzed using SPSS 26.0. All statistical tests will be two-sided, and a *p*-value of less than 0.05 will be considered statistically significant. Measurement data conforming to a normal distribution will be expressed as mean ± standard deviation (x ± s). Comparisons among groups will be performed *via* one-way analysis of variance, using the Student–Newman–Keuls q-test for pairwise comparisons. Variables that do not follow a normal distribution will be analyzed with the Kruskal–Wallis H-test. Categorical and count data will be described as frequency or percentage and compared among groups using the chi-square or Fisher's test.

Discussion

Although perioperative ERAS measures can facilitate accelerated recovery in patients, there is still much room for improvement in preventing and treating postoperative gastrointestinal dysfunction and analgesia, as well as reducing

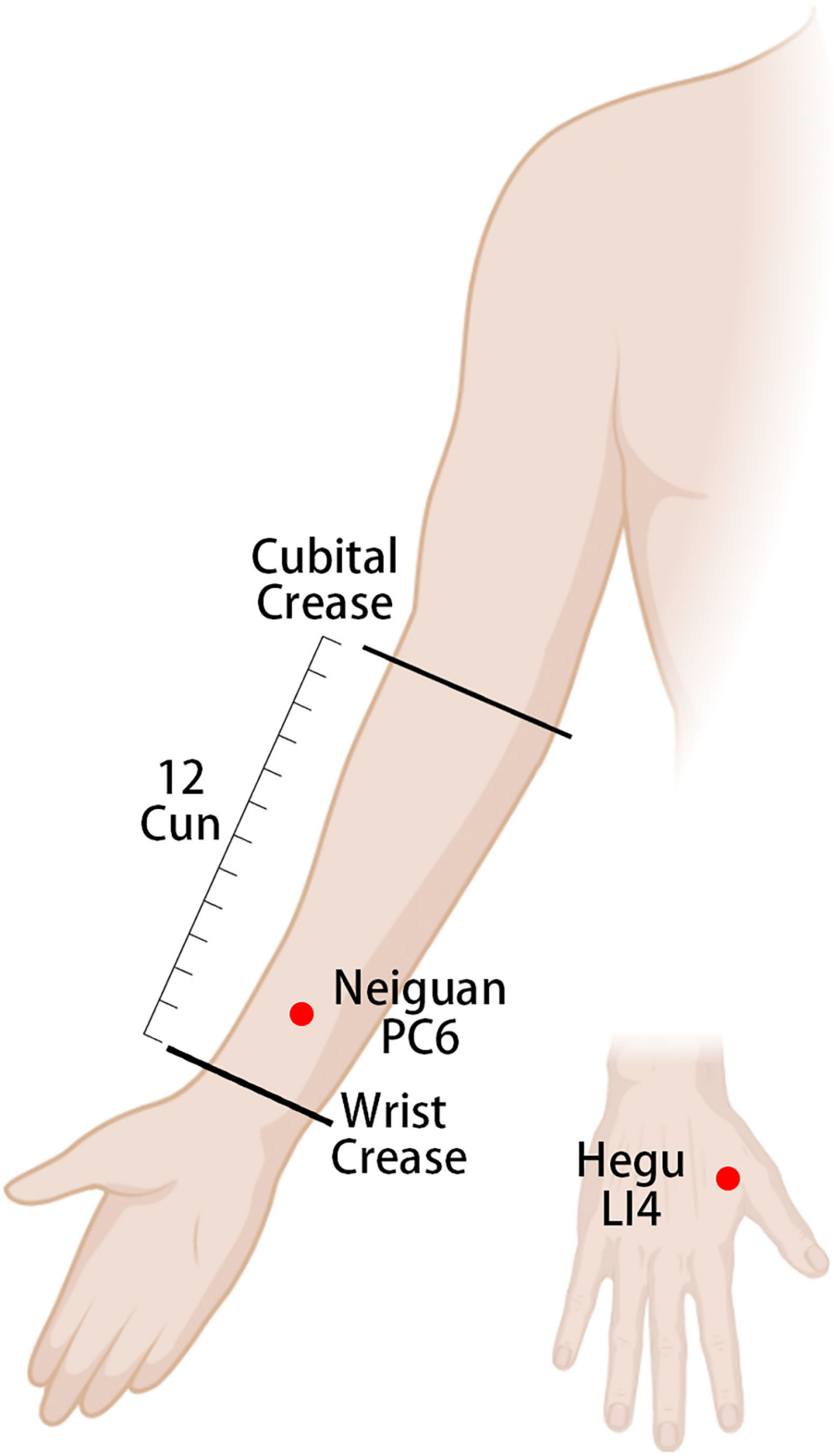

FIGURE 3
Location of upper extremity acupoints and electrode connection. PC6 (Neiguan) on the volar aspect of the forearm, 2 cun on the wrist crease, and between the palmaris longus tendon and the flexor carpi radialis tendon. LI4 (Hegu) between the first and second metacarpals, at the midpoint of the radial aspect of the second metacarpal. A set of electrodes is attached at the red dot (electroacupuncture).

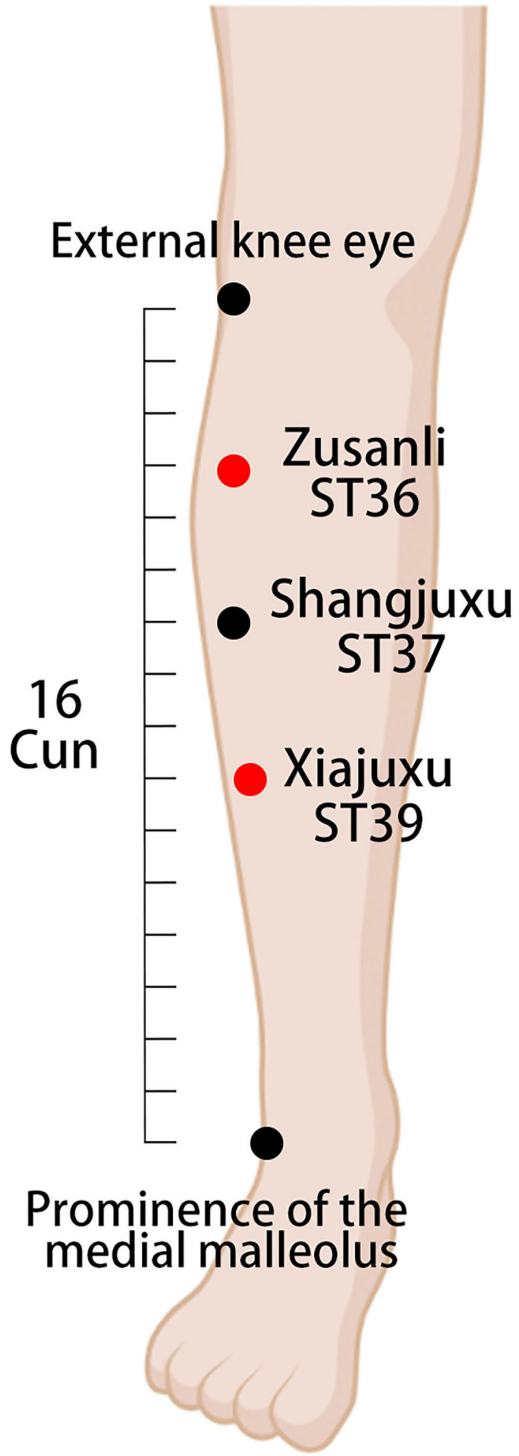

FIGURE 4
Location of lower extremity acupoints and electrode connection. ST36 (Zusanli) on the lateral lower leg, 3 cun under the external knee eye. ST37 (Shangjuxu) on the lateral lower leg, 6 cun under the external knee eye and 3 cun under the ST36 (Zusanli) acupoint. ST39 (Xiajuxu) on the lateral lower leg, 9 cun under the external knee eye. A set of electrodes is attached at the red dot (electroacupuncture).

the length of hospital stay (23–25). Acupuncture can contribute to postoperative multimodal analgesia. Postoperative analgesia is one of the core components of ERAS. While opioids remain the conventional option for postoperative pharmacological analgesia, they are also associated with nausea, vomiting, and other complications. Thus, reducing opioid use can help patients recover sooner. Many studies have been conducted on the mechanism of acupuncture analgesia through electrophysiology, neurochemistry, molecular biology, and brain imaging investigations (26–28). Patient recovery is also adversely affected by postoperative nausea and vomiting (29), which may be effectively prevented by Neiguan (PC6) stimulation (30, 31).

Previous studies have demonstrated the efficacy of acupuncture in rapid postoperative rehabilitation (32). However, there may be concerns that the presence of surgical wounds after abdominal surgery and the possible changes in the structure and state of abdominal organs after abdominal surgery can affect the manipulation and safety of acupuncture. Therefore, previous clinical experience and research for acupuncture treatment after abdominal surgery have primarily selected acupoint stimulation on the distal limbs (24). Studies have shown that Zusanli (ST36), Shangjuxu (ST37), and Xiajuxu (ST39) stimulation can effectively improve gastrointestinal transit by reducing local inflammation of the intestinal musculature (33). Thus, based on the plethora of available clinical evidence for the use of EA at distal extremity acupoints, we have proposed in the present study the adjunctive use of TEAS, which is safer than EA for the stimulation of abdominal acupoints. Moreover, the selected bilateral Liangmen (ST21) and Daheng (SP15) of the abdomen are unconventional locations for incisions during abdominal surgery. Daheng (SP15) is a pair of acupoints belonging to the spleen meridian, and Liangmen (ST21) is a pair of acupoints belonging to the stomach meridian. These acupoints are more convenient to use; they are also antiemetic and promote gastrointestinal peristalsis, relieve abdominal pain, and have other effects (34, 35).

Therefore, this parallel-group randomized controlled trial aims to compare the efficacy and safety of three acupoint stimulation methods and two EA instrument waveforms for facilitating rapid recovery after abdominal surgery. The results of this study will inform the development of an objective and unified standard for EA and TEAS as indispensable components of ERAS.

Limitation

This trial will not include a sham control arm, and a placebo response and effect analysis is lacking. Nevertheless, some studies on the use of acupuncture for gastrointestinal symptoms have shown that EA may have more significant benefits than sham acupuncture, despite a placebo effect (36, 37).

Data availability statement

The datasets are not readily available as they are currently under the protection of WCHSU. Data for use or analysis following study completion will be available from the corresponding author on reasonable request.

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Review Board of West China Hospital of Sichuan University. The participants provided written informed consent to participate in this study.

Author contributions

HaL and CD contributed equally to this article. HaL and QW conceived the idea for this study. CD participated in the design and drafted the manuscript. L-yL and H-mX are responsible for recruiting subjects. X-yH and NL contributed to the final version of the manuscript. HoL and QW are responsible for monitoring this study. All authors contributed to the manuscript revision, read, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.1017375/full#supplementary-material

References

1. Bragg D, El-Sharkawy AM, Psaltis E, Maxwell-Armstrong CA, Lobo DN. Postoperative ileus: Recent developments in pathophysiology and management. *Clin Nutr.* (2015) 34:367–76. doi: 10.1016/j.clnu.2015.01.016

2. Gan TJ, Belani KG, Bergese S, Chung F, Diemunsch P, Habib AS, et al. Fourth consensus guidelines for the management of postoperative nausea and vomiting. *Anesth Analg.* (2020) 131:411–48. doi: 10.1213/ANE.0000000000004833

3. Zaydfudim VM. Postoperative complications after major abdominal operations. *Surgery.* (2021) 169:1017. doi: 10.1016/j.surg.2020.10.006

4. Ren QP, Luo YL, Xiao FM, Wen TF, Wu MH, Juan W, et al. Effect of enhanced recovery after surgery program on patient-reported outcomes and function recovery in patients undergoing liver resection for hepatocellular carcinoma. *Medicine.* (2020) 99:e20062. doi: 10.1097/MD.0000000000020062

5. Liu Y, May BH, Zhang AL, Guo X, Lu C, Xue CC, et al. Acupuncture and related therapies for treatment of postoperative ileus in colorectal cancer: a systematic review and meta-analysis of randomized controlled trials. *Evid Based Compl Alternat Med.* (2018) 2018:3178472. doi: 10.1155/2018/3178472

6. Shah S, Godhardt L, Spofford C. Acupuncture and postoperative pain reduction. *Curr Pain Headache Rep.* (2022) 26:453–8. doi: 10.1007/s11916-022-01048-4

7. Chen KB, Huang Y, Jin XL, Chen GF. Electroacupuncture or transcutaneous electroacupuncture for postoperative ileus after abdominal surgery: a systematic review and meta-analysis. *Int J Surg.* (2019) 70:93–101. doi: 10.1016/j.ijsu.2019.08.034

8. Oztas B, Iyigun E. The effects of two different electrical stimulation methods on the pain intensity of the patients who had undergone abdominal surgery with a midline incision: randomized controlled clinical trial. *Contemp Nurse.* (2019) 55:122–38. doi: 10.1080/10376178.2019.1628650

9. Lam WL, Wang J, Yeung WF, Cheung CW, Chan KKL, Ngan HYS, et al. A combination of electroacupuncture and auricular acupuncture for postoperative pain after abdominal surgery for gynaecological diseases: a randomized controlled trial. *Phytomedicine.* (2022) 104:154292. doi: 10.1016/j.phymed.2022.154292

10. Li WJ, Gao C, An LX Ji YW, Xue FS, Du Y. Perioperative transcutaneous electrical acupoint stimulation for improving postoperative gastrointestinal function: a randomized controlled trial. *J Integr Med.* (2021) 19:211–8. doi: 10.1016/j.joim.2021.01.005

11. Liu MY, Wang CW, Wu ZP Li N. Electroacupuncture for the prevention of postoperative gastrointestinal dysfunction in participants undergoing vascular laparotomy under general anesthesia: a randomized controlled trial. *Chin Med.* (2017) 12:5. doi: 10.1186/s13020-016-0122-9

12. Zhu F, Yin S, Zhu X, Che D, Li Z, Zhong Y, et al. Acupuncture for relieving abdominal pain and distension in acute pancreatitis: a systematic review and meta-analysis. *Front Psychiatry.* (2021) 12:786401. doi: 10.3389/fpsyt.2021.786401

13. Liu XL, Tan JY, Molassiotis A, Suen LK, Shi Y. Acupuncture-point stimulation for postoperative pain control: a systematic review and meta-analysis

of randomized controlled trials. *Evid Based Compl Alternat Med.* (2015) 2015:657809. doi: 10.1155/2015/657809

14. Li HJ, Zhao Y, Wen Q, Liu J, Lü JQ, Liu MY, et al. Comparison of clinical effects of electroacupuncture of abdominal and limb acupoints in the treatment of acute pancreatitis. *Zhen Ci Yan Jiu.* (2018) 43:725–9.

15. Zhang K, Gao C, Li C, Li Y, Wang S, Tang Q, et al. Acupuncture for acute pancreatitis: a systematic review and meta-analysis. *Pancreas.* (2019) 48:1136–47. doi: 10.1097/MPA.0000000000001399

16. Law SK, Lowe S, Law SM, Giaconi JA, Coleman AL, Caprioli J. Prospective evaluation of acupuncture as treatment for glaucoma. *Am J Ophthalmol.* (2015) 160:256–65. doi: 10.1016/j.ajo.2015.04.033

17. Zhu D, Bai L, Zhang X, Xu X, Zhang J. Research progress on quantification of electroacupuncture parameters. *Zhongguo Zhen Jiu.* (2015) 35:525–8. doi: 10.13703/j.0255-2930.2015.05.032

18. Huang DE, Qin Y, Lin MN, Lai XL. Clinical efficacy of different waves of electroacupuncture on knee osteoarthritis and its effect on TGF-β1 in joint fluid. *Zhongguo Zhen Jiu.* (2020) 40:370–4. doi: 10.13703/j.0255-2930.20190422-0005

19. Zhu J, Xu Q, Zou R, Wu W, Wang X, Wang Y, et al. Distal acupoint stimulation versus peri-incisional stimulation for postoperative pain in open abdominal surgery: a systematic review and implications for clinical practice. *BMC Compl Altern Med.* (2019) 19:192. doi: 10.1186/s12906-019-2583-8

20. Ljungqvist O, Scott M, Fearon KC. Enhanced recovery after surgery: a review. *JAMA Surg.* (2017) 152:292–8. doi: 10.1001/jamasurg.2016.4952

21. Committee CNSA, Committee CISM. *China National Standard Nomenclature and Location of Meridian Points (GB/T 12346-2021).* Beijing: China Standards Press. (2021).

22. van Bree SH, Bemelman WA, Hollmann MW, Zwinderman AH, Matteoli G, El Temna S, et al. Identification of clinical outcome measures for recovery of gastrointestinal motility in postoperative ileus. *Ann Surg.* (2014) 259:708–14. doi: 10.1097/SLA.0b013e318293ee55

23. McLeod RS, Aarts MA, Chung F, Eskicioglu C, Forbes SS, Conn LG, et al. Development of an enhanced recovery after surgery guideline and implementation strategy based on the knowledge-to-action cycle. *Ann Surg.* (2015) 262:1016–25. doi: 10.1097/SLA.0000000000001067

24. Penfold JA, Wells CI, Du P, Bissett IP, O'Grady G. Electrical stimulation and recovery of gastrointestinal function following surgery: a systematic review. *Neuromodulation.* (2019) 22:669–79. doi: 10.1111/ner.12878

25. Hajibandeh S, Hajibandeh S, Bill V, Satyadas T. Meta-analysis of enhanced recovery after surgery (ERAS) protocols in emergency abdominal surgery. *World J Surg.* (2020) 44:1336–48. doi: 10.1007/s00268-019-05357-5

26. Hauck M, Schröder S, Meyer-Hamme G, Lorenz J, Friedrichs S, Nolte G, et al. Acupuncture analgesia involves modulation of pain-induced gamma oscillations and cortical network connectivity. *Sci Rep.* (2017) 7:16307. doi: 10.1038/s41598-017-13633-4

27. Cui X, Liu K, Xu D, Zhang Y, He X, Liu H, et al. Mast cell deficiency attenuates acupuncture analgesia for mechanical pain using c-kit gene mutant rats. *J Pain Res.* (2018) 11:483–95. doi: 10.2147/JPR.S152015

28. Zhao ZQ. Neural mechanism underlying acupuncture analgesia. *Prog Neurobiol.* (2008) 85:355–75. doi: 10.1016/j.pneurobio.2008.05.004

29. Skolnik A, Gan TJ. Update on the management of postoperative nausea and vomiting. *Curr Opin Anaesthesiol.* (2014) 27:605–9. doi: 10.1097/ACO.0000000000000128

30. Lee A, Chan SK, Fan LT. Stimulation of the wrist acupuncture point PC6 for preventing postoperative nausea and vomiting. *Cochrane Database Syst Rev.* (2015) 2015:Cd003281. doi: 10.1002/14651858.CD003281.pub4

31. Kim YH, Kim KS, Lee HJ, Shim JC, Yoon SW. The efficacy of several neuromuscular monitoring modes at the P6 acupuncture point in preventing postoperative nausea and vomiting. *Anesth Analg.* (2011) 112:819–23. doi: 10.1213/ANE.0b013e31820f819e

32. Xin C, Sun JH. The value of acupuncture-moxibustion in enhance recovery after surgery. *Zhongguo Zhen Jiu.* (2020) 40:679–82. doi: 10.13703/j.0255-2930.20190501-0005

33. Yang NN, Ye Y, Tian ZX, Ma SM, Zheng Y, Huang J, et al. Effects of electroacupuncture on the intestinal motility and local inflammation are modulated by acupoint selection and stimulation frequency in postoperative ileus mice. *Neurogastroenterol Motil.* (2020) 32:e13808. doi: 10.1111/nmo.13808

34. Jie Ma, Yunxiao Wang, Dan Fan, Chen H. Clinical study of acupoint catgut embedding in the treatment of chronic functional constipation. *J Sichuan Trad Chin Med.* (2015) 10:161–2.

35. Jian-hua. S. Clinical observation of acupuncture plus flash cupping for gastroparesis in senile type 2 diabetes. *Shanghai J Acu-mox.* (2018) 37:1132–35. doi: 10.13460/j.issn.1005-0957.2018.10.1132

36. Liu Z, Yan S, Wu J, He L, Li N, Dong G, et al. Acupuncture for chronic severe functional constipation: a randomized trial. *Ann Intern Med.* (2016) 165:761–9. doi: 10.7326/M15-3118

37. Wang CP, Kao CH, Chen WK, Lo WY, Hsieh CL, A. single-blinded, randomized pilot study evaluating effects of electroacupuncture in diabetic patients with symptoms suggestive of gastroparesis. *J Altern Complement Med.* (2008) 14:833–9. doi: 10.1089/acm.2008.0107

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Clinical evidence of acupuncture and moxibustion for irritable bowel syndrome: A systematic review and meta-analysis of randomized controlled trials

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Background: Acupuncture and moxibustion have been widely used in the treatment of Irritable Bowel Syndrome (IBS). But the evidence that acupuncture and moxibustion for IBS reduction of symptom severity and abdominal pain, and improvement of quality of life is scarce.

Methods: PubMed, Embase, Cochrane Library, Web of Science, Chinese National Knowledge Infrastructure (CNKI), Chinese Scientific Journals Database (VIP), Wanfang Database, China Biomedical Literature Service System (SinoMed), and unpublished sources were searched from inception until June 30, 2022. The quality of RCTs was assessed with the Cochrane Collaboration risk of bias tool. The strength of the evidence was evaluated with the Grading of Recommendations Assessment, Development and Evaluation system (GRADE). Trial sequential analysis (TSA) was conducted to determine whether the participants in the included trials had reached optimal information size and whether the cumulative data was adequately powered to evaluate outcomes.

Results: A total of 31 RCTs were included. Acupuncture helped reduce the severity of symptoms more than pharmaceutical drugs (MD, −35.45; 95% CI, −48.21 to −22.68; I^2 = 71%). TSA showed the cumulative Z score crossed O'Brien-Fleming alpha-spending significance boundaries. Acupuncture wasn't associated with symptom severity reduction (SMD, 0.03, 95% CI, −0.25 to 0.31, I^2 = 46%), but exhibited therapeutic benefits on abdominal pain (SMD, −0.24; 95% CI, −0.48 to −0.01; I^2 = 8%) compared to sham acupuncture. Moxibustion show therapeutic benefits compared to sham moxibustion on symptom severity (SMD, −3.46, 95% CI, −5.66 to −1.27, I^2 = 95%) and abdominal pain (SMD, −2.74, 95% CI, −4.81 to −0.67, I^2 = 96%). Acupuncture (SMD, −0.46; 95% CI, −0.68 to −0.24; I^2 = 47%) and the combination of acupuncture

and moxibustion (SMD, −2.00; 95% CI, −3.04 to −0.96; I^2 = 90%) showed more benefit for abdominal pain compared to pharmacological medications as well as shams. Acupuncture (MD, 4.56; 95% CI, 1.46–7.67; I^2 = 79%) and moxibustion (MD, 6.97; 95% CI, 5.78–8.16; I^2 = 21%) were more likely to improve quality of life than pharmaceutical drugs.

Conclusion: Acupuncture and/or moxibustion are beneficial for symptom severity, abdominal pain and quality of life in IBS. However, in sham control trials, acupuncture hasn't exhibited robust and stable evidence, and moxibustion's results show great heterogeneity. Hence, more rigorous sham control trials of acupuncture or moxibustion are necessary.

Systematic review registration: https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=262118, identifier CRD42021262118.

KEYWORDS

irritable bowel syndrome, acupuncture, moxibustion, complementary and alternative medicine, abdominal pain, quality of life

Key points

- Numerous IBS patients seek substitute medical help from acupuncture and moxibustion. It is essential for physicians to understand the evidence supporting acupuncture and moxibustion as a treatment for IBS.
- In this systematic review and meta-analysis, acupuncture and/or moxibustion are beneficial for symptom severity, abdominal pain and quality of life. The strength of the evidence ranges from very low to high.
- Acupuncture hasn't exhibited robust and stable evidence, and moxibustion's results show great heterogeneity. However, high quality of sham-controlled trials are necessary to consolidate these results.

Introduction

Irritable bowel syndrome (IBS) is the most common functional bowel disorder, which characterized by abdominal pain or discomfort association with altered stool form or frequency (1, 2). The disorder affects 5–17.5% of general population and has significant effect on quality of life and social function (2–4). Moreover, The pain and discomfort of abdominal and other impairment from IBS contribute to significant healthcare resource consumption and workplace absenteeism (5–7). The biopsychosocial mechanisms that explain abdominal pain and disordered bowel habits in IBS are multifaceted, including genetic predisposition, adverse childhood events, psychological factors, and changes in the enteric nervous system, which regulates intestinal motor, sensory, mucosal barrier, and secretory responses (1). Due to the complexity of the underlying mechanism, effective and safe medications are still in research. Most of the drugs recommended for treating the condition only focus on symptom alleviation, and antispasmodics, tricyclic antidepressants and selective serotonin reuptake inhibitors were found to have low to moderate quality of evidence by the American College of Gastroenterology Task Force. They also found some of these agents have a risk of ischemic colitis and cardiovascular events (8).

Patients and healthy providers are frequently dissatisfied with the existing pharmacological drugs and may seek complementary and alternative medicine (CAM) for help (9). Acupuncture and moxibustion, also termed energy-healing therapies, are two of the most widely utilized CAM therapies worldwide. Several systematic reviews and meta-analyses of acupuncture and moxibustion have been performed, however, the primary outcomes of these reviews were based on adequate relief rate or total response. IBS symptom severity score and abdominal pain, which are recommended by the FDA to assess the therapeutic effect (10), were rarely assessed as the primary or secondary outcomes in this meta-analysis. In addition, some conflicting results from previous meta-analysis still couldn't provide a definitive conclusion, for example, relative to sham acupuncture, real acupuncture had no significant benefit for symptom severity, but patients receiving real acupuncture reported greater improvements of IBS symptoms compared with patients receiving pharmacological therapies (11). It has been several years since the publication of the most recent meta-analysis for acupuncture and moxibustion in the treatment of IBS. More rigorous randomized controlled trials (RCTs) of acupuncture and related therapies have been published in recent years, for instance, a multicenter RCT of acupuncture published in 2021 found that patients with IBS who received acupuncture therapy experienced a significant reduction in IBS symptom severity and an improvement in quality of life (12). What's not mentioned in the previous meta-analysis is the sample size estimation, which is as important as sample size calculation in RCTs. And none of the previous meta-analysis had corrected the

increased risk of type I errors caused by parse data and repeated significance testing on accumulating data.

In light of the conflicting results, limitations of previous reviews, the increasing number of RCTs of acupuncture or moxibustion used in IBS, and the ensuring need for critical evacuation, a systematic review and meta-analysis of the available evidence is essential. The specific research questions were as follows: do acupuncture or moxibustion contribute to reducing symptom severity and abdominal pain, and improving the quality of life compared with pharmacological medications or sham control?

Methods

Protocol and guidance

This systematic review and meta-analysis followed Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA), and the protocol was registered in PROSPERO with the ID of CRD42021262118.

Inclusion criteria

We considered trials to be eligible if they enrolled adults (age ≥18) with IBS; if they compared acupuncture or moxibustion with sham control or pharmacological medications (when other therapies were also given, they had to be the same dosage in all groups); if they provided information in symptom severity measured by the IBS symptom severity scale (IBS-SSS), abdominal pain, or IBS quality of life (IBS-QOL); and if they were randomized controlled trials.

Exclusion criteria

We excluded studies that were case reports, case series, or observational studies; if the participants were pregnant or lactating women; if the intervention included laser acupuncture, non-invasive electrostimulation (i.e., using electrodes on the skin rather than needles to stimulate acupuncture points), transcutaneous electrical nerve stimulation, and acupressure; and if studies compared two types of acupuncture techniques or acupuncture with other traditional treatments. All conference proceedings, guidelines, dissertations, commentaries, and letters were excluded.

Outcomes

The primary outcome was symptom severity, measured by the Irritable Bowel Syndrome Symptom Severity Scale (IBS-SSS). Secondary outcomes were abdominal pain and quality of life as measured by Irritable Bowel Syndrome Quality of Life (IBS-QOL).

Search strategy

One of the authors (YYM) conducted the search of several databases: PubMed, Embase, Web of Science, Cochrane Library, the Chinese National Knowledge Infrastructure Database (CNKI), Wanfang Database, China Science and Technology Journal Database (VIP) and China Biomedical Literature Service System (SinoMed) from database inception to June 30, 2022. ClinicalTrials.gov was also searched to identify ongoing or unpublished eligible trials. The search strategy consists of 3 components: clinical condition (Irritable Bowel Syndrome), intervention (acupuncture or moxibustion) and study design (randomized controlled trial). To maximize the search of relevant articles, existing systematic reviews were examined to identify additional studies. Language restrictions were not applied.

Study selection

The searching results were exported to Endnote for screening and removing duplicates. Two reviewers (RKH and ZK) independently reviewed the titles and abstracts to identify any relevant studies. Citations deemed potentially relevant by either screener were advanced to second-stage full-text review. Then, full text reports were retrieved and screened for eligibility. Any discrepancies between the reviews were handled through discussion or consultation with a third party (SM) until consensus was reached. The flowchart illustrated the process of literature review and study selection (Figure 1).

Data collection process

Two independent researchers (YYM and RKH) used a standard data extraction form to extract data from the included trials. We extracted the following information from included studies: study title, first author, demographic data, details of the interventions, and outcomes.

Assessment of risk of bias and quality of evidence

Quality of all included trials was assessed independently by two investigators (YYM and SM) who recorded the method used to generate the randomization schedule and allocation concealment; whether blinding was implemented for participants, prescribers and outcome assessors; whether there was evidence of incomplete outcome data, and whether there

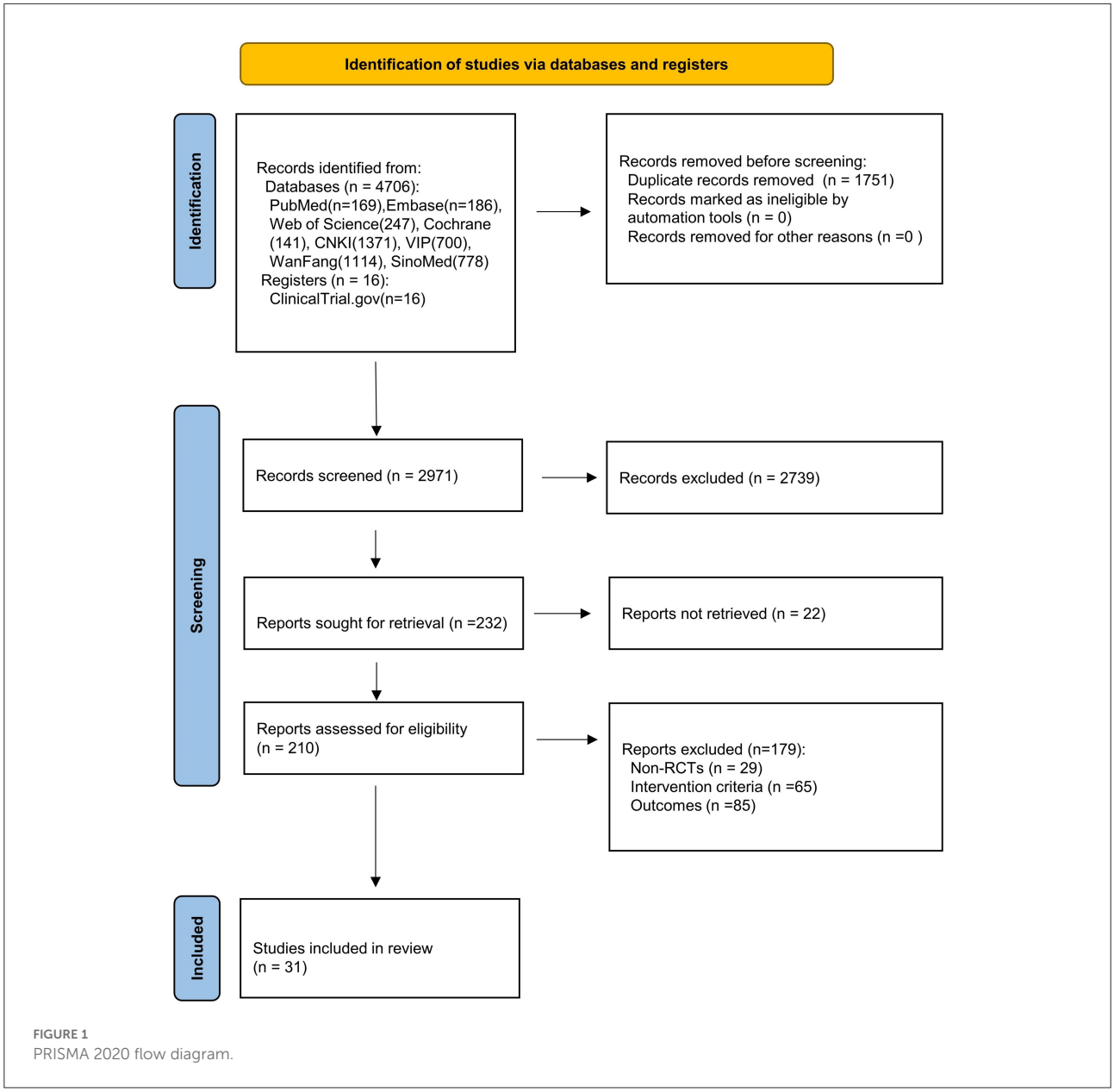

FIGURE 1
PRISMA 2020 flow diagram.

were any selective outcome reporting data available. In addition, they examined the quality of evidence for outcomes using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach.

Data synthesis

RevMan (version 5.4.4; the Cochrane Collaboration) and Stata (version 16; Stata Corp, LLC) were used to perform statistical analyses. As the outcomes we selected were continuous variables, mean differences (MD) and 95% confidence intervals (CI) were calculated for variables with the same scale (e.g., symptom severity as measured by IBS-SSS and quality of life as measured by IBS-QOL). For continuous outcomes with different scales (e.g., different measures of abdominal pain), the standardized mean difference (SMD) and 95% confidence intervals (CI) were calculated. Heterogeneity was assessed using the I^2 test. Fixed effects models were used to pool outcomes if significant heterogeneity was not present ($I^2 < 50\%$), otherwise, random effects models were applied. The possibility of small study effects was assessed by the Egger and Begg tests.

Trial sequential analysis

Trial sequential analysis (TSA) was conducted to determine whether the cumulative data has significant power to evaluate

TABLE 1 Characteristics of randomized controlled trials.

