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Metabolic factors in erectile dysfunction

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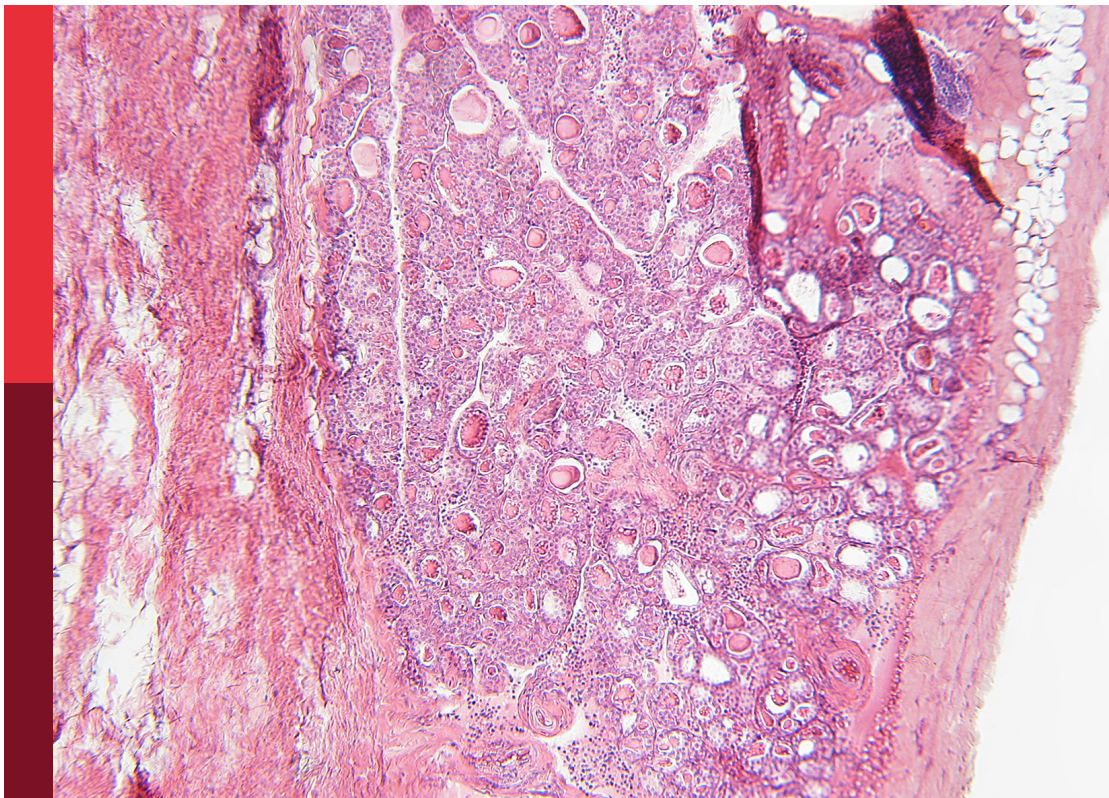

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Metabolic factors in erectile dysfunction

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Editorial: Metabolic factors in erectile dysfunction

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KEYWORDS

erectile dysfunction, diabetes mellitus, hypertension, obesity, metabolic syndrome, chronic liver disease

Editorial on the Research Topic
Metabolic factors in erectile dysfunction

Erectile dysfunction (ED) is a common male sexual dysfunction that seriously affects the life quality of patients. The worldwide prevalence of ED is predicted to reach 322 million cases by 2025 (1). The causes of ED are mainly divided into vasculogenic, neurogenic, endocrinological, drug-induced depression, systemic diseases and general ill health, local penile factors, or psychological problem. Among these causes, the increasing incidence of obesity, diabetes mellitus (DM), hyperlipidemia, hypertension, cardiovascular diseases, and metabolic syndrome has prompted studies to focus on the relationship between metabolic factors and erectile function.

Metabolic factors such as metabolic syndrome, cardiovascular disease, and obesity are risk factors for ED (2). Meanwhile, ED is a marker for metabolic conditions, preceding adverse metabolic events by several years. Therefore, healthcare providers encountering ED should screen for cardiovascular diseases or metabolic syndrome. Causality and associated mechanisms are difficult to establish because ED and these metabolic conditions also share risk factors such as age, hypertension, DM, insulin resistance, smoking, and increased BMI. Hence, the journal has organized this Research Topic: *Metabolic Factors and Erectile Dysfunction*.

In the Research Topic, a total of 13 articles were published, including 8 original research articles and 5 reviews. It involves metabolic factors such as DM, age, hypothalamic–pituitary–adrenal axis activity, serum 25(OH)D levels, idiopathic pulmonary fibrosis and chronic liver disease, and their mechanisms associated with erectile function.

As one of the important causes of ED, DM has attracted the attention of researchers. There are two studies focusing on DM-ED of the 8 original articles. A cross-sectional study from Ethiopia revealed that up to 83.8% of patients with ED have different levels of ED. Thus, they suggested that routine screening and management for ED in patients with DM should be part of the routine medical care, particularly for adult male patients and those with poor glycemic control. Another cross-sectional study comparing the characteristics between DM-induced erectile dysfunction (DM-ED) and non-DM-induced erectile dysfunction Chinese populations revealed that age,

height, BMI, fasting blood glucose, follicle-stimulating hormone, triglycerides, total testosterone, and triglyceride-glucose index significantly differed between the DMED and non-DMED populations. Therefore, the above-mentioned metabolic factors play an important role in the occurrence of DM-ED.

In the study on age-related erectile dysfunction (A-ED), Zhou et al. used transcriptome analyses and bioinformatics methods to predict the possible lncRNA-miRNA-mRNA regulatory network that regulated the occurrence of ED in the elderly. Then they concluded that LncRNA ENSRNOT00000029245 possibly regulated downstream mRNAs leading to apoptosis in the cavernous tissue, fibrosis, and endothelial dysfunction, which ultimately caused ED. These findings provide seminal insights into the molecular biology of A-ED, which could spur the development of effective therapeutics.

Psychogenic erectile dysfunction (pED) is also an important type of ED. Psychological stress and its two stress response systems, the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS), are closely related to pED. Xu et al. first studied the changes in perceived stress and two neural stress systems in pED patients. Their results suggested that the interrelation between ANS and HPA axis activity might enhance our comprehension of how stress affected the physical and mental health of pED patients.

And in other studies of ED, Yuan et al. uncovered the genetic links of ED and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS). Finally, NQO1 was identified as a key genetic link between ED and CP/CPPS. It was predominately enriched in corpus cavernosum endothelial cell, and correlated with other male urogenital and immune system diseases tightly. They identified the genetic profiles as well as corresponding regulatory network underlying interaction between ED and CP/CPPS via multi-omics analysis. These findings expanded a new understanding for the molecular mechanism of ED with CP/CPPS.

Zhang et al. investigated the connection between serum 25(OH) D and carotid artery intima-media thickness (CIMT) in men with ED. The serum level of 25(OH)D and IIEF-5 score were positively correlated, while the CIMT values and IIEF-5 score were negatively correlated. And they concluded that the level of serum 25(OH)D should be analyzed in men with ED, especially in patients with vasculogenic ED, and supplementation is recommended for those who were with vitamin D deficiency.

In a study on cisplatin, Yin et al. found that cisplatin treatment could induce ED, by affecting the content of endothelial and smooth muscle and causing the senescence-associated secretory phenotype in cavernous nerve (CN). Their results indicate that cisplatin treatment should be considered as a risk factor for ED. Clinicians should pay more attention to the erectile function of cancer patients who receive cisplatin treatment.

Several studies have found that ED is associated with interstitial lung disease. However, the causal relationship between idiopathic pulmonary fibrosis (IPF) and ED risk remains unclear. Zhang et al. conducted a two-sample Mendelian randomization (MR) study aiming to reveal the causal effect of IPF on ED risk and concluded that IPF may increase the ED risk of the European population.