Study ID	Location	Number of patients	IBS details		Intervention			Control			Mean age (SD)		Gender (Male/Female)	
			Definition	Type	Method	Duration (weeks)	Follow-up (weeks)	Method	Duration (weeks)	Follow-up (weeks)	Intervention	Control	Intervention	Control
Jing et al. (12)	China	137	Rome III	IBS-C	Acupuncture, 30 min, 3/weeks	6	18	Polyethylene glycol, 20 g, qd	6	18	46.34 (14.32)	47.02 (13.59)	33/59	17/28
Jing et al. (12)	China	382	Rome III	IBS-D	Acupuncture, 30 min, 3/weeks	6	18	Pinaverium, 50 mg, tid	6	18	45.72 (12.52)	46.99 (12.48)	146/106	71/59
Li et al. (27)	China	70	Rome III	IBS-D	Acupuncture, 20 min, 5/weeks	8	–	Trimebutine maleate, 0.2 g tid	8	–	38.15 (7.85)	38.45 (8.22)	12/23	13/22
Pei et al. (31)	China	519	Rome III	IBS-C/D	Acupuncture, 30 min, 3/weeks	6	18	Polyethylene glycol, 10 g, qd (IBS-C); Pinaverium, 50 mg, tid (IBS-D)	6	18	45.89 (13.01)	47.00 (12.73)	177/165	88/87
Wenjiao (36)	China	80	Rome III	IBS-D	Acupuncture, 30 min, 3/weeks	6	18	Pinaverium, 50 mg, tid	6	18	46.38 (11.47)	47.49 (12.39)	22/18	19/21
Guojuan (17)	China	70	Rome IV	IBS-D	Acupuncture, 30 min, 5/weeks	4	–	Pinaverium, 50 mg, tid	4	–	39.3 (11.5)	38.4 (13.5)	16/19	13/22
Lei (24)	China	104	Rome III	IBS-D	Acupuncture, 30 min, 5/weeks	4	–	Pinaverium, 50 mg, tid live combined Bifidobacterium, Lactobacillus and Enterococcus powder, 2 g, tid	4	–	40.71 (10.84)	41.55 (11.62)	24/30	23/27
Hongming et al. (20)	China	70	Rome III	IBS-D	Acupuncture, 30 min, 5/weeks	16	24	Pinaverium, 50 mg, tid Bifidobacterium capsules, 0.84 g, tid	16	24	44.38 (8.61)	44.05 (8.72)	16/19	17/18
Yu et al. (40)	China	58	Rome III	IBS-D	Acupuncture, 30 min, 3/weeks	4	12	Pinaverium, 50 mg, tid	4	12	41 (11)	39 (12)	14/16	11/17
Jing et al. (22)	China	77	Rome III	IBS-D	Acupuncture, 30 min, 3/weeks	6	–	Pinaverium, 50 mg, tid	6	–	46 (13)	48 (13)	24/27	13/13
Wenjing and Yingjie (37)	China	80	Rome III	IBS-D	Acupuncture	4	–	Pinaverium, 50 mg, tid	4	–	30.0 (6.2)	32.2 (5.4)	12/28	15/25
Lixia et al. (26)	China	60	Rome III	IBS-C	Acupuncture, 30 min, 5/weeks	4	12	lactulose oral liquid, 15 ml, tid	4	12	44 (12)	44 (14)	11/19	9/21

(Continued)

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TABLE 1 (Continued)

Study ID	Location	Number of patients	IBS details		Intervention			Control			Mean age (SD)		Gender (Male/Female)	
			Definition	Type	Method	Duration (weeks)	Follow-up (weeks)	Method	Duration (weeks)	Follow-up (weeks)	Interven-tion	Control	Interven-tion	Control
Hao et al. (18)	China	70	Rome III	IBS-D	Acupuncture, 30 min, 3–4/weeks	4	–	Pinaverium, 50 mg, tid	4	–	39.1 (11.8)	37.9 (11.5)	15/20	18/17
Sun et al. (34)	China	63	Rome III	IBS-D	Acupuncture	4	–	Pinaverium, 50 mg, tid	4	–	38.81 (11.80)	38.59 (11.45)	13/18	20/12
Bin et al. (15)	China	54	Rome II	IBS-D	Acupuncture, 30 min, 3/weeks	8	–	Pinaverium, 50 mg, tid	8	–	–	–	12/15	13/14
Mak et al. (29)	Hong Kong	80	Rome III	IBS-D	Acupuncture, 30 min, 1/weeks	10	16	Sham acupuncture, 30 min, 1/weeks	10	16	50.85 (11.57)	50.83 (14.15)	20/20	18/22
Lowe et al. (28)	Canada	79	Rome I	-	Acupuncture, 30 min, 2/weeks	4	12	Sham acupuncture, 30 min, 2/weeks	4	12	42 (15)	43 (15)	7/36	10/26
Xia et al. (38)	China	80	Rome III	IBS-D	Acupuncture, 40 min; Bifidobacterium tetralogy capsule, 4.5 g, tid	4	–	Sham acupuncture, 40 min; Bifidobac-terium tetralogy capsule, 4.5 g, tid	4	–	40.0 (13.2)	37.6 (12.6)	23/17	21/19
Park and Cha (30)	South Korea	42	Rome III	–	Acupuncture, 25 min, 2/week	4	–	Sham acupuncture, 25 min, 2/week	4	–	22.26 (3.23)	21.48 (2.73)	–	–
Lembo et al. (21)	USA	153	Rome II	Any	Acupuncture	3	–	Sham acupuncture	3	–	37.5 (14.6)	38.9 (14.1)	–	–
Wei et al. (35)	China	80	Rome IV	IBS-D	Moxibustion, every other day	4	–	Pinaverium, 50 mg, tid	4		43 (4)	43 (5)	16/24	18/22
Yanli (39)	China	52	NA	IBS-D	Moxibustion, 30 min, bid plus Loperamide 2 mg, bid	2	–	Loperamide 2 mg, bid	2	–	41.33 (1.14)	22/30 (M/F)	–	–
Lingjun et al. (25)	China	80	Rome III	IBS-D	Moxibustion, qd	4	–	Trimebutine maleate, 0.2 g tid	4	–	41.33 (1.14)	44.05 (1.14)	12/28	14/26
Di et al. (16)	China	97	Rome III	IBS-D	Moxibustion, qd	4	12	Loperamide 2 mg, tid	4	12	43.11 (13.22)	44.53 (12.63)	21/28	26/22
Shouqin (33)	China	60	Rome III	IBS-D	Moxibustion, 45 min Pinaverium, 50 mg, tid	4	8	Pinaverium, 50 mg, tid	4	8	35.68 (8.25)	26.23 (7.82)	13/17	11/19

(Continued)

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TABLE 1 (Continued)

Study ID	Location	Number of patients	IBS details		Intervention			Control			Mean age (SD)		Gender (Male/Female)	
			Definition	Type	Method	Duration (weeks)	Follow-up (weeks)	Method	Duration (weeks)	Follow-up (weeks)	Interven-tion	Control	Interven-tion	Control
Haoran et al. (19)	China	60	Rome II	IBS-D	Moxibustion, 30 min	2	–	Loperamide, 2 mg, bid	2	–	48.3 (12.5)	46.8 (13.2)	23/7	24/6
Jun (23)	China	100	Rome III	IBS-D	Acupuncture and moxibustion, 30 min	4	–	Pinaverium, 50 mg, tid	4	–	44.8 (9.5)	45.3 (10.2)	21/29	20/30
Qian et al. (32)	China	111	Rome IV	IBS-C/D	Acupuncture and moxibustion, 20 min,	4	–	Trimebutine maleate, 0.2 g tid	4	–	47.00 (2.50)	46.80 (2.70)	25/31	23/32
Anastasi et al. (14)	USA	29	Rome II	NA	Acupuncture and moxibustion	4	–	Sham acupuncture and moxibustion	4	–	47.1	34.3	6/9	5/10
Shen et al. (43)	China	65	Rome III	IBS-D	Acupuncture, 3 times per week, 30 min	8	–	Sham acupuncture, 3 times per week, 30 min	8	–	38.61 (11.57)	43.28 (13.64)	18/15	16/16
Bao et al. (42)	China	104	Rome III	IBS-D	Moxibustion, 3 times per week, 30 min	6	24	Sham moxibustion, 3 times per week, 30 min	6	24	47.6 (11.9)	45.2 (14.7)	23/29	28/24
Wang et al. (41)	China	76	Rome IV	IBS-D	Moxibustion, 3 times per week, 30 min	6	12	Sham moxibustion, 3 times per week, 30 min	6	12	250.21 (12.21)	44.26 (15.10)	21/17	20/18

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TABLE 2 Grade recommendation of acupuncture and moxibustion for IBS with different outcomes at the time of treatment ending.

Certainty assessment							No. of patients		Effect		Certainty	importance
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Acupuncture	Control therapy	Relative (95% CI)	Absolute (95% CI)		
Acupuncture vs. pharmacological medications for symptom severity at the time of treatment ending (assessed with: IBS-SSS)												
10	Randomized trials	Very serious[a]	Not serious	Not serious	Not serious	Strong association	968	599	–	MD **35.45 fewer** (48.21 fewer to 22.68 fewer)	⨁⨁⨁◯ Moderate	
Moxibustion vs, pharmacological medications for symptom severity at the time of treatment ending (assessed with: IBS-SSS)												
1	Randomized trials	Very serious[a]	Not serious	Not serious	Not serious	None	40	40	–	MD **59.75 lower** (71.47 lower to 48.03 lower)	⨁⨁◯◯ Low	
Moxibustion vs. placebo moxibustion for symptom severity at the time of treatment ending (assessed with IBS-SSS)												
2	Randomized trials	Not serious	Not serious	Not serious	Not serious	None	90	90	–	SMD **3.46 SD lower** (5.66 lower to 1.27 lower)	⨁⨁⨁⨁ High	
Acupuncture vs. pharmacological medications for abdominal pain at the time of treatment ending												
7	Randomized trials	Very serious[a]	Not serious	Not serious	Not serious	Strong association	562	366	–	SMD **0.35 SD lower** (0.48 lower to 0.21 lower)	⨁⨁⨁◯ Moderate	
Acupuncture vs. sham acupuncture for abdominal pain at time of treatment ending												
4	Randomized trials	Serious[a]	Serious[b]	Not serious	Not serious	None	144	137	–	SMD **0.24 SD lower** (0.48 lower to 0.01 lower)	⨁⨁◯◯ Low	
Moxibustion vs. pharmacological medications for abdominal pain at the time of treatment ending												
3	Randomized trials	Very serious[a]	Not serious	Not serious	Not serious	None	96	96	–	SMD **0.75 SD lower** (1.04 lower to 0.46 lower)	⨁⨁◯◯ Low	
Acupuncture and moxibustion vs. pharmacological medications for abdominal pain at the time of treatment ending												
2	Randomized trials	Very serious[a]	Serious[c]	Not serious	Not serious	None	106	105	–	SMD **2 SD lower** (3.04 lower to 0.96 lower)	⨁◯◯◯ Very low	
Acupuncture and moxibustion vs. sham acupuncture and sham moxibustion for abdominal pain at the time of treatment ending												
1	Randomized trials	Not serious	Not serious	Not serious	Very serious[d]	None	14	15	–	MD **1.12 lower** (1.78 lower to 0.46 lower)	⨁⨁◯◯ Low	

(Continued)

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TABLE 2 (Continued)

No. of studies	Study design	Certainty assessment					No. of patients		Effect		Certainty	importance
		Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Acupuncture	Control therapy	Relative (95% CI)	Absolute (95% CI)		
Acupuncture vs. pharmacological medications for quality of life at the time of treatment ending (assessed with: IBS-QOL)												
7	Randomized trials	Very serious[a]	Not serious	Not serious	Not serious	Strong association	838	497	–	MD **4.56 higher** (1.46 higher to 7.67 higher)	⨁⨁⨁◯	Moderate
Moxibustion vs. pharmacological medications for quality of life at the time of treatment ending (assessed with: IBS-QOL)												
3	Randomized trials	Very serious[a]	Not serious	Not serious	Not serious	None	119	118	–	MD **6.97 higher** (5.78 higher to 8.16 higher)	⨁⨁◯◯	Low

CI, confidence interval; MD, mean difference; SMD, standardized mean difference; [a] High risk of blinding and allocation concealment; [b] Consequence was influenced when one of study was removed; [c] Heterogeneity: I-squire 90%; [d] Sample size is small and only one trial is available.

outcomes. In meta-analysis, TSA can be used to assess the likely influence of future trials on the pooled findings and estimate the point at which further studies are not likely to change the pooled findings (13). In our research, TSA (version 0.9.5.10 Beta, Copenhagen Trial unit, https://ctu.dk/tsa/) was performed with an overall 5% risk of type I error and 80% power.

Subgroup analyses and sensitively analyses

The studies were categorized by the type of intervention (acupuncture, moxibustion or the combination of acupuncture and moxibustion). Subgroup analyses were performed according to comparators (e.g., pharmacological medications including pinaverium, trimebutine, loperamide and others). Sensitive analyses were conducted by systematically omitting one of the trials.

Results

Eligible studies and study characteristics

A total of 4,706 records were identified through different database searches and 16 records were searched from ClinicalTrial.gov, from which 1,751 duplicate publications were removed and 2,940 articles were excluded for not meeting the inclusion criteria. Thirty-one eligible RCTs (12, 14–43) were included in the final meta-analysis, and details are shown in Table 1.

Among the 31 trials included, 9 (21.43%) were sham controlled and 22 (78.57%) were open-label trials. Acupuncture was compared to pharmacological medications in fourteen of the 22 open-label trials, and in one trial, participants were divided into IBS-D and IBS-C groups, with pinaverium and polyethylene glycol serving as corresponding control interventions (12). Six of the 22 open-label trials compared moxibustion to medications, while two of the 22 trials compared the combination of acupuncture and moxibustion to pharmaceuticals. Seven sham controlled trials compared real acupuncture to sham acupuncture, Bifidobacterium tetralogy capsules were used with the same dosage between real and sham groups in one trial (38), another sham controlled trial compared real acupuncture plus real moxibustion to sham acupuncture and sham moxibustion (14), and two sham controlled trials compared moxibustion to sham moxibustion. Seven sham controlled studies were distinguished for their high quality, since each of the 7 domains of risk of bias was deemed to have a low risk. Detection bias existed in the residual trials due to participants not being blinded to the treatments and the primary and secondary outcomes being subjective. Therefore, it was

thought that there was a high risk of bias in the 22 open-label trials (Figure 2).

Primary outcome: Symptom severity

In terms of symptom severity at the time of treatment ending, pooled results from 9 articles containing 10 open-label trials showed a significant association between acupuncture and a reduction in symptom severity compared to pharmacological medications, along with substantial heterogeneity and moderate certainty (MD, −35.45; 95% CI, −48.21 to −22.68; $I^2 = 71\%$) (Figure 3 and Table 2). The exclusion of one outlier study (31) reduced heterogeneity with no significant change in results (MD, −39.30; 95% CI, −49.44 to −29.17; $I^2 = 39\%$). In trial sequential analysis (TSA), though the information size of participants with symptom severity didn't exceed the required information size (RIS), the cumulative Z score crossed O'Brien-Fleming alpha-spending significance boundaries (sample size, 1,567; RIS, 5,490) (Figure 4). Funnel plot analysis showed no asymmetry and suggested no publication bias (Figure 5). In addition, neither the Egger test ($P = 0.191$) (Figure 6) nor the Begg test ($P = 0.929$) (Supplementary Figure 1) detected any significant small study effects. Moreover, the result was robust in sensitivity analyses by systematically omitting one of the trials (Figure 7). At the time of follow-up, pooled results also indicated that acupuncture was superior to pharmacological medications on IBS symptom severity (MD, −23.8; 95% CI, −32.28 to −15.32, $I^2 = 24\%$) (Supplementary Figure 2). However, the therapeutic benefits of acupuncture on symptom severity are not exhibited when compared to sham acupuncture (SMD, 0.03, 95% CI, −0.25 to 0.31, $I^2 = 46\%$) (Supplementary Figure 3). With respect to moxibustion, results from one trial showed the association of symptom severity reduction and moxibustion rather than pharmacological medications with low certainty (MD, −59.75; 95% CI, −71.47 to −48.03) (Supplementary Figure 4). Results from two sham-controlled trials show a significant association between moxibustion and a reduction in symptom severity when compared to placebo moxibustion along with substantial heterogeneity and high certainty, not only at the time of treatment ending (SMD, −3.46, 95% CI, −5.66 to −1.27, $I^2 = 95\%$) but also at the time of follow-up (SMD, −4.07, 95% CI, −6.08 to −1.34, $I^2 = 96\%$) (Supplementary Figures 5, 6).

Subgroup analyses revealed that acupuncture was superior to pinaverium (MD, −45.24; 95% CI, −58.23 to −32.25; $I^2 = 32\%$) and pinaverium plus Bifidobacterium (MD, −33.49; 95% CI, −54.38 to −12.60; $I^2 = 64\%$) for symptom severity reduction (Figure 3). It also showed that there was no significant difference in symptom severity reduction between acupuncture and other medications like polyethylene glycol and lactulose oral liquid in patients with IBS-C (MD, −8.55; 94% CI, −19.80 to 2.71; $I^2 = 0\%$) (Figure 3).

Secondary outcomes: Abdominal pain and quality of life

Data from 7 RCTs demonstrated that acupuncture is linked with a reduction in abdominal pain compared to pharmacological medications with heterogeneity and moderate certainty (SMD, −0.35; 95% CI, −0.48 to −0.21; $I^2 = 47\%$) (Supplementary Figure 7). Trial sequential analysis showed that the sample size of abdominal pain didn't transcend the required information size (RIS), but the cumulative Z score crossed O'Brien-Fleming alpha-spending significance boundaries (sample size, 928; RIS, 1,260) (Supplementary Figure 8).

Pooled results from four sham-controlled studies revealed a significant benefit of real acupuncture over sham acupuncture for the relief of abdominal pain, along with small heterogeneity and low certainty (SMD, −0.24; 95% CI, −0.48 to −0.01; $I^2 = 8\%$) (Supplementary Figure 9). Three open-label trials on moxibustion showed that the reduction of abdominal pain was associated with moxibustion in comparison with pharmacological medications without heterogeneity and low certainty (SMD, −0.75; 95% CI, −1.04 to −0.46; $I^2 = 0$) (Supplementary Figure 10). Pooled results from two trials found that the reduction of abdominal pain was associated with a combination of acupuncture and moxibustion rather than pharmacological medications with considerable heterogeneity and very low certainty (SMD, −2.00; 95% CI, −3.04 to −0.96; $I^2 = 90\%$) (Supplementary Figure 11). A single sham-controlled trial revealed that acupuncture and moxibustion are superior to sham acupuncture and sham moxibustion with low certainty (MD, −1.12; 95% CI, −1.78 to −0.46) (Supplementary Figure 12). Two sham-controlled trials also show a significant association between moxibustion and a reduction in abdominal pain when compared to placebo moxibustion along with substantial heterogeneity at the time of treatment ending (SMD, −2.74, 95% CI, −4.81 to −0.67, $I^2 = 96\%$) and follow-up (SMD, −2.87, 95% CI, −4.75 to −0.99, $I^2 = 95\%$) (Supplementary Figures 13, 14).

Regarding quality of life (QOL) at the time of treatment ending, 6 papers containing 7 trials analyzing 1,335 participants contained data demonstrating the association between improvement of quality of life and acupuncture compared to pharmacological medications with substantial heterogeneity and moderate certainty (MD, 4.56; 95% CI, 1.46–7.67; $I^2 = 79\%$) (Supplementary Figure 15). The exclusion of two outliers (18, 24) reduced heterogeneity without reversing change in effect (MD, 2.37; 95% CI, 0.16–4.59; $I^2 = 43\%$). At the time of follow-up, pooled results also suggested that acupuncture is superior to pharmacological medications on improvement of quality of life (MD, 4.33; 95% CI, 2.54–6.11; $I^2 = 0\%$) (Supplementary Figure 16). Three open-label trials revealed that the improvement of QOL was associated with moxibustion compared with pharmacological medications with small

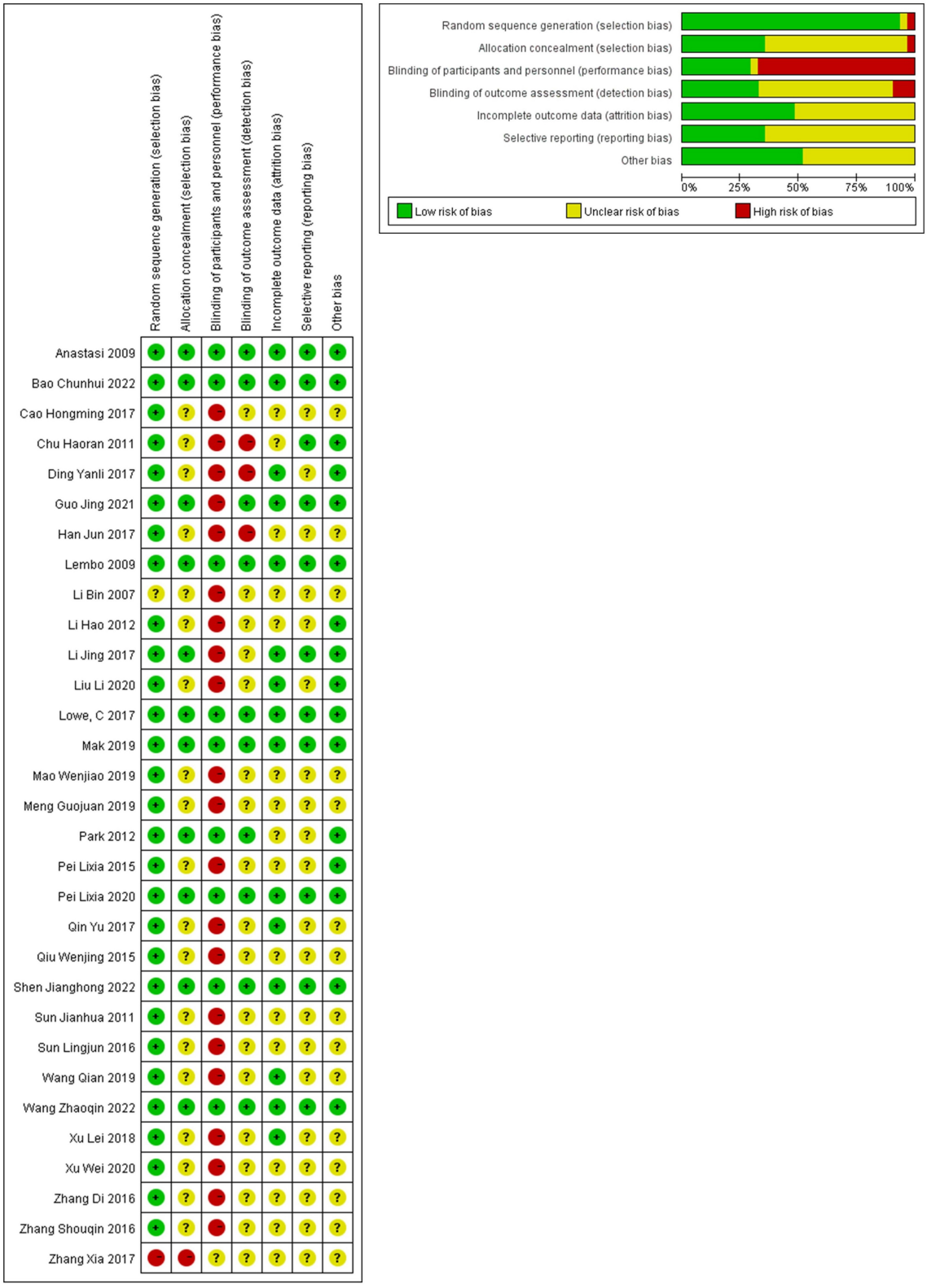

FIGURE 2
Summary of risk of bias.

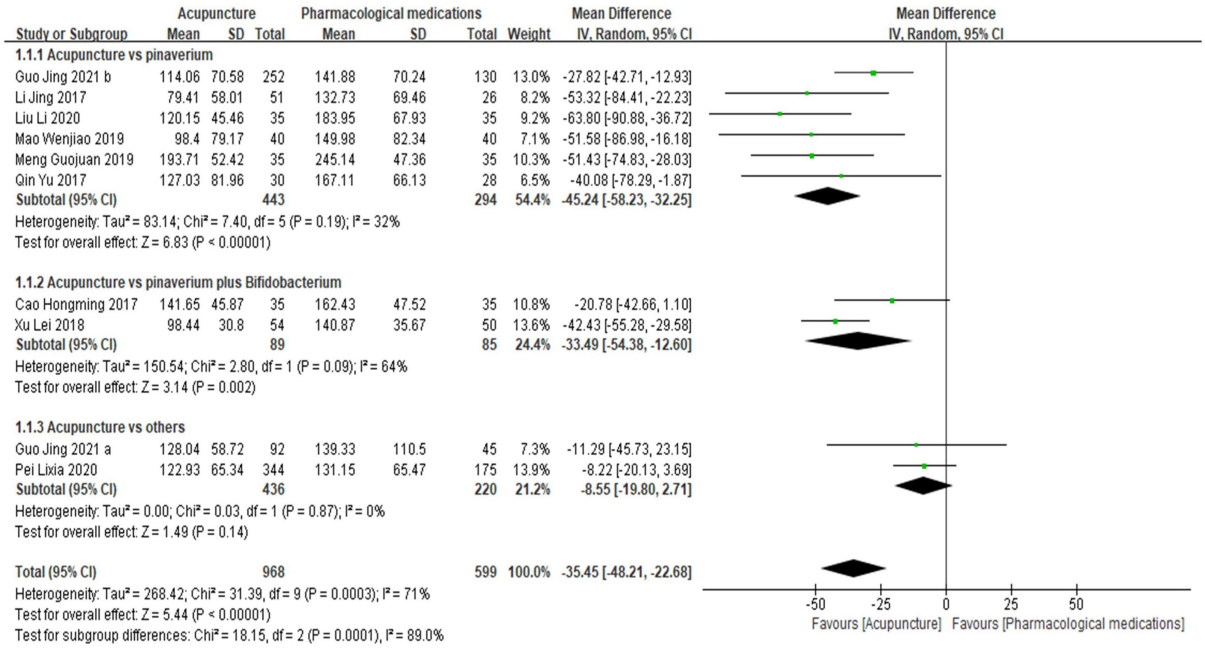

FIGURE 3
Forrest plot of acupuncture vs. pharmacological medications for symptom severity at the time of treatment ending. Results are shown by using the random-effect model with mean difference and 95% confidence intervals (CI).

heterogeneity and low certainty (MD, 6.97; 95% CI, 5.78–8.16, $I^2 = 21\%$) (Supplementary Figure 17).

Discussion

In this meta-analysis of 31 randomized controlled trials, acupuncture or moxibustion were found to be beneficial for IBS symptom severity, abdominal pain and quality of life. The present study updated the synthesis of the current evidence and suggests that acupuncture or moxibustion could reduce symptom severity and abdominal pain, and improve quality of life with low to high certainty of evidence (Table 2).

Principal findings and comparison with other studies

Although several systematic review and meta-analysis on acupuncture and moxibustion has been conducted (11, 44–47), primary outcome assessment of these studies has been limited to effectiveness rate. The continuous outcome from one of these reviews used Chinese Medicine symptoms integral with weighted mean difference (WMD) to estimate the efficacy (45). However, Chinese Medicine symptoms integral to IBS were rarely used in the clinical trials. The IBS series scales, such as the symptom severity scale (IBS-SSS) and quality of life (IBS-QOL), are widely used worldwide. Hence, symptom severity measured by IBS-SSS and quality of life measured by IBS-QOL are necessary to be assessed. Abdominal pain in the included trials was measured by Likert classification or visual analog scoring, the standardized scales should receive more application. Therefore, IBS series scales, such as IBS-SSS which including abdominal pain, are recommended in further research. Another essential issue which is not discussed in previous review is the increased type I error rate due to sparse data and repeated significance testing when updating meta-analysis with new trials.

Consistent with previous researches (11, 48), our results showed that acupuncture is linked with a significant reduction in symptom severity and abdominal pain in open-label studies. However, there may be an overestimation of treatment response in these trials due to a lack of blinding (48), since the therapeutic benefits of acupuncture on symptom severity are not exhibited when compared to sham acupuncture. Our analysis shows that real acupuncture is related to a reduction in abdominal pain compared to sham control, which differs from the findings of the previous review (11), owing to the inclusion of the latest published trials (38). However, the interesting finding was reversed when the sensitive analysis was conducted by omitting one of the trials (38), which indicated the result wasn't robust.

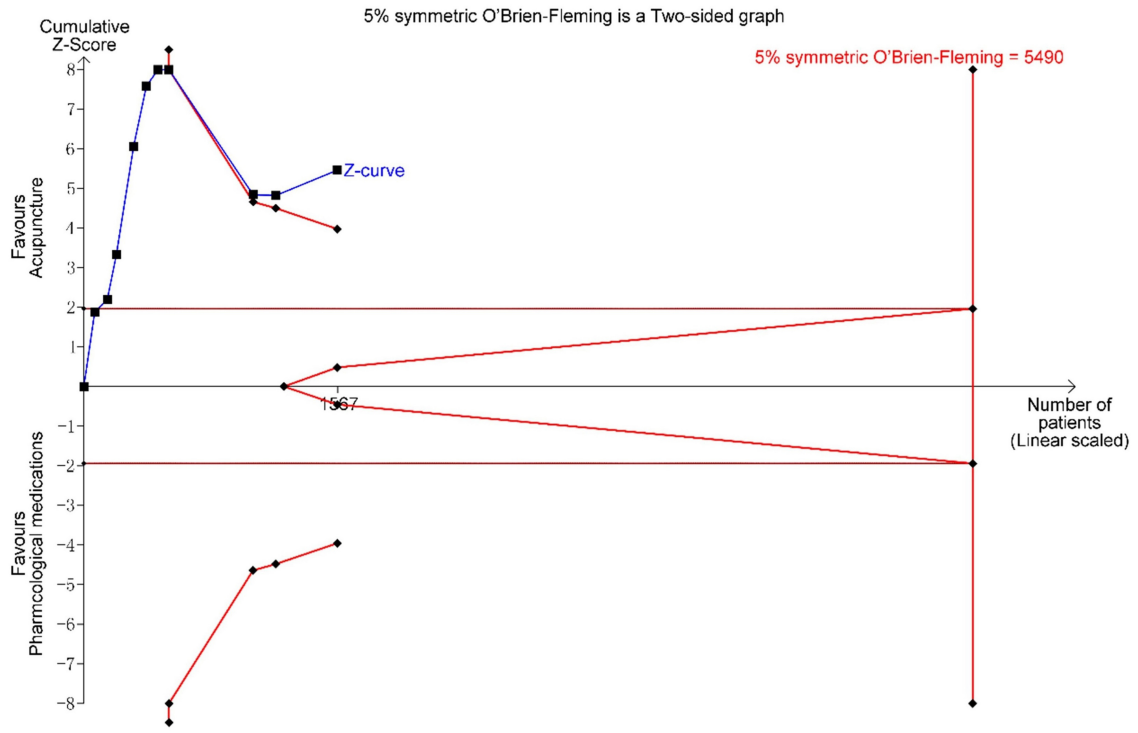

FIGURE 4
Trial sequential analysis (TSA) for symptom severity. Trial sequential analysis (TSA) of 10 trials comparing acupuncture with pharmacological medications for symptom severity in patients with IBS. The TSA shows that the information size is insufficient, but the cumulative Z score crossed O'Brien-Fleming alpha-spending significance boundaries. The evidence is sufficient to identify the effect of intervention. A required information size of 5,490 was calculated using $\alpha = 0.05$ (two sided), ß = 0.20 (power 80%).

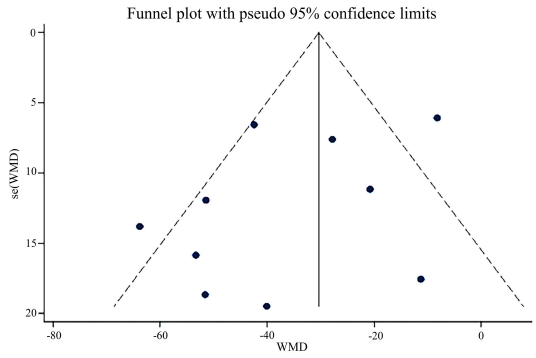

FIGURE 5
Funnel plot of acupuncture vs. pharmacological medications on IBS symptom severity at the time of treatment ending.

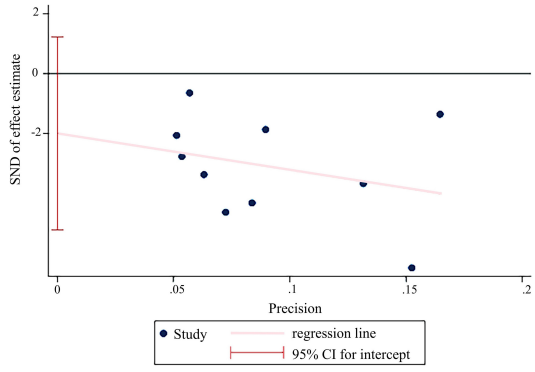

FIGURE 6
Egger test of acupuncture vs. pharmacological medications on IBS symptom severity at the time of treatment ending (Egger test, *P = 0.191*).

What should be noted is that the sham control used in these trials, which involves skin penetrating needles inserted at non-acupuncture points, may have potential weak physiological activity that could influence the outcome and lead to a more feasible intervention response (11), particularly in the IBS population where high placebo responses are common (49). The ongoing randomized sham-controlled trials [NCT04276961 (50), ChiCTR2100044762] will clarify the efficacy of acupuncture compared with sham-acupuncture. And

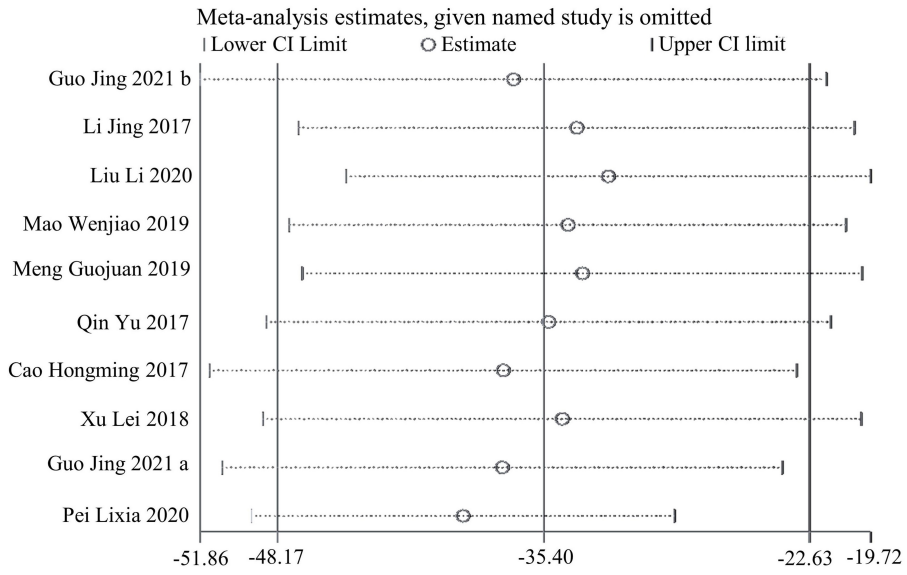

FIGURE 7
Sensitive analysis of acupuncture vs. pharmacological medications on IBS symptom severity at the time of treatment ending.

more shams, which are unlikely to have physiological effects, would be necessary for further research.

An exciting result, which is different from acupuncture's result, is found in moxibustion. On the one hand, our results show that moxibustion, like the pooled open-label trials result of acupuncture, is linked with a significant reduction in symptom severity and abdominal pain; on the other hand, the real and sham-controlled trials of moxibustion show benefits in symptom severity, which differ from the pooled sham-control trials result of acupuncture. Although the number of rigorous sham-controlled trial is limited and heterogeneity is great, ongoing RCTs (51) and the registered RCT with ID ChiCTR2100046852 may provide additional evidence of moxibustion's effectiveness for IBS treatment. Hence, more rigorous sham-controlled trials should be conducted in the future to enhance their reliability.

The major innovation that differentiated this research from prior reviews was the trial sequence analysis on IBS symptom severity and abdominal pain. Due to the potential for additional bias, heterogeneity in various features of the design and conduct of the included trials, and an inflated type I error rate, it is reasonable to interpret a meta-analysis with a higher level of skepticism than a single randomized controlled trial (52). Though the number of patients included in our study is much smaller than the calculated optimal information size, the cumulative Z score crossed O'Brien-Fleming alpha-spending significance boundaries for the outcomes of symptom severity and abdominal pain. Trial sequence analysis shows that there is enough evidence to show that acupuncture is better than pharmaceutical drugs when it comes to reducing symptoms and abdominal pain in open-label trials. More research is unlikely to change this result.

For a meta-analysis to provide definitive evidence, it must meet the basic requirements of a well-designed, adequately powered, and rigorously executed single randomized controlled trial (52). However, the majority of studies comparing acupuncture or moxibustion to pharmacological therapy are not blind. And expectation effects, which are defined as the impact of expectations on subjective outcomes, may differ between acupuncture and drug treatment (53, 54). As a result of the lack of blinding and differential expectations, it is impossible to determine whether any of the reported benefits of acupuncture are due to a larger biological effect of acupuncture needling compared to drugs, or the impact of the trial participants' greater expectation of benefit from acupuncture (11). Therefore, we recommend that future acupuncture and moxibustion studies should focus on sham-controlled trials. Further research should consider these questions as a research direction for some of the ideas we'd like to pursue but haven't been able to because of a lack of related trials, such as the effect difference of acupuncture and moxibustion between different subtypes of IBS and different regions.

Strengths and limitations

This systematic review and meta-analysis possesses a number of methodological strengths. We followed the Cochrane Collaboration's recommendations and were registered in PROSPERO under the number CRD42021262118. This study

also included a rigorous assessment of the quality of evidence using the GRADE approach. In addition, trial sequence analysis was used to evaluate the required information size and interim monitoring boundaries, which could decrease the probability of type I error.

Nonetheless, several limitations are unavoidable. First, the methodologic quality of the included trials was generally low due to a lack of blinding and allocation concealment, which limited the credibility of the results and contributed to a poorer evidence grade. Second, outcomes in our research were limited to symptom severity and quality of life, the possibility of a risk of selective bias should be considered. Thirdly, abdominal pain in the RCTs was measured by different classification scales, and the effect size was assessed with standard mean difference (SMD), which may have decreased the accuracy of the effect size. In light of these limitations, more sham-controlled trials of acupuncture or moxibustion are required to detect the treatment response and long-term prognosis.

Conclusions

The findings of this systematic review and meta-analysis suggest that acupuncture and/or moxibustion are beneficial for symptom severity, abdominal pain and quality of life in IBS. The effects of acupuncture and moxibustion should be better known by more doctors and patients and widely used in clinical practice. However, in sham control trials, acupuncture hasn't exhibited robust and stable evidence, and moxibustion's results show great heterogeneity. Hence, more rigorous sham control trials of acupuncture or moxibustion are necessary.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author/s.

Author contributions

SH and ZB conceived the study. HW and SQ designed the protocol. YY performed the literature search. KR and KZ selected the studies. YY and KR extracted the relevant information. YY and MS synthesized the data. YY wrote the first draft of the paper. All the authors critically revised successive drafts of the paper and approved the final version.

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Conflict of interest

Authors SH, HW, SQ, and HZ reported receiving grants the National Natural Science Foundation of China, internal funding from the Traditional Chinese Medicine Bureau of Guangdong Province during the conduct of the study and internal funding from the Guangdong Provincial Hospital of Chinese Medicine for Specific Research and Guangzhou University of Chinese Medicine.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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References

1. Ford AC, Sperber AD, Corsetti M, Camilleri M. Irritable bowel syndrome. *Lancet.* (2020) 396:1675–88. doi: 10.1016/S0140-6736(20)31548-8

2. Mearin F, Lacy BE, Chang L, Chey WD, Lembo AJ, Simren M, et al. Bowel disorders. *Gastroenterology.* (2016) 18:S0016-5085(16)00222-5. doi: 10.1053/j.gastro.2016.02.031

3. Sperber AD, Dumitrascu D, Fukudo S, Gerson C, Ghoshal UC, Gwee KA, et al. The global prevalence of IBS in adults remains elusive due to the heterogeneity of studies: a rome foundation working team literature review. *Gut.* (2017) 66:1075–82. doi: 10.1136/gutjnl-2015-311240

4. Oka P, Parr H, Barberio B, Black CJ, Savarino EV, Ford CA. Global prevalence of irritable bowel syndrome according to Rome III or IV criteria: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol.* (2020) 5:908–17. doi: 10.1016/S2468-1253(20)30217-X

5. Zhang F, Xiang W, Li CY Li CS. Economic burden of irritable bowel syndrome in China. *World J Gastroenterol.* (2016) 22:10450–60. doi: 10.3748/wjg.v22.i47.10450

6. Flacco ME, Manzoli L, De Giorgio R, Gasbarrini A, Cicchetti A, Bravi F, et al. Costs of irritable bowel syndrome in European countries with universal healthcare coverage: a meta-analysis. *Eur Rev Med Pharmacol Sci.* (2019) 23:2986–3000.

7. Maxion-Bergemann S, Thielecke F, Abel F, Bergemann R. Costs of irritable bowel syndrome in the UK and US. *Pharmacoeconomics.* (2006) 24:21–37. doi: 10.2165/00019053-200624010-00002

8. Brandt LJ, Chey WD, Foxx-Orenstein AE, Schiller LR, Schoenfeld PS, Spiegel BM, et al. An evidence-based position statement on the management of irritable bowel syndrome. *Am J Gastroenterol.* (2009) 104(Suppl. 1):S1–35. doi: 10.14309/00000434-200901001-00002

9. Wu JC. Complementary and alternative medicine modalities for the treatment of irritable bowel syndrome: facts or myths? *Gastroenterol Hepatol.* (2010) 6:705–11.