In the five review papers, the main focus is on the research progress of pathological mechanism and treatment of ED. Song et al. presented a comprehensive overview of the current molecular pathogenesis of CN injury-induced ED. Song et al. introduced the physiological basis of erectile function and the pathophysiological changes in ED and summarized the current knowledge on the expression, biological functions, and molecular mechanisms of miRNAs in ED, especially the potential of miRNA-targeted therapies to improve ED. Pang et al. reviewed the diagnosis of ED and related physical therapy methods, and explored the pathogenesis of ED. In their opinion, these treatment methods could help many ED patients recover fully or partially from ED within the next few decades. A review conducted by Wang et al. summarized the recent advances on exosome therapy with animal models of ED, and proposed the prospect of future research in order to provide a basis for clinical trials and clinical translation. These reviews can help us better understand the occurrence, development and treatment progress of ED.

In addition to the above reviews, there is a literature review by Zang et al. explored the relationship between chronic liver diseases (CLDs) and ED. As we all know, liver is an important metabolic organ in the human body and is involved in the regulation of many metabolic factors. CLDs frequently result in the abnormal metabolism of sex hormones, glucose, and lipids and mental and psychological illnesses, all of which are significant risk factors for ED. The prevalence of ED in male patients with CLDs ranges from 24.6% to 85.0%. The mechanism by which liver damage affects penile erectile function is not fully understood. The review concluded that male patients with CLDs often have decreased testosterone levels, increased estrogen levels, and other sex hormone metabolism disorders, which may be reasons for the decline of erectile function.

However, metabolic factors are not limited to the factors discussed in our Research Topic. Other metabolic factors still need to be explored, such as high lipid levels, high prolactin, and thyroid hormone levels. In addition, whether ED is caused by a single metabolic factor or a variety of metabolic factors must be further elaborated. Finally, even though researchers performed a deep exploration about the Research Topic, the exact mechanics between metabolic factors and ED remains to be further studied.

Author contributions

XM: Writing – original draft, Writing – review & editing. KR: Writing – review & editing. JC: Writing – review & editing.

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.

Publisher's note

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ORIGINAL RESEARCH
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Comparative Transcriptome Analyses of Geriatric Rats Associate Age-Related Erectile Dysfunction With a lncRNA-miRNA-mRNA Regulatory Network

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Background: The key regulatory roles of long non-coding RNAs (lncRNAs) in age-related erectile dysfunction (A-ED) are unknown.

Aim: This study aimed to identify putative lncRNAs that regulate age-related erectile dysfunction *via* transcriptome analyses, and to predict their specific regulatory routes *via* bioinformatics methods.

Methods: 22 geriatric male SD rats were divided into age-related erectile dysfunction (A-ED) and negative control (NC) groups after evaluations of intracavernous pressure (ICP). By comparative analysis of transcriptomes of cavernosal tissues from both groups, we identified differentially expressed lncRNAs, miRNAs, and mRNAs. Seven differentially expressed lncRNAs were selected and further verified by quantitative real-time polymerase chain reactions (RT-qPCR). The construction of the lncRNA-miRNA-mRNA network, the Gene Ontology (GO) term enrichment, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed in Cytoscape.

Results: From comparative transcriptome analyses of A-ED and NC groups, 69, 29, and 364 differentially expressed lncRNAs, miRNAs, and mRNAs were identified respectively. Differentially expressed lncRNAs were culled to seven, which were all verified by qPCR. Three of these lncRNAs (ENSRNOT00000090050, ENSRNOT00000076482, and ENSRNOT00000029245) were used to build regulatory networks, of which only ENSRNOT00000029245 was successful. Moreover, GO and KEGG analyses demonstrated that these lncRNAs possibly regulated muscle myosin complex, muscle cell cellular homeostasis, and ultimately erectile function in rats through PI3K-Akt, fluid shear stress, and atherosclerosis pathways.

Conclusion: Our study identified differentially expressed lncRNAs, miRNAs, and mRNAs through comparisons of transcriptomes of geriatric rats. An identified lncRNA verified by qPCR, was used to construct a lncRNA-miRNA-mRNA regulatory network. LncRNA

ENSRNOT00000029245 possibly regulated downstream mRNAs through this regulatory network, leading to apoptosis in the cavernous tissue, fibrosis, and endothelial dysfunction, which ultimately caused ED. These findings provide seminal insights into the molecular biology of aging-related ED, which could spur the development of effective therapeutics.

Keywords: lncRNA (long non-coding RNA), erectile dysfunction, aging, bioinformatics, miRNA, RNA sequence analyses

INTRODUCTION

Erectile dysfunction (ED) is an inability to either achieve or maintain a satisfactory penile erection for sexual activity. It is more common in middle-aged and elderly men, especially those over 40 years of age (1). Moreover, most ED patients have an organic etiology (2). ED is not only associated with aging, but also with cardiovascular and endocrine diseases, and neurogenic illnesses (3).

By 2050, 22% of the global population is predicted to have become over 60 years of age (4), and this will result in more ED patients, whose current prevalence rate is 2% and 86% for men under 40 and over 80 years of age respectively (5). Yet by 2025, 332 million people are predicted to have suffered from ED (6). Oral selective phosphodiesterase type 5 inhibitors (PDE5-Is) are common first-line therapies. They inhibit the conversion of cyclic guanosine monophosphate (cGMP) to cyclic guanosine monophosphate(GMP), leading to relaxation of vascular and cavernosal smooth cells, and this ultimately maintains an erection. Vacuum erection devices (VED), intracavernosal injections (ICI), and penile implants are used as second-line therapies. Nevertheless, they have a variety of shortcomings, resulting in the current unsatisfactory clinical treatment of ED (2). Hence, further investigations on the molecular mechanisms of ED are not only needed but could also lead to the development of effective therapies.

Senescence is a major risk factor for various chronic diseases, including type 2 diabetes mellitus (T2DM), cardiovascular disease, and cancer (7). Senescence is a programmed progression caused by accumulated DNA, protein, and lipid damage (8). Relatedly, endothelial dysfunction, which is closely related to erectile dysfunction, occurs due to aging (9–11). Specifically, accumulated damage due to aging leads to dysfunction of the vascular endothelium, which in turn leads to vasculogenic erectile dysfunction (12).

Long non-coding ribonucleic acids (lncRNAs) are ribonucleic acids (RNA) that are both longer than 200 base pairs and not translated into proteins (13, 14). They play important roles in transcriptional regulation, the formation of subcellular structures, epigenetic gene regulation, and programmed cellular development (15, 16). In cardiovascular diseases, lncRNAs regulate downstream biological functions by positive and negative modulatory effects, playing roles as miRNA sponges and co-expression with miRNAs (17). LncRNAs have been associated with ED (18, 19). For example, in diabetes mellitus-induced erectile dysfunction, lncRNA MALAT1 functioned as a sponge for miR-206 to upregulate the expression of VEGFA, thus boosting bone marrow-derived mesenchymal stem cells (BM-MSCs) differentiation into endothelial cells for rectification of ED (20). However, there exists a paucity of studies on this topic. To fill this research gap, we aimed to elucidate possible key roles of lncRNA in age-related ED (A-ED) by constructing a lncRNA-miRNA-mRNA regulatory network *via* comparative transcriptome analyses.

Abbreviations: ED, Erectile Dysfunction; A-ED, Age-related Erectile dysfunction; PDE5-Is, phosphodiesterase type 5 inhibitors; GO, Gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; qPCR, Quantitative real-time PCR; MAP, Mean arterial pressure; ICP, Intracavernous pressure; lncRNAs, Long non-coding ribonucleic acids.

METHODS

Animals Procedures

We used 22 geriatric male SD rats (20 months) purchased from the Experimental Animal Center of Nanjing Medical University, and our research was approved by the Animal Care and Use Committee of the First Affiliated Hospital of Nanjing Medical University. The animals were anesthetized with pentobarbital natrium (40 mg/kg) by intraperitoneal injection.