10. FDA. *Guidance for Industry Irritable Bowel Syndrome-Clinical Evaluation of Drugs for Treatment. U.S. Department of Health and Human Services; Food and Drug Administration; Center for Drug Evaluation and Research (CDER).* New Hampshire: The Daily Journal of the United States Government (2012).

11. Eric M, Susan WL, Ke C, Min LS, Xueyong S, Lixing L, et al. Acupuncture for irritable bowel syndrome: systematic review and meta-analysis. *Am J Gastroenterol.* (2012) 107:835–848. doi: 10.1038/ajg.2012.66

12. Jing G, Jian-hua S, Lu C, Hao G, Guo-hui Y, Rongrong S, et al. Bidirectional regulation of acupuncture——subgroup analysis of a multicenter randomized controlled trial of acupuncture with Tiaoshen Jianpi for irritable bowel syndrome. *Chin Acupunct Moxibustion.* (2021) 41:845–850. doi: 10.13703/j.0255-2930.20201111-k0001

13. Wetterslev J, Thorlund K, Brok J, Gluud C. Trial sequential analysis may establish when firm evidence is reached in cumulative meta-analysis. *J Clin Epidemiol.* (2008) 61:64–75. doi: 10.1016/j.jclinepi.2007.03.013

14. Anastasi JK, McMahon DJ, Kim HG. Symptom management for irritable bowel syndrome a pilot randomized controlled trial of acupuncture/moxibustion. *Gastroenterol Nurs.* (2009) 32:243–55. doi: 10.1097/SGA.0b013e3181b2c920

15. Bin L, Lijuan Y, Yi F. Curative effect of acupuncture on diarrheogenic irritable bowel syndrome. *J Beijing Univ Tradit Chin Med.* (2007) 14:19–21.

16. Di Z, Huajun W, Peng L, Yuxin D, Xinhua W, Binbin Y, et al. Clinical observation of back-shu and front-mu points of large intestine with mild moxibustion in irritale bowel syndrome. *Zhejiang J Int Tradit Chin Western Med.* (2016) 26:1096–8.

17. Guojuan M. Acupuncture treatment for depressive symptom in diarrhea-predominant irritable bowel syndrome: a randomized controlled study. *J Acupunct Tuina Sci.* (2019) 17:422–6. doi: 10.1007/s11726-019-1138-3

18. Hao L, Lixia P, Junling Z, Jianhua S. Comparative observation on therapeutic effects between acupuncture and western medicine for diarrhea-predominant irritable bowel syndrome. *Chin Acupunct Moxibustion.* (2012) 32:69–682. doi: 10.13703/j.0255-2930.2012.08.006

19. Haoran C, Xueyong H, Xuejun L, Hongliang C, Yixia D. Clinical research of moxibustion for diarrhea-predominant irritable bowel syndrome. *J Anhui Univ Chin Med.* (2011) 30:33–5.

20. Hongming C, Deying Z, Shuiqing L. Clinical effect of acupuncture therapy of strengthening spleen and soothing live for 35 cases of diarrhea-predominant irritable bowel syndrome with liver depression and spleen deficiency. *J Gansu Univ Chin Med.* (2017) 34:60–3. doi: 10.16841/j.issn1003-8450.2017.05.19

21. Lembo AJ, Conboy L, Kelley JM, Schnyer RS, McManus C, Quilty MT, et al. A treatment trial of acupuncture in IBS patients. *Am J Gastroenterol.* (2009) 104:1489–1497. doi: 10.1038/ajg.2009.156

22. Jing L, Jin L, Jianhua S, Zhizhong R, Dake X, Hao G, et al. Acupuncture with regulating mind and spleen for diarrhea irritable bowel syndrome and sleep quality: a randomized controlled trial. *Chin Acupunct Moxibustion.* (2017) 37:9–13.

23. Jun H. Clinical observation of chinese medicine for diarrhea-predominant irritable bowel syndrome. *Chin Manipul Rehabil Med.* (2017) 8:48–9.

24. Lei X. Clinical observation of acupuncture therapy of strengthening spleen and soothing liver for 54 cases of diarrhea-predominant irritable bowel syndrome. *J N Chin Med.* (2018) 50:112–5. doi: 10.13457/j.cnki.jncm.2018.02.032

25. Lingjun S, Lili F, Wei Q, Yanceng G, Jie S, Qiang S. Effect of heat-sensitive moxibustion combined with trimebutine on expressions of SP.SP and VIP in Patients With IBS-D. *World Latest Med Informat.* (2016) 16:17–18+30. doi: 10.3969/j.issn.1671-3141.2016.93.009

26. Lixia P, Li Z, Jianhua S, Xiaoliang W, Lu C. Constipation predominant irritable bowel syndrome treated with acupuncture for regulating the mind and strengthening the spleen:a randomized controled teial. *Chin Acupunct Moxibustion.* (2015) 35:1095–8. doi: 10.13703/j.0255-2930.2015.11.00

27. Li L, Li-Jun H, Zhi-Min S. Clinical observation of acupuncture at points of liver meridian of foot jueyin in treating diarrhea-predominant irritable bowel syndrome. *J Guangzhou Univ Trad Chin Med.*(2020) 37:279–84. doi: 10.13359/j.cnki.gzxbtcm.2020.02.016

28. Lowe C, Aiken A, Day AG, Depew W, Vanner JS. Sham acupuncture is as efficacious as true acupuncture for the treatment of IBS: a randomized placebo controlled trial. *Neurogastroenterol Motility.* (2017) 29. doi: 10.1111/nmo.13040

29. Mak ADP, Chung VCH, Yuen SY, Tse YK, Wong SYS, Ju Y, et al. Noneffectiveness of electroacupuncture for comorbid generalized anxiety disorder and irritable bowel syndrome. *J Gastroenterol Hepatol.* (2019) 34:1736–42. doi: 10.1111/jgh.14667

30. Park HJ, Cha C. The effect of korean hand acupuncture on young, single korean students with irritable bowel syndrome. *Gastroenterol Nurs.* (2012) 35:403–14. doi: 10.1097/SGA.0b013e318274b1f2

31. Pei L, Geng H, Guo J, Yang G, Wang L, Shen R, et al. Effect of acupuncture in patients with irritable bowel syndrome: a randomized controlled trial. *Mayo Clin Proc.* (2020) 95:1671–83. doi: 10.1016/j.mayocp.2020.01.042

32. Qian W, Kanjun C, Ansheng Y. Therapeutic effects and mechanism of acupuncture on hepatic spleen deficicecy and irritable bowel syndrome. *Chin J Integ Tradit Western Med Digest.* (2019) 27:520–4.

33. Shouqin Z. Therapy of integrated medicine in the treatment of diarrhea predominant irritable bowel syndrome for 30 cases. *Guang J Chin Med.* (2016) 31:2860–2.

34. Sun JH, Wu XL, Xia C, Xu LZ, Pei LX, Li H, et al. Clinical evaluation of Soothing Gan and invigorating Pi acupuncture treatment on diarrhea-predominant irritable bowel syndrome. *Chin J Integrat Med.* (2011) 17:780–5. doi: 10.1007/s11655-011-0875-z

35. Wei X, Songbai F, Aixia T, Xiangwu D, Yanyan H. Therapeutic observation of heat-sensitive moxibustion combined with pinaverium bromide for diarrhea-predominant irritable bowel syndrome and its effects on serum NGF and TNF-α. *Shanghai J Acupunct Moxibustion.* (2020) 39:1094–8. doi: 10.13460/j.issn.1005-0957.2020.09.1094

36. Wenjiao M. Clinical obsevation of acupuncture therapy for 40 cases of diarrhea-predominant irritable bowel syndrome. *Jiangsu J Tradit Chin Med.* (2019) 51:63–65. doi: 10.3969/j.issn.1672-397X.2019.09.022

37. Wenjing Q, Yingjie W. Clinical observation on subcutaneous needling treating 40 cases of diarrhea-irritable bowel syndrome. *World J Integ Tradit Western Med.* (2015) 10:691–3. doi: 10.13935/j.cnki.sjzx.150533

38. Xia Z, Huanhuan J, Hanqing Z, Wenze D, Changbai L, Li W, Jinwang K. Clinical observation of bifidobacterium tetralogy capsule combined with acupuncture and acupuncture for irritable bowel syndrome. *Hebei Med J.* (2017) 39:1857-1859. doi: 10.3969/j.issn.1002-7386.2017.12.029

39. Yanli D. Clinical observation of moxibustion combined with loperamide for irritable bowel synrome. *J Clin Med Pract.* (2017) 21:173–4. doi: 10.7619/jcmp.201724074

40. Yu Q, Wei Y, Shuxiong L, Chunfang Y, Zemin Z. Clinical effect of abdominal acupuncture for diarrhea irritable bowel syndrome. *Chin Acupunct Moxibustion.* (2017) 37:1265–8. doi: 10.13703/j.0255-2930.2017.12.003

41. Wang Z, Xu M, Shi Z, Bao C, Liu H, Zhou C, et al. Mild moxibustion for irritable bowel syndrome with diarrhea (IBS-D): a randomized controlled trial. *J Ethnopharmacol.* (2022) 289:115064. doi: 10.1016/j.jep.2022.115064

42. Bao C, Wu L, Shi Y, Shi Z, Jin X, Shen J, et al. Long-term effect of moxibustion on irritable bowel syndrome with diarrhea: a randomized clinical trial. *Therap Adv Gastroenterol.* (2022) 15:17562848221075131. doi: 10.1177/17562848221075131

43. Shen J, Ye Y, Zhu K, Li S. Acupuncture for diarrhea-predominant irritable bowel syndrome: a randomized control study. *World J Acupunct Moxibustion.* (2022) 32:123–30. doi: 10.1016/j.wjam.2022.02.003

44. Pei LX, Zhang XC, Sun JH, Geng H, Wu LX. [Meta analysis of acupuncture-moxibustion in treatment of irritable bowel syndrome]. *Zhongguo Zhen Jiu.* (2012) 32:957–60. doi: 10.13703/j.0255-2930.2012.10.028

45. Guixing X, Biqing H, Jun X. Systematic review of acupuncture therapies for irritable bowel syndrome. *Chin Arch Tradit Chin Med.* (2016) 34:2171–2174. doi: 10.13193/j.issn.1673-7717.2016.09.035

46. Wei X, Wen Y, Wei Y, Liang X, Ma X, Zhang B, et al. External therapy of traditional Chinese medicine for treating irritable bowel syndrome with diarrhea: a systematic review and meta-analysis. *Front Med.* (2022) 9:940328. doi: 10.3389/fmed.2022.940328

47. Dai Y-Q, Weng H, Wang Q, Guo X-J, Wu Q, Zhou L, et al. Moxibustion for diarrhea-predominant irritable bowel syndrome: a systematic review and meta-analysis of randomized controlled trials. *Compl Ther Clin Pract.* (2022) 46:101532. doi: 10.1016/j.ctcp.2021.101532

48. Wu IXY, Wong CHL, Ho RST, Cheung WKW, Ford AC, Wu JCY, et al. Acupuncture and related therapies for treating irritable bowel syndrome: overview of systematic reviews and network meta-analysis. *Therap Adv Gastroenterol.* (2019) 12:1756284818820438. doi: 10.1177/1756284818820438

49. Shah E, Pimentel M. Placebo effect in clinical trial design for irritable bowel syndrome. *J Neurogastroenterol Motil.* (2014) 20:163–70. doi: 10.5056/jnm.2014.20.2.163

50. Zhao J, Chen M, Wang X, Ye K, Shi S, Li H, et al. Efficacy of acupuncture in refractory irritable bowel syndrome: study protocol for a randomised controlled trial. *BMJ Open.* (2021) 11:e045655. doi: 10.1136/bmjopen-2020-045655

51. Bao C, Zhang J, Liu J, Liu H, Wu L, Shi Y, et al. Moxibustion treatment for diarrhea-predominant irritable bowel syndrome: study protocol for a randomized controlled trial. *BMC Compl Altern Med.* (2016) 16:408. doi: 10.1186/s12906-016-1386-4

52. Devereaux PJ, Beattie WS, Choi PT, Badner NH, Guyatt GH, Villar JC, et al. How strong is the evidence for the use of perioperative beta blockers in non-cardiac surgery? Systematic review and meta-analysis of randomised controlled trials. *BMJ.* (2005) 331:313–21. doi: 10.1136/bmj.38503.623646.8F

53. O'Connell NE, Wand BM. Goldacre B. Interpretive bias in acupuncture research?: a case study. *Eval Health Prof.* (2009) 32:393–409. doi: 10.1177/0163278709353394

54. Manheimer E, Linde K, Lao L, Bouter LM, Berman MB. Meta-analysis: acupuncture for osteoarthritis of the knee. *Ann Intern Med.* (2007) 146:868–77. doi: 10.7326/0003-4819-146-12-200706190-00008

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Acupressure versus parecoxib sodium in acute renal colic: A prospective cohort study

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Background: Here provides a complementary treatment, acupressure at the Qiu acupoint, a novel acupoint, which potentially alleviates renal colic.

Materials and methods: 90 patients were included in this study. Acupressure-group patients (n = 46) were administered acupressure at the Qiu acupoint following a preset protocol. Parecoxib sodium-group patients (n = 44) were administered parecoxib sodium (40 mg) (*via* the direct intravenous route). The visual analog scale (VAS) was used to evaluate pain intensity at baseline and at 1, 5, 10, 20, 30, and 120 min after initiating the intervention. Linear mixed effects model was performed to detect the rate of decrease of VAS per time and their covariant effect on the efficacy of acupressure.

Results: No significant statistical differences in baseline data and VAS scores were observed. The acupressure group obtained lower VAS scores at the 1st, 5th, 10th, and 20th minute than the parecoxib sodium group after initiating the intervention (mean: 4.33 vs. 7.61, mean difference (MD): 3.29, 95% CI: 0.23, 2.84; mean: 2.65 vs. 7.61, MD: 4.96, 95% CI: 4.44, 5.49; mean: 1.63 vs. 6.59, MD: 4.96, 95% CI: 4.48, 5.44; mean: 1.26 vs. 3.64 MD: 2.38, 95% CI: 1.87, 2.88; $P < 0.05$). The markedly effective rate was similar between the two groups. The linear mixed effects model demonstrated that acupressure at the Qiu point was significantly faster than parecoxib sodium in decreasing VAS scores with an estimate of −2.05 (95% CI: −2.51, −1.59, p = 0.000), especially within 10 minutes with an estimate of 0.18 (95% CI: 0.12, 0.25, p = 0.000).

Conclusion: Acupressure at the Qiu acupoint is significantly faster than parecoxib sodium in decreasing VAS scores within 10 minutes.

Clinical trial registration: http://www.chictr.org.cn/, identifier 2100047168.

KEYWORDS

acupressure, parecoxib sodium, renal colic, alternative therapy, NSAIDs

1. Introduction

Acute renal colic is a common symptom presented to the emergency and urology departments, with lifetime risks of 12 and 6% in men and women, respectively (1). Among patients with acute abdominal pain, 31.18% of the cases are caused by acute renal colic (2). Acute renal colic reportedly affects 5–15% of the UK population, often requiring emergent administration (3). Non-steroidal anti-inflammatory drugs (NSAIDs) and opioids are the most common pharmacotherapies for acute renal colic (4). NSAIDs have proven to be effective drugs for acute renal colic and have been recommended by the European Association of Urology guidelines (5). However, the adverse and financial burdens posed by these painkillers should be taken into consideration. NSAIDs are associated with approximately 30% of hospital admissions for drug-related adverse events (6). In particular, older patients are vulnerable to NSAID side effects, such as gastrointestinal toxicity, cardiovascular adverse effects, and nephrotoxicity (7). Furthermore, healthy financial data generated in Australia demonstrated that opioid prescriptions are expected to reach approximately three million by 2030, thus raising Australian healthcare system costs from AUD$ 25.2 million to AUD$ 72.4 million (8). Hence, there is an urgent need to identify an alternative treatment with reduced painkiller-induced side effects and financial burdens.

Although acupressure has been proven to be effective in patients with pain caused by cancers, premenstrual syndrome, and labor (9–11), there is limited evidence corroborating the utility of acupressure for acute renal colic. Herein, we propose a novel alternative treatment, namely, acupressure at the Qiu acupoint. The Qiu acupoint was discovered by YQ, a urologist at the First Affiliated Hospital of Guangzhou University of Chinese Medicine, during his extensive clinical practice in treating acute renal colic for decades. The location of the Qiu acupoint is described in detail in the Section "2 Materials and methods." Our previous clinical observations revealed that acupressure at the Qiu acupoint is advantageous (12). However, strong evidence is still lacking; hence, we performed a prospective cohort study on acupressure at the Qiu acupoint for acute renal colic.

2. Materials and methods

2.1. Study design

This prospective cohort study was conducted at the First Affiliated Hospital of Guangzhou University of Chinese Medicine between June and October 2021. This trial was registered at the Chinese Clinical Trial Registry ChiCTR (ChiCTR2100047168) and approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou University of Chinese Medicine (ethical approval number: NO. ZYYECK [2020] 083). This study was performed and drafted according to the STROBE statement.

2.2. Enrollment criteria

Patients were enrolled if they fulfilled the following inclusion criteria: (1) sudden lumbar and abdominal pain; (2) accompanying percussion pain of the kidney region's affected side; (3) paleness, weakness, nausea, vomiting, and sweating accompanying pain; (4) perineal radiation pain; (5) lower urinary tract symptoms (frequent urination, urgent urination, and pain); (6) anal-irritation symptoms; and (7) microscopic or naked-eye hematuria. Patients who provided consent to participate were included in the study, and their ages ranged from 18 to 65 years.

2.3. Exclusion criteria

Patients with the following conditions were excluded: (1) lumbar and abdominal pain not calculous-related; (2) unconsciousness, mental disease, complications with other serious diseases or unstable vital signs, or inability to cooperate; (3) pregnancy, preparation for pregnancy, or lactation; (4) lumbar skin damage, ulceration, or severe urinary tract infection; (5) allergy to NSAIDs or previous history of gastrointestinal bleeding, active gastric ulcer, or related contraindications prohibited in drug instructions; and (6) history of taking oral calcium blockers and α-receptor blockers within 4 days before inclusion in the study.

2.4. Location of Qiu acupoint

The Qiu acupoint lies one thumb width (body size equal to 1.3 inches) down and one thumb width inside the lumbocostal point. The lumbocostal point is an intersection point crossed by the twelfth floating rib and erector spinae. Herein, we describe a simple method to find the Qiu acupoint. The lumbocostal point was pressed with one thumb, and the other thumb was subsequently pressed on the erector spinae in a position directly facing the thumb that pressed the lumbocostal point. Thereafter, the thumb that pressed on the erector spinae was moved down a distance of one thumb width (body size equal to 1.3 inches) along with the erector spinae, and the position

Abbreviations: VAS, visual analog scale; OR, odds ratio; CI, confidence interval; NSAIDs, non-steroidal anti-inflammatory drugs; MD, mean difference; PSM, propensity score matching.

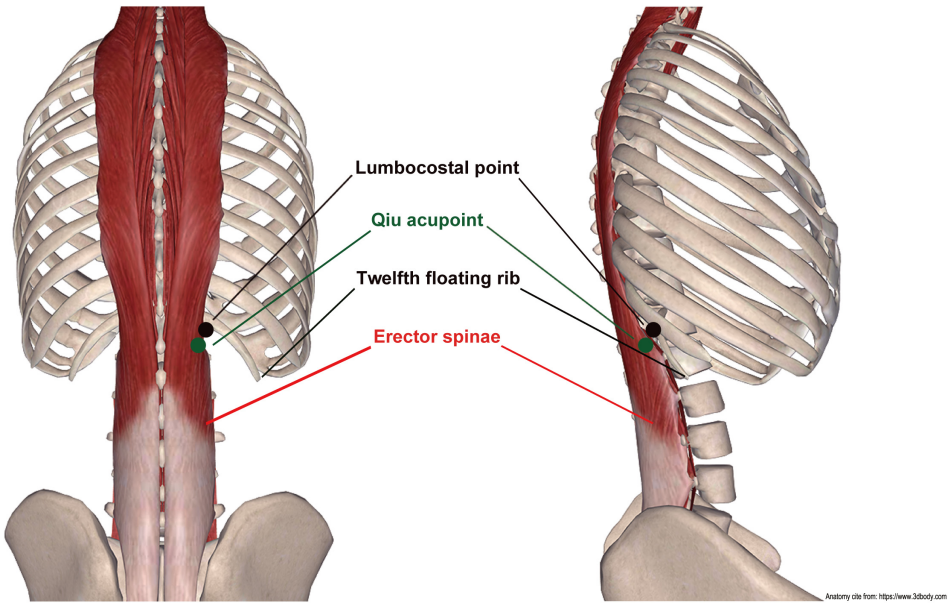

FIGURE 1
Anatomical location of Qiu acupoint (26).

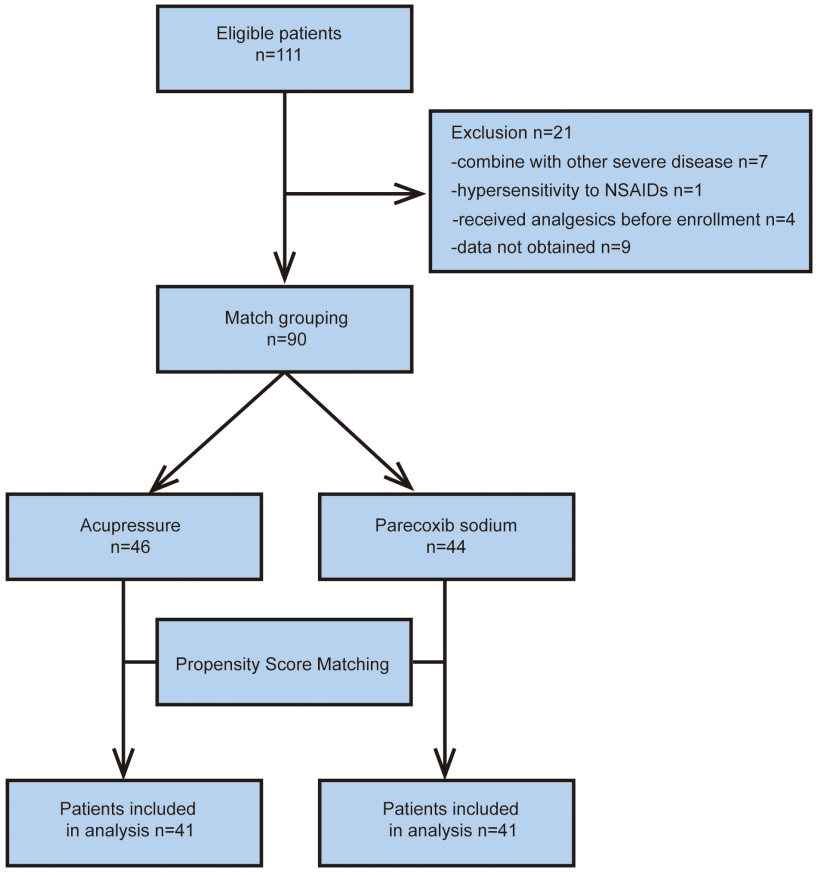

FIGURE 2
Flow chart of the study.

TABLE 1 Baseline demographic and clinical characteristics of enumeration data.

Categorical variable[a]	Before PSM					After PSM				
	Acupressure group (n = 46)		Parecoxib sodium group (n = 44)		P-value	Acupressure group (n = 41)		Parecoxib sodium group (n = 41)		P-value
	No.	%	No.	%		No.	%	No.	%	
Age					0.941					1.000
≤39	13	28.3	11	25		10	24.4	10	24.4	
≥40, ≤59	31	67.4	31	70.5		29	70.7	29	70.7	
≥60	2	4.3	2	4.5		2	4.9	2	4.9	
Gender					0.361					0.821
Male	26	56.5	29	65.9		24	58.5	26	63.4	
Female	20	43.5	15	34.1		17	41.5	15	36.6	
Pain site					0.544					0.653
Left	28	60.9	24	54.5		26	63.4	23	56.1	
Right	18	39.1	20	45.5		15	36.6	18	43.9	
Stone location					0.774					0.856
Renal pelvis and upper ureter	18	39.1	18	40.9		16	39.0	17	41.5	
Middle ureter	10	21.7	7	15.9		9	22.0	7	17.1	
Lower ureter	18	39.1	19	43.2		16	39.0	17	41.5	
Stone size					0.834					1.000
<6 mm	7	15.2	6	13.6		6	14.6	6	14.6	
≥6 mm <10 mm	27	58.7	24	54.5		24	58.5	24	58.5	
≥ 10 mm	112	26.1	14	31.8		11	26.8	11	26.8	
Continuous[b] variable	**Mean**	**SD**	**Mean**	**SD**	0.925	**Mean**	**SD**	**Mean**	**SD**	0.834
Age	45.5	9.0	45.6	9.2		46.1	8.5	45.7	9.4	

[a] Categorical variables were analysis by *chi-square* tests.
[b] Continuous variables were analysis by *t*-tests.

of the thumb was thus above the Qiu point. The location of the Qiu acupoint is shown in **Figure 1** and **Supplementary Video**.

2.5. Exposure

Patients in the acupressure group were administered acupressure at the Qiu acupoint. The detailed protocol is as follows: (1) The patient was placed in a lateral position with the painful side facing upward. (2) The Qiu acupoint was pressed in a force direction of 45° oblique to the spine with the thumb. (3) The power of the thumb was gradually increased for 1 min. The correct sign was the sensation of soreness and distention in the patient. Patients in the parecoxib sodium group were administered parecoxib sodium 40 mg resuspended in 20 ml 0.9% sodium chloride *via* the direct intravenous route.

2.6. Outcomes

The primary outcome was pain intensity, measured using a visual analog scale (VAS) that was 100-mm linear. VAS score ranges from 0 to 10 and every 10-mm represented 1 score. A score of 0 means no pain, and a score of 10 means the most excruciating pain. The VAS score was recorded by the investigator before the intervention and at 1, 5, 10, 20, 30, and 120 min after the intervention. The secondary outcomes were (1) onset time, defined as the time taken to achieve a 50% decrease in VAS score, and (2) the markedly effective rate, defined as the proportion of patients achieving a >60% VAS-score decrease within 30 min relative to each patient's baseline. Adverse events were recorded at treatment initiation and until the patient was discharged from the hospital. Demographic and clinical data were collected from all the patients.

TABLE 2 Outcomes of each group.

	Before PSM								After PSM							
	Acupressure group (n = 46)		Parecoxib sodium group (n = 44)			95% CI		P[a]-value	Acupressure group (n = 41)		Parecoxib sodium group (n = 41)			95% CI		P-value
	Mean	SD	Mean	SD	Mean difference	Lower limit	Upper limit		Mean	SD	Mean	SD	Mean difference	Lower limit	Upper limit	
VAS before treatment	7.60	0.86	7.61	0.87	−0.01	−0.37	0.36	0.978	7.68	0.82	7.61	0.89	0.73	−0.30	0.45	0.700
1 min VAS	4.33	1.23	7.61	0.87	−3.28	−3.74	−2.84	0.000	4.39	1.28	7.61	0.89	−3.22	−3.70	−2.73	0.000
5 min VAS	2.65	1.54	7.61	0.87	−4.96	−5.49	−4.44	0.000	2.71	1.60	7.61	0.89	−4.90	−5.47	−4.33	0.000
10 min VAS	1.63	1.36	6.59	0.84	−4.96	−5.44	−4.48	0.000	1.66	1.39	6.59	0.87	−4.93	−5.43	−4.42	0.000
20 min VAS	1.26	1.44	3.64	0.89	−2.38	−2.88	−1.87	0.000	1.24	1.48	3.66	0.88	−2.41	−2.95	−1.87	0.000
30 min VAS	1.22	1.43	1.55	1.17	−0.33	−0.88	0.22	0.238	1.20	1.47	1.51	1.21	−0.32	−0.91	0.27	0.289
120 min VAS	1.46	1.36	1.14	1.05	0.32	−0.19	0.83	0.216	1.41	1.41	1.15	1.06	0.27	−0.28	0.82	0.334
Onset time (min)	1.15	0.36	15.80	1.89	14.64	14.08,	15.21	0.000	1.17	0.38	15.68	1.89	14.51	13.91,	15.11	0.000
Recurrence in 24 h	Number		Number		Proportion difference				Number		Number		Proportion difference			
Yes	10		8						10		8					
No	36		36					0.673	31		33					0.790
Proportion of Recurrence in 24 h	0.22		0.18		0.04	−0.15	0.21		0.24		0.20		0.04	−0.15	0.24	

[a] Categorical variables were analysis by *chi-square* tests; Continuous variables were analysis by *t*-tests.

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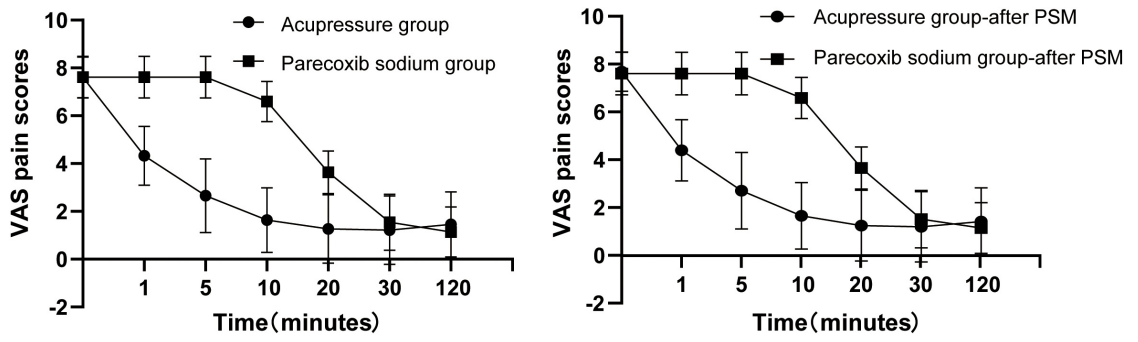

FIGURE 3
Visual analog scale (VAS) decrease between the acupressure group and parecoxib sodium group before and after propensity score matching (PSM).

2.7. Propensity score matching

To adjust the potential confounding factors and baseline. PSM analysis was conducted to reduce these biases, PSM was conducted by creating a new control group from original control group in a way of selecting better matched subjects depending on a similar probability to receive the treatment. To achieve this, matching variables including gender, age, pain site which is defined as left side or right side (detected by ultrasound or X-ray), stone location which is defined as renal pelvis and upper ureter, middle ureter and lower ureter, and stone size which is defined as size <6 mm, 6 mm ≤ size <10 mm, and size ≥1 mm (detected by ultrasound or X-ray) were obtained as independent variables, and groups (control or treatment) were obtained as dependent variables. Those data were analyzed by logistic regression to find out the correlation of each variable with the selected treatment (13). The tolerance in PSM was 0.05 according to the previous report (14).

2.8. Statistical analysis

Variables are expressed as the mean ± standard deviation or values with 95% confidence intervals (CIs). All statistical analyses were performed using SPSS (version 25.0; IBM, Armonk, NY, USA). Student's *t*-test was used to compare the continuous variables in baseline of demographic and clinical characteristics between acupressure group and parecoxib sodium group, the chi-square test was used to compare the categorical variables in baseline of demographic and clinical characteristics, VAS decrease, and markedly effective rate in 30 min between acupressure group and parecoxib sodium group, and the linear mixed effects models were used to compare the rate of decrease of VAS within 10 min between acupressure group and parecoxib sodium group and their covariant effect on the efficacy of acupressure. Statistical significance was set at $P < 0.05$. No data were missed.

3. Results

3.1. Baseline information

A total of 111 patients who met the inclusion criteria were included in the study. Twenty patients were excluded for the following reasons: (1) comorbid severe disease, (2) hypersensitivity to NSAIDs, (3) administration of analgesics before enrollment, and (4) refusal to participate. Ninety patients were divided into two cohorts. Forty-six patients were assigned to the acupressure group and 44 to the parecoxib sodium group. After PSM, both acupressure group and parecoxib sodium group were 41 patients. All patients in the two groups completed the study, and data were obtained for further analysis. Detailed information is shown in the flowchart in **Figure 2**. There were no statistical differences between the two groups in the following baseline characteristics: age, sex, pain site, stone location, and stone size. Details of the baseline characteristics are shown in **Table 1**.

3.2. Effects on VAS-decrease rate and markedly effective rate

As shown in **Table 2**, pre-treatment VAS scores were comparable between the acupressure and parecoxib sodium groups ($P = 0.961$). The median and IQR for the baseline of VAS in the acupressure and parecoxib sodium group were both 8, 1 (before PSM) and 8, 1 (after PSM). Both groups exhibited significant decreases in VAS scores before or after PSM. However, the acupressure group yielded lower VAS scores at the 1st, 5th, 10th, and 20th minute than the parecoxib sodium group (mean: 4.33 vs. 7.61, mean difference (MD): −3.28, 95% CI: −3.74, −2.84; mean: 2.65 vs. 7.61, MD: −4.96, 95% CI: −5.49, −4.44; mean: 1.63 vs. 6.59, MD: −4.96, 95% CI: −5.44, −4.48; mean: 1.26 vs. 3.64 MD: −2.38, 95% CI:−2.88, −1.87), $P < 0.05$ before PSM; (mean: 4.39 vs. 7.61, MD: −3.22, 95%

TABLE 3 Markedly effective rate in 30 min.

	Before PSM					After PSM				
	Acupressure group (n = 46)	Parecoxib sodium group (n = 44)	Proportion difference	95% CI		Acupressure group (n = 41)	Parecoxib sodium group (n = 41)	Proportion difference	95% CI	
				Lower limit	Upper limit				Lower limit	Upper limit
VAS decrease ≥ 60%	40	39				36	36			
VAS decrease < 60%	6	5				5	5			
Proportion	0.869	0.886	−0.017	−0.173	0.143	0.878	0.878	0.000	−0.167	0.167

Analysis by *chi-square* tests: $P > 0.05$.

CI: −3.70, −2.73; mean: 2.71 vs. 7.61, MD: −4.90, 95% CI: −5.47, −4.33; mean: 1.66 vs. 6.59, MD: −4.93, 95% CI: −5.43, −4.42; mean: 1.24 vs. 3.66 MD: −2.41, 95% CI: −2.95, −1.87) $P < 0.05$ after PSM. There was no significant difference in VAS score between the two groups at the 30th and 120th minute (Figure 3). The onset time (time taken to achieve a 50% VAS-score decrease relative to each patients' VAS baseline) was remarkably different between the two groups (1.15 min of acupressure vs. 15.80 min of parecoxib sodium, MD: 14.64, 95% CI: 14.08, 15.21, $P < 0.001$) before PSM and (1.17 min of acupressure vs. 15.68 min of parecoxib sodium, MD: 14.51, 95% CI: 13.91, 15.11, $P < 0.001$) after PSM. These results indicate that acupressure achieved a faster remission of renal colic than parecoxib sodium within 20 min after administration. After the 20th minute, the groups exhibited the same efficacy. Furthermore, the recurrence rate within 24 h was almost the same (0.22 vs. 0.18 95% CI: −0.15, 0.21 before PSM, 0.24 vs. 0.20 95% CI: −0.15, 0.24 after PSM, $P > 0.05$). The markedly effective rate, which was defined as the proportion of patients achieving a >60% decrease within 30 min relative to each patient' s baseline, was similar between the two groups (86.9% for acupressure vs. 88.6% for parecoxib sodium, 95% CI: −0.173, 0.143 before PSM, 87.8 vs. 87.8%, after PSM, 95% CI: −0.167, 0.167 Table 3). The adverse effects are shown in Table 4.

3.3. Linear mixed effects regression

Further linear mixed effects regression analysis was conducted to investigate the rate of decrease of VAS within 10 min between acupressure group and parecoxib sodium group and their covariant effect on the efficacy of acupressure. Covariant including age, sex, pain site (right or left), stone location, and stone size were enrolled in this model. Linear mixed effects regression was performed and the results are shown in Table 5. In this model, the estimate of intercept represented that the VAS score was 7.61 (95% CI: 6.75, 8.47, $p = 0.000$) under no any other factors. The acupressure group

TABLE 4 Adverse effects.

Adverse effects	Acupressure group (n = 46)	Parecoxib sodium group (n = 44)
Nausea and vomiting	0	0
Bloating	0	1
Narcolepsy	0	0
Dizziness	0	1
Rash	0	0
Itching/rash/bleeding at press point	0	0
Total	0	2

TABLE 5 The estimation of visual analog scale (VAS) decreasing rate in linear mixed effects models.

Parameters	Estimate	SE.	Df.	t	Significance	95% CI	
						Lower limit	Upper limit
Intercept	7.61	0.44	617	17.34	0.000	6.75	8.47
Acupressure[a]	−2.05	0.23	617	−8.75	0.000	−2.51	−1.59
Male[b]	0.26	0.13	617	1.96	0.051	0.00	0.52
Pain site: Left[c]	−0.20	0.13	617	−1.46	0.144	−0.46	0.07
Stone location: Renal pelvis and upper ureter	−0.25	0.14	617	−1.76	0.079	−0.53	0.03
Stone location: Middle ureter[d]	0.32	0.18	617	1.80	0.072	−0.03	0.67
Stone size: < 6 mm	0.01	0.22	617	0.04	0.968	−0.42	0.44
Stone size: 6 mm ≤ size < 10 mm[e]	0.12	0.15	617	0.85	0.398	−0.16	0.41
Age	0.01	0.01	617	1.20	0.230	−0.01	0.02
Time 1[f]	−0.34	0.24	617	−14.55	0.000	−0.39	−0.30
Time 2[g]	0.31	0.02	617	12.34	0.000	0.26	0.36
Acupressure * Time 1	−0.15	0.03	617	−4.38	0.000	−0.21	−0.08
Parecoxib sodium * Time 1	–	–	–	–	–	–	–
Acupressure * Time 2	0.18	0.03	617	5.29	0.000	0.12	0.25
Parecoxib sodium* Time 2	–	–	–	–	–	–	–

[a] Compared with the parecoxib sodium group, the same below the interaction models.
[b] Compared with female, the same below the interaction models.
[c] Compared with the pain site: Right.
[d] Compared with the renal pelvis, upper ureter and lower ureter.
[e] Compared with the group of <6 mm and group of ≥10 mm, the same below the interaction models.
[f] Time 1 was continuous variable equal to 0, 1, 5, 10, 20, 30, and 120 min.
[g] Time 2 was continuous variable equal to 0, 0, 0, 0, 10, 20, and 110 min.

could significantly affect the VAS decreasing rate and gained an estimate of −2.05 (95% CI: −2.51, −1.59, p = 0.000) lower than the parecoxib Sodium group. Other covariant such as gender, age, pain site, stone location, and stone size showed no remarkable effect on VAS decreasing rate. The estimate of Time 1 represented the rate of change of VAS per minute among the controls before minute 10 and gained an estimate of −0.34 (95% CI: −0.39, −30, p = 0.000) which meant that is a decrease of 0.34 per minute. Time 2 represented the change of the before minute 10 rate of change of VAS per minute among the controls after minute 10 and gained an estimate of 0.31 (95% CI: 0.26, 0.36, p = 0.000). In another word, that is a decrease of 0.03 (−0.34 + 0.31) per minute past minute 10 among the controls. The interactive model found the estimate of Acupressure * Time 1 represented a difference in rate of change of VAS between the acupressure and the controls before minute 10 with an estimate of −0.15 (95% CI: −0.21, −0.08, p = 0.000), which indicated that acupressure is associated with a further 0.15 decrease per minute compared to the controls. The full rate of decrease for acupressure group before minute 10 is −0.15–0.34 = −0.49 per minute. However, parecoxib sodium group* Time 2 showed a better effect on VAS decreasing rate than acupressure group. The co-effect of them gained an estimate of 0.18 (95% CI: 0.12, 0.25, p = 0.000). It also meant the full rate of change among the acupressure group is 0.18–0.15 + 0.31–0.34 = 0.00 per minute after minute 10.

4. Discussion

With the widespread use of painkillers, including NSAIDs and opioids, side effects, such as nausea, vomiting, constipation, and drowsiness, have also received increasing attention. Thus, an increasing number of alternative therapies have been developed for acute renal colic. Treatment involving active warming of the abdomen and lower back region has been reported to be effective for renal colic (15). Acupuncture also plays an important role in renal colic, a phenomenon that has been proven in several clinical trials (16, 17). Acupressure, which is very similar to acupuncture, is an ancient Chinese treatment. It performs an analgesic function through the stimulation of acupoints using acute pressure, called acupressure. According to Chinese medicine, pressing these acupoints potentially regulates the balance of yin and yang through meridians (18). Current

studies have demonstrated that neurotransmitters, such as serotonin, and the increase in pain threshold mediate the analgesic mechanism through the stimulation of acupoints (18–21).

Like other acupoints, the Qiu acupoint also exerts its effect through acute pressure. However, the Qiu acupoint is not the traditional acupoint that goes through the meridians; it is similar to the A-shi point, which reflects a specific pain area beyond the meridian acupoint, treated using alternative therapies, especially acupuncture and acupressure.

Our data demonstrated that acupressure at the Qiu acupoint achieves a similar effect to that of parecoxib sodium; excitingly, Qiu acupoint has a remarkably rapid effect. In our investigation, VAS scores decreased significantly in the 1st min in the acupressure group; however, an almost similar effect between the same groups was observed in the 30th min. This result is consistent with that obtained by Kaynars et al. (16), in which the acupuncture group experienced a drastic decrease after 10 min compared with the diclofenac and acetaminophen groups. Coincidentally, several studies have reported on analgesic treatment in locations near the Qiu acupoint. Gul and Gul (22) injected sterile water into the triangular area bound by the 12th costal margin, iliac crest, and vertebral spine for renal colic, and it had an effect similar to that of diclofenac sodium by muscle injection. Aydin et al. (23) performed a pilot clinical feasibility study and found that erector spinae plane block with 0.25% bupivacaine achieved a better effect than NSAIDs. The trigger point, which is described as a point located at an area bound by the costal margin, vertebral spine, and iliac crest, has been reported to be effective for renal colic by injecting lidocaine (24, 25). The Qiu acupoint lies near the middle of the triangular area bound by the 12th costal margin, iliac crest, and vertebral spine. This evidence reveals that a certain mechanism exists for analgesia in this triangular area. According to our study, acupressure at the Qiu acupoint appears easy to perform and does not require injection equipment or any medicine. In linear mixed effects regression analysis, we determined the rate of decrease of VAS within 10 min between acupressure group and parecoxib sodium group and their covariant effect on the efficacy of acupressure at the Qiu acupoint and found that acupressure had a better VAS decreased rate within 10 min compared to parecoxib sodium while there was no effect in other clinical factors including age, sex, pain site, stone location, and stone size. These result considered the co-effect of other variants and convinced the result that acupressure at the Qiu acupoint has a quicker Analgesic effect within 10 min compared to parecoxib sodium.

Acupressure has been considered a low-cost, low-risk, environmental- and patient-friendly treatment in China. More importantly, the alternative treatment potentially curtails the addictiveness of many analgesics, such as morphine, and side effects caused by NSAIDs. However, the worldwide promotion of acupressure still faces many challenges. First, there was a lack of high-quality evidence. In addition, the habit of using painkillers is not easy to alter. Thus, we hope our study provides some evidence regarding the utility of acupressure in treating acute renal colic.

Indeed, there are certain limitations to our study. First, the sample size was insufficient, and only a single center was used. We hope that a multi-center study or randomized controlled trials (RCTs) will be performed in the future to strengthen our conclusions. Second, bias was inevitably generated because of the nature of our cohort study compared with that of RCTs. Third, the investigation time of the protocol was limited to 30 min, which was based on the NSAID onset time. The duration of analgesia was also assessed at the 120th min. However, it might have been better to increase the detection time to the 60th and 90th minutes, thus rendering the study more complete.

5. Conclusion

In this study, we demonstrated that acupressure at the Qiu acupoint had a significant effect on reducing pain caused by renal colic, similar to that of parecoxib, but without significant adverse effects. Acupressure was significantly faster than parecoxib sodium in decreasing VAS scores within 10 min. Overall, the foregoing evidence presents an alternative therapy for acute renal colic.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou University of Chinese Medicine (ethical approval number: No. ZYYECK [2020] 083). Written informed consent for participation was not required for this study under the emergency clinic situation.

Author contributions

HoL, YQ, and SX conceived and designed the study. CC and ZZ performed the study, collected and analyzed clinical data. CC wrote and revised the manuscript. ML, ZW, HaL, HM, JW, and MC participated

in discussing the manuscript. All the authors approved the final version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.968433/full#supplementary-material

References

1. Bultitude M, Rees J. Management of renal colic. *BMJ.* (2012) 345:e5499. doi: 10.1136/bmj.e5499

2. Cervellin G, Mora R, Ticinesi A, Meschi T, Comelli I, Catena F, et al. Epidemiology and outcomes of acute abdominal pain in a large urban emergency department: retrospective analysis of 5,340 cases. *Ann Transl Med.* (2016) 4:362. doi: 10.21037/atm.2016.09.10

3. Gandhi A, Hashemzehi T, Batura D. The management of acute renal colic. *Br J Hosp Med.* (2019) 80:C2–6. doi: 10.12968/hmed.2019.80.1.C2

4. Cupisti A, Pasquali E, Lusso S, Carlino F, Orsitto E, Melandri R. Renal colic in pisa emergency department: epidemiology, diagnostics and treatment patterns. *Intern Emerg Med.* (2008) 3:241–4. doi: 10.1007/s11739-008-0145-z

5. Pathan S, Mitra B, Cameron P. A systematic review and meta-analysis comparing the efficacy of nonsteroidal anti-inflammatory drugs, opioids, and paracetamol in the treatment of acute renal colic. *Eur Urol.* (2018) 73:583–95. doi: 10.1016/j.eururo.2017.11.001

6. Bindu S, Mazumder S, Bandyopadhyay U. Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: a current perspective. *Biochem Pharmacol.* (2020) 180:114147. doi: 10.1016/j.bcp.2020.114147

7. Wongrakpanich S, Wongrakpanich A, Melhado K, Rangaswami J. A Comprehensive review of non-steroidal anti-inflammatory drug use in the elderly. *Aging Dis.* (2018) 9:143–50. doi: 10.14336/ad.2017.0306

8. Ackerman I, Zomer E, Gilmartin-Thomas J, Liew D. Forecasting the future burden of opioids for osteoarthritis. *Osteoarthr Cartilage.* (2018) 26:350–5. doi: 10.1016/j.joca.2017.11.001

9. Armour M, Ee C, Hao J, Wilson T, Yao S, Smith C. Acupuncture and acupressure for premenstrual syndrome. *Cochrane Database Syst Rev.* (2018) 8:CD005290. doi: 10.1002/14651858.CD005290.pub2

10. He Y, Guo X, May B, Zhang A, Liu Y, Lu C, et al. Clinical evidence for association of acupuncture and acupressure with improved cancer pain: a systematic review and meta-analysis. *JAMA Oncol.* (2020) 6:271–8. doi: 10.1001/jamaoncol.2019.5233

11. Smith C, Levett K, Collins C, Dahlen H, Ee C, Suganuma M. Massage, reflexology and other manual methods for pain management in labour. *Cochrane Database Syst Rev.* (2018) 3:CD009290. doi: 10.1002/14651858.CD009290.pub3

12. Zhigang WY. Analysis of the superiority of qiu acupoint in the treatment of acute renal colic. *J Chengdu Univ Tradit Chin Med.* (2018) 02:45–8. doi: 10.13593/j.cnki.51-1501/r.2018.02.045

13. Kane L, Fang T, Galetta M, Goyal D, Nicholson K, Kepler C, et al. Propensity score matching: a statistical method. *Clin Spine Surg.* (2020) 33:120–2. doi: 10.1097/bsd.0000000000000932

14. Zhu Y, Xia X, Gao L, Zhu X, Diao W, Liang Z, et al. Prognostic implications of human papillomavirus type 16 status in non-oropharyngeal head and neck cancer: a propensity score matching analysis. *Ann Transl Med.* (2019) 7:759. doi: 10.21037/atm.2019.11.72

15. Davenport K, Timoney A, Keeley F. Conventional and alternative methods for providing analgesia in renal colic. *BJU Int.* (2005) 95:297–300. doi: 10.1111/j.1464-410X.2005.05286.x

16. Koyuncu F, Buldu İ, Tekinarslan E, Tepeler A, Karatağ T, İstanbulluoğlu M, et al. Comparison of the efficacy of diclofenac, acupuncture, and acetaminophen in the treatment of renal colic. *Am J Emerg Med.* (2015) 33:749–53. doi: 10.1016/j.ajem.2015.02.033

17. Lee Y, Lee W, Chen M, Huang J, Chung C, Chang L. Acupuncture in the treatment of renal colic. *J Urol.* (1992) 147:16–8. doi: 10.1016/s0022-5347(17)37121-5

18. Luo D, Wang X, He J. A comparison between acute pressure block of the sciatic nerve and acupressure: methodology, analgesia, and mechanism involved. *J Pain Res.* (2013) 6:589–93. doi: 10.2147/jpr.S47693

19. Lee I, Cheon S, Park J. Central and peripheral mechanism of acupuncture analgesia on visceral pain: a systematic review. *Evid Based Complement Alternat Med.* (2019) 2019:1304152. doi: 10.1155/2019/1304152

20. Qiao L, Guo M, Qian J, Xu B, Gu C, Yang Y. Research advances on acupuncture analgesia. *Am J Chin Med.* (2020) 48:245–58. doi: 10.1142/s0192415x20500135

21. Wang W, Shao X, Shag B. [Influence of "Sancai" acupuncture treatment on plasma and urine SP, 5-HT levels of renal colic patients]. *Zhen Ci Yan Jiu.* (2013) 38:152–7.

22. Gul A, Gul M. Intracutaneous sterile water injection for pain relief during extracorporeal shock wave lithotripsy: comparison with diclofenac sodium. *Urolithiasis.* (2020) 48:103–8. doi: 10.1007/s00240-019-01147-9

23. Aydin M, Tekin E, Ahiskalioglu E, Ates I, Karagoz S, Aydin O, et al. Erector spinae plane block vs non-steroidal anti-inflammatory drugs for severe renal colic pain: a pilot clinical feasibility study. *Int J Clin Pract.* (2021) 75:e13789. doi: 10.1111/ijcp.13789

24. Eken C, Durmaz D, Erol B. Successful treatment of a persistent renal colic with trigger point injection. *Am J Emerg Med.* (2009) 27:252.e3–4. doi: 10.1016/j.ajem.2008.06.026

25. Iguchi M, Katoh Y, Koike H, Hayashi T, Nakamura M. Randomized trial of trigger point injection for renal colic. *Int J Urol.* (2002) 9:475–9. doi: 10.1046/j.1442-2042.2002.00504.x

26. 3Dbody. *Premium Medical Image Digital Content*. Available online at: https://www.3dbody.com (accessed October 10, 2021).

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Suggesting a mechanism for acupuncture as a global percutaneous needle fasciotomy that respects tensegrity principles for treating fibromyalgia

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Acupuncture is a minimally invasive therapeutic method that uses small caliber needles while inserting them through the skin into various areas of the body. Some empirical studies find evidence to support the use of acupuncture as a treatment for certain medical conditions, however, this peculiar practice is widely considered as the domain of alternative and non-evidence-based medicine. Several mechanisms have been suggested in an attempt to explain the therapeutic action of acupuncture, but the way in which acupuncture alleviates chronic non-cancer pain or psychosomatic and psychiatric disorders is not fully understood. A recent study suggested a theoretical model (coined "Fascial Armoring") with a cellular pathway to help explain the pathogenesis of myofascial pain/fibromyalgia syndrome and functional psychosomatic syndromes. It proposes that these syndromes are a spectrum of a single medical entity that involves myofibroblasts with contractile activity in fascia and aberrant extracellular matrix (ECM) remodeling, which may lead to widespread mechanical tension and compression. This can help explain diverse psycho-somatic manifestations of fibromyalgia-like syndromes. Fascia is a continuous interconnected tissue network that extends throughout the body and has qualities of bio-tensegrity. Previous studies show that a mechanical action by needling induces soft tissue changes and lowers the shear modulus and stiffness in myofascial tissue. This hypothesis and theory paper offers a new mechanism for acupuncture therapy as a global percutaneous needle fasciotomy that respects tensegrity principles (tensegrity-based needling), in light of the theoretical model of "Fascial Armoring." The translation of this model to other medical conditions carries potential to advance therapies. These days opioid overuse and over-prescription are ubiquitous, as well as chronic pain and suffering.

KEYWORDS

acupuncture, fascial armoring, fibromyalgia, mechanism, myofibroblast, needling, pain, treatment

1. Introduction

Acupuncture is a traditional Chinese treatment method using fine needles that are inserted into various areas of the body. The origins of this strange practice date back several thousand years to the far East, with the canonical text established in the first century BC and known as the Inner Classic of the Yellow Emperor (1). Suggested mechanisms to explain acupuncture include neurohormonal pathways and induction or release of anti-nociceptive

substances, activation of endogenous opioid mechanisms, stimulation of neuropeptide gene expression, the gate control theory, immunomodulation, reflex and spinal nerve stimulation followed by autonomic modulation, mechanical cellular signaling pathways, psychological or placebo effect, and "Qi energy" (1–5). While several explanations exist for its mode of action, acupuncture is often perceived as non-scientific. Until relatively recently, many in the west considered it the Chinese equivalent of voodoo (1). Despite the existence of some empirical evidence to support this therapeutic method for certain medical conditions, many physicians do not recommend acupuncture to their patients due to lack of a comprehensive theoretical model to explain it. Indeed, the treatment should be based on the pathophysiology of the disease. Often grouped with other alternative methods under one eastern umbrella, acupuncture stands out of the rest because it appears to be a minimally invasive surgical intervention of the fascia.

A recent study suggested a theoretical model (coined "Fascial Armoring") with a cellular pathway to help explain the mechanism and pathogenesis of "functional somatic syndromes" (6). Syndromes such as "fibromyalgia," "chronic fatigue syndrome," "somatic symptoms disorder," "myofascial pain syndrome," and other psychosomatic/functional/"non-specific" conditions are suggested to be on a spectrum of a single medical entity (6–8). The underlying pathophysiology of these syndromes is suggested to involve, among several things, myofibroblasts in fascia that possess contractile activity with a positive feedback loop of force generation (6). This abnormality can lead to mechanical tension in the fascial tensegrity system and compression of anatomical structures, which can help explain the symptomatology and manifestations of fibromyalgia-like syndromes. Fibromyalgia is a chronic-widespread-pain-and-fatigue disorder with an unknown mechanism or cause, with much stigma. It affects 2–6 percent of the population (7) and is often managed by primary care physicians and rheumatologists. Current treatments are insufficient, causing frustration among patients and clinicians alike. No cure is known to exist. The opioid crisis in our society is ongoing, suffering is prevalent, and the social and economic burden of chronic pain conditions is high. In this paper the theoretical model of Fascial Armoring is presented in order to then discuss a new mechanism of action for acupuncture.

The following discussion is based on a literature review and aims to offer a new mechanism for acupuncture as a global percutaneous needle fasciotomy that respects tensegrity principles in light of the framework of Fascial Armoring. According to this suggested model, a decompressive mechanical treatment that applies multiple needles in different areas of the tensegrity system might be utilized to treat the fascial abnormality in myofascial pain/fibromyalgia syndrome and functional psychosomatic syndromes. Insertion of multiple needles, while letting the system completely rest, might allow internal fascial forces to tear fibers and release tensions in the system (6). It is a more yielding approach with less pain. In addition, compared to western dry needling (which elicits pain and may not properly treat the underlying disease), more compliance is expected from patients using this method. The holistic view of acupuncture aligns with the continuity and pervasiveness of fascia. In the long term, a method that respects tensegrity principles is expected to be much more effective as a treatment and prophylaxis of fibromyalgia-like diseases.

Abbreviations: α-SMA, alpha smooth muscle actin; DNA, deoxyribonucleic acid; ECM, extracellular matrix; TBN, tensegrity-based needling.

The key points setting the ground for this paper's proposed hypothesis are as follows:

- Acupuncture is an intriguing ancient technique, considered by many as alternative medicine, and still lacks a clear mechanism to explain its practical application and theory.
- Fascia and proto/myo/fibroblasts phenotypes may have a major role in the pathophysiology of "non-specific" pain and psychosomatic syndromes.
- Fibromyalgia patients are found to have pathological changes in their extracellular matrix (ECM) and myofascial tissue.
- The theoretical model of Fascial Armoring integrates biomechanical properties of fascia and biotensegrity principles to propose a treatment for fibromyalgia-like conditions.
- A new mechanism of action for needling therapy was recently offered, whereby needling fascia relieves mechanical abnormalities in myofascial tissue and in the network of myo/fibroblasts, while taking into consideration the concept of tensegrity.

2. A brief explanation of the theoretical model of fascial armoring

In order to unfold the therapeutic action of acupuncture as a global percutaneous needle fasciotomy that respects tensegrity principles, we must first look at the model of fascial armoring and the process of myofibroblast force generation in the bio-tensegrity system of fascia. Fascial armoring is a theoretical model that was developed to help explain the pathogenesis of fibromyalgia-like syndromes (6). Functional psychosomatic syndromes are suggested to be on a spectrum of a single medical entity (7, 8), which may share a common rheuma-psycho-neurological mechanism (6). The fascial armoring model focused on the rheumatological facet of this medical entity. It suggests that these syndromes are driven, in part, by myofibroblast-generated-biotensegrity-tension, i.e., a network of myo/fibroblasts with contractile activity in fascia causing a connective tissue abnormality of the fascial biotensegrity system, leading to widespread mechanical compression (6).

The cellular pathway of the pathophysiology of fibromyalgia was suggested to involve mechano-signaling of myo/fibroblast phenotype cells in fascia (e.g., integrin and focal adhesion, Rho-associated-kinase, alpha smooth muscle actin, and transforming growth factor beta pathways) (6). Widespread mechanical tension and fascial rigidity might help explain several of fibromyalgia's manifestations such as: pain, distribution of pain, decreased pressure pain threshold, chronic fatigue, morning stiffness, cardiovascular and metabolic abnormalities, autonomic abnormalities, small fiber neuropathy, close association with hypermobility syndrome, various somatic symptoms, absence of clear inflammation, silent imaging investigations, and other phenomena (e.g., occasional complete resolution soon after surgery) (6). The following findings are the basis for the theoretical model of fascial armoring: (i) Myofibroblasts can cause long-term contractures in tissue; they synthesize alpha-smooth-muscle-actin (α-SMA) fibers that allow them to lock in tension in the ECM in a slip and ratchet mechanism (9). Tomasek et al. described the complex biological and mechanosensitive activity of myofibroblasts and their stress shielding. Forces generated in ECM are maintained and reinforced over time by matrix remodeling and

collagen deposition, accompanied by simultaneous myofibroblast contraction and force generation (9). (ii) Fascia forms a continuous network throughout the body and has qualities of tensegrity (or biotensegrity) (6, 10–12). Tensegrity is an architectural term describing how compressive and tensile element forces enable the dynamic stabilizing of one connected structure (10, 11). The term "biotensegrity" integrates complex biological aspects of living systems into a tensegrity biomechanical model where each "separate part" of the system is valued with relation to the whole (12). (iii) Fascia can transmit forces in the body to a distance with mechanical forces able to travel along myofascial chains (13). (iv) Fascia is able to actively contract (14). Myofibroblasts are present in fascia of normal individuals and can cause long term pathological contracture as shown in studies of Schleip and Klingler (15). (v) Soft tissue fibroblasts form a widespread interconnected cellular network with potentially critically overlooked physiological and functional importance (16).

These findings help form a theoretical model of fascial armoring-the creation of generalized tension and compression due to a widespread myofibroblast network in a stiff and rigid fascial system. Empirical evidence in support of the fascia-based pathogenesis of fibromyalgia, with widespread compression, can be found in studies such as:

1) A study by Katz et al. found significantly higher intramuscular pressures in trapezius muscle of fibromyalgia patients. The mean value of intramuscular pressure in the patient group was 33.48 mmHg compared to 12.23 mmHg in controls (17). The authors of the study stated that the burden of the pressure abnormality might contribute to diffuse muscle pain in fibromyalgia and may be an intrinsic feature of the disease. Therefore, fibromyalgia as a disease of exclusively central neuroplastic pain should be reconsidered (17).
2) Biopsies of fibromyalgia cases indicate definite and non-specific muscle changes which are suggested to be due to chronic muscle contraction and ischemia of unknown etiology (18).
3) Decreased peripheral blood flow in fibromyalgia patients was found, which suggests there are functional disturbances in their cardiovascular system (19).
4) In muscles of fibromyalgia patients, increased DNA fragmentation and ultrastructural changes are suggested to be secondary to chronic muscle contraction (20).
5) In a biopsy study of fibromyalgia patients, peripheral fibroblast transforming growth factor-β gene expression is significantly higher compared to controls (21).
6) The protein expression of genes involved in ECM turnover and oxidative metabolism has a differential expression in fibroblast of fibromyalgia patients, which could explain the inflammatory status of these patients (22).
7) A significantly lower amount of intramuscular collagen was found in fibromyalgia patients, which may cause a lower threshold for muscle micro-injury and result in non-specific signs of muscle pathology (23).

An elaboration and analysis with further empirical evidence that support fibromyalgia as an entity involving widespread mechanical compression can be found in a recent study (6). According to the model of fascial armoring, unhealthy lifestyle (e.g., sedentarism, western diet, obesity, and mechanical factors such as tight clothes) is suggested to be a major contributor to "functional-psycho/somatic syndromes" by stimulating fascial myofibroblasts. Based on the model, any factor that induces proto/myofibroblasts in fascia may increase risk for, or lead to, a fibromyalgia-like condition. It was hypothesized that a sever manifestation of fascial armoring might manifest as a mild-to-moderate-global-chronic-compartment-like syndrome, which may help explain various organic findings in fibromyalgia suggesting long-standing low-grade widespread ischemia (6).

3. Hypothesis

A treatment that uses multiple needles inserted in various areas of the body while respecting the complex properties of fascia is hypothesized to modulate the fascial bio-tensegrity system, thereby relieving mechanical tension in myofascial and subcutaneous tissue (6). I suggest acupuncture acts mechanically as a global percutaneous needle fasciotomy that respects the biotensegral properties of fascia (i.e., "tensegrity-based needling") and can shift the state of the system to a more balanced state. Studies show that inserting dry needles has mechanical effects on fascia and alleviates pain (24–32). Modulating the bio-tensegrity structure and fascia may be a way to relieve mechanical pressure and treat the underlying fascial abnormality in fibromyalgia-like diseases (6). Tensegrity-based needling/acupuncture (needling while respecting qualities of tensegrity) is expected to lower the shear modulus of fascia over multiple sessions, and ultimately lead to significant effects on myofibroblasts in fascia through mechanosensitive signaling and changes in tissue stiffness (6). Fibromyalgia, even though currently defined as a central neuroplastic pain disorder, is dependent on peripheral input of nociception. Therefore, I reason that acupuncture might be able to treat the fascial abnormality of fibromyalgia-like diseases to help induce and sustain remission.

4. Methods

This work is based on a narrative review that used key phrases on fascia, myofascial pain, fibromyalgia, and acupuncture. The databases used were MEDLINE, EMBASE, COCHRANE, PEDro, and medRxiv. Systematic searches for keywords on fascia, fibromyalgia, acupuncture, and myofascial pain, were done between September 2020 and 6 February 2022. Non-systematic searches for keywords on related topics were done in PUBMED. Inclusion/exclusion based on title and abstract, then full text inspection. Items off topic and foreign language were excluded. A data charting form was used to abstract key data, as well as a summary document that was updated in an iterative process-that document evolved into this review. A total of 831 items were included in the literature of this study for the purpose of offering a new hypothesis and theory on acupuncture based on the model of fascial armoring.

5. Needling

Empirical studies suggest needling exerts mechanical effects on fascia and a mechanical action of needling itself relieves pain (24–26). Needling therapy lowers the shear modulus and stiffness of myofascial tissue (27–29). Langevin et al. show that the insertion of

needles into fascia has important effects on surrounding tissue and cells (30–32). Fibroblasts contract upon release of tension in attached collagen lattices (33). Fibroblasts create tension in the attached matrix and when tension is released cells undergo contraction with a rapid contraction of the collagen matrix (9). It was suggested that needling may help relieve mechanical tension from the fascial fiber-cellular network and modulate the bio-tensegrity structure as a possible treatment for fibromyalgia (6). Evidence for the efficacy of needling techniques in relieving chronic pain and myofascial pain conditions can be found in literature (34–38). Two main types of mechanical needling therapy (without injection) are found in the literature which are: (i) western dry needling, and (ii) traditional Chinese medicine's acupuncture. I would like to discuss these two methods in light of principles of the bio-tensegrity model of facial armoring, a framework called tensegrity-based needling (TBN) (6).

5.1. Dry needling

Dry needling is a relatively new minimally invasive method developed in modern medicine. It still lacks a comprehensive theory to fully explain its mode of action in myofascial and "non-specific" or psychosomatic diseases, mainly because these conditions lack a clear pathophysiology and mechanism themselves. Ideally, any treatment method should theoretically conform to the pathophysiology of the disease that it aims to treat. Some empirical studies support western dry needling, although most of the studies examine its short/medium-term effects, whereas the long-term effects are still unclear (36, 39–41).

Western dry needling can be seen as a variation of trigger point injection (without the injection) or an uncomplicated variation of acupuncture. Used often to treat myofascial pain and trigger/tender points, dry needling can be extremely painful for the patient (29), some might even call it abusive. In dry needling for myofascial trigger-points, a needle is often inserted forcefully and repeatedly deep into the trigger/tender point during a session (e.g., fast-in and fast-out/pistoning/sparrow pecking maneuver, possibly in a fan or cone shape), with the purpose of "destroying" the trigger point or trying to induce edema, positive inflammation, and self-healing processes; or aiming to relieve the pain through spinal cord afferent signaling or other mechanisms (42–45). However, as many clinicians' experiences might indicate, these mysterious painful points recur a short while after treatment too often, if not in the same site, then in a different one. Interestingly, sometimes pain migrates.

Many patients are deterred from repeating this type of treatment due to extreme pain elicited by needling (called "post-needling soreness") (46). If the trigger point recurs, either in the same spot or a different one, the needling procedure can be repeated. However, in cases where the patient's pain eventually becomes diffuse and widespread, the underlying diagnosis can be altered to a psychologically driven pain or "chronic widespread pain"/"fibromyalgia," for which the treatment nowadays is often psychological or psychiatric. Cognitive behavioral therapy is one such modality for diffuse pain (47). Noteworthy, chronic widespread pain is not considered by some certified clinicians as a real organic medical condition to treat (48). Fibromyalgia is one example of such a prevalently neglected and stigmatized medical condition (where many certified clinicians actually think that the patient is imagining the pain).

5.2. Acupuncture

Acupuncture is an important therapeutic method in East Asian medicine. Its theoretical and philosophical framework differs from that of the modern West. East Asian medicine tries to formulate a qualitative diagnosis in the overall entirety of a person's state, signs, and behaviors. The west's biomedical approach aspires to achieve a scientific and measurable quantitative assessment of an organic biological process (1). Eastern philosophy, such as yin-yang and Qi, is integrated into acupuncture and is one of the reasons for the difficulty of accepting it in western thought and science (1). Moreover, acupuncture uses a network like view of the human body with channels of flow of energy that can be needled in certain points to correct an imbalance or to modulate internal elemental life energies. Multiple needles are gently inserted in various area of the body, sometimes in a distance from the chief area of complaint. The scientific measurable justification for the theory and philosophy behind acupuncture is not an issue for traditional eastern thought and practitioners (1).

Evidence supporting the use of acupuncture in chronic pain and "psychosomatic" conditions can be found in literature (49–53). The mechanism of acupuncture is not fully understood, and many theories have been proposed to explain it, e.g., neurohormonal pathways and induction or release of anti-nociceptive substances, activation of endogenous opioid mechanisms, stimulation of neuropeptide gene expression, immunomodulation, the gate control theory, mechanical cellular signaling pathways, Qi energy, psychological effect, placebo effect, and so forth (1–5).

Overall, acupuncture is considered safe when performed by a competent trained practitioner (1, 54, 55). In rare circumstances, acupuncture can cause complications such as those associated with any other type of needle use (dizziness, minor hemorrhage, pain, infection, or more serious events such as pneumothorax) (1, 56–58). Adverse events usually occur when the acupuncture practitioner has inadequate training (1).

5.3. Tensegrity-based needling

Tensegrity-based needling (TBN) was suggested as a treatment method based on the facial armoring model of myofascial pain/fibromyalgia syndrome (6). It utilizes a tensegrity network conceptual framework to treat "functional psycho/somatic" or "non-specific" pain conditions. Tensegrity states that structures (or tensegrity systems) are stabilized by continuous tension with discontinuous compression, and function as one connected system (10–12). TBN suggests that needling therapy should take into consideration the bio-tensegrity qualities of fascia and its interconnectedness and continuity throughout the body. Needles are inserted in different areas of the system in order to lower global fascial stiffness and aim to collapse the tension of the system in a synchronized and directed manner (6).

The forces imposed in muscles are distributed over the tissue as a whole and transmitted by intramuscular connective tissue (59). Skeletal muscles are linked by fascial tissue and form a body-wide network of multidirectional myofascial continuity (60). A 2004 study suggests that soft tissue fibroblasts form an extensively interconnected cellular network, suggesting they may have important overlooked physiological functions (16). Fascia has contractile

abilities and contains contractile cells (proto/myofibroblasts) (14, 15). A fascial myo/fibroblast network was suggested to cause long term contracture and tensegrity tension in fascia; therefore, needle insertion throughout this network is expected to cause mechanical disruption of the ECM's fiber-cellular network and modulate abnormal tensions in the fascial system (6).

TBN is based on three elements of fascial armoring. These are (i) tensegrity mindset in the continuity of the fascial system (ii) trigger points and satellites (iii) a proto/myo/fibroblast population (network) creating tension, contracture, and stress shielding.

The mechanical action of needling has physiological and therapeutic importance: studies suggest the needle insertion itself relieves pain (25, 26, 37), which might suggest needling creates a focus for a mechanical tearing action on fascia (6). Needling exerts mechanical effects on tissues (30–32). Under the framework of fascial armoring, the tension and shear stress inside connective tissue pull on the point of fine needle insertion until the sum force vectors (or horizonal components) at that point is eliminated or needle is removed (6). Eliminating tensional forces and cutting off fascial fibers causes cells to rapidly contract and subsequently lose their stress fibers and adhesion complexes (9). Myofascial trigger points and satellites were suggested to be manifestations of myofibroblast populations in fascia with mechanical tensegrity links (6). Myo/fibroblasts create tension in the attached matrix; when tension is released cells undergo contraction with rapid contraction of the ECM (9). Studies observe changes in contractility and stiffness in fascia, including "needle grasp" phenomenon when needles are inserted (30–32). Needle delivery systems deform and move soft tissue and organs (61, 62). These mechanical effects may suggest that inserting a needle causes windup of connective tissue and modulation of the fascial system's fiber-cellular network, guided by its internal forces and contractile cells. Studies suggest needling affects facia both at the insertion point and further at a distance (3, 32, 61, 63). Damage to fascia inflicted by a needle might actually allow for eliminating tensions between tense nodes in the network of (myofibroblasts) myofascial trigger-points and satellites. If inserted deep enough, it might also free edematous fluid, hyaluronic acid, and other factors trapped within the layers (e.g., serotonin). Micro-dialysis studies of trapezius muscle show myofascial pain syndrome patients have increased concentration of nociceptive and inflammatory substances in myofascial tissue (e.g., substance P, interleukins, tumor necrosis factor alpha, etc.) (64–66), and fibromyalgia patients have higher levels of lactate and pyruvate in myofascial tissue (67). Relieving mechanical tension might improve prefusion and lymphatic drainage, leading to better clearance of nociceptive and inflammatory substances (6). While one study suggests needling can improve blood perfusion (68), another study showed infiltration of trigger points can improve symptoms of intermittent claudication (69).

In short, TBN is expected to gently change the dynamics of the tensegrity structure and shift the state of the system to a more balanced state. The theoretical end purpose for this treatment would be to progressively collapse the tension of the system in a concentric way throughout multiple sessions by passively inserting multiple needles and then allowing the network of cells to lower global fascial stiffness beneath the threshold necessary for myofibroblasts activity (∼10–20 kPa) (6). One study suggested tissue stiffness of ∼20 kPa is the threshold for robust myofibroblast α-SMA synthesis (70). Upon complete freeing of tension in the attached matrix, myofibroblasts undergo apoptosis with reduction of α-SMA (71). Each intrafascial/subcutaneous needling treatment should be adapted to the current state of the bio-tensegrity structure. Ignoring tensegrity principles is expected to lead to more pain, either in the immediate, short, or long term. As an example, to illustrate this model, limb amputation is expected to lead to high risk of chronic pain due to a new imbalance in the fascial system (6). Studies suggest needling lowers myofascial tissue stiffness as measured by shear wave elastography (27–29). Releasing tensions in ECM might also relieve stretch lesions along sensory/sympathetic nerves (6). The more stress-shielding is present, the longer it should take until significant symptomatic improvement is achieved. Under this framework, needling therapy actively breaks the connections in the tensegrity-like network of fascia and then allows for new healthier connections to form *via* natural remodeling of the ECM (6). After modulation of the tensegrity structure, fascia will regenerate *via* myo/fibroblasts. Studies show fascia regenerates within several months after fasciectomy/fasciotomy (72, 73).

Under the framework of tensegrity and fascial armoring, one might consider first needling the dorsal foot and foreleg in plantar fasciitis, for example, if connective tissue is pulling the plantar fascia and triggering pain. Thus, aiming to treat the underlying tensional abnormality in the connective tissue, and not simply the symptoms of pain. Importantly, needling the foreleg would change other areas of the tensegrity system, because tensegrity domes are made of many nodes- and changing one node changes the dome. **Figures 1**, **2** depict the framework of the fascial armoring model as a geodesic dome to illustrate fascia as a complex connected system susceptible to imbalance.

Realizing the tensegrity qualities of fascia, it seems that a trigger point with severe localized pain may not necessarily be the source of pathological tension because myofibroblasts stress shield their area from tension. Targeting only the symptom of pain during treatment is expected to lead to an endless chase of migrating pain whose location is determined by dynamics of the bio-tensegrity system (6). Tension may have a tendency of developing near hard and angled surfaces with sharp force-gradients. Tension and myofibroblasts in connective tissue will be present, but pain depends on the degree of sensory nerve involvement (6). Developing a therapeutic method that treats such a complex dynamic affair is not a trivial matter.

6. Discussion

In 1815 Dr. William Balfour described his method of "curing" patients with "rheumatism" (i.e., fibromyalgia) (74). Balfour applied bandages to treat patients with fibromyalgia or chronic "non-specific" pain at the area of complaint and saw immediate improvement in pain, joint range of motion, and ability to move. However, some patients returned a short while later with pain that migrated to a different area. The "cure," as it seems, was only symptomatic and temporary (e.g., the case of Mrs. M. pp. 179–180) (74). Mechanical signals feed input into the myofibroblast positive feedback cascade of transforming growth factor beta-1 and α-SMA synthesis, which can lead to further contraction and tension in the ECM in a detrimental loop (9). This suggests that Balfour's method modulates the tensegrity dynamics but in an opposite way compared to needling and acupuncture (6). Bandages (as well as taping and compression techniques) can modulate the tensegrity structure and/or induce

FIGURE 1
A geodesic model of the bio-tensegrity structure. Each sheet may represent an area of fascia in the system. Each "sheet" has its spring constant set by fascial qualities and myofibroblast generated forces. Mechanical forces can travel to other areas through connections in the structure and myofascial chains. With permission from PACIFIC DOMES Inc. www.eventdome.wordpress.com. Reprinted from https://eventdome.files.wordpress.com/2010/07/bm-multi-colored.jpg, with permission from Sequoia Miller from Pacific Domes Inc.