Erectile Evaluation

After anesthesia, dissections were made along the penis midline to expose the carotid artery. A neck artery was cannulated and a heparinized 25-gauge needle was inserted into the corpora cavernosum of the penis to measure both mean arterial pressure (MAP) and ICP. Electrical stimulation was set at 5 V, 15 Hz, pulse width 0.2ms, and the stimulation lasted for 1 min. The BL-420S Biological Functional System (Chengdu Taimeng Technology Co, LTD, Chengdu, China) was used to measure pressure and generate electrical stimulation. Each experiment was done in triplicate. The ratio of the maximal ICP to the corresponding MAP (ICP/MAP) was calculated to estimate the erectile function. The corpus cavernosum tissue was isolated and stored -80°C for further experiments.

Masson Trichrome Staining

Experiments were carried out as previously described (21). Penile tissue was fixed with 4% paraformaldehyde and embedded in paraffin. Masson trichrome staining was used to observe, under 200x magnification, then evaluate the ratio of smooth muscle to collagen area in penile tissue.

Total RNA Isolation and Transcriptome Sequencing (RNA-seq)

The rats were divided into geriatric A-ED (erectile dysfunction) and NC (negative control) groups based on the erectile evaluation results. ICP/MAP > 35% was defined as the geriatric NC group, and ICP/MAP < 35% was defined as the geriatric ED group. After stripping the urethra and other connective tissue from the penis, the cavernous tissue was retained. Cavernous tissue was minced into small pieces with a low-temperature grinder (Servicebio, KZ-III-F) under grinder parameters of 60Hz & -10°C. Then, total RNA was extracted from pieces of cavernous tissues or cells using a TRIzol (Invitrogen, Carlsbad, CA, USA) based protocol. RNA concentration and purity were determined *via* a NanoDrop 2000 spectrophotometer (Madison, WI, USA). Ribosomal RNA (rRNA) was separated from total RNA and RNA integrity was analyzed *via* an Agilent RNA 6000 Nano Kiton an Agilent 2100 Bioanalyzer. Residual total RNA was broken into short fragments for 2 min, then reverse transcribed into cDNA with random hexamers as primers *via* a SuperScript III First-Strand Synthesis protocol (Invitrogen). Synthetized products were purified using a QIAquick PCR Purification Kit (QIAGEN). DNA fragment ends were repaired and ligated with poly (A) tails to connect them to Illumina sequencing adapters. Then, the second strand was digested and the appropriate fragment was identified for PCR amplification. The cDNA sequencing library was made and subsequently sequenced using an Illumina HiSeq TM 4000 platform (Gene Denovo Biotechnology Co., Guangzhou, China). Only reads that met the following criteria were used as raw data (1) reads did not contain adapters; (2) reads did not contain more than 10% of unknown nucleotides (N); (3) reads did not contain more than 50% of low quality (Q-value ≤ 20) bases. These reads were aligned to Ribosome RNA (rRNA) and mapped to a rat reference genome using HISAT2 (22).

Quantitative Real-Time PCR

We extracted total RNA from previously isolated corpus cavernosum tissues using a Trizol (Invitrogen, USA) based protocol. Then, cDNA was synthesized using HiScript® III All-in-one RT SuperMix Perfect for qPCR (Vazyme, China). Next, we utilized the StepOne Plus Real-Time PCR system (Applied Biosystems, USA) to perform qPCR. Fold changes in mRNA expression were calculated using the 2−ΔΔCt method and normalized against β-actin with ABI Step One software version 2.1. PCR primer sequences were synthesized by TSINGKE Biological Technology (Nanjing, China) and are listed in **Supplementary Table S1**.

Bioinformatic Analysis

Transcriptome data were normalized and analyzed in R using the edgR package. Then three validated lncRNAs were selected (ENSRNOT00000090050, ENSRNOT00000076482, and ENSRNOT00000029245). For prediction analysis, we defined differently expressed miRNAs and mRNAs as logFC >2 and p <0.05. MiRNAs target prediction tools TargetScan, miRcode, and miRDB were used to predict miRNAs targets. All relevant annotations of miRNAs were derived from miRbase.