FIGURE 2
A bio-tensegrity structure with imbalance. Nerves can be affected by internal fascial forces and sense tension to send pain signals. This happens more in weaker areas of the structure, i.e., areas that are pulled by myofascial tissue and myofibroblast generated forces, for example, as seen on the right side of the structure when the dome is facing the observer. Needling the painful area does not address the underlying cause for the pain, which may be increased tension generated by the left side of the structure (when facing the observer). To balance the structure, one should needle the contralateral side to pain as well: the side to which the structure leans and which indicates more stress shielding and force generation. In this model, pain and somatic manifestations are a symptom of imbalance rather that the problem to simply target anatomically. With permission from, and photographed by, Aaron Neilson-Belman AaronNeilsonBelman.com. Reprinted from https://hippievanman.com/photos (accessed 2021).

myofibroblasts, matrix remodeling, and stress shielding, but fail to resolve the overall tension. TBN, on the other hand, is expected to slowly release the tensions from the tensegrity system, lower tissue stiffness, and, over time, treat the patient's underlying fascial tensegrity abnormality. TBN, when done correctly, is expected to effectively treat- and perhaps even completely relieve- the underlying disease, according to the fascial armoring model.

Many controlled trials of acupuncture in the research literature are described as lacking strong evidence (38, 53). Noteworthy, establishing a proper control group is difficult in such studies. Finding a suitable placebo is somewhat challenging, and as it seems, any sham needling is not inert with regards to tensegrity dynamics. Moreover, some studies adopted a western non-holistic approach while using a fixed, drug-like, well-defined study protocol of needling

a single acupuncture point for a particular symptom, even though this approach has little relevance to the methods of an acupuncture practitioner in clinic (1).

The terminology regarding dry needling in literature can benefit from being more consistent. In this paper, the term "dry needling" refers to any technique that suggests one should insert needles into points/areas of pain as the only strategy of the therapy, without embracing a holistic view of the body or the bio-tensegrity system. This encompasses "intramuscular manual therapy" or "trigger point dry needling" and any other technique that sees "non-specific" pain as the problem to target anatomically rather than a symptom of imbalance in a complex fiber-cellular network of fascia. A guiding principle of TBN is that imbalance in fascia should be balanced by modulating multiple areas of the tensegrity system simultaneously (*via* mechanical needling) to minimize any exacerbation and carefully shift the imbalance of the system toward a more balanced state. Geodesic tensegrity domes are made of many nodes- and to change a node is to change the dome. In this discussion, a geodesic tensegrity dome is a parable for the (fascio)musculoskeletal system, for the purpose of simplicity. As biology does not separate or segregate itself into different medical specialties, an imbalanced tensegrity structure is likely to be associated with an imbalanced mind (6). The "body" and the "mind" are one Being, one flesh.

6.1. Acupuncture as tensegrity-based needling (TBN)

Studies find needling lowers shear wave modulus of myofascial tissue (29). Systematic reviews and meta-analyses find acupuncture alleviates pain and stiffness in fibromyalgia and in myofascial pain syndrome (34, 36, 38). The proposition that acupuncture may be a form of TBN is derived from the marked similarity between the two methods and the holistic view of the body they both embrace. The following points suggest acupuncture may function as TBN:

(1) In traditional eastern acupuncture, several needles are often used simultaneously and in multiple areas of the body, as recommended by TBN. Between five and fifteen needles are typically used in acupuncture treatment, with the point combinations varying over the course of the sessions (1).

(2) Acupuncture points adhere to symmetry of the human body. Symmetry during needling reflects the understanding that affecting one side of the tensegrity structure can affect the reciprocal contralateral side of the structure, and a modulation of one side alone can exacerbate any pre-existing imbalance. Inserting a needle into one point on the midline respects right-left symmetry but not anterior-posterior or cranio-caudal chains of force transmission. Needling bilateral points off the midline respects left-right symmetry. The skeleton is mostly symmetrical across the mid-sagittal plane, but internal organs and their fascia are not necessarily so.

(3) Needles are often inserted into a distant site further away from the chief area of complaint (75, 76), or sometimes in the contralateral side (77). This is a guiding principle in TBN. Since myofibroblasts stress shield themselves and remodel ECM, a painful point is not necessarily the source of pathological tension but rather may be a sign of a weak area that is being pulled by more distant myofascial tissue (*via* myofascial or biotensegral channels or chains). In acupuncture therapy for plantar fasciitis, for example, needles are often applied in the dorsal side of the foot and in the foreleg (points designated BL 57, BL 60, ST 36, SP 5, etc.) (78, 79). According to TBN, the foreleg and dorsal side of the foot, as well as more proximal points in the leg, are reciprocal areas that should be needled because they act to pull the plantar fascia and cause tension and pain. A study indicated that tightness in the gastrocnemius and hamstrings is associated with plantar fasciitis (80, 81). In lateral epicondylitis, points in the wrist and the elbow are needled, including in the anterior surface of the upper limb (82). In "neuropathic/neuroplastic" limb pain, needles are inserted into the contralateral limb to relieve pain (77). In tension-type headache, adjacent points can be used, bilaterally, including the adjacent large myofascial tissue of the trapezius and sternocleidomastoid (83). In acupuncture theory, headaches can be treated by placing pins in the hands (1). The rationale for this, even though may sound peculiar to a reader who is not familiar with the concept of tensegrity, is that the myofascial tissue in the upper limbs is suggested to pull the fascia of the head and transmit the tension through myofascial chains and tensegrity connections. Fascia is continuous from the trunk across the upper and lower limbs, thus capable of affecting range of motion. Fascia connects the ankle and hip not only anatomically, but also mechanically (84). While one study demonstrated existence of an anatomical continuity between all muscles of the flexor region of the upper limb (85), there is evidence in support of direct serial tissue continuity from the neck and shoulder area to the forearm (86). Reportedly, an anatomical myofascial continuum exists between the neck, head, and eyes (87). Most human skeletal muscles are directly linked by fascia and connective tissue (13).

(4) Acupuncture points are distributed in a higher density in areas of bone protrusions (88) and are distributed in a systemic network-like relationship of channels (88–90). Needling points adjacent to bone protrusions and solid curved surfaces is a general principle of TBN because those are the areas where tension and/or myofibroblasts tend to develop, even if asymptomatic or subclinical (6). Solid surfaces cause more pressure and stronger mechanical signals when fascia is chronically pressed against them in a person subjected to factors such as sedentary behavior and tight clothing. Chronic mechanical stimuli then lead to induction of myofibroblasts and their cascade of contracture. The development of myofibroblasts and fascial armoring will also depend on the type of tissue and its mass at the involved area. Mapping areas and points adjacent to hard surfaces and myofascial tissue, while avoiding important structures such as nerves and blood vessels, while also considering myofascial chains, might correlate to a certain degree with the distribution and relationship of acupuncture points. A study aimed to evaluate the correlation between trigger points and acupuncture points for pain (91). It found a high degree of correspondence (approximately 70 percent) between these two sets of points. The close correlation suggests that trigger points and acupuncture points for pain represent the same phenomenon and may be explained in terms of the same underlying mechanisms (91). Non-trigger-point needling might influence muscular and neurovascular structures as well as the connective tissue. Noteworthy, trigger

points and acupuncture points should not be expected to correspond entirely under this framework, because of the complexity of the tensegrity system. Tension developing in one area will often have a reciprocal area to be needled (even if not painful). For example, needling the area of the mastoid process (bilaterally) might require needling the back, the legs, or the middle of the forehead in the future even if the forehead is mostly flat, and even if the forehead does not have a painful trigger point. This is because releasing tension from the back of the head can shift imbalance to the front of the head, especially in a person with myopic vision overuse or a person overusing myopic vision during the needling session. The topography of acupuncture points might reflect tensegrity tension and its dynamics, and not simply myofascial painful points. Pain is a symptom of the entity; it is not the entity. Worth noting, the area of the abdomen does not contain many bone protrusions (although the anterior superior iliac spine should not be overlooked), however, bloating and sedentary behavior, as well as poor diet, belts and tight clothes, might lead to fibroblast-to-myofibroblast differentiation (with downstream physiological consequences). Fibrosis occurs in immobilized contracted muscles if they are maintained in the flexed position (59, 92). Sitting maintains abdominal muscles in the more flexed position. Psychological stress can potentially increase chronic abdominal muscle tone, thereby exacerbating the issue. Integrating the enteric nervous system and gastrointestinal tract complicates this model, though, overall, bloating acts to feed mechanical stimuli into the myofibroblast cascade of this model. **Figure 3** shows the vicious cycle of fascial armoring and factors that modulate the myofibroblast cycle of force generation and tissue contracture (**Figure 3**). Poor diet can have biochemical effects and effects on the microbiome to induce myofibroblasts (93–95). Needling the abdominal fascia or Linea alba might require reciprocal needling in all four limbs and head to maintain balance in the fascial system.

(5) In acupuncture, needles are inserted once in a session, and patients are allowed to fully relax and rest. Manipulation of a needle once inserted is performed momentarily and minimally, only if necessary. Needle twisting may be utilized to induce a mechanical response by myofibroblast contraction around the needle. Twisting the needle winds up ECM and fascia around the needle causing increased tension momentarily. Myofibroblasts have stretch activated calcium channels, and intracellular calcium and myofibroblast contractility are mechanistically linked (6, 96, 97). The direction of twisting can increase or decrease tension in the fascia, depending on the forces in it and the direction of twist. In certain cases, twisting the needle after insertion can help release tensions, for example, in cases where fascia has become very fibrotic with much remodeling and stress shielding. If a torque force exists in an intertwined fascia, twisting might help relieve it. Nonetheless, increasing tension *via* twisting in specific points will modulate the tensegrity forces around that area (and along that chain), and then, the needle will tear fibers throughout the session, as explained in the findings section. Encountering microscopic blood vessels/capillaries throughout the tearing process of fascia might lead to minor bleeding by the end of the session. The more bleeding and erythema, the stronger indication it is that much tearing of fascia occurred (assuming a blood vessel was not accidentally punctured). Too much bleeding is a sign of unconstrained modulation of fascia.

(6) Pain is something to avoid, not induce. As is seems, the pain and post-needling soreness of western dry needling is not a complication of the technique but rather it is a direct result. An important principle of TBN which is adhered to by acupuncture is allowing the myofascial system to rest during treatment. Elicited pain and the accompanied muscle contractions of patients during a "dry needling pistoning maneuver" is absolutely avoided. Rest and relaxation are of special importance during treatment. The fascio-musculo-skeletal system needs to be relaxed during treatment according to TBN, as well as the relaxation of the mind. When there is rest and relaxation, the internal fascial forces guide the process of fascial tensional release, which is a key aspect of TBN.

(7) The order of sequential needle insertion is a factor that is given attention in acupuncture (98). In TBN, if imbalance in the tensegrity structure is detected in the right upper part of the body, for example, it might require needling the right lower limb, left lower limb, and left upper limb, working around the structure to collapse the tension in a controlled fashion. If, for example, imbalance and pain is detected in the lower limb (e.g., following below knee amputation), needling might be done in the head, unilateral upper limb, contralateral upper limb, and contralateral lower limb, while working around the weak area of the structure. The back and thoracolumbar fascia should be needled to help relieve tension traveling down the lower limb. Determining the order of needling is complex under this framework and requires further understanding. Much research is required before translating this oversimplified discussion into clinical practice.

(8) Systemic symptoms require needling in multiple points throughout the body (98, 99). According to fascial armoring, functional-somatic or non-specific symptoms such as fatigue can be a sign of widespread fascial compression or a global chronic exertional compartment-like syndrome. Fascia is an interconnected fiber-cellular network like a woven net, with myofibroblast nodes/populations that create tension, and trigger points and satellites (6). Tension in a woven net cannot be released by cutting one string.

Even if a series of needling treatments does not induce complete relaxation in the tensegrity structure, it most likely makes the body more receptive to relaxation because it decreases mechanical tension and sympathetic tone. Sympathetic tone can be generated by pain and by fascial forces or stretch lesions in the nerves embedded in fascia (6). The bio-tensegrity structure is dynamic and each patient's structure and the imbalance in it is unique to that individual at that point in time. Therefore, each needling treatment should be adapted according to the current state of the patient's tensegrity status and modified according to the response to treatment. In other words, one needling protocol does not fit all, and does not fit one at all times. A reader who is familiar with acupuncture probably understood this concept already. Clinical examination is important and should be done prior to each needling session. Developing a method to monitor the state of the body's fascial tensegrity structure by palpation would be an astounding achievement for modern medicine. The purpose would be to palpate and monitor the fascial system and to feel its qualities, not the cardiac pulse. Although, a weak string like cardiac

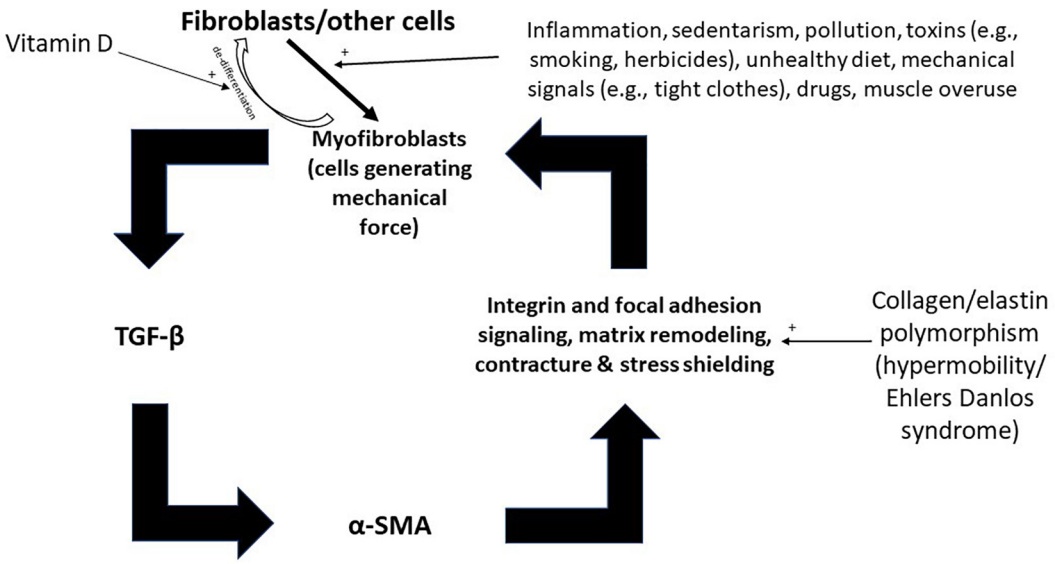

FIGURE 3
Inputs into the positive feedback loop of fascial armoring (fascial rigidity and biotensegrity tension). Fibroblasts, adipocytes, and other cell-types can differentiate to myofibroblasts. Empirical studies show various factors influence fibroblast-to-myofibroblast differentiation and α-SMA synthesis, such as: immobility, infection/inflammation, diet, herbicides, drugs, epi/genetics, etc. (6). Once myofibroblasts activity is initiated, a positive feedback loop can stimulate and maintain fascial armoring (i.e., lead to a chronic fascial bio-tensegrity pathology driven by a myo/fibroblast network of contracting cells in connective tissue). Open arrows with a plus sign indicate upregulation/stimulation. TGF-β, transforming growth factor beta; α-SMA, alpha smooth muscle actin.

radial pulse might be a marker for a widespread compression by an abnormal bio-tensegrity structure. A disease that is an "exertional chronic compartment-like syndrome of the whole body" should be easily felt and evaluated *via* palpation by a skillful individual (if one is aware of what to look for). Placing only two fingers on the wrist might not be sufficient to detect lateral movement or changes of the fascia beneath the skin during palpation.

Non-ideal modulation of the tensegrity structure, resulting from needling in a wrong way, would lead to exacerbation of pain and symptoms, either immediately or in the long-term. The therapeutic process should be gradual and progressive spanned over multiple sessions because changing the tensegrity structure too quickly may cause serious harm (6). Some suggest needles should remain for 20–30 min during each session, with a frequency of approximately two sessions per week (83). Fascia might have enough hidden potential elastic energy to cause bone fracture (6). A sudden change in fascia might shift tensegrity forces and exacerbate an imbalance (100).

Choice of needle depth: the precise depth of needling is an important variable. De-qi (needle grasp) describes a successful needling (31). It is a mechanical phenomenon. It may actually be the phenomenological representation of myo/fibroblast rapid contractions in connective-tissue (31) which is mediated by smooth muscle actin and calcium-myosin mediated contraction (6). Myo/fibroblasts have stretch activated calcium channels, and intracellular calcium and myo/fibroblast contractility are mechanistically linked (96, 97). Needle insertion can induce temporary contraction and increase the local tension. Following this increase in tension, if let rest, the needle will tear fascial fibers and slowly release tension (6). To "puncture the bone without damaging the tendons, to puncture the tendons without damaging the muscles, to puncture the muscle without damaging the "pulse," and to puncture the "pulse" without damaging the skin" (Huangdi Neijing Suwen), seems to suggest that a therapist should be able to target and modulate the tensegrity system with caution and precision (the term "pulse" refers to fascia under this framework). If the needle does not penetrate the proper layer, the tensegrity structure will not be able to release its internal tensions in a healthy manner. If inserted too deep, it may cause harm. The angle of insertion affects depth. Worth noting, the repeated action of needle penetration in dry needling is neither very careful nor delicate and should be avoided, according to this theory. The long-term consequences of dry needling while ignoring tensegrity dynamics are not entirely known.

6.2. "Meridians": A bio-tensegrity map?

The idea of meridians and their mechanical significance is interesting under this framework. It may be possible that the fascial tensegrity structure tends to have a conserved topography of tensional forces. Variations in this topography may also exist. **Figure 2** depicts a tensegrity structure with imbalance whereby imbalance in the structure can manifest in different anatomical areas due to myofascial forces traveling in the system. Any specific imbalance in the fascial system would require needling in reciprocal areas. Imbalance or asymmetry in the tensegrity structure might have a tendency to reach certain energetic states which are stabilized by abnormal myofascial tensegrity forces. Also, the specific state of the tensegrity structure will be affected by elements of the musculoskeletal system. The skeleton is somewhat conserved between individuals but still has variations. Also, hypertrophy of a certain muscle group can cause imbalance in the structure. If, hypothetically, meridians serve as a topographical guide for the location of needling that can alleviate compression and shift the tensegrity dynamics to a more balanced state, it will further support the notion that acupuncture acts as TBN. If meridians correspond (to a degree) with myofascial/tensegrity chains, acupuncture as TBN

would make even more sense. Any therapy that modulates the bio-tensegrity structure would have to take fascia's complex dynamics into consideration while understanding that forces can be transmitted to a distance *via* myofascial chains. Myofibroblasts can be arranged in nodes or in lines, and lines of force are found to exist in tissue (101–106). For example, cardiac myofibroblasts tend to align their shapes and forces with the long axis of a wavy substrate (107). Myofibroblasts reorient themselves toward the direction of a stretch force exerted on them (108). Substrate concavity and convexity also affect mechanics of cells (109).

The ability of tensegrity forces to flow freely throughout this fiber-cellular network and in the system of fascia (or the "pulse") might be related to the idea of a palpated "Qi energy." This notion is presented assuming traditional Chinese philosophy accepts that even the metaphysical can have a physical representation in the body. If mechanical needling affects a person's "Qi," then "Qi" should have a mechanical aspect in the body. The Body-Mind-Spirit is one Being. This discussion is not aimed to validate or refute the idea of Qi energy or the whole philosophical background of acupuncture.

6.3. Acupuncture for fibromyalgia: Should we expect relapses until remission?

Since studies suggest fibromyalgia is overall dependent on peripheral input (110), interventions such as TBN/acupuncture aiming to target peripheral tissue have a role for central components of fibromyalgia as well as for treating their fascial abnormality. Allodynia and hyperalgesia in fibromyalgia can be improved or abolished by removal of peripheral impulse input (110). Mechanical hyperalgesia in fibromyalgia patients is likely a consequence of abnormal input from sensitized peripheral tissue receptors and resultant central sensitization. Abnormally sustained impulse input from muscle receptors in fibromyalgia patients and/or muscular ischemia appear to be a relevant mechanism for chronic muscle pain in such patients (110). Research has yet to establish if enhanced responses to somatic and cutaneous stimuli in fibromyalgia result from facilitating mechanisms within the brain, spinal sensitization maintained by tonic impulse input from somatic tissues, or abnormal mechanisms of descending facilitation from the brain to the spinal cord and/or somatic tissues (110). Nevertheless, recognizing the elevated intramuscular pressure as a possible mechanism for hypoperfusion and subsequent diffuse muscle pain in fibromyalgia suggests a therapeutic role for reducing intramuscular pressure as an intervention (17).

In the fascial armoring model, hyperalgesia would likely be affected by the state of nociceptors in abnormal fascia. Chronic nociceptor and mechanoreceptor activation, mechanical compression of neural structures, increased circulating inflammatory signals, and cytokine induced peripheral sensitization, are some of the pathways possibly leading to long term changes in the brain of these patients. Relieving a fascial biotensegrity abnormality and its noxious stimuli may be relevant for neurobiology by affecting input to the central nervous system including the somatosensory cortex, amygdala, brainstem, and insular cortex, reducing physical-emotional stress, affecting pathways of peripheral and central sensitization (including spinal and dorsal root ganglion), reduce microglia activation, and conditioned pain modulation, among many other related processes and paradigms neuroscience is familiar with. Treating their fascia properly is also expected to lead to less "pain catastrophizing" and should be more therapeutically effective than, for example, acceptance and commitment therapy.

Clinical trials show a beneficial effect of acupuncture for fibromyalgia (34, 38). According to the fascial armoring model, relapse is expected to happen after treatment due to the proto/myofibroblasts that remain in fascia (6). Myofibroblasts contract and lock in tension in the ECM over several hours (9). Relapses might occur a short while after each treatment. This will happen until global tension is eliminated or decreased enough to downregulate α-SMA and cause myofibroblast de-differentiation or apoptosis (6). A randomized controlled trial studied dry needling and extracorporeal shockwave therapy and measured their effect on the stiffness and shear modulus of fascia using shear wave elastography (27). Results showed both modalities significantly lowered the stiffness of fascia as measured by shear wave elastography. The shear modulus measured 1 month after treatment was significantly lower than pre-treatment values, and these values corresponded with lower patient reported pain and pressure pain thresholds. However, when measured at 3 months post-treatment, the shear modulus had increased above 1-month post-treatment values. Stiffness seemed to recover to a degree, perhaps reflecting the start of recurrence. The authors noted that the mechanisms of elastic recovery after the extracorporeal shockwave therapy and dry needling treatment are unclear (27).

According to fascial armoring, as long as proto/myofibroblasts are present in fascia, and as long as lifestyle continues to feed input into the cascade of fascial armoring, relapse of tension, stiffness, pain, and increased shear wave measurements is expected to occur due to matrix remodeling and myofibroblast force generation. Based on fascial armoring, TBN therapy (and any needling therapy) initiates a process of tensegrity modulation that should be then supported and continued by the patient following treatment. Release of tension from of the bio-tensegrity structure is a dynamic ongoing process. Therefore, movement, physical exercise, maintaining proper posture, and relaxation techniques are required especially during the period following each needling session and as a lifestyle intervention.

6.4. Suggesting a role for acupuncture in the prevention of chronic widespread pain and psychosomatic disorders

The dynamic nature of ECM synthesis as an ongoing process suggests that homeostasis and balance normally exist between matrix synthesis and degradation (remodeling), between myofibroblast induction and de-differentiation, and between cellular force generation and tensional release. It is a delicate interplay of multiple factors (e.g., collagen, elastin, matrix metalloproteinases, growth factors, etc.). If any imbalance develops in the fascial bio-tensegrity structure, it can help maintain and propel the myofibroblast cascade of contracture *via* mechanosensitive signals, therefore driving the positive feedback loop of myofibroblast contractions and stress shielding (6). Studies show a close association between hypermobility/Ehlers-Danlos syndrome and fibromyalgia/psychosomatic disease (111–114). Chronic widespread pain, fibromyalgia, and hypermobility syndrome pain, share common features and perhaps a common pathophysiology (6). Patients with Ehlers-Danlos/hypermobility syndrome have widespread ECM

disarray and an increase in myofibroblasts (115–117). According to the fascial armoring model these patients are expected to have a tendency to develop an imbalance in the tensegrity system because of the altered qualities of their fascia (6). In this model, an imbalanced tensegrity structure with laxity in its frame is expected to collapse more easily. The link between a collagen polymorphism and chronic widespread pain becomes more intuitive once we consider the properties of fascia.

The multifactorial interplay of fascia suggests that needling might be utilized as a method to maintain fascia in a low stiffness state. As mentioned, studies show needling lowers fascial stiffness and its shear modulus (27–29), and that myofibroblast synthesis of α-SMA is regulated by substrate rigidity (70). In disorders where fascia and tensegrity have a part in the pathophysiology, it might be useful to use TBN as a prophylaxis and maintenance. The aim would be to maintain the shear modulus of soft/myofascial tissue below the threshold of myofibroblast activity (6). Prophylaxis seems a more optimal way than trying to treat late-stage advanced disease. "Fibromyalgia," due to high cut-off for diagnosis chosen by the American College of Rheumatology, is by definition a late-stage advanced disease likely to be more resistant to many treatment modalities and monotherapies. Since lifestyle (sedentarism, tight clothes, diet, pollution, and smoking, etc.) is suggested to be a major part of the etiology of fibromyalgia-like syndromes by inducing fascial myofibroblasts, lifestyle changes should be part of the treatment and prevention (6). Needling alone may not be enough for the cure and maintenance of remission. As long as lifestyle factors stimulate the cascade of fascial myofibroblasts, symptoms are expected to recur.

6.5. The problem with dry needling in light of bio-tensegrity

Because the mechanism of trigger/tender points themselves is not entirely understood, the theoretical scientific validity of western dry needling for such conditions remains just as questionable. The frequency of recurrence and possible migration of pain to different anatomical locations in the long term have not been well studied. Many studies of dry needling aim to examine the local effect of needling on a certain type of pain syndrome in a certain anatomical location, while the long-term consequences are still unclear (37, 39–41, 118–123).

It was recently suggested that trigger points and "non-specific" pains might arise due to myofibroblasts with contractile activity in the fascial bio-tensegrity system (6). These can affect nearby nerves, meaning, a painful spot is not necessarily the origin and cause of the underlying problem. Therefore, needle treatment should also be applied further away from the location of chief pain and aim to collapse the underlying tension of the bio-tensegrity system in a concentric manner. It should be performed while working according to a trigger point/satellite and myofibroblast-tensegrity-network model. The method of dry needling does not utilize these ideas, but rather "aiming and shooting at the pain" is the main strategy of this technique. According to TBN, negating one painful node in the system is not sufficient to treat the underlying problem. On the contrary: when done improperly, even if improving the local myofascial pain that it aims to treat by freeing the nerve from local tethering or tension, this false treatment might add tensions to other nodes in the structure and exacerbate the overall imbalance, and lead to other seemingly unrelated pains and symptoms. Beginning the relaxation process further away from the focus of pain while using multiple needles is sensible under this framework. It slowly and progressively starts to relax the nodes in the satellite-relationship model. In line with the bio-tensegrity model, one should recognize that the peripheries carry tension affecting the focus and should not be disregarded simply because they seem asymptomatic.

According to fascial armoring, the in-and-out maneuver stimulates local myofibroblasts to rapidly contract (*via* calcium influx and mechanosensitive stretch receptors) and leads to increased tension in the area. Increased tension leads to increase in pain. Forcefully causing edema and/or minor hemorrhage might affect fascia after multiple iterations of needle insertion. With so much pro-active destruction of fascial tissue ultimately lowering local tensions, it unfortunately neglects any conception of bio-tensegrity dynamics. Meanwhile, permitting needles to remain in fascia with patience might allow the tensegrity system to re-align according to its internal forces and release tensions in the fascial network. Rest and relaxation should allow the high-density of myofibroblasts in the network to pull and tear fascia around the needle, delicately and independently. It is also worth noting that some studies in literature have defined certain complications of treatments as "unrelated" rather than "unexplained" (124). Defining adverse events as "unrelated" may be problematic, especially if a sufficient explanation is not provided for the subjective interpretation of that phenomenon. Studies of dry needling should reflect on this fact because dry needling affects the bio-tensegrity structure, even at more distant seemingly unrelated locations.

Langevin et al. provide important evidence on acupuncture points and meridians and their relationship with connective tissue, along with evidence on the complex mechano-cellular effects of acupuncture (31, 32, 125). The effect of needling and needle manipulation on fibroblast and connective tissue is documented in studies (126, 127). As substrate rigidity and matrix mechanics deeply influence myofibroblasts and other cell types (128–132), acupuncture may be able to affect vital biomechanical, physiological, and cellular processes. The fascial connective tissue system constitutes a widespread network throughout the body, including intermuscular and subcutaneous tissue layers, and is continuous with more specialized connective tissues such as perineurium, periosteum, perimysium, peritoneum, and pleura (6, 31, 60, 85). The material surrounding blood vessels and lymphatics includes interstitial connective tissue (31). The myodural bridge connects the rectus capitis posterior with the dura (133, 134) and there is evidence that the meninges and the brain function as one network (135–138). Modulating interstitial connective tissue would cause biomechanical, vasomotor and neuromodulatory effects (31). Abnormal biotensegrity forces affecting spinal or intracranial fascia are expected not to be physiologically immaterial. Changing the fascia changes the bio-tensegrity system; and changing bio-tensegrity dynamics is expected to change the "mind" (6). The body and the mind are one being.

7. Conclusion

Acupuncture and TBN share several characteristics. While acupuncture may act as a global percutaneous needle fasciotomy that

respects tensegrity principles, it is reasonable to suggest there are several mechanisms working in parallel in acupuncture, TBN being another mode of action in this ancient practice. Understanding the mechanism of acupuncture as tensegrity-based needling and how it can treat resistant chronic pain syndromes such as fibromyalgia may open a new avenue for medical research and practice. Examining this model in the context of other medical conditions may prove useful. In the modern era, western medicine (and humanity) aspires to follow evidence-based science; further research is needed to help reveal the mechanisms by which acupuncture targets and treats chronic non-cancer pain and functional-psychosomatic medical conditions.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

SP solely contributed to the study conception and design, material preparation, data collection, data curation, conceptualization, integration of information, formal analysis investigation, methodology, visualization, resources, writing—original draft, writing—review and editing, contributed to the article, and approved the submitted version.

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Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Kaptchuk T. Acupuncture: theory, efficacy, and practice. *Ann Intern Med.* (2002) 136:374–83. doi: 10.7326/0003-4819-136-5-200203050-00010

2. Cabioğlu M, Cetin B. Acupuncture and immunomodulation. *Am J Chin Med.* (2008) 36:25–36. doi: 10.1142/S0192415X08005552

3. Langevin H, Konofagou E, Badger G, Churchill D, Fox J, Ophir J, et al. Tissue displacements during acupuncture using ultrasound elastography techniques. *Ultrasound Med Biol.* (2004) 30:1173–83. doi: 10.1016/j.ultrasmedbio.2004.07.010

4. Lu L, Zhang Y, Tang X, Ge S, Wen H, Zeng J, et al. Evidence on acupuncture therapies is underused in clinical practice and health policy. *BMJ.* (2022) 376:e067475. doi: 10.1136/bmj-2021-067475

5. Wen J, Chen X, Yang Y, Liu J, Li E, Liu J, et al. Acupuncture medical therapy and its underlying mechanisms: a systematic review. *Am J Chin Med.* (2021) 49:1–23.

6. Plaut S. Scoping review and interpretation of myofascial pain/fibromyalgia syndrome: an attempt to assemble a medical puzzle. *PLoS One.* (2022) 17:e0263087. doi: 10.1371/journal.pone.0263087

7. Firestein G, Kelley W. *Kelley's textbook of rheumatology*. 9th Edn. Philadelphia, PA: Elsevier (2013).

8. Wessely S, Hotopf M. Is fibromyalgia a distinct clinical entity? Historical and epidemiological evidence. *Baillieres Best Pract Res Clin Rheumatol.* (1999) 13:427–36. doi: 10.1053/berh.1999.0032

9. Tomasek J, Gabbiani G, Hinz B, Chaponnier C, Brown R. Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat Rev Mol Cell Biol.* (2002) 3:349–63. doi: 10.1038/nrm809

10. Caldeira P, Fonseca S, Paulo A, Infante J, Araújo D. Linking tensegrity to sports team collective behaviors: towards the group-tensegrity hypothesis. *Sports Med Open.* (2020) 6:24. doi: 10.1186/s40798-020-00253-y

11. Tadeo I, Berbegall A, Escudero L, Alvaro T, Noguera R. Biotensegrity of the extracellular matrix: physiology, dynamic mechanical balance, and implications in oncology and mechanotherapy. *Front Oncol.* (2014) 4:39. doi: 10.3389/fonc.2014.00039

12. Scarr G. Biotensegrity: What is the big deal? *J Bodyw Mov Ther.* (2020) 24:134–7. doi: 10.1016/j.jbmt.2019.09.006

13. Wilke J, Krause F, Vogt L, Banzer W. What is evidence-based about myofascial chains: a systematic review. *Arch Phys Med Rehabil.* (2016) 97:454–61. doi: 10.1016/j.apmr.2015.07.023

14. Schleip R, Gabbiani G, Wilke J, Naylor I, Hinz B, Zorn A, et al. Fascia is able to actively contract and may thereby influence musculoskeletal dynamics: a histochemical and mechanographic investigation. *Front Physiol.* (2019) 10:336. doi: 10.3389/fphys.2019.00336

15. Schleip R, Klingler W. Active contractile properties of fascia. *Clin Anat.* (2019) 32:891–5. doi: 10.1002/ca.23391

16. Langevin H, Cornbrooks C, Taatjes D. Fibroblasts form a body-wide cellular network. *Histochem Cell Biol.* (2004) 122:7–15. doi: 10.1007/s00418-004-0667-z

17. Katz R, Leavitt F, Small A, Small B. Intramuscular pressure is almost three times higher in fibromyalgia patients: a possible mechanism for understanding the muscle pain and tenderness. *J Rheumatol.* (2021) 48:598–602. doi: 10.3899/jrheum.191068

18. Kalyan-Raman U, Kalyan-Raman K, Yunus M, Masi A. Muscle pathology in primary fibromyalgia syndrome: a light microscopic, histochemical and ultrastructural study. *J Rheumatol.* (1984) 11:808–13.

19. Morf S, Amann-Vesti B, Forster A, Franzeck U, Koppensteiner R, Uebelhart D, et al. Microcirculation abnormalities in patients with fibromyalgia – measured by capillary microscopy and laser fluxmetry. *Arthritis Res Ther.* (2005) 7:R209–16. doi: 10.1186/ar1459

20. Sprott H, Salemi S, Gay R, Bradley L, Alarcón G, Oh S, et al. Increased DNA fragmentation and ultrastructural changes in fibromyalgic muscle fibres. *Ann Rheum Dis.* (2004) 63:245–51. doi: 10.1136/ard.2002.004762

21. Evdokimov D, Kreß L, Dinkel P, Frank J, Sommer C, Üçeyler N. Pain-associated mediators and axon pathfinders in fibromyalgia skin cells. *J Rheumatol.* (2020) 47:140–8. doi: 10.3899/jrheum.190248

22. Ramírez-Tejero J, Martínez-Lara E, Peinado MÁ, Del Moral ML, Siles E. Hydroxytyrosol as a promising ally in the treatment of fibromyalgia. *Nutrients.* (2020) 12:2386. doi: 10.3390/nu12082386

23. Gronemann S, Ribel-Madsen S, Bartels E, Danneskiold-Samsoe B, Bliddal H. Collagen and muscle pathology in fibromyalgia patients. *Rheumatology.* (2004) 43:27–31. doi: 10.1093/rheumatology/keg452

24. Cummings T, White A. Needling therapies in the management of myofascial trigger point pain: a systematic review. *Arch Phys Med Rehabil.* (2001) 82:986–92. doi: 10.1053/apmr.2001.24023

25. Frost F, Jessen B, Siggaard-Andersen J. A control, double-blind comparison of mepivacaine injection versus saline injection for myofascial pain. *Lancet.* (1980) 1:499–500. doi: 10.1016/s0140-6736(80)92761-0

26. Lewit K. The needle effect in the relief of myofascial pain. *Pain.* (1979) 6:83–90. doi: 10.1016/0304-3959(79)90142-8

27. Luan S, Zhu Z, Ruan J, Lin C, Ke S, Xin W, et al. Randomized trial on comparison of the efficacy of extracorporeal shock wave therapy and dry needling in myofascial trigger points. *Am J Phys Med Rehabil.* (2019) 98:677–84. doi: 10.1097/PHM.0000000000001173

28. Maher R, Hayes D, Shinohara M. Quantification of dry needling and posture effects on myofascial trigger points using ultrasound shear-wave elastography. *Arch Phys Med Rehabil.* (2013) 94:2146–50. doi: 10.1016/j.apmr.2013.04.021

29. Sánchez-Infante J, Bravo-Sánchez A, Jiménez F, Abián-Vicén J. Effects of dry needling on muscle stiffness in latent myofascial trigger points: a randomized controlled trial. *J Pain.* (2021) 22:817–25. doi: 10.1016/j.jpain.2021.02.004

30. Fox J, Gray W, Koptiuch C, Badger G, Langevin H. Anisotropic tissue motion induced by acupuncture needling along intermuscular connective tissue planes. *J Altern Complement Med.* (2014) 20:290–4. doi: 10.1089/acm.2013.0397

31. Langevin H, Churchill D, Cipolla M. Mechanical signaling through connective tissue: a mechanism for the therapeutic effect of acupuncture. *FASEB J.* (2001) 15:2275–82. doi: 10.1096/fj.01-0015hyp

32. Langevin H, Churchill D, Fox J, Badger G, Garra B, Krag M. Biomechanical response to acupuncture needling in humans. *J Appl Physiol.* (2001) 91:2471–8. doi: 10.1152/jappl.2001.91.6.2471

33. Tomasek J, Haaksma C, Eddy R, Vaughan M. Fibroblast contraction occurs on release of tension in attached collagen lattices: dependency on an organized actin cytoskeleton and serum. *Anat Rec.* (1992) 232:359–68. doi: 10.1002/ar.1092320305

34. Deare J, Zheng Z, Xue C, Liu J, Shang J, Scott S, et al. Acupuncture for treating fibromyalgia. *Cochrane Database Syst Rev.* (2013) 2013:CD007070. doi: 10.1002/14651858.CD007070.pub2

35. Furlan A, van Tulder M, Cherkin D, Tsukayama H, Lao L, Koes B, et al. Acupuncture and dry-needling for low back pain. *Cochrane Database Syst Rev.* (2005) 1:CD001351. doi: 10.1002/14651858.CD001351.pub2

36. Li X, Wang R, Xing X, Shi X, Tian J, Zhang J, et al. Acupuncture for myofascial pain syndrome: a network meta-analysis of 33 randomized controlled trials. *Pain Physician.* (2017) 20:E883–902.

37. Liu L, Huang Q, Liu Q, Thitham N, Li L, Ma Y, et al. Evidence for dry needling in the management of myofascial trigger points associated with low back pain: a systematic review and meta-analysis. *Arch Phys Med Rehabil.* (2018) 99:144–52.e2. doi: 10.1016/j.apmr.2017.06.008

38. Zhang X, Chen H, Xu W, Song Y, Gu Y, Ni G. Acupuncture therapy for fibromyalgia: a systematic review and meta-analysis of randomized controlled trials. *J Pain Res.* (2019) 12:527–42. doi: 10.2147/JPR.S186227

39. Gattie E, Cleland J, Snodgrass S. The effectiveness of trigger point dry needling for musculoskeletal conditions by physical therapists: a systematic review and meta-analysis. *J Orthop Sports Phys Ther.* (2017) 47:133–49. doi: 10.2519/jospt.2017.7096

40. Liu L, Huang Q, Liu Q, Ye G, Bo C, Chen M, et al. Effectiveness of dry needling for myofascial trigger points associated with neck and shoulder pain: a systematic review and meta-analysis. *Arch Phys Med Rehabil.* (2015) 96:944–55. doi: 10.1016/j.apmr.2014.12.015

41. Hu H, Gao H, Ma R, Zhao X, Tian H, Li L. Is dry needling effective for low back pain?: A systematic review and PRISMA-compliant meta-analysis. *Medicine.* (2018) 97:e11225. doi: 10.1097/MD.0000000000011225

42. De Meulemeester K, Calders P, Cagnie B. Exploring the underlying mechanisms of action of dry needling: what is the immediate effect on muscle electrophysiology? An experimental randomized controlled trial. *Am J Phys Med Rehabil.* (2022) 101:18–25. doi: 10.1097/PHM.0000000000001732

43. Hsieh Y, Chou L, Joe Y, Hong C. Spinal cord mechanism involving the remote effects of dry needling on the irritability of myofascial trigger spots in rabbit skeletal muscle. *Arch Phys Med Rehabil.* (2011) 92:1098–105. doi: 10.1016/j.apmr.2010.11.018

44. Perreault T, Fernández-de-Las-Peñas C, Cummings M, Gendron B. Needling interventions for sciatica: choosing methods based on neuropathic pain mechanisms-a scoping review. *J Clin Med.* (2021) 10:2189. doi: 10.3390/jcm10102189

45. Rajkannan P, Vijayaraghavan R. Dry needling in chronic abdominal wall pain of uncertain origin. *J Bodyw Mov Ther.* (2019) 23:94–8. doi: 10.1016/j.jbmt.2018.01.004

46. Martín-Pintado-Zugasti A, Mayoral Del Moral O, Gerwin R, Fernández-Carnero J. Post-needling soreness after myofascial trigger point dry needling: current status and future research. *J Bodyw Mov Ther.* (2018) 22:941–6. doi: 10.1016/j.jbmt.2018.01.003

47. Lumley M, Schubiner H, Lockhart N, Kidwell K, Harte S, Clauw D, et al. Emotional awareness and expression therapy, cognitive behavioral therapy, and education for fibromyalgia: a cluster-randomized controlled trial. *Pain.* (2017) 158:2354–63. doi: 10.1097/j.pain.0000000000001036

48. Sluka K, Clauw D. Neurobiology of fibromyalgia and chronic widespread pain. *Neuroscience.* (2016) 338:114–29. doi: 10.1016/j.neuroscience.2016.06.006

49. Linde K, Allais G, Brinkhaus B, Fei Y, Mehring M, Shin B, et al. Acupuncture for the prevention of tension-type headache. *Cochrane Database Syst Rev.* (2016) 4:CD007587. doi: 10.1002/14651858.CD007587.pub2

50. Linde K, Allais G, Brinkhaus B, Fei Y, Mehring M, Vertosick E, et al. Acupuncture for the prevention of episodic migraine. *Cochrane Database Syst Rev.* (2016) 2016:CD001218. doi: 10.1002/14651858.CD001218.pub3

51. Vickers A, Vertosick E, Lewith G, MacPherson H, Foster N, Sherman K, et al. Acupuncture for chronic pain: update of an individual patient data meta-analysis. *J Pain.* (2018) 19:455–74. doi: 10.1016/j.jpain.2017.11.005

52. Smith C, Armour M, Lee M, Wang L, Hay P. Acupuncture for depression. *Cochrane Database Syst Rev.* (2018) 3:CD004046. doi: 10.1002/14651858.CD004046.pub4

53. Yuan Q, Wang P, Liu L, Sun F, Cai Y, Wu W, et al. Acupuncture for musculoskeletal pain: a meta-analysis and meta-regression of sham-controlled randomized clinical trials. *Sci Rep.* (2016) 6:30675. doi: 10.1038/srep30675

54. Vincent C. The safety of acupuncture. *BMJ.* (2001) 323:467–8. doi: 10.1136/bmj.323.7311.467

55. Yamashita H, Tsukayama H, White A, Tanno Y, Sugishita C, Ernst E. Systematic review of adverse events following acupuncture: the Japanese literature. *Complement Ther Med.* (2001) 9:98–104. doi: 10.1054/ctim.2001.0446

56. Bensoussan A, Myers S, Carlton A. Risks associated with the practice of traditional Chinese medicine: an Australian study. *Arch Fam Med.* (2000) 9:1071–8. doi: 10.1001/archfami.9.10.1071

57. Ernst E, White A. Life-threatening adverse reactions after acupuncture? A systematic review. *Pain.* (1997) 71:123–6. doi: 10.1016/s0304-3959(97)03368-x

58. Yamashita H, Tsukayama H, Tanno Y, Nishijo K. Adverse events related to acupuncture. *JAMA.* (1998) 280:1563–4. doi: 10.1001/jama.280.18.1563-a

59. Williams P, Goldspink G. Connective tissue changes in immobilised muscle. *J Anat.* (1984) 138:343–50.

60. Wilke J, Schleip R, Yucesoy C, Banzer W. Not merely a protective packing organ? A review of fascia and its force transmission capacity. *J Appl Physiol.* (2018) 124:234–44. doi: 10.1152/japplphysiol.00565.2017

61. Li A, Liu Y, Plott J, Chen L, Montgomery J, Shih A. Multi-bevel needle design enabling accurate insertion in biopsy for cancer diagnosis. *IEEE Trans Biomed Eng.* (2021) 68:1477–86. doi: 10.1109/TBME.2021.3054922

62. Li A, Putra K, Chen L, Montgomery J, Shih A. Mosquito proboscis-inspired needle insertion to reduce tissue deformation and organ displacement. *Sci Rep.* (2020) 10:12248. doi: 10.1038/s41598-020-68596-w

63. Langevin H, Churchill D, Wu J, Badger G, Yandow J, Fox J, et al. Evidence of connective tissue involvement in acupuncture. *FASEB J.* (2002) 16:872–4. doi: 10.1096/fj.01-0925fje

64. Ernberg M, Hedenberg-Magnusson B, Alstergren P, Kopp S. The level of serotonin in the superficial masseter muscle in relation to local pain and allodynia. *Life Sci.* (1999) 65:313–25. doi: 10.1016/s0024-3205(99)00250-7

65. Moraska A, Hickner R, Kohrt W, Brewer A. Changes in blood flow and cellular metabolism at a myofascial trigger point with trigger point release (ischemic compression): a proof-of-principle pilot study. *Arch Phys Med Rehabil.* (2013) 94:196–200. doi: 10.1016/j.apmr.2012.08.216

66. Shah J, Gilliams E. Uncovering the biochemical milieu of myofascial trigger points using in vivo microdialysis: an application of muscle pain concepts to myofascial pain syndrome. *J Bodyw Mov Ther.* (2008) 12:371–84. doi: 10.1016/j.jbmt.2008.06.006

67. Gerdle B, Söderberg K, Salvador Puigvert L, Rosendal L, Larsson B. Increased interstitial concentrations of pyruvate and lactate in the trapezius muscle of patients with fibromyalgia: a microdialysis study. *J Rehabil Med.* (2010) 42:679–87. doi: 10.2340/16501977-0581

68. Dong Z, Shun-Yue L, Shu-You W, Hui-Min M. Evaluation of influence of acupuncture and electro-acupuncture for blood perfusion of stomach by laser Doppler blood perfusion imaging. *Evid Based Complement Alternat Med.* (2011) 2011:969231. doi: 10.1093/ecam/nep050

69. Dorigo B, Bartoli V, Grisillo D, Beconi D. Fibrositic myofascial pain in intermittent claudication. Effect of anesthetic block of trigger points on exercise tolerance. *Pain.* (1979) 6:183–90. doi: 10.1016/0304-3959(79)90125-8

70. Goffin J, Pittet P, Csucs G, Lussi J, Meister J, Hinz B. Focal adhesion size controls tension-dependent recruitment of alpha-smooth muscle actin to stress fibers. *J Cell Biol.* (2006) 172:259–68. doi: 10.1083/jcb.200506179

71. Kural M, Billiar K. Myofibroblast persistence with real-time changes in boundary stiffness. *Acta Biomater.* (2016) 32:223–30. doi: 10.1016/j.actbio.2015.12.031

72. Moog P, Buchner L, Cerny M, Schmauss D, Megerle K, Erne H. Analysis of recurrence and complications after percutaneous needle fasciotomy in Dupuytren's disease. *Arch Orthop Trauma Surg.* (2019) 139:1471–7. doi: 10.1007/s00402-019-03247-y

73. Soreide E, Murad M, Denbeigh J, Lewallen E, Dudakovic A, Nordsletten L, et al. Treatment of Dupuytren's contracture: a systematic review. *Bone Joint J.* (2018) 100-B:1138–45. doi: 10.1302/0301-620X.100B9.BJJ-2017-1194.R2

74. Balfour W. Observations on the pathology and cure of rheumatism. *Edinb Med Surg J.* (1815) 11:168–87.

75. Hopwood V. *Acupuncture in physiotherapy: Key concepts and evidence-based practice*. Oxford: Elsevier Butterworth-Heinemann (2004).

76. Tan R, Rush S. *Twelve and twelve in acupuncture: advanced principles and techniques*. San Diego, CA: Richard Tan Publishing (1991).

77. Guo Q, Di Z, Tian H, Zhang Q. Contralateral acupuncture for the treatment of phantom limb pain and phantom limb sensation in oncologic lower limb amputee: a case report. *Front Neurosci.* (2021) 15:713548. doi: 10.3389/fnins.2021.713548

78. Karagounis P, Tsironi M, Prionas G, Tsiganos G, Baltopoulos P. Treatment of plantar fasciitis in recreational athletes: two different therapeutic protocols. *Foot Ankle Spec.* (2011) 4:226–34. doi: 10.1177/1938640011407320

79. Wang W, Liu Y, Jiao R, Liu S, Zhao J, Liu Z. Comparison of electroacupuncture and manual acupuncture for patients with plantar heel pain syndrome: a randomized controlled trial. *Acupunct Med.* (2021) 39:272–82. doi: 10.1177/0964528420947739

80. Harty J, Soffe K, O'Toole G, Stephens M. The role of hamstring tightness in plantar fasciitis. *Foot Ankle Int.* (2005) 26:1089–92. doi: 10.1177/107110070502601215

81. Labovitz J, Yu J, Kim C. The role of hamstring tightness in plantar fasciitis. *Foot Ankle Spec.* (2011) 4:141–4. doi: 10.1177/1938640010397341

82. Shin K, Kim J, Lee S, Shin M, Kim T, Park H, et al. Acupuncture for lateral epicondylitis (tennis elbow): study protocol for a randomized, practitioner-assessor blinded, controlled pilot clinical trial. *Trials.* (2013) 14:174. doi: 10.1186/1745-6215-14-174

83. Melchart D, Streng A, Hoppe A, Brinkhaus B, Witt C, Wagenpfeil S, et al. Acupuncture in patients with tension-type headache: randomised controlled trial. *BMJ.* (2005) 331:376–82. doi: 10.1136/bmj.38512.405440.8F

84. Nordez A, Gross R, Andrade R, Le Sant G, Freitas S, Ellis R, et al. Non-muscular structures can limit the maximal joint range of motion during stretching. *Sports Med.* (2017) 47:1925–9. doi: 10.1007/s40279-017-0703-5

85. Stecco A, Macchi V, Stecco C, Porzionato A, Ann Day J, Delmas V, et al. Anatomical study of myofascial continuity in the anterior region of the upper limb. *J Bodyw Mov Ther.* (2009) 13:53–62. doi: 10.1016/j.jbmt.2007.04.009

86. Wilke J, Krause F. Myofascial chains of the upper limb: a systematic review of anatomical studies. *Clin Anat.* (2019) 32:934–40. doi: 10.1002/ca.23424

87. Prabu Raja G, Shyamasunder Bhat N, Marie Cruz A, Prabhu A, Fernandes S, Naaz N. The anatomical myofascial continuum between the neck and eyes. *Clin Anat.* (2022) 35:340–6. doi: 10.1002/ca.23835

88. Focks C, März U, Hosbach I. *Atlas of acupuncture*. Edinburgh: Churchill Livingstone/Elsevier (2008).

89. Veith, I. Huang Ti Nei ching Su Wên: The Yellow Emperor's classic of internal medicine. Chapters 1-34 translated from the Chinese, with an introductory study by Ilza Veith. Berkeley, CA: University of California Press (1972).

90. Yang Z, Liu L. *The Yellow Emperor's Inner Transmission of Acupuncture*. Hong Kong: The Chinese University of Hong Kong Press (2020).

91. Melzack R, Stillwell D, Fox E. Trigger points and acupuncture points for pain: correlations and implications. *Pain.* (1977) 3:3–23. doi: 10.1016/0304-3959(77)90032-X

92. Sasabe R, Sakamoto J, Goto K, Honda Y, Kataoka H, Nakano J, et al. Effects of joint immobilization on changes in myofibroblasts and collagen in the rat knee contracture model. *J Orthop Res.* (2017) 5:1998–2006. doi: 10.1002/jor.23498

93. Wan R, Weissman J, Grundman K, Lang L, Grybowski D, Galiano R. Diabetic wound healing: The impact of diabetes on myofibroblast activity and its potential therapeutic treatments. *Wound Repair Regen.* (2021) 29:573–81. doi: 10.1111/wrr.12954

94. Wolfson B, Zhang Y, Gernapudi R, Duru N, Yao Y, Lo P, et al. A high-fat diet promotes mammary gland myofibroblast differentiation through MicroRNA 140 downregulation. *Mol Cell Biol.* (2017) 37:e00461–16. doi: 10.1128/MCB.00461-16

95. Yang W, Zhang S, Zhu J, Jiang H, Jia D, Ou T, et al. Gut microbe-derived metabolite trimethylamine N-oxide accelerates fibroblast-myofibroblast differentiation and induces cardiac fibrosis. *J Mol Cell Cardiol.* (2019) 134:119–30. doi: 10.1016/j.yjmcc.2019.07.004

96. Arora P, Bibby K, McCulloch C. Slow oscillations of free intracellular calcium ion concentration in human fibroblasts responding to mechanical stretch. *J Cell Physiol.* (1994) 161:187–200. doi: 10.1002/jcp.1041610202

97. Castella L, Buscemi L, Godbout C, Meister J, Hinz B. A new lock-step mechanism of matrix remodelling based on subcellular contractile events. *J Cell Sci.* (2010) 123:1751–60. doi: 10.1242/jcs.066795

98. Kluger B, Rakowski D, Christian M, Cedar D, Wong B, Crawford J, et al. Randomized, controlled trial of acupuncture for fatigue in parkinson's disease. *Mov Disord.* (2016) 31:1027–32. doi: 10.1002/mds.26597

99. Mist S, Jones K. Randomized controlled trial of acupuncture for women with fibromyalgia: group acupuncture with traditional chinese medicine diagnosis-based point selection. *Pain Med.* (2018) 19:1862–71. doi: 10.1093/pm/pnx322

100. Wu L. Nonlinear finite element analysis for musculoskeletal biomechanics of medial and lateral plantar longitudinal arch of Virtual Chinese Human after plantar ligamentous structure failures. *Clin Biomech.* (2007) 22:221–9. doi: 10.1016/j.clinbiomech.2006.09.009

101. Padhi A, Singh K, Franco-Barraza J, Marston D, Cukierman E, Hahn K, et al. Force-exerting perpendicular lateral protrusions in fibroblastic cell contraction. *Commun Biol.* (2020) 3:390. doi: 10.1038/s42003-020-01117-7

102. Verjee L, Midwood K, Davidson D, Essex D, Sandison A, Nanchahal J. Myofibroblast distribution in Dupuytren's cords: correlation with digital contracture. *J Hand Surg Am.* (2009) 34:1785–94. doi: 10.1016/j.jhsa.2009.08.005

103. VandeBerg J, Rudolph R, Gelberman R, Woodward M. Ultrastructural relationship of skin to nodule and cord in Dupuytren's contracture. *Plast Reconstr Surg.* (1982) 69:835–44. doi: 10.1097/00006534-198205000-00021

104. Bisson M, McGrouther D, Mudera V, Grobbelaar A. The different characteristics of Dupuytren's disease fibroblasts derived from either nodule or cord: expression of alpha-smooth muscle actin and the response to stimulation by TGF-beta1. *J Hand Surg Br.* (2003) 28:351–6. doi: 10.1016/s0266-7681(03)00135-9

105. Ng C, Hinz B, Swartz M. Interstitial fluid flow induces myofibroblast differentiation and collagen alignment in vitro. *J Cell Sci.* (2005) 118:4731–9. doi: 10.1242/jcs.02605

106. Ma H, Macdougall L, GonzalezRodriguez A, Schroeder M, Batan D, Weiss R, et al. Calcium signaling regulates valvular interstitial cell alignment and myofibroblast activation in fast-relaxing boronate hydrogels. *Macromol Biosci.* (2020) 20:e2000268. doi: 10.1002/mabi.202000268

107. Soiné J, Hersch N, Dreissen G, Hampe N, Hoffmann B, Merkel R, et al. Measuring cellular traction forces on non-planar substrates. *Interface Focus.* (2016) 6:20160024. doi: 10.1098/rsfs.2016.0024

108. Cirka H, Monterosso M, Diamantides N, Favreau J, Wen Q, Billiar K. Active traction force response to long-term cyclic stretch is dependent on cell pre-stress. *Biophys J.* (2016) 110:1845–57. doi: 10.1016/j.bpj.2016.02.036

109. Vassaux M, Milan J. Stem cell mechanical behaviour modelling: substrate's curvature influence during adhesion. *Biomech Model Mechanobiol.* (2017) 16:1295–308. doi: 10.1007/s10237-017-0888-4

110. Staud R. Peripheral pain mechanisms in chronic widespread pain. *Best Pract Res Clin Rheumatol.* (2011) 25:155–64. doi: 10.1016/j.berh.2010.01.010

111. Acasuso-Díaz M, Collantes-Estévez E. Joint hypermobility in patients with fibromyalgia syndrome. *Arthritis Care Res.* (1998) 11:39–42. doi: 10.1002/art.1790110107

112. Sacheti A, Szemere J, Bernstein B, Tafas T, Schechter N, Tsipouras P. Chronic pain is a manifestation of the Ehlers-Danlos syndrome. *J Pain Symptom Manage.* (1997) 14:88–93. doi: 10.1016/s0885-3924(97)00007-9

113. Gedalia A, Press J, Klein M, Buskila D. Joint hypermobility and fibromyalgia in schoolchildren. *Ann Rheum Dis.* (1993) 52:494–6. doi: 10.1136/ard.52.7.494

114. Chen G, Olver J, Kanaan R. Functional somatic syndromes and joint hypermobility: a systematic review and meta-analysis. *J Psychosom Res.* (2021) 148:110556. doi: 10.1016/j.jpsychores.2021.110556

115. Chiarelli N, Zoppi N, Ritelli M, Venturini M, Capitanio D, Gelfi C, et al. Biological insights in the pathogenesis of hypermobile Ehlers-Danlos syndrome from proteome profiling of patients' dermal myofibroblasts. *Biochim Biophys Acta Mol Basis Dis.* (2021) 1867:166051. doi: 10.1016/j.bbadis.2020.166051

116. Zoppi N, Chiarelli N, Binetti S, Ritelli M, Colombi M. Dermal fibroblast-to-myofibroblast transition sustained by αvß3 integrin-ILK-Snail1/Slug signaling is a common feature for hypermobile Ehlers-Danlos syndrome and hypermobility spectrum disorders. *Biochim Biophys Acta Mol Basis Dis.* (2018) 1864:1010–23. doi: 10.1016/j.bbadis.2018.01.005

117. Chiarelli N, Zoppi N, Venturini M, Capitanio D, Gelfi C, Ritelli M, et al. Matrix metalloproteinases inhibition by doxycycline rescues extracellular matrix organization and partly reverts myofibroblast differentiation in hypermobile ehlers-danlos syndrome dermal fibroblasts: a potential therapeutic target? *Cells.* (2021) 10:3236. doi: 10.3390/cells10113236

118. Llamas-Ramos R, Pecos-Martín D, Gallego-Izquierdo T, Llamas-Ramos I, Plaza-Manzano G, Ortega-Santiago R, et al. Comparison of the short-term outcomes between trigger point dry needling and trigger point manual therapy for the management of chronic mechanical neck pain: a randomized clinical trial. *J Orthop Sports Phys Ther.* (2014) 44:852–61. doi: 10.2519/jospt.2014.5229

119. Mejuto-Vázquez M, Salom-Moreno J, Ortega-Santiago R, Truyols-Domínguez S, Fernández-de-Las-Peñas C. Short-term changes in neck pain, widespread pressure pain sensitivity, and cervical range of motion after the application of trigger point dry needling in patients with acute mechanical neck pain: a randomized clinical trial. *J Orthop Sports Phys Ther.* (2014) 44:252–60. doi: 10.2519/jospt.2014.5108

120. Calvo-Lobo C, Pacheco-da-Costa S, Martínez-Martínez J, Rodríguez-Sanz D, Cuesta-Álvaro P, López-López D. Dry needling on the infraspinatus latent and active myofascial trigger points in older adults with nonspecific shoulder pain: a randomized clinical trial. *J Geriatr Phys Ther.* (2018) 41:1–13. doi: 10.1519/JPT.0000000000000079

121. Geist K, Frierson E, Goudiss H, Kitchen H, Wilkins M, Pruszynski D, et al. Short-term effects of dry needling at a spinal and peripheral site on functional outcome measures, strength, and proprioception among individuals with a lateral ankle sprain. *J Bodyw Mov Ther.* (2021) 26:158–66. doi: 10.1016/j.jbmt.2020.12.021

122. Kalia V, Mani S, Kumar S. Short-term effect of myofascial trigger point dry-needling in patients with Adhesive Capsulitis. *J Bodyw Mov Ther.* (2021) 25:146–50. doi: 10.1016/j.jbmt.2020.10.014

123. Fernández-De-Las-Peñas C, Plaza-Manzano G, Sanchez-Infante J, Gómez-Chiguano G, Cleland J, Arias-Buría J, et al. Is dry needling effective when combined with other therapies for myofascial trigger points associated with neck pain symptoms?

A systematic review and meta-analysis. *Pain Res Manag.* (2021) 2021:8836427. doi: 10.1155/2021/8836427

124. Mishra A, Skrepnik N, Edwards S, Jones G, Sampson S, Vermillion D, et al. Efficacy of platelet-rich plasma for chronic tennis elbow: a double-blind, prospective, multicenter, randomized controlled trial of 230 patients. *Am J Sports Med.* (2014) 42:463–71. doi: 10.1177/0363546513494359

125. Langevin H, Yandow J. Relationship of acupuncture points and meridians to connective tissue planes. *Anat Rec.* (2002) 269:257–65. doi: 10.1002/ar.10185

126. Langevin H, Bouffard N, Churchill D, Badger G. Connective tissue fibroblast response to acupuncture: dose-dependent effect of bidirectional needle rotation. *J Altern Complement Med.* (2007) 13:355–60. doi: 10.1089/acm.2007.6351

127. Langevin H, Bouffard N, Badger G, Churchill D, Howe A. Subcutaneous tissue fibroblast cytoskeletal remodeling induced by acupuncture: evidence for a mechanotransduction-based mechanism. *J Cell Physiol.* (2006) 207:767–74. doi: 10.1002/jcp.20623

128. Kayal C, Moeendarbary E, Shipley R, Phillips J. Mechanical response of neural cells to physiologically relevant stiffness gradients. *Adv Healthc Mater.* (2020) 9:e1901036. doi: 10.1002/adhm.201901036

129. Gu Y, Ji Y, Zhao Y, Liu Y, Ding F, Gu X, et al. The influence of substrate stiffness on the behavior and functions of Schwann cells in culture. *Biomaterials.* (2012) 33:6672–81. doi: 10.1016/j.biomaterials.2012.06.006

130. Yip A, Iwasaki K, Ursekar C, Machiyama H, Saxena M, Chen H, et al. Cellular response to substrate rigidity is governed by either stress or strain. *Biophys J.* (2013) 104:19–29. doi: 10.1016/j.bpj.2012.11.3805

131. Doss B, Pan M, Gupta M, Grenci G, Mège R, Lim C, et al. Cell response to substrate rigidity is regulated by active and passive cytoskeletal stress. *Proc Natl Acad Sci U.S.A.* (2020) 117:12817–25. doi: 10.1073/pnas.1917555117

132. Discher D, Janmey P, Wang Y. Tissue cells feel and respond to the stiffness of their substrate. *Science.* (2005) 310:1139–43. doi: 10.1126/science.1116995

133. Zheng N, Yuan X, Chi Y, Liu P, Wang B, Sui J, et al. The universal existence of myodural bridge in mammals: an indication of a necessary function. *Sci Rep.* (2017) 7:8248. doi: 10.1038/s41598-017-06863-z

134. Jiang W, Zhang Z, Yu S, Sun J, Ding S, Ma G, et al. Scanning electron microscopic observation of myodural bridge in the human suboccipital region. *Spine.* (2020) 45:E1296–301. doi: 10.1097/BRS.0000000000003602

135. Bose A, Basu R, Maulik M, Das Sarma J. Loss of Cx43-mediated functional gap junction communication in meningeal fibroblasts following mouse hepatitis virus infection. *Mol Neurobiol.* (2018) 55:6558–71. doi: 10.1007/s12035-017-0861-3

136. Grafstein B, Liu S, Cotrina M, Goldman S, Nedergaard M. Meningeal cells can communicate with astrocytes by calcium signaling. *Ann Neurol.* (2000) 47:18–25.

137. Mercier F, Hatton G. Immunocytochemical basis for a meningeo-glial network. *J Comp Neurol.* (2000) 420:445–65. doi: 10.1002/(sici)1096-9861(20000515)420:4<445::aid-cne4>3.0.co;2-3

138. Mercier F, Hatton G. Connexin 26 and basic fibroblast growth factor are expressed primarily in the subpial and subependymal layers in adult brain parenchyma: roles in stem cell proliferation and morphological plasticity? *J Comp Neurol.* (2001) 431:88–104. doi: 10.1002/1096-9861(20010226)431:1<88::aid-cne1057>3.0.co;2-d

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Safety and efficacy of 650 nm invasive laser acupuncture on non-specific chronic low back pain: A protocol for a multicenter randomized placebo-controlled trial

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Background: We aim to obtain clinical trial data regarding the safety, efficacy, and usefulness of invasive laser acupuncture (ILA) for non-specific chronic low back pain (NSCLBP) through a randomized placebo-controlled trial.

Methods: Our clinical trial will be an assessor- and patient-blinded, prospective, parallel-arm, multi-center, randomized placebo-controlled clinical trial. One hundred and six participants with NSCLBP will be allocated evenly to the 650 ILA or control group. All participants will receive education on exercise and self-management. The 650 ILA group will undergo 650 nm ILA for 10 min, and the control group will undergo sham ILA for 10 min per visit, twice a week for 4 weeks, at bilateral GB30, BL23, BL24, and BL25. The primary outcome will be the proportion of responders (≥30% reduction in pain visual analogue scale [VAS] without increased use of painkillers) at 3 days after the intervention ends. The secondary outcomes will include changes in the scores of the VAS, European Quality of Life Five Dimension Five Level scale, and Korean version of the Oswestry Disability Index at 3 days after the intervention ends and 8 weeks after the intervention ends.

Discussions: The results of our study will provide clinical evidence concerning the safety and efficacy of 650 nm ILA for the management of NSCLBP.

Clinical trial registration: https://cris.nih.go.kr/cris/search/detailSearch.do?search_lang=E&focus=reset_12&search_page=M&pageSize=10&page=undefined&seq=21591&status=5&seq_group=21591, identifier KCT0007167.

KEYWORDS

safety, efficacy, non-specific chronic low back pain, study protocol, randomized controlled trial, laser acupuncture

Introduction

Low back pain (LBP), defined as discomfort and pain, localized below the costal margin and above the inferior gluteal folds with or without referred lower extremity pain, is a symptom rather than a specific disease (1, 2). It is a major cause of disability in daily living. Functional restrictions and consequent disabilities pose a huge economic burden for individuals and the society (3). Most LBPs are non-specific (commonly cited as 90–95%) (4), and non-specific chronic LBP (NSCLBP) is defined as that which cannot be attributed to a known specific pathology (e.g., structural deformity, tumor, osteoporosis, infection, radicular syndrome, fracture, cauda equina syndrome, or ankylosing spondylitis) and persists for >3 months (1, 2, 5–7).

Treatment guidelines for patients with NSCLBP recommend the use of exercise therapy, antidepressants, psychosocial interventions, and non-steroidal anti-inflammatory drugs (2, 5, 6). Non-pharmacological treatments, such as exercise and self-management, are preferred to pharmacological interventions in the management of NSCLBP (2, 7–9). Physical modalities such as transcutaneous electrical nerve stimulation and low level laser therapy (LLLT) have a low quality evidence (6, 8) and acupuncture has mixed support and received conflicting recommendations (2, 5, 8).

Low level laser therapy is a light source intervention that creates no vibration, heat, or sound and stimulates photochemical or non-thermal cellular processes (10). LLLT is currently used to treat musculoskeletal disorders, such as back pain (11). The possible underlying mechanisms for the pain reduction effects of LLLT include its inhibition of neural function, anti-inflammatory effect, and connective tissue repair ability which have been demonstrated by a number of experiments (12–14). Laser acupuncture (LA) is the irradiation of a low-intensity laser at the acupoints using laser pointer devices (15). Whether LLLT, including LA, is effective for LBP when compared with sham laser remains controversial. A Cochrane systematic review of LLLT on non-specific LBP in 2008 decried the lack of sufficient data to either refute or support the efficacy of LLLT for the management of LBP (16). In contrast, a meta-analysis suggested that LLLT was an effective treatment for reducing pain in patients with NSCLBP (17, 18). Glazov et al. reported that LLLT alone or in combination with other therapies might effectively reduce pain for up to 3 months in NSCLBP without adverse effects (19).

While LA is a non-invasive therapy that uses laser-emitting devices, invasive LA (ILA) is conducted concurrently with invasive acupuncture and focused laser irradiation utilizing an acupuncture needle attached to a laser instrument (20). Our previous pilot randomized controlled trial (RCT) revealed that 650 nm ILA with the same ILA parameters (650 nm wavelength, 20 mW power, and 50 Hz frequency), treatment acupoint, and treatment schedule as in this study significantly improved pain and pain-related functional limitations in patients with NSCLBP at the end of the intervention (21). These potential effects of 650 nm ILA on NSCLBP requires validation using a rigorous RCT with a large sample size. Therefore, we aim to obtain clinical evidence on the safety and efficacy of 650 nm ILA for the management of NSCLBP. In a situation where is no high-quality evidence supporting the efficacy of LLLT and acupuncture, the results of this study will provide clinical evidence of the use of ILA for NSCLBP and thus promote the use of ILA in the treatment of NSCLBP.

Abbreviations: AE, adverse event; CRC, clinical research coordinator; eCRF, electronic case report form; EQ-5D-5L, European quality of life five dimension five level scale; FAS, full analysis set; ILA, invasive laser acupuncture; IRB, institutional review board; LA, laser acupuncture; LBP, low back pain; LLLT, low-level laser therapy; NSCLBP, non-specific chronic low back pain; ODI, Oswestry disability index; PI, principal investigator; PPS, per-protocol set; RCTs, randomized controlled trials; SAE, serious adverse event; SOPs, standard operating procedures; SPIRIT, standard protocol items: recommendations for interventional trials; VAS, visual analogue scale.

Materials and analysis

Aims

1) We will investigate the clinically persistent pain-reduction effect of 650 nm ILA for NSCLBP at 3 days after the end of intervention.
2) We will investigate the pain reduction, functional limitation improvement, and quality of life improvement effects of 650 nm ILA for NSCLBP.
3) We will investigate the safety of 650 nm ILA in patients with NSCLBP.

Hypothesis

1) The 650 ILA group will have a significantly higher proportion of responders than the control group at 3 days after the end of intervention.
2) The 650 nm ILA will show improvements in pain intensity, quality of life, and functional limitation in patients with NSCLBP.
3) The 650 nm ILA would be a safe treatment for patients with NSCLBP.

Study design and setting

Our study was approved by the Ministry of Food and Drug Safety (Medical Device Approval No. 1322) and was registered with the Clinical Research Information Service (registration No. KCT0007167). Our study complies with the Korean Good Clinical Practice guidelines and the principles of the Declaration of Helsinki. We have written the manuscript in accordance with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) reporting checklist (22).

This clinical trial will be an assessor and patient–blinded, prospective, parallel-arm, multi-center, randomized placebo-controlled clinical trial. In total, 106 eligible participants will be randomized evenly into the 650 ILA or control group (n = 53 in each group). All participants will receive education on exercise and self-management. Participants in the 650 ILA group will undergo real 650 nm ILA for 10 min, while those in the control group will undergo sham ILA for 10 min. The treatment will be administered once per visit, twice a week for 4 weeks, at bilateral Gallbladder 30 (GB30; Huantiao), Bladder 23 (BL23; Shenshu), Bladder 24 (BL24; Qihaishu), and Bladder 25 (BL25; Dachangshu).

The primary outcome will be the proportion of responders (≥30% reduction in pain visual analogue scale [VAS] without increased use of painkillers) at 3 days after the intervention ends. The secondary outcome will be changes in the scores of the VAS, European Quality of Life Five Dimension Five Level scale (EQ-5D-5L), and Korean version of the Oswestry Disability Index (ODI) at 3 days after the intervention ends and 8 weeks after the intervention ends.

The clinical trial design is shown in Table 1.

Recruitment

We will recruit participants at the Kyung Hee University Korean Medicine Hospital and Dongshin University Gwangju Korean Medicine Hospital in the Republic of Korea *via* the use of posters, local newspapers, and the internet in hospitals and communities. Interested people will receive an explanation of this study from the clinical research coordinator (CRC) upon visiting the hospital and will provide written informed consent prior to participation.

At each visit, the CRC will explain the following visit schedule and will adjust the visit schedules for each participant to facilitate participation.

Inclusion criteria

(1) Adults aged between 19 and 70 years; (2) has NSCLBP lasting for at least the preceding 3 months, and LBP that occurred more than 14 days a month; (3) has no change in history of medications for 4 weeks before screening. For participants with medications prescribed and used for NSCLBP, usage shall be at a stable dose in the 4 weeks prior to the baseline visit; (4) moderate pain (100 mm VAS scores for pain had a range of 35–74) (23) at screening; and (5) has sufficient fluency in Korean to perform valid assessments.

Exclusion criteria

(1) Participants has progressive neurological deficits or radicular pain; (2) has a serious disease (diabetic neuropathy or cancer, severe kidney, liver, cerebrovascular, or cardiovascular disease); (3) has a serious spinal pathology (cauda equina syndrome, cancer, inflammatory spondylitis, recent vertebral fracture, or spinal infection); (4) has a LBP caused by rheumatoid arthritis, ankylosing spondylitis, gout, trauma, or fibromyalgia; (5) has a history of treatment for mental illness (depression, dementia, schizophrenia, or epilepsy) or drug/alcohol dependency in the 6 months preceding

TABLE 1 SPIRIT statement showing the enrollment, interventions, and data collection.

	Study period							
	Enrollment	Allocation	Post-allocation					Close-out
Timepoint	Screening		Visit 1–2	Visit 3–4	Visit 5–6	Visit 7–8	Visit 9	Visit 10
	Week		1	2	3	4	4 + 3 days	12
Enrollment								
Informed consent	X							
Sociodemographic profile	X							
Medical history	X							
Vital signs	X	X	X	X	X	X	X	X
Inclusion/Exclusion criteria	X							
Allocation		X						
Visual analogue scale	X							
Interventions								
Invasive laser acupuncture (sham or 650 nm)			X	X	X	X		
Education on self management and exercise			X	X	X	X		
Assessments								
Change of medical history			X	X	X	X	X	X
Safety assessment (Incidence of AEs)			X	X	X	X	X	X
Clinical laboratory test	X						X	
Visual analogue scale			X				X	X
European quality of life five dimension five level scale			X				X	X
Scores for the Korean version of the Oswestry disability index level scale			X				X	X

screening; (6) has moderate or severe depression (scored ≥ 23 points on a Korean version of Beck depression inventory-II) (24) at screening; (7) has contraindications for ILA, such as presence of electronic medical devices, severe skin disease in the lumbar region, blood clotting abnormalities, or presence of metallic devices in the lumbar vertebrae; (8) has a history of lumbar spinal surgery within 1 year or scheduled procedures during the trial; (9) participation for the purpose of social insurance or compensation; (10) concurrent participation in another trial; (11) Pregnant or having a plan for pregnancy; and (12) not suitable for ILA and our rescue regimen.

Dropout and violation criteria

The dropout criteria are as follows: (1) incomplete data that can affect the results of the trial; (2) decision to discontinue participation in this trial by the institutional review board (IRB) or principal investigator (PI) owing to the inability of the participant to participate in this study or the occurrence of a side effects requiring long-term treatment; (3) withdrawal of consent; or (4) occurrence of a serious adverse event (SAE) causing requirement of hospitalization or surgery, serious disability, or death. Participants who meet the dropout criteria will be discontinued from participating in our clinical trial.

The violation criteria are as follows: (1) participating in less than six of the eight treatment sessions (<75% compliance with the intervention protocol); and (2) serious deviation in implementation or critical errors in the protocol.

The participants who meet the violation and dropout criteria will be excluded from the per protocol set (PPS) analysis.

Ethics

This protocol (version. 1.0) was approved by the Ministry of Food and Drug Safety (date: March 16, 2022; Medical Device Approval # 1322). It was approved by the IRB of Kyung Hee University Korean Medicine Hospital (date: April 22, 2022; approval No.: KOMCIRB 2022-03-004-001) and Dongshin University Gwangju Korean Medicine Hospital (date: March 22, 2022; approval No.: DSGOH-2022-002). Participants and their companions will be informed of the study purpose and risks. All participants will provide written informed consent prior to participation.

Randomization and allocation

The investigator will carry out a screening interview, and then the assessor will conduct baseline assessment. The 106 enrolled participants will be randomly allocated evenly to the 650 ILA or control group (n = 53 per group). The serial numbers will be generated by stratified block randomization using SAS® version 9.4 (SAS Institute Inc., Cary, NC, USA). The serial numbers will be packed in opaque envelopes and stored in a cabinet with two locks.

The investigator managing the serial numbers will open the envelopes and assign participants to the investigator who will conduct the intervention.

Implementation

The investigator managing the serial numbers will generate the randomization sequence and distribute participants to the groups. The CRC will enroll the participants.

Blinding

The practitioner will insert the acupuncture needles into GB30, BL23, BL24, and BL25 and then operate the laser emitting device according to the treatment method of each group (650 ILA group, 20 mW power for 10 min; control group, 0 mW power for 10 min). Therefore, the practitioner who will conduct the intervention would know the group assignment of participant. Owing to the practitioner unblinding, we will adopt an assessor and patient-blinded design using sham ILA. During the course of the study, all investigators except those who will perform the intervention and manage the serial number codes will be blinded. However, if necessary, such as in the event of SAE, unblinding will be permitted under the IRB approval. This study will only include individuals without predetermined positions or competing interests.

Interventions

Trained Korean medical doctors will administer the treatment. The investigators who will carry out the interventions will undergo joint training to ensure adherence to our protocol. ILA treatment will be conducted using a laser emitting device (Ellise; Wontech Co. Ltd., Daejeon, Republic of Korea) comprising an optical fiber-coupled laser diode (InGaAlP), a sterile acupuncture needle with optical fibers inserted therein, and a laser output device (**Figure 1**).

The acupuncture needles will be vertically inserted into GB30, BL23, BL24, and BL25 and then the laser (650 ILA group, 20 mW power for 10 min; control group, 0 mW power for 10 min) will be turned on. The ILA parameters will be 50 Hz frequency, 20 mW power, 63.69 W/cm^2 power density, 12 J/point energy dose, 38216.56 J/cm^2 energy density, and pulse type wave. Participants will receive treatment for 10 min per visit, twice a week for 4 weeks. Based on previous RCTs investigating the efficacy of LLLT for NSCLBP (25, 26), the control group will receive the same procedure as the 650 ILA group. No significant differences in sound, feeling, or observation will be expected between the two groups. Hence, all participants will be blinded. The ILA treatment methods have been described in more details in our previous pilot study (20).

All participants will receive education on exercise and self-management during the treatment period (Visit 1–Visit 8). We will provide all participants with acetaminophen (500 mg), which can be taken when the pain is severe as a rescue regimen.

At each visit, the medical condition of the participants will be monitored to ensure that they adhere to the trial procedure. All participants will be expected to comply with the intervention protocol. However, the assessment and treatment schedule may be changed upon the request of the participant or in accordance with the judgment of the PI.

During the study period, all participants will not be permitted to receive other treatments (complementary and alternative therapies, physical therapy, or pharmacological treatments not allowed in our

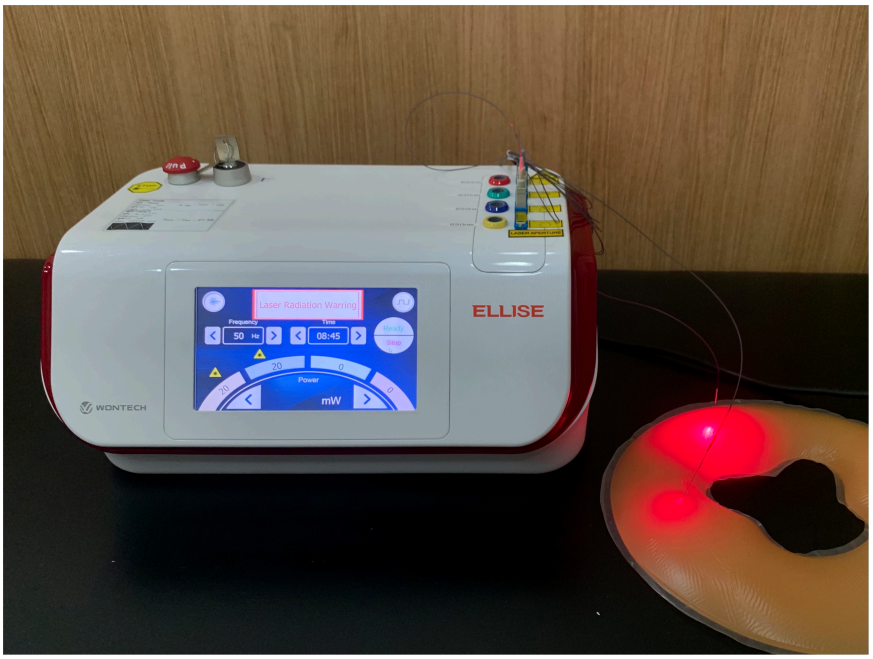

FIGURE 1
Invasive laser acupuncture (ILA) therapy.

trial) for improving NSCLBP symptoms. However, they will be allowed to use pharmacological and non-pharmacological treatments for improving other symptoms.

Outcome measurements

The primary outcome will be the between-group differences of the proportions of responders at 3 days after the intervention ends. A responder is defined as a participant who responds with more than 30% decrease in baseline VAS score without an increase in baseline use of painkillers (27, 28).

The secondary outcomes will include the between-group differences of changes in VAS, EQ-5D-5L, and ODI at 3 days after the treatment ends and 8 weeks after the treatment ends.

The VAS is a self-reported scale, usually a 100-mm-long straight line marked 0 for no pain and 100 for pain as intense as it could be (29). It is widely used to assess pain severity in LBP trials (30).

The EQ-5D is a generic assessment tool for measuring health-related quality of life (31). The EQ-5D-5L is a new version of the EQ-5D that extends the level of each dimension from three to five (32).

The ODI includes nine questions about interference with several physical activities and one question about pain intensity (33). We used the Korean version of the ODI, which excluded sexual life from the original ODI and has been validated (34).

Adverse events

Adverse events (AEs) are unintentional and undesirable symptoms, signs, or diseases that appear during or after treatment in a clinical trial. In our pilot study, no AEs and SAEs were associated with laser irradiation (21). AEs that could occur in our trial include bleeding, pallor, local hematoma, skin irritation, objective worsening of pain, and dizziness or fainting. All the AEs and SAEs would be recorded in detail, including the potential causalities between the intervention and AE, degree of severity, time of occurrence, and any measures taken to improve AE by the CRC. They will be reported to the IRB. The occurrence rate of SAEs and AEs will be compared between the two groups in the safety assessment. Participants who will experience AEs and SAEs related to our intervention will be compensated according to the relevant regulations.

Quality control

Our protocol has been developed and revised severally by experts in LA, NSCLBP, and statistics. Before the study, all investigators will be trained severally to fully understand the standard operating procedures (SOPs) of this trial and the protocol. An independent clinical research associate will monitor our study and check all the trial documents. Any revision of this protocol will be reviewed and approved by the Ministry of Food and Drug Safety and the IRB of Kyung Hee University Korean Medicine Hospital and Dongshin University Gwangju Korean Medicine Hospital.

Sample size estimation

In our previous pilot study (21), the difference in proportion of responders between 650 ILA (93% [14/15]) and control groups (40% [6/15]) was 53%. To obtain sufficient clinical data, we determined the sample size assuming an expected responder proportion of 30% in the control group and 60% in the 650 ILA group, a two-sided alpha level of 0.05, and a statistical power of 0.8. Under these assumptions,

a total of 84 (42 per group) participants will be required. Estimating a maximum dropout rate of 20%, 106 participants (53 in each group) will be required in our trial.

$$\frac{n = (z_{\alpha/2}\sqrt{2\bar{p}\bar{q}} + z_{\beta}\sqrt{p_1q_1 + p_2q_2})^2}{(p_t - p_c)^2} =$$

$$\frac{(1.96\sqrt{2 \times 0.45 \times 0.55} + 0.842\sqrt{0.30 \times 0.70 + 0.60 \times 0.40})^2}{(0.60 - 0.30)^2}$$

$$\approx 42$$

Statistical analysis

The final data will be analyzed by a biostatistician not involved in the execution of our trial. The primary analysis population for assessing the efficacy of the intervention will be a full analysis set (FAS), while the supplementary analysis population will be a PPS. The results of the FAS and PPS analyses will be compared and reflected in the efficacy evaluation. "Multiple Imputation" method will be used to obtain the missing value. All analyses will be performed at the 5% (two-sided) significance level using SAS® version 9.4 (SAS institute. Inc., Cary, NC, USA) software. We will not perform interim analyses.

Baseline characteristics and variables will be compared between the two groups. Categorical data will be compared using the Fisher's exact test or chi-square test, while continuous data will be compared using the Wilcoxon's rank sum test or independent *t*-test.

The difference in responder proportions will be tested using the chi-square test or Fisher's exact test. The degrees of changes in the VAS, EQ-5D-5L, and ODI scores at 3 days after the treatment ends and 8 weeks after the intervention ends, relative to the baseline score, between the groups will be evaluated using analysis of covariance with baseline scores as covariates. Within each group, changes in VAS, EQ-5D-5L, and ODI scores at each time will be analyzed using one-way analysis of variance and a paired *t*-test or Wilcoxon signed rank test. Sub-analyses will be performed according to each institution.

A safety assessment will be conducted for all SAEs and AEs that will occur during the trial period. The incidences of SAEs and AEs will be compared between the two groups using chi-square test or Fisher's exact test. A comparative analysis will be carried out between the groups for participants who will be out of normal range for a clinical laboratory test.

Confidentiality and data management

All documents will be classified and logged with identification codes, but the names will remain concealed.

All identification records will be kept confidential and will not be accessible without IRB approval. All data will be recorded in the electronic case report forms (eCRFs) by the CRC and checked by a investigator, who will not be involved in the execution of this trial. Electronic data will be securely stored in the eCRFs using the myTrial data management system (NIKOM, Republic of Korea). Data access will be protected by user name and password. The data manager will have online access to the all data, whereas institutions will have access only to data related to their own institution. The data manager and the statistician will have online access to the entire database. The data coordinating center in the Korea Institute of Oriental Medicine will be unaffiliated with the sponsor and devoid of competing interests. No access to the data will be granted to anyone not approved by the IRB. In addition, raw data will be stored for 3 years after the completion of the trial. Participants will voluntarily offer informed written consent for the dissemination of their personal information.

Discussion

The efficacy of LA for musculoskeletal pain is majorly determined by the energy dosage applied (35). Energy penetration through the skin is affected by the scatter, reflection, and absorption of energy by skin structures. As the acupoints are thought to be located in the myofascial layer, the low energy transmission of non-invasive LA may not be fully effective in stimulating acupoints (15). However, the 650 nm wavelength ILA used in this study is safe and can compensate for the scatter, reflection, and absorption of light by the skin and enhance energy transmission, because the laser is emitted at the acupuncture needle's tip after being placed beneath the skin. The design of this study, including the ILA intervention (i.e., treatment acupoint and laser wavelength), treatment schedules, outcome measurements, and sample size, is based on that of our previous pilot study (21). In our previous pilot clinical trial (21), 650 nm ILA at bilateral GB30, BL23, BL24, and BL25 showed significant improvement in the VAS and ODI scores at the intervention endpoint and ODI score at 4 weeks after the intervention ends, compared with sham laser in patients with NSCLBP. Therefore, BL23, BL24, BL25, and GB30 were selected for treatment acupoints and 650 nm for laser wavelength in this study.

The clinical assessments used in LBP trials are often highly subjective and correlate poorly with symptoms. The responder index could be sensitive to clinically meaningful treatment effects and could mitigate the placebo effect (27). The preliminary developed responder index for chronic low back pain was at least 30% improvement in pain, with an improvement of at least 30% in patient global assessment and no worsening in function (27). We adopted responder proportions (≥30% relief on the VAS without analgesics increase) used in a recent chronic LBP clinical trial as a primary outcome (28). Four core outcome domains for LBP trial are pain intensity, number of deaths, health-related quality of life, and physical functioning (36, 37). We used change in VAS scores to measure the pain intensity, ODI scores to measure the physical functioning, and EQ-5D-5L scores to evaluate the health-related quality of life.

Considering the results of this study, 650 nm ILA is expected to show safety, clinically significant improvement, pain reduction, and improvement of functional limitation and quality of life in patients with NSCLBP. This is important for the clinical use of 650 nm ILA and development of optimal laser parameters for the treatment of NSCLBP.

This protocol has certain limitations. First, we will not use different treatment methods of LA for NSCLBP treatment. The factors that influence the efficacy of LA are selected acupoints, wavelength, and energy dose. Various treatment methods of LA differ according to wavelength, energy dose, and acupoints for treating NSCLBP (16–19, 38). Since 650 nm ILA parameters, including the wavelength, power, energy dose, and acupoints, used in our pilot study show significant pain reduction at the intervention endpoint, we adopted only the 650 nm ILA parameters used in our pilot study in this study. Thus, further studies should be conducted to investigate the optimal parameters. Second, since most participants in our pilot

study had moderate pain at the baseline and the high dropout rate owing to pain increase during the study period in the case of patients with severe pain was concerning, our study will include only patients with moderate NSCLBP, not those with severe NSCLBP. Third, since the practitioner will operate the laser emitting device according to the treatment method of each group (650 ILA group, 20 mW power for 10 min; control group, 0 mW power for 10 min), we cannot adopt practitioner blinding. Therefore, we will adopt an assessor- and patient-blinded study design.

Nevertheless, the findings of this study would suggest clinical evidence concerning the safety and efficacy of 650 nm ILA for the management of NSCLBP, thereby establishing the basis for further investigation. It would contribute to increasing the availability of laser and promoting the development of optimal laser treatment method in the management of LBP.

Ethics statement

This protocol (version. 1.0) was approved by the Ministry of Food and Drug Safety (date: March 16, 2022; Medical Device Approval # 1322). It was approved by the IRB of Kyung Hee University Korean Medicine Hospital (date: April 22, 2022; approval No.: KOMCIRB 2022-03-004-001) and Dongshin University Gwangju Korean Medicine Hospital (date: March 22, 2022; approval No.: DSGOH-2022-002). Participants and their companions will be informed of the study purpose and risks. All participants will provide written informed consent prior to participation.

Author contributions

J-HK and CY were responsible for designing and conceiving the trial, preparing the manuscript, supervising the entire clinical trial process, and writing and revising the final manuscript. J-HK, JY, G-CP, A-RK, JK, DN, and YH participated in the data collection and were in charge of the recruitment, treatment, and evaluation of the patients. B-KK was responsible for planning the data analysis and analyzing final data from the trial. All authors reviewed and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Airaksinen O, Brox J, Cedraschi C, Hildebrandt J, Klaber-Moffett J, Kovacs F, et al. Chapter 4. European guidelines for the management of chronic nonspecific low back pain. *Eur Spine J.* (2006) 15(Suppl 2):S192–300. doi: 10.1007/s00586-006-1072-1

2. Maher C, Underwood M, Buchbinder R. Non-specific low back pain. *Lancet.* (2017) 389:736–47. doi: 10.1016/S0140-6736(16)30970-9

3. Hoy D, March L, Brooks P, Blyth F, Woolf A, Bain C, et al. The global burden of low back pain: estimates from the Global Burden of Disease 2010 study. *Ann Rheum Dis.* (2014) 73:968–74. doi: 10.1136/annrheumdis-2013-204428

4. Bardin L, King P, Maher C. Diagnostic triage for low back pain: a practical approach for primary care. *Med J Aust.* (2017) 206:268–73. doi: 10.5694/mja16.00828

5. Oliveira C, Maher C, Pinto R, Traeger A, Lin C, Chenot J, et al. Clinical practice guidelines for the management of non-specific low back pain in primary care: an updated overview. *Eur Spine J.* (2018) 27:2791–803. doi: 10.1007/s00586-018-5673-2

6. Qaseem A, Wilt T, McLean R, Forciea M. Clinical Guidelines Committee of the American College of Physicians, Denberg TD, et al. Noninvasive treatments for acute, subacute, and chronic low back pain: a clinical practice guideline from the American College of Physicians. *Ann Intern Med.* (2017) 166:514–30. doi: 10.7326/M16-2367

7. Krenn C, Horvath K, Jeitler K, Zipp C, Siebenhofer-Kroitzsch A, Semlitsch T. Management of non-specific low back pain in primary care – a systematic overview of recommendations from international evidence-based guidelines. *Prim Health Care Res Dev.* (2020) 21:e64. doi: 10.1017/S1463423620000626

8. Meroni R, Piscitelli D, Ravasio C, Vanti C, Bertozzi L, De Vito G, et al. Evidence for managing chronic low back pain in primary care: a review of recommendations from high-quality clinical practice guidelines. *Disabil Rehabil.* (2021) 43:1029–43. doi: 10.1080/09638288.2019.1645888

9. Foster N, Anema J, Cherkin D, Chou R, Cohen S, Gross D, et al. Prevention and treatment of low back pain: evidence, challenges, and promising directions. *Lancet.* (2018) 391:2368–83. doi: 10.1016/S0140-6736(18)30489-6

10. Basford J. Low-energy laser therapy: controversies and new research findings. *Lasers Surg Med.* (1989) 9:1–5. doi: 10.1002/lsm.1900090103

11. Clijsen R, Brunner A, Barbero M, Clarys P, Taeymans J. Effects of low-level laser therapy on pain in patients with musculoskeletal disorders: a systematic review and meta-analysis. *Eur J Phys Rehabil Med.* (2017) 53:603–10. doi: 10.23736/S1973-9087.17.04432-X

12. Sakurai Y, Yamaguchi M, Abiko Y. Inhibitory effect of low-level laser irradiation on LPS-stimulated prostaglandin E2 production and cyclooxygenase-2 in human gingival fibroblasts. *Eur J Oral Sci.* (2000) 108:29–34. doi: 10.1034/j.1600-0722.2000.00783.x

13. Skinner S, Gage J, Wilce P, Shaw RM. A preliminary study of the effects of laser radiation on collagen metabolism in cell culture. *Aust Dent J.* (1996) 41:188–92. doi: 10.1111/j.1834-7819.1996.tb04854.x

14. Chow R, Armati P. Photobiomodulation: implications for anesthesia and pain relief. *Photomed Laser Surg.* (2016) 34:599–609. doi: 10.1089/pho.2015.4048

15. Chon T, Mallory M, Yang J, Bublitz S, Do A, Dorsher P. Laser acupuncture: a concise review. *Med Acupunct.* (2019) 31:164–8. doi: 10.1089/acu.2019.1343

16. Yousefi-Nooraie R, Schonstein E, Heidari K, Rashidian A, Pennick V, Akbari-Kamrani M, et al. Low level laser therapy for nonspecific low-back pain. *Cochrane Database Syst Rev.* (2008) 2:CD005107. doi: 10.1002/14651858.CD005107.pub4

17. Huang Z, Ma J, Chen J, Shen B, Pei F, Kraus V. The effectiveness of low-level laser therapy for nonspecific chronic low back pain: a systematic review and meta-analysis. *Arthritis Res Ther.* (2015) 17:360. doi: 10.1186/s13075-015-0882-0

18. Yeum H, Hong Y, Nam D. Low-level laser therapy including laser acupuncture for non-specific chronic low back pain: systematic review and meta-analysis. *J Acupunct Res.* (2021) 38:8–19. doi: 10.13045/jar.2020.00283

19. Glazov G, Yelland M, Emery J. Low-level laser therapy for chronic non-specific low back pain: a meta-analysis of randomised controlled trials. *Acupunct Med.* (2016) 34:328–41. doi: 10.1136/acupmed-2015-011036

20. Kim J, Na C, Park G, Lee J. Effects of different wavelengths of invasive laser acupuncture on chronic non-specific low back pain: a study protocol for a pilot randomized controlled trial. *Trials.* (2021) 22:118. doi: 10.1186/s13063-021-05038-6

21. Kim J, Na C, Cho M, Park G, Lee J. Efficacy of invasive laser acupuncture in treating chronic non-specific low back pain: a randomized controlled trial. *PLoS One.* (2022) 17:e0269282. doi: 10.1371/journal.pone.0269282

22. Chan A, Tetzlaff J, Altman D, Laupacis A, Gøtzsche P, Krleža-Jerić K, et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. *Ann Intern Med.* (2013) 158:200–7. doi: 10.7326/0003-4819-158-3-201302050-00583

23. Boonstra A, Schiphorst Preuper H, Balk G, Stewart R. Cut-off points for mild, moderate, and severe pain on the visual analogue scale for pain in patients with chronic musculoskeletal pain. *Pain.* (2014) 155:2545–50. doi: 10.1016/j.pain.2014.09.014

24. Park K, Jaekal E, Yoon S, Lee S, Choi K. Diagnostic utility and psychometric properties of the Beck Depression Inventory – II Among Korean adults. *Front Psychol.* (2019) 10:2934. doi: 10.3389/fpsyg.2019.02934

25. Glazov G, Yelland M, Emery J. Low-dose laser acupuncture for non-specific chronic low back pain: a double-blind randomised controlled trial. *Acupunct Med.* (2014) 32:116–23. doi: 10.1136/acupmed-2013-010456

26. Shin J, Ku B, Kim J, Lee Y, Kang J, Heo H, et al. Short-term effect of laser acupuncture on lower back pain: a randomized, placebo-controlled, double-blind trial. *Evid Based Complement Alternat Med.* (2015) 2015:808425. doi: 10.1155/2015/808425

27. Simon L, Evans C, Katz N, Bombardier C, West C, Robbins J, et al. Preliminary development of a responder index for chronic low back pain. *J Rheumatol.* (2007) 34:1386–91.

28. Gilligan C, Volschenk W, Russo M, Green M, Gilmore C, Mehta V, et al. An implantable restorative-neurostimulator for refractory mechanical chronic low back pain: a randomized sham-controlled clinical trial. *Pain.* (2021) 162:2486–98. doi: 10.1097/j.pain.0000000000002258

29. Huskisson E. Measurement of pain. *J Rheumatol.* (1982) 9:768–9. doi: 10.1016/S0140-6736(74)90884-8

30. Chiarotto A, Maxwell L, Ostelo R, Boers M, Tugwell P, Terwee C. Measurement properties of visual analogue scale, numeric rating scale, and pain severity subscale of the brief pain inventory in patients with low back pain: a systematic review. *J Pain.* (2019) 20:245–63. doi: 10.1016/j.jpain.2018.07.009

31. Kim M, Cho Y, Uhm W, Kim S, Bae S. Cross-cultural adaptation and validation of the Korean version of the EQ-5D in patients with rheumatic diseases. *Qual Life Res.* (2005) 14:1401–6. doi: 10.1007/s11136-004-5681-z

32. Herdman M, Gudex C, Lloyd A, Janssen M, Kind P, Parkin D, et al. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). *Qual Life Res.* (2011) 20:1727–36. doi: 10.1007/s11136-011-9903-x

33. Fairbank J, Pynsent P. The Oswestry disability index. *Spine.* (2000) 25:2940–52. doi: 10.1097/00007632-200011150-00017

34. Jeon C, Kim D, Kim S, Kim D, Lee H, Park H. Validation in the cross-cultural adaptation of the Korean version of the Oswestry Disability Index. *J Korean Med Sci.* (2006) 21:1092–7. doi: 10.3346/jkms.2006.21.6.1092

35. Law D, McDonough S, Bleakley C, Baxter G, Tumilty S. Laser acupuncture for treating musculoskeletal pain: a systematic review with meta-analysis. *J Acupunct Meridian Stud.* (2015) 8:2–16. doi: 10.1016/j.jams.2014.06.015

36. Froud R, Patel S, Rajendran D, Bright P, Bjørkli T, Buchbinder R, et al. A systematic review of outcome measures use, analytical approaches, reporting methods, and publication volume by year in low back pain trials published between 1980 and 2012: respice, adspice, et prospice. *PLoS One.* (2016) 11:e0164573. doi: 10.1371/journal.pone.0164573

37. Chiarotto A, Deyo R, Terwee C, Boers M, Buchbinder R, Corbin T, et al. Core outcome domains for clinical trials in non-specific low back pain. *Eur Spine J.* (2015) 24:1127–42. doi: 10.1007/s00586-015-3892-3

38. Yeum H, Hong Y, Nam D. Effective low-level laser therapy including laser acupuncture treatment conditions for non-specific chronic low back pain: systematic review and meta-analysis. *J Acupunct Res.* (2021) 38:85–95. doi: 10.13045/jar.2020.00311

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Warm needle acupuncture for osteoarthritis: An overview of systematic reviews and meta-analysis

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Background: Osteoarthritis (OA) is a chronic disease that is a major cause of pain and functional disability. Warm needle acupuncture (WA) therapy has been widely used to treat OA. This overview summarizes the evidence from systematic reviews (SRs) and assesses the methodological quality of previous SRs that evaluated the use of WA therapy for OA.

Methods: We searched electronic databases to identify SRs that evaluated the efficacy of WA therapy for OA. Two reviewers independently extracted data and assessed the methodological quality of the reviews according to the A Measurement Tool to Assess Systematic Reviews (AMSTAR 2) tool. The reporting quality was assessed using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis 2020 (PRISMA 2020) guidelines. The quality of evidence was assessed according to the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach.

Results: Fifteen SRs were included in this study. WA therapy was more effective than control conditions for the treatment of OA. The results of the AMSTAR 2 tool showed that the methodological quality of all included studies was critically low. The items with the lowest scores were item 2 (reporting the protocol), item 7 (listing excluded studies and justifying the exclusions), and item 16 (including conflicts of interest). Regarding the PRISMA guidelines, 2 SRs exhibited greater than 85% compliance. The overall quality of evidence in the included SRs ranged from "very low" to "moderate."

Conclusion: This overview shows that WA therapy was more effective than the control treatment for OA. However, the methodological quality of the reviews was low, indicating the need for improvements in the collection of evidence. Future studies are needed to collect high-quality evidence regarding the use of WA for OA.

Systematic review registration: https://www.researchregistry.com/, Research Registry (reviewregistry1317).

KEYWORDS

acupuncture, moxibustion, warm needle acupuncture, osteoarthritis, overview, systematic review

Introduction

Osteoarthritis (OA) is a common chronic disease and a main symptom of joint stiffness, instability, and weakness. It usually occurs in middle-aged (between 50 and 60 years of age) people, and in particular, it occurs more often among women than men (1, 2). According to research results, the costs directly incurred by OA are billions of US dollars per year (3, 4). Therefore, the treatment of OA is significant for reducing pain in patients and alleviating the socioeconomic burden.

Traditional medicine has been used for thousands of years to treat numerous diseases and has been used to relieve pain and improve the function of the knee joint in OA patients (5, 6). Acupuncture is one of the options for treating OA (7, 8). WA is one type of acupuncture combined with moxibustion (9). The heat of the needle is transmitted to the deep part of the acupoint through the needle, which helps reduce pain and improve function. Recently, the number of studies using warm needle acupuncture (WA) for the treatment of musculoskeletal pain has increased, and the quality of the studies has gradually improved (5, 10). Systematic review (SR) is performed on a particular topic in order to provide a comprehensive and unbiased clinical evidence based on rigorous studies (11). One recent SR analyzed 66 randomized controlled trials (RCTs) and showed beneficial effects of WA for OA (12).

An overview of SRs is a method for compiling evidence and synthesizing the results of various SRs (13, 14). The greater the amount of information gathered, the better the quality of evidence that can be provided for clinical work. An overview of SRs on traditional Chinese medicine (TCM) for knee OA (15) and acupuncture for knee OA has been published recently (8, 16), which concluded that TCM generally appears to be effective for the treatment of knee OA. Nevertheless, the effectiveness of WA as a treatment for OA has not been thoroughly evaluated.

The purpose of this study was to summarize the efficacy of WA in the treatment of OA presented in SRs and to evaluate the methodological quality of the SRs.

Methods

We followed the Preferred Reporting Items for Overviews of SRs (PRIOR) statement (17). This overview was registered in the Research Registry (reviewregistry1317) (18).

Data sources and search strategy

An electronic literature search was conducted in PubMed, the Cochrane Register of Controlled Trials (CENTRAL), Embase, three Chinese databases (CNKI, VIP, and Wanfang), and six Korean databases (Research Information Service System (RISS), the Korean Studies Information Services System (KISS), Korean Medical Database (KMbase), DBPIA, (Korean Traditional Knowledge Portal) KTKP, KoreaMed, and Oriental Medicine Advanced Searching Integrated System (OASIS)) from their inception to January 2023. The search terms were ("warm needle acupuncture" OR "wen zhen" OR "warm acupuncture" OR "warm needle moxibustion") AND ("osteoarthritis") AND ("systematic review" OR "Meta-analysis") in Korean, Chinese, and English. The search terms and websites of 12 databases are described in Supplementary 1.

Inclusion and exclusion criteria

Types of studies

SRs and meta-analyses of randomized controlled trials (RCTs) or quasi-RCTs that used WA for OA were included.

Population

Studies of participants diagnosed with OA. There were no restrictions regarding sex or age.

Intervention and comparators

Studies that used WA as an intervention to treat OA were included regardless of types of comparators. Moreover, studies in which WA was combined with other therapies were also included.

Outcomes

SRs reporting on patient health outcomes were included. The studies included data on at least one outcome evaluating the total treatment effect and clinical symptom of interest.

Study selection and data extraction

Two reviewers (JHJ and TYC) separately assessed the citations obtained during the search, and full-text publications from potentially relevant SRs were retrieved and appraised for inclusion. One reviewer (JHJ) extracted the data using a standardized form. Two reviewers (JHJ and TYC) independently evaluated the retrieved data, and any differences were addressed through discussions between the two authors (SP and MSL) and were resolved by discussion. The data extracted from the reviews included the first author, publication year, data search, number of trials included, interventions, comparators, outcomes, direction of effect, overall risk of bias, conclusion, and adverse events. An assessment of the methodological quality for each included SR was also conducted.

Overlap calculation of the reviews

The degree of overlap of the original literature for SRs was assessed by creating citation metrics for SRs. We calculated the "corrected covered area" (CCA) index (19, 20). The measure of overlap dividing the frequency of repeated occurrences of the index publication in other reviews by the product of index publications and reviews is reduced by the number of index publications. Calculation formulas were calculated as $CCA = (N - r)/(rc - r)$, where N is the number of included publications in evidence synthesis (this is the sum of the ticked boxes in the citation matrix), r is the number of rows (number of index publications), and c is the number of columns (number of reviews) (supplement overlap). The calculation results lower than 5 can be considered a "slight overlap," 6–10 can be considered a "moderate overlap," 11–15 can be considered a "high overlap," and greater than or equal to 15 can be considered a "very high overlap."

Methodological quality assessment

The quality of the included SRs was evaluated using the Assessing the Methodological Quality of Systematic Reviews 2 (AMSTAR 2) tool (21). There were 16 evaluation items. The reporting was assessed as being sufficiently reported and performed (Yes), insufficiently reported (Partial Yes), or not reported (No). The overall confidence in the results of the review was rated as follows: critically low quality (more than one critical flaw with or without non-critical weaknesses), low quality (one critical flaw with or without non-critical weaknesses), moderate quality (more than one non-critical weakness), and high quality (zero or one non-critical weakness). The AMSTAR 2 tool was used by two authors (JHJ and TYC). If there was a disagreement, the other authors (SP and MSL) resolved the disagreement.

Reporting quality assessment

We used the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA 2020) checklist (22). There were 27 element items that evaluated SR reporting quality. "Yes," "Partial yes," or "NO" were used to respond to each item. We reported the results as a ratio.

Certainty of evidence

The quality of outcomes of the included SRs was evaluated by the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) tool[1] (23, 24). If the GRADE tool was not used in SRs, we evaluated the strength of evidence from primary trials. The assessment of the included SRs was independently carried out by the reviewers. The five categories of GRADE influenced (i.e., downgraded or upgraded) the quality of evidence and included risk of bias, inconsistency, indirectness, imprecision, and publication bias. The quality of evidence of SRs was rated as "high," "moderate," "low," and "very low." Evidence based on RCTs began as high quality. Two authors (JHJ and TYC) assessed the quality of evidence. Disagreements were resolved by discussion with a third author (MSL).

Data synthesis and analysis

Narrative synthesis was provided because of the high heterogeneity. The results of the WA intervention were also narratively summarized in more detail from the included SRs, and the direction of effects was calculated. Such a detailed form included the features of the intervention, methodological quality, and quality of evidence.

1 https://www.gradepro.org/

Results

Study selection

Twelve database searches identified 161 potentially relevant studies, with 39 repeated studies removed. Of the remaining 122 studies, 93 studies were excluded due to lack of relation, review, protocol, and RCT designs. A total of 29 studies were obtained after retrieval. After the final reading of the full texts, 15 SRs (12, 25–38) were included in this review. The details of the SR selection screening process are shown in Figure 1. The list of excluded studies and reasons for exclusion are shown in Supplementary 2.

Characteristics of the included studies

Fourteen SRs (25–38) were conducted in China. They were published between 2015 and 2022, of which 40% were published in 2019. Thirteen SRs (25–33, 35–38) were published in Chinese, and two SRs (12, 34) were published in English. The SRs included between 8 and 66 primary studies. In total, the reviews included 155 different RCTs and 2 clinical control trials (CCTs). The total number of participants in the SRs was 13,940 participants. Five SRs (25, 27, 36–38) evaluated the included studies using the Jadad scale, nine SRs (12, 26, 28–30, 32–35) evaluated studies using the Cochrane risk of bias (ROB) tool, and one SR (31) did not mention an evaluation tool. All SRs conducted a meta-analysis as a statistical approach. The outcomes included in the SRs varied widely; however, they mainly focused on the overall total effective rate, visual analog scale (VAS) scores, the LKSS, and the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). One SR (12) focused on 3 outcomes: total effective rate, pain, and function. Five SRs (12, 27, 30, 33, 35) assessed the adverse effects of WA treatment of OA. Nine SRs (25–27, 29–34, 37) arrived at a clearly positive conclusion, four SRs (12, 28, 35, 36) were neither positive nor negative, and one SR (38) drew a negative conclusion.

Various comparisons among the studies in the included SRs included WA versus Western medicine (12, 25–27), WA versus traditional medicine (acupuncture, electroacupuncture, and EA) (34–38), WA versus all types of therapies (including traditional medicine and Western medicine) (32, 33), WA plus Western medicine versus Western medicine (12, 28–30), and WA plus all types of therapies vs. all types of therapies (31). The data from the included SRs are summarized in Table 1.

Overlap of reviews

A total of 15 SRs (12, 25–38) were included in this review. N indicates 245, r indicates 157, and c indicates 15. The formula $CCA = (245–157)/(15 \times 157–157) = 0.04$ indicates slight overlap. The overlap matrix is shown in Supplementary 3.

Outcomes

Fifteen SRs (12, 25–38) summarized the evidence on the effectiveness of WA alone or in combination with Western medicine or traditional medicine in reducing pain and improving the total effective rate, function, and WOMAC total score. The outcomes from the included SRs are summarized and presented in Table 2.

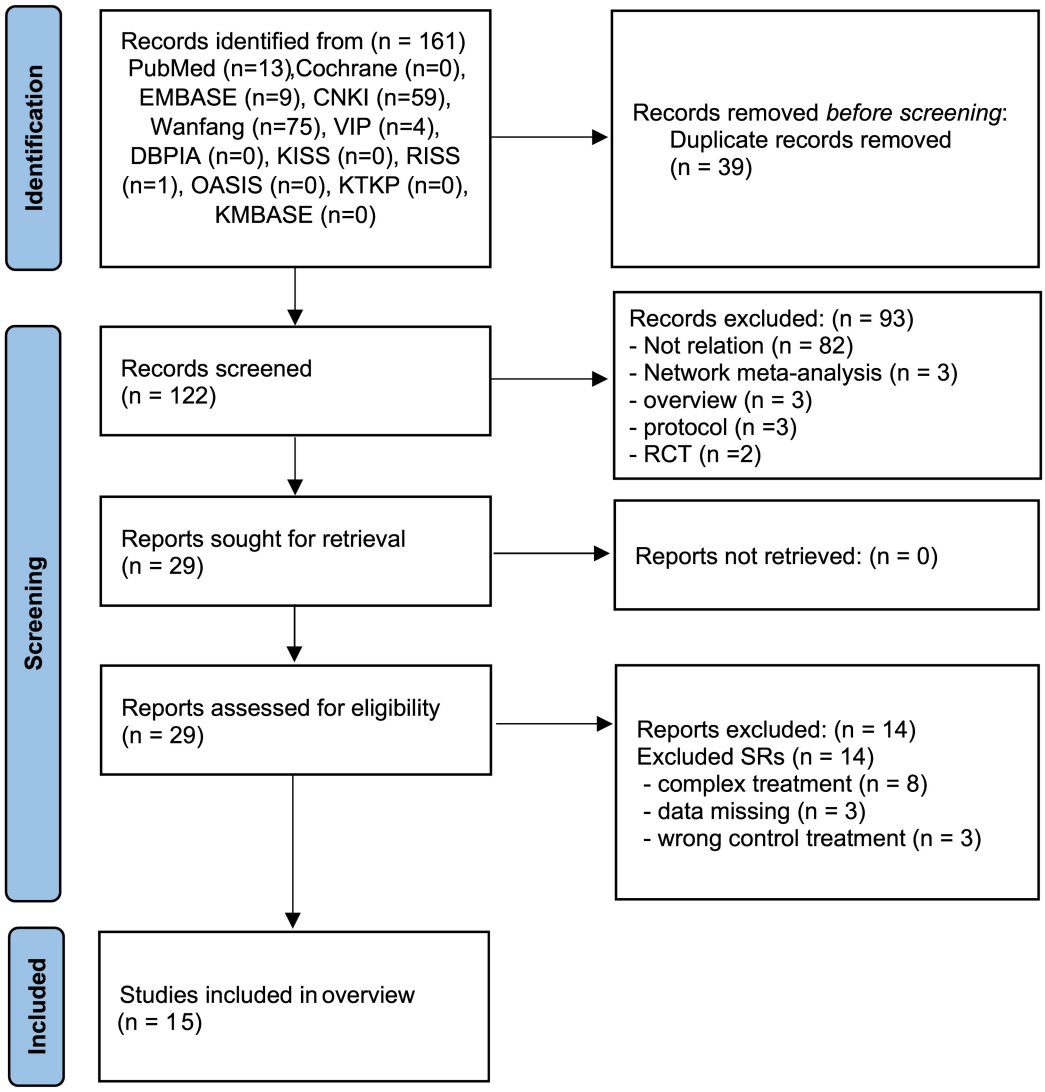

FIGURE 1
PRISMA diagram for the included studies. RCT, randomized controlled trials; SR, systematic review.

Total effective rate

Fourteen SRs (12, 25–30, 32–38) suggested that the total effective rate of WA alone or combined with other therapies in OA patients was superior to that in the control group. One SR (30) with the largest sample size included 66 RCTs with 6,231 patients treatment, and a comparison of the effects of WA or WA plus WM group versus control group results showed a greater effect in the intervention group than in the control group. In most studies, WA was effective for OA. However, two SRs (35, 38) reported no significant differences between WA and EA.

Pain

Seven SRs (28, 31, 32, 34–36, 38) reported VAS scores. Four SRs (29, 31, 32, 34) had positive results, and three SRs (35, 36, 38) had negative results. Three SRs (28, 35, 36) reported the Lysholm score (LKSS) meta-analysis and showed that there was a significant difference between the WA alone or combined with other therapy groups and the control group. One SR (12) reported pain, which included the VAS, LKSS, and WOMAC (pain score). The SRs of the results were neither positive nor negative.

Function

One SR (12) evaluated the effects of WA alone or WA plus WM in the intervention group on function compared to WM. The analysis results of this SR showed that the intervention group was significantly improved compared with controls.

WOMAC total score

Seven SRs (28, 31, 32, 34–36, 38) reported the WOMAC total score. The meta-analysis showed the effects of WA alone or combined with other therapies on the WOMAC total score. However, four SRs (28, 31, 32, 34) failed to show that WA had superior effects compared with EA on the WOMAC.

Adverse events

Of all 15 SRs, five SRs (12, 27, 30, 33, 35) mentioned adverse events. The major symptoms reported in the WA treatment groups were skin burns. Most of the RCTs included in the SRs reported no adverse events. Four SRs (12, 30, 33, 35) reported

TABLE 1 Summary of systematic review studies of warm needle acupuncture for knee osteoarthritis.

First author (year) [ref]	Data search	Number of trials included (total sample size)	Intervention	Comparator	Outcomes	Direction of effect*	Overall risk of bias of primary studies	Methodological quality of SRs (AMSTAR 2)	Conclusion (Quote)	AEs
Feng (2019) (25)	Jun 2018	8 (582)	WA	WM	Total effective rate	+	Jadad (High)	Critically low	… positive effective…	No
Guo (2018) (26)	Oct 2017	11 (930)	WA	WM	(1) Total effective rate	(1) +	Cochrane ROB (High)	Critically low	…effective…	No
					(2) WOMAC	(2) +				
Lu (2015) (27)	Dec 2014	8 (811)	WA	WM	(1) Total effective rate	(1) +	Jadad (High)	Critically low	…conclude be an effect...	Yes
					(2) AEs	(2) +				
Kong (2019) (28)	Mar 2018	21 (1810)	WA + WM	WM	(1) Total effective rate	(1) +	Cochrane ROB (High)	Low	… improve the efficiency…	No
					(2) Pain (VAS)	(2) +				
					(3) WOMAC	(3) +				
					(4) LKSS	(4) +				
Cao (2019) (29)	Mar 2018	9 (807)	WA + WM	WM	Total effective rate	+	Cochrane ROB (High)	Critically low	…effect…	No
Jun (2022) (12)	May 2022	66 (6231)	WA or WA + WM	WM	(1) Total effective rate	(1) +	Cochrane ROB (High)	Moderate	…have some distinct advantage…	Yes
					(2) Pain	(2) +/–				
					(3) Function	(3) +/–				
					(4) QoL	(4) +				
Jiang (2019) (30)	Jul 2018	20 (18 RCT, 2 CCT) (1719)	WA + WM	WM	Total effective rate	+	Cochrane ROB (High)	Critically low	…significant effect…	Yes
Chen (2019) (31)	Jul 2018	19 (1943)	WA or WA + WM	No limited (TDP, WM)	(1) Pain (VAS)	(1) +	n.r.	Critically low	…can increase efficacy…	No
					(2) WOMAC total	(2) +				
Huang (2021) (32)	Feb 2020	18 (1209)	WA	No limited (AT, EA, Moxa, WM)	(1) Total effective rate	(1) +	Cochrane ROB (High)	Low	…effective…	No
					(2) Pain (VAS)	(2) +				
					(3) WOMAC	(3) +				

(Continued)

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TABLE 1 (Continued)

First author (year) [ref]	Data search	Number of trials included (total sample size)	Intervention	Comparator	Outcomes	Direction of effect*	Overall risk of bias of primary studies	Methodological quality of SRs (AMSTAR 2)	Conclusion (Quote)	AEs
Luo (2019) (33)	Oct 2017	10 (819)	WA	No limited (AT, EA, Moxa, WM)	Total effective rate	+	Cochrane ROB (High)	Critically low	… positive effective…	Yes
Jin (2022) (34)	Oct 2021	8 (399)	WM	TCM	(1) Total effective rate	(1) +	Cochrane ROB (High)	Low	… better overall… efficacy	No
					(2) Pain (VAS)	(2) +				
					(3) WOMAC	(3) +				
Li (2021) (35)	Dec 2019	17 (1515)	WA	AT or EA	(1) Total effective rate	(1) +/–	Cochrane ROB (High)	Low	…superior to EA…	Yes
					(2) Pain (VAS)	(2) –				
					(3) WOMAC	(3) +/–				
					(4) LKSS	(4) –				
Zhang (2018) (36)	Jun 2018	12 (1176)	WA	AT or EA	(1) Total effective rate	(1) +	Jadad (High)	Critically low	…no…significant difference…	No
					(2) Pain (VAS)	(2)–				
					(3) WOMAC	(3) –				
					(4) LKSS	(4) +				
Ou (2018) (37)	Jul 2017	7 (530)	WA	AT	Total effective rate	+	Jadad (High)	Critically low	… positive effective…	No
Wu (2016) (38)	Nov 2014	11 (772)	WA	EA	(1) Total effective rate	(1)–	Jadad (High)	Critically low	… is not superior to that of EA...	No
					(2) Pain (VAS)	(2)–				
					(3) WOMAC	(3)–				

AT, acupuncture; AEs, adverse events; CCT, controlled clinical trials; HM, herbal medicine; IA, intra-articular injection; LKSS, Lysholm score; KOA, knee osteoarthritis; n.r., not reported; RCT, randomized controlled trials; ROB, risk of bias; VAS, visual analog scale; WA, warm acupuncture; western medicine; WOMAC, Western Ontario and McMaster Universities Osteoarthritis index.

*Total effective rate = (number of markedly effective cases + number of effective cases)/total cases.

*Relied on the original author's judgment, (+): overall positive; (−): negative; (+/−): unclear.

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TABLE 2 Certainty of evidence in included systematic review with GRADE approach.

First author (year) [ref]	Outcomes	Study design	Number of studies (Total sample size)	Effect (95% CI)	*p*-value	Certainty of evidence
Feng (2019) (25)	Total effective rate	WA vs. WM	8 (582)	OR 4.10 [2.51, 6.71]	*< 0.00001*	Low
Guo (2018) (26)	Total treatment effect	WA vs. WM	11 (925)	OR 4.54 [3.02, 6.82]	*< 0.00001*	Low
	WOMAC total	WA vs. WM	2 (150)	SMD -0.68 [-1.02, -0.35]	*< 0.00001*	Very low
Lu (2015) (27)	Total effective rate	WA vs. WM	8 (811)	RR 1.37 [1.27, 1.48]	*< 0.0001*	Very low
	Total effective rate (long term)	WA vs. WM	2 (175)	RR 1.16 [1.04, 1.29]	*= 0.008*	Very low
	Total effective rate (short term)	WA vs. WM	4 (339)	RR 2.31 [1.57, 3.41]	*< 0.00001*	Low
	Adverse events	WA vs. WM	2 (176)	RR 0.20 [0.05, 0.75]	*= 0.02*	Low
Kong (2019) (28)	Total treatment effect	WA+WM vs. WM	16 (1445)	OR 4.20 [2.80, 6.32]	*< 0.00001*	Moderate
	Pain (VAS)	WA+WM vs. WM	11 (979)	MD -1.53 [-1.96, -1.11]	*< 0.00001*	Very low
	LKSS	WA+WM vs. WM	8 (753)	MD 19.7 [13.76, 25.18]	*< 0.00001*	Very low
	WOMAC total	WA+WM vs. WM	3 (312)	MD -10.25 [-15.61, -4.90]	*0.0002*	Very low
Cao (2019) (29))	Total effective rate	WA+WM vs. WM	9 (786)	RR 1.16 [1.10, 1.22]	*< 0.00001*	Moderate
Jun (2022) (12)	Total treatment effect	WA vs. WM (Drug)	24 (2278)	RR 1.22 [1.17, 1.27]	*< 0.001*	Low
		WA vs. WM (Injection)	5 (465)	RR 0.99 [0.91, 1.09]	NS	Low
		WA + WM (Drug) vs. WM (Drug)	8 (646)	RR 1.27 [1.18, 1.35]	*< 0.001*	Very Low
		WA + WM (Injection) vs. WM (Injection)	25 (2238)	RR 1.1.5 [1.11, 1.19]	*< 0.001*	Low
	Pain	WA vs. WM (Drug)	10 (874)	SMD -2.65 [-3.92, -1.38]	*= 0.01*	Very low
		WA vs. WM (Injection)	8 (726)	SMD -0.01 [-0.57, 0.55]	NS	Very low
		WA + WM (Drug) vs. WM (Drug)	2 (168)	SMD -5.85 [-7.84, -3.85]	*< 0.001*	Very low
		WA + WM (Injection) vs. WM (Injection)	19 (1795)	SMD -1.68[-2.07, -1.29]	*< 0.001*	Very low
	Function	WA vs. WM (Drug)	13 (1354)	SMD -1.79 [-2.31, -1.26]	*< 0.001*	Very low
		WA vs. WM (Injection)	6 (547)	SMD -0.6 [-1.59, 0.39]	NS	Very low
		WA + WM (Drug) vs. WM (Drug)	4 (364)	SMD -1.45 [-3.11, 0.22]	*< 0.001*	Very low
		WA + WM (Injection) vs. WM (Injection)	22 (2012)	SMD -1.40 [-1.72, -1.08]	*< 0.001*	Very low
Jiang (2019) (30)	Total treatment rate	WA+WM vs. WM	20 (1719)	OR 4.45 [3.12, 6.35]	*< 0.00001*	Moderate
Chen (2019) (31)	Pain (VAS)	WA + other therapies vs. all type therapies	5 (450)	WMD -2.20 [-3.34, -1.06]	*< 0.05*	Very low
	WOMAC total	WA + other therapies vs. all type therapies	5 (418)	WMD -0.67 [-1.27, -0.07]	*< 0.05*	Very low
Huang (2021) (32)	Total effective rate	WA vs. all type therapies	17 (1109)	OR 3.41 [2.27, 5.13]	*< 0.00001*	Moderate
	Pain (VAS)	WA vs. all type therapies	10 (663)	MD -0.93 [-1.20, -0.67]	*< 0.00001*	Low
		WA vs. AT	4 (232)	MD -1.23 [-1.62, -0.84]	*< 0.00001*	Very low
		WA vs. EA	2 (130)	MD -0.63 [-1.09, -0.17]	*= 0.007*	Low
		WA vs. Moxa	3 (233)	MD -0.80 [-0.90, -0.70]	*< 0.00001*	Low
		WA vs. WM	1 (68)	MD -1.30 [-2.00, -0.60]	*= 0.0003*	Low
	WOMAC	WA vs. all type therapies	6 (430)	MD -8.91 [-12.58, -5.23]	*< 0.00001*	Low
		WA vs. AT	2 (172)	MD -14.11 [-20.89, -7.33]	*< 0.0001*	Very low
		WA vs. EA	2 (130)	MD -4.42 [-15.27, 6.44]	NS	Very low
		WA vs. WM	2 (128)	MD -8.29 [-9.50, -7.08]	*< 0.0001*	Low

(Continued)

TABLE 2 (Continued)

First author (year) [ref]	Outcomes	Study design	Number of studies (Total sample size)	Effect (95% CI)	*p*-value	Certainty of evidence
Luo (2019) (33)	Total treatment effect	WA vs. all type therapies	10 (819)	OR 5.22 [3.45, 7.89]	*< 0.00001*	Low
Jin (2022) (34)	Total effective rate	WA vs. TCM	8 (795)	RR 1.18 [1.06, 1.33]	*= 0.0004*	Low
	Daily activities	WA vs. TCM	2 (129)	MD -4.31 [-10.90, 2.28]	NS	Low
	Pain (VAS)	WA vs. TCM	6 (427)	MD -1.06 [-1.61, -0.51]	*= 0.0002*	Very low
	WOMAC	WA vs. TCM	6 (427)	MD -6.93 [-12.14, -1.72]	*= 0.009*	Low
Li (2021) (35)	Total effective rate	WA vs. AT	10 (872)	OR 3.44 [2.25, 5.27]	*< 0.00001*	Moderate
		WA vs. EA	8 (643)	OR 0.91 [0.58, 1.43]	NS	Low
	Pain (VAS)	WA vs. AT	3 (178)	MD -1.40 [-1.83, 0.96]	*< 0.00001*	Low
		WA vs. EA	5 (374)	MD 0.82 [-0.08, 1.72]	NS	Very low
	WOMAC	WA vs. EA	4 (279)	MD 0.98 [-1.76, 3.71]	NS	Very low
	LKSS	WA vs. AT	2 (114)	MD 17.36 [13.40, 21.32]	*< 0.00001*	Low
Zhang (2018) (36)	Total effective rate	WA vs. TCM	12 (1176)	RR 1.04 [1.00, 1.08]	*= 0.06*	Very low
	Pain (VAS)	WA vs. TCM	7 (563)	SMD -0.48 [-1.26, 0.30]	NS	Very low
	LKSS	WA vs. AT	4 (359)	SMD -1.68 [-2.03, -1.32]	*< 0.001*	Low
	WOMAC total	WM vs. EA	4 (299)	SMD 0.69 [0.46, 0.92]	NS	Very low
Ou (2018) (37)	Total effective rate	WA vs. AT	7 (530)	OR 5.07 [2.85, 9.04]	*< 0.00001*	Low
Wu (2016) (38)	Total effective rate	WA vs. EA	11(772)	OR 1.21 [0.81, 1.80]	NS	Very low
	Pain (VAS)	WA vs. EA	5 (339)	SMD 0.25 [-0.11, 0.61]	NS	Very low
	WOMAC	WA vs. EA	4 (279)	SMD 0.08 [-0.15, 0.32]	NS	Very low

AT, acupuncture; GRADE, Grades of Recommendations, Assessment, Development, and Evaluation; LKSS, Lysholm Knee Score Scale; MD, mean difference; Moxa moxibustion; OR, odd ratio; ROB, risk of bias; RR, risk ratio; SMD, standard mean difference; TCM, traditional Chinese medicine; VAS, visual analog scale; WA, warm needle acupuncture.

that serious adverse events did not occur. One SR (27) indicated that the incidence of adverse events in the WA treatment groups was lower than that in the control groups, which indicated that WA was a safe therapy for OA.

Methodological quality of the included systematic reviews

The results of the AMSTAR 2 tool showed that the included SRs were critically low quality, low quality, or moderate (Figure 2; Supplementary 4). Ten SRs (25–27, 29–31, 33, 36–38) were considered to have critically low quality, four SRs (28, 32, 34, 35) were considered to have low quality, and one SR (12) was considered to have moderate quality. All of the SRs reported the inclusion of PICO components (item 1). None of the SRs provided a complete list of excluded studies with reasons (item 7). Some SRs were evaluated with a partial yes in three domains (e.g., items 4 and 8).

Seven domains (items 2, 4, 7, 9, 11, 13, and 15) of the AMSTAR 2 tool were critical domains. For item 2, 14 of the SRs (25–38) provided a registry protocol, and one SR (12) was registered with PROSPERO and published protocol. For item 4, six SRs (12, 28, 30, 31, 34, 35) searched core databases (PubMed, the Cochrane Library, and Embase) and related intervention databases. However, nine SRs (25–27, 29, 32, 33, 36–38) lacked a search of the core databases. For item 7, none of the SRs provided the excluded studies and explained the reason for exclusion. For item 9, 13 SRs (25–30, 32–37) described the bias, one SR insufficiently reported bias (38), and one SR performed the assessment, but the results were not described. For item 11, all of the SRs performed a meta-analysis. For item 13, six SRs (12, 28, 33, 35–37) took the risk of bias into account when discussing the results and drew a conclusion with caution. For item 15, all of the SRs investigated publication bias and analyzed its potential effects on the results of the review.

Report quality of included systematic reviews

To assess the reporting quality of the included SRs, we used the PRISMA 2020 checklist (22). Figure 3 shows the reporting quality assessment results of the included SRs. Item 1 (title), item 2 (abstract), item 4 (objects), item 8 (selection process), item 19 (results of individual studies), item 20 (results of syntheses), and item 21 (reporting biases) were reported adequately (100%). Item 15 (describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome), item 22 (certainty of evidence), and item 24 (registration and protocol) of results reported insufficient description. Overall, two SRs (12, 34) exhibited over 85% compliance. The results are shown in Supplementary 5.

FIGURE 2
Quality evaluation using AMSTAR 2. AMSTAR2 was used to critically appraise the reporting quality of each included SR. The overall confidence of each SR was graded as "high" (no or non-critical weakness in all items), "moderate" (more than one non-critical weakness among all the items), "low" (one critical flaw with or without non-critical weakness), or "critically low" (more than one critical flaw with or without non-critical weakness). Q1: Did the research questions and inclusion criteria for the review included the components of PICO?; Q2: Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?; Q3: Did the review authors explain their selection of the study designs for inclusion in the review?; Q4. Did the review authors use a comprehensive literature search strategy?; Q5: Did the review authors perform study selectin in duplicate?; Q6: Did the review authors perform data extraction in duplicate?; Q7: Did the review authors provide a list of excluded studies and justify the exclusions?; Q8: Did the review authors describe the included studies in adequate detail?; Q9: Did the review authors used a satisfactory technique for assessing the risk of bias (ROB) in individual studies that were included in the review?; Q10: Did the review authors report on the sources of funding for the studies included in the review?; Q11: If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results? Q12: If meta-analysis was performed, did the review authors assess the potential impact of ROB in individual studies on the results of the meta-analysis or other evidence synthesis?; Q13: Did the review authors account for ROB in individual studies when interpreting/ discussing the results of the review?; Q14: Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?; Q15: If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?; Q16: Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?. CL: critically low; L: low; M: moderate; PT: partial yes.

Certainty of evidence

We evaluated the quality of outcomes extracted from the included studies. Table 2 shows the level of evidence quality of the studies reported. The quality of evidence for outcomes evaluated by the GRADE approach ranged from very low to moderate (Supplementary 6). The risk of bias and imprecision mainly accounted for the downgrade. The quality of evidence was moderate for 5 outcomes (8.92%), low for 21 outcomes (35.21%), and very low for 32 outcomes (55.17%).

Discussion

This overview of SRs was intended to summarize the features and evaluate the quality of methodological, reporting bias, and evidence from included SRs about the efficacy of WA in OA. Fifteen SRs reported that intervention groups using WA alone or WA plus other therapies showed symptom improvements compared with control groups (32, 35, 36, 38). WA treatment was safer than control treatment, and serious adverse events did not occur; however, the evidence of safety based on the included reviews was not sufficient since certain data were missing. Most of the SRs were associated with a high risk of bias, rated moderate to very low with the GRADE approach, and rated critically low with the AMSTAR 2 tool. Thus, it is not possible to draw a clear conclusion. Future research involving large sample sizes and high-quality studies are needed. Regarding the reporting quality of the results, only 2 SRs (12, 34) exhibited over 85% compliance.

All included studies had average reporting quality, according to the PRISMA 2020 checklist. The 6 element items (items 1, 2, 4, 8, 19, 20, and 21) were complete. Only two SRs reached 85.2% (34) and 100% ((12) compliance. Most of the included SRs were on knee OA and were conducted and published in China. In future studies, the reasonable

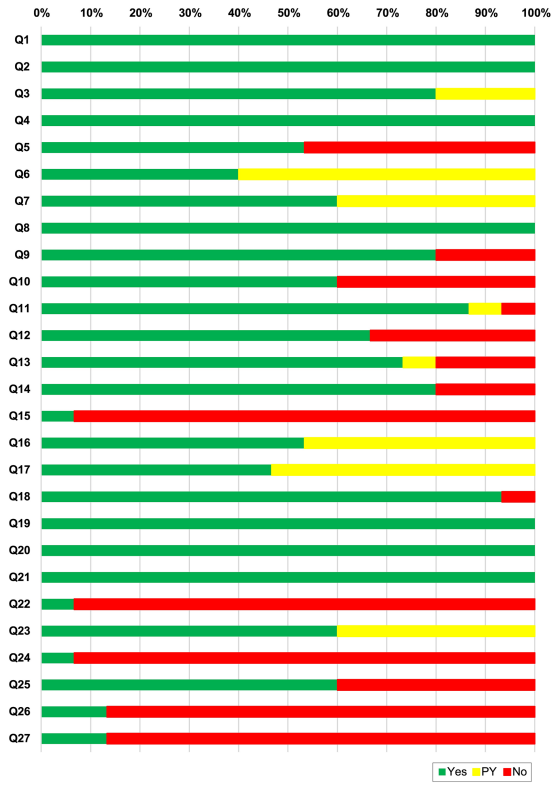

FIGURE 3
PRISMA 2020 checklist results for each item. Q1. Title; Q2. Abstract; Q3. Rationale; Q4. Objectives; Q5. Eligibility criteria; Q6. Information sources; Q7. Search strategy; Q8. Selection process; Q9. Data collection process; Q10. Data items; Q11. Study risk of bias assessment; Q12. Effect measures; Q13. Synthesis methods; Q14. Reporting bias assessment; Q15. Certainty assessment; Q16. Study selection; Q17. Study characteristics; Q18. Risk of bias in studies; Q19. Results of individual studies; Q20. Results of syntheses; Q21. Reporting biases; Q22. Certainty of evidence; Q23. Discussion; Q24. Registration and protocol; Q25. Support; Q27. Availability of data, code and other materials.

utilization of the Consolidated Standards of Reporting Trials (CONSORT) (39) and PRISMA (22) checklists will improve the reporting quality of SRs and meta-analyses, which will reduce potential selection bias.

In nine SRs, the methodological quality was critically low because there were deficits in the critical items of the AMSTAR 2 tool, which included items 2 (registration protocol), 7 (list of excluded studies), and 16 (potential source of conflicts of interest). For item 2, only one SR (12) reported rates in the protocol and recording section. Preregistration helps to promote transparency, minimize potential biases in reporting and reviewing, reduce duplication of effort among groups, and keep service requests current. For item 7, an exclusion list is recommended because without this list, authors can arbitrarily exclude RCTs that differ from their desired results (21). Nevertheless, as the AMSTAR 2 tool is a more rigorous assessment tool than the previous version, the evaluation results should be interpreted by considering that the methodological quality of the published SRs was underestimated. A major reason for downgrading the evidence in the GRADE tool was that most of the included SRs were assessed as having a risk of bias and inconsistency across categories. The major reasons for this quality of evidence assessment were that randomization and blinding methods were not described and there was high heterogeneity.

This overview has some limitations. First, the SRs were dependent on RCTs published in China. The results of this review are not applicable or generalizable to other studies conducted elsewhere. In the future, clinical research should be actively conducted in countries other than China so that WA treatment for OA can be actively used in various ways. Second, the evaluation tools (AMSTAR, PRISMA, and GRADE) that were used were subjective. Two independent reviewers provided the evaluation, and the results were checked; nevertheless, they may have been their own judgment included in the assessment of each factor. Third, this overview was limited to the use of AMSTAR 2 to evaluate the methodological quality of the SRs. Consequently, the quality of the included SRs was not assessed. Future research should use the Risk of Bias in Systematic reviews (ROBIS) tool (40) to evaluate risk of bias and the PRISMA checklist (22) to evaluate the reporting characteristics of the included SRs.

In conclusion, WA or WA plus other therapies was more effective than the control conditions. However, the methodological quality of most of the included systematic reviews was critically low. Therefore, future studies should report SRs according to reporting guidelines, such as the PRISMA 2020 checklist, to improve the methodological quality and quality of evidence. This overview will help improve the evidence-based treatment and acupuncture evaluation system and facilitate research conducted by clinicians and scientific researchers.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

JJ and ML: conceptualization, methodology, investigation, and writing—original draft. JJ: software, visualization, and project administration. T-YC and SP: validation and writing—review and editing. JJ and T-YC: formal analysis and resources. SP and ML: data curation and supervision. ML: funding acquisition. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.971147/full#supplementary-material

References

1. Glyn-Jones, S, Palmer, AJ, Agricola, R, Price, AJ, Vincent, TL, Weinans, H, et al. Osteoarthritis. *Lancet*. (2015) 386:376–87. doi: 10.1016/S0140-6736(14)60802-3

2. Pereira, D, Ramos, E, and Branco, J. Osteoarthritis. *Acta Medica Port*. (2015) 28:99–6. doi: 10.20344/amp.5477

3. Buckwalter, JA, Saltzman, C, and Brown, T. The impact of osteoarthritis: implications for research. *Clin Orthop Relat Res*. (2004) 427:S6–S15. doi: 10.1097/01.blo.0000143938.30681.9d

4. London, NJ, Miller, LE, and Block, JE. Clinical and economic consequences of the treatment gap in knee osteoarthritis management. *Med Hypotheses*. (2011) 76:887–92. doi: 10.1016/j.mehy.2011.02.044

5. Wang, X, Wang, X, Hou, M, Wang, H, and Ji, F. Warm-needling moxibustion for knee osteoarthritis:a randomized controlled trial. *Zhongguo Zhen Jiu*. (2017) 37:457–62. doi: 10.13703/j.0255-2930.2017.05.001

6. Yuan.Z.F., Li, G, Jun-tao, Y, Min, F, and Yao, CW. Effects of acupuncture and chinese massage on the functions of knee flexors and extensors in patients with knee osteoarthritis: randomized controlled trial. *World J Acupunct Moxibus* (2010) 20:29–36.

7. French, HP, Abbott, JH, and Galvin, R. Adjunctive therapies in addition to land-based exercise therapy for osteoarthritis of the hip or knee. *Cochrane Database Syst Rev*. (2022) 2022:Cd011915. doi: 10.1002/14651858.CD011915.pub2

8. Li, J, Li, YX, Luo, LJ, Ye, J, Zhong, DL, Xiao, QW, et al. The effectiveness and safety of acupuncture for knee osteoarthritis: an overview of systematic reviews. *Medicine (Baltimore)*. (2019) 98:e16301. doi: 10.1097/MD.0000000000016301

9. Lee, JH, Jo, HR, Kim, SH, Lee, YS, Park, SW, Moon, SR, et al. A review on the characteristics of temperature variation in warm needle. *J Korean Med*. (2019) 40:112–38. doi: 10.13048/jkm.19031

10. Chung, JY, Choi, DY, Woo, HS, and Kang, SK. Review of clinical trials on warming acupuncture for musculoskeletal pain diseases -a systematic revie. *J Korean Acupunct Moxi Soc*. (2009) 26:11–8.

11. Lasserson, TJ, Thomas, J, and Higgins, JPT. Starting a review In: JPT Higgins and J Thomas, editors. *Cochrane Handbook for Systematic Reviews of Intervention*. Oxford, UK: Wiley Blackwell (2019)

12. Jun, JH, Choi, TY, Robinson, N, Park, JY, Jun, EY, Kim, KH, et al. Warm needle acupuncture for osteoarthritis: a systematic review and meta-analysis. *Phytomedicine*. (2022) 106:154388. doi: 10.1016/j.phymed.2022.154388

13. Becker, LA, and Oxman, AD. Overviews of reviews In: JP Higgins and S Green, editors. *Cochrane Handbook for Systematic Reviews of Interventions*. West Sussex, UK: Wiley-Blackwell (2008). 607–31.

14. Smith, V, Devane, D, Begley, CM, and Clarke, M. Methodology in conducting a systematic review of systematic reviews of healthcare interventions. *BMC Med Res Methodol*. (2011) 11:15. doi: 10.1186/1471-2288-11-15

15. Yang, M, Jiang, L, Wang, Q, Chen, H, and Xu, G. Traditional Chinese medicine for knee osteoarthritis: an overview of systematic review. *PLoS One*. (2017) 12:e0189884. doi: 10.1371/journal.pone.0189884

16. Chen, J, Liu, A, Zhou, Q, Yu, W, Guo, T, Jia, Y, et al. Acupuncture for the treatment of knee osteoarthritis: an overview of systematic reviews. *Int J Gen Med*. (2021) 14:8481–94. doi: 10.2147/IJGM.S342435

17. Gates, M, Gates, A, Pieper, D, Fernandes, RM, Tricco, AC, Moher, D, et al. Reporting guideline for overviews of reviews of healthcare interventions: development of the PRIOR statement. *BMJ*. (2022) 378:e070849. doi: 10.1136/bmj-2022-070849

18. Jun, JH, and Lee, MS. Warm needle acupuncture for osteoarthritis: an overview of systematic reviews and meta-analysis. https://www.researchregistry.com/browse-the-registry#registryofsystematicreviewsmeta-analyses/registryofsystematicreviewsmeta-analysesdetails/6229a69d3fe622001ffba1ae (2022).

19. Bougioukas, KI, Diakonidis, T, Mavromanoli, AC, and Haidich, AB. ccaR: a package for assessing primary study overlap across systematic reviews in overviews. *Res Synth Methods*. (2022). 1–12. doi: 10.1002/jrsm.1610

20. Pieper, D, Antoine, SL, Mathes, T, Neugebauer, EA, and Eikermann, M. Systematic review finds overlapping reviews were not mentioned in every other overview. *J Clin Epidemiol*. (2014) 67:368–75. doi: 10.1016/j.jclinepi.2013.11.007

21. Shea, BJ, Reeves, BC, Wells, G, Thuku, M, Hamel, C, Moran, J, et al. AMSTAR 2: a critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both. *BMJ*. (2017) 358:j4008. doi: 10.1136/bmj.j4008

22. Page, MJ, McKenzie, JE, Bossuyt, PM, Boutron, I, Hoffmann, TC, Mulrow, CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. (2021) 372:n71. doi: 10.1136/bmj.n71

23. Brozek, JL, Akl, EA, Alonso-Coello, P, Lang, D, Jaeschke, R, Williams, JW, et al. Grading quality of evidence and strength of recommendations in clinical practice guidelines. Part 1 of 3. An overview of the GRADE approach and grading quality of evidence about interventions. *Allergy*. (2009) 64:669–77. doi: 10.1111/j.1398-9995.2009.01973.x

24. Liu, J-P. GRADE methods in traditional medicine. *Integ Med Res*. (2022) 11:100836. doi: 10.1016/j.imr.2022.100836

25. Feng, P, Gao, YL, Yang, J, Xu, J, Su, YL, et al. Meta analysis of clinical efficacy of acupuncture and moxibustion in the treatment of knee osteoarthritis. *Sci tech inf. Gansu*. (2019) 48:87–9.

26. Guo, KY, and Chen, DL. Efficacy of needle warming therapy for treatment of knee osteoarthritis: a systematic review. *J Tradit Chin Orthop Traumatol*. (2018) 30:17–20.

27. Lu, W. Needle warming moxibustion versus oral western medicine for knee osteoarthritis: a systematic review and meta-analysis. *J Trauma Emergency*. (2015). 2:30–4.

28. Kong, YY, Jing, MY, Qin, Y, Chen, PJ, and Ren, KJ. Effect of warm acupuncture combined with sodium hyaluronate injection on knee osteoarthritis. *World Latest Med Inf*. (2019) 19:35. doi: 10.19613/j.cnki.1671-3141.2019.20.013

29. Cao, J, Zhou, Q, and Qian, QR. Clinical curative effect of combianton therpy of acupuncture and intra-articular injection of sodium hyaluronate versus monotherapy of intra-articular injection of sodium hyaluronate for knee osteoarthritis: a systematic review. *J Tradit Chin Orthop Trauma*. (2019) 31:33–7.

30. Jiang, H, and Zhang, S. Meta-analysis of warm acupuncture combined with sodium hyaluronate therapy in treatment of knee osteoarthritis. *Chin Med Herald*. (2019) 16:154–8.

31. Chen, YL, Zhou, QL, Ji, SL, Sun, WP, and Chen, JT. Efficacy and safety of warming needle moxibustion in treating knee osteoarthritis: a meta -analysis. *Chin J Ethnomed Ethnopharm*. (2019) 28:45–50.

32. Huang, ZL, Shi, SN, He, JJ, Gao, HY, and Hong, ZQ. Meta-analysis of trherpeutic effect of warm acupuncture and moxibustion on knee osteoarthritis of ynag deficiency and cold coagulation and research on regularity of acupoint selection. *Rehabilit Med*. (2021) 31:341–50. doi: 10.3724/sp.J.1329.2021.04012

33. Luo, M, Tan, J, Zhu, YL, Sun, L, Lan, W, Zhu, ZZ, et al. Meta analysis of therapeutic effect of warm acupuncture and moxibustion on knee osteoarthritis. *Hunan J Tradit Chin Med*. (2019) 35:120–3. doi: 10.16808/j.cnki.issn1003-7705.2019.03.058

34. Jin, S, and Guan, X. A systematic review and meta-analysis of the comparative curative effects of warm acupuncture and other traditional Chinese medicines in the treatment of knee osteoarthritis. *Ann Palliat Med*. (2022) 11:708–16. doi: 10.21037/apm-21-3972

35. Li, XJ, Lei, MN, Jiang, ZM, Zhao, LN, Hu, L, and Wu, ZJ. A meta analysis of warming needle acupuncture in the treatment of knee osteoarthritis. *Henan Tradit Chin Med*. (2021) 41:250–60. doi: 10.16367/j.issn.1003-5028.2021.02.0059

36. Zhang, JW. Meta analysis of randomized controlled trials of warming needle moxibustion for knee osteoarthritis. *Clin J Tradit Chin Med*. (2018) 30:2050–4.

37. Ou, YJJ, Liu, ZF, Wu, Y, Cai, WP, and Gu, X. Meta-analysis of clincial efficacy of warm acupuncture and moxibustion in the treatment of knee osteoarthrits. Hunan. *J Tradit Chin Med*. (2018) 34:128–9. doi: 10.16808/j.cnki.issn1003-7705.2018.04.062

38. Wu, Y, Xie, ZY, Sun, SQ, Li, HN, and Tan, T. Meta-analysis of curative effect of needle warming moxibustion and electroacupuncture on knee osteoarthritis. *Shandong J Tradit Chin Med*. (2016) 35:320–4.

39. Schulz, KF, Altman, DG, and Moher, DGroup C. CONSORT 2010 statement: updated guidelines for reporting parallel group randomized trials. *Ann Intern Med*. (2010) 152:726–32. doi: 10.7326/0003-4819-152-11-201006010-00232

40. Whiting, P, Savovic, J, Higgins, JP, Caldwell, DM, Reeves, BC, Shea, B, et al. ROBIS: a new tool to assess risk of bias in systematic reviews was developed. *J Clin Epidemiol*. (2016) 69:225–4. doi: 10.1016/j.jclinepi.2015.06.005

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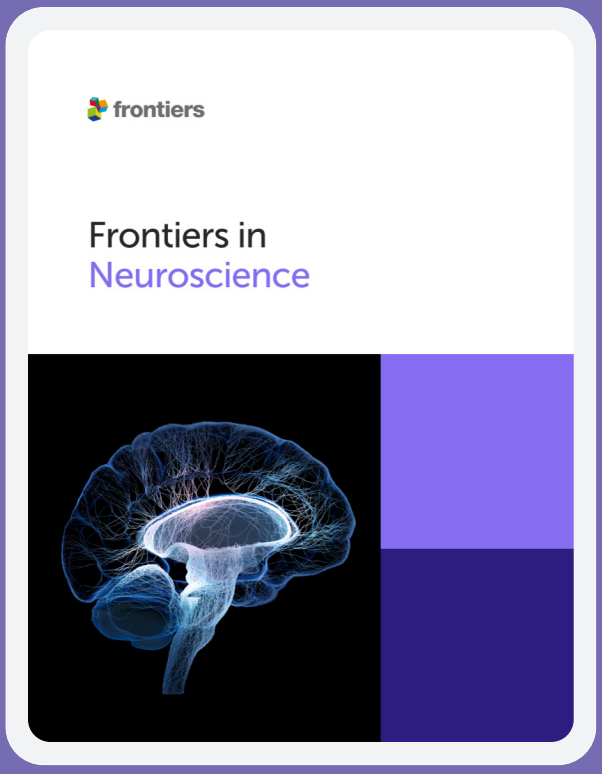

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