The network was constructed based on ceRNA theory as previously described (21). (1) Spearman rank correlation coefficient (SCC) was used to evaluate expression correlation between miRNAs-mRNAs or miRNA-lncRNAs. RNA interaction pairs that had SCC<0 were defined as either negatively co-expressed lncRNA-miRNA or mRNA-miRNA pairs. All RNAs were differentially expressed. The mRNA and lncRNA were predicted as miRNA target genes. (2) Pearson correlation coefficient (PCC) was used to assess expression correlations between lncRNA and mRNA. ceRNA interaction pairs that had PCC > 0.5 were defined as co-expressed lncRNA-mRNA pairs. RNA interaction pairs of mRNA and lncRNA were termed as negatively or postively co-expressed with a common miRNA. Pairs of mRNA and lncRNA were targeted negatively or positively co-expressed with a common miRNA. (3) The significance of common miRNA sponges differences between the two genes was tested by a hypergeometric cumulative distribution function test. The filtering condition was p< 0.05. Finally, all eligible RNA interaction pairs were collected and combined into a network, which was visualized in Cytoscape 3.8.2 (http://www.cytoscape.org/).

Gene ontology (GO, http://www.geneontology.org/) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg) pathway analysis of differentially expressed mRNAs were performed using the R package clusterProfiler. An alpha level of p<0.05 was used to test statistical significance.

Cell Culture

The rat vascular endothelium cell line (RAOEC) was purchased from Shanghai Jingkang Biological Co., LTD (Shanghai, China) and cultured at 37 °CC in 5% CO_2. The endothelial cell lines were characterized by double-immunofluorescent staining for biomarkers CD31 and vWF (Proteintech, China). DMEM medium, supplemented with 10% FBS, and 100 units/mL penicillin, were used in cell culture (Gibco).

Induction and Evaluation of Senescence

ROAEC cells were treated with hydrogen peroxide (H_2O_2) to induce senescence (23, 24). Cells were seeded onto 6-well plates (10^6/well) in a full growth medium. We choose the optimized concentration (100 μM) to build a cellular aging model according to the references (25). After 24 hours, the cells were treated with H_2O_2 (100μM) and DMSO (0.01%) respectively for 24 hours. Senescence-associated β-galactosidase (sa-β-Gal) staining was performed using a β-galactosidase enzyme assay kit (Beyotime, China) between the H_2O_2-treated group and the DMSO group.

Statistical Analysis

All data analyses were done using SPSS software (SPSS V 24.0). T-tests were used to determine the statistical significance of differences between two groups of parametric data whereas Mann-Whitney tests were used for nonparametric data. Statistical significances of GO and KEGG analyses were

determined using Fisher's exact tests. An alpha level of $p<0.05$ was used for all statistical tests.

RESULTS

Erectile Function Evaluation

Among 22 rats, we selected ICP/MAP less than 0.35 as the low-pressure group and ICP/MAP greater than 0.35 as the high-pressure group, and when combined with the results of the Masson staining, 22 rats were divided into A-ED group and NC group (n=7, n=15). There was no significant difference in MAP between the A-ED and NC groups (p=0.3777). However, the ratio of ICP/MAP was lower in the A-ED than the NC group (**Figures 1A, B**). Masson trichromatic staining results showed that the smooth muscle/collagen ratio was significantly lower in the A-ED than the NC group (**Figures 1C, D**). Thus, this A-ED rat model was valid for studies on age-related erectile dysfunction.

Results of RNA-seq Data

A total of 5,307 lncRNAs, 1,322 miRNAs, and 22,050 mRNAs were identified by sequencing. The expression levels of lncRNAs in six samples were assessed by FPKM, and did not show a deviated distribution of mRNAs nor lncRNAs (**Figures 2A, B**). Scatterplots (**Figures 2C, D**) demonstrated high reproducibility of samples from each group.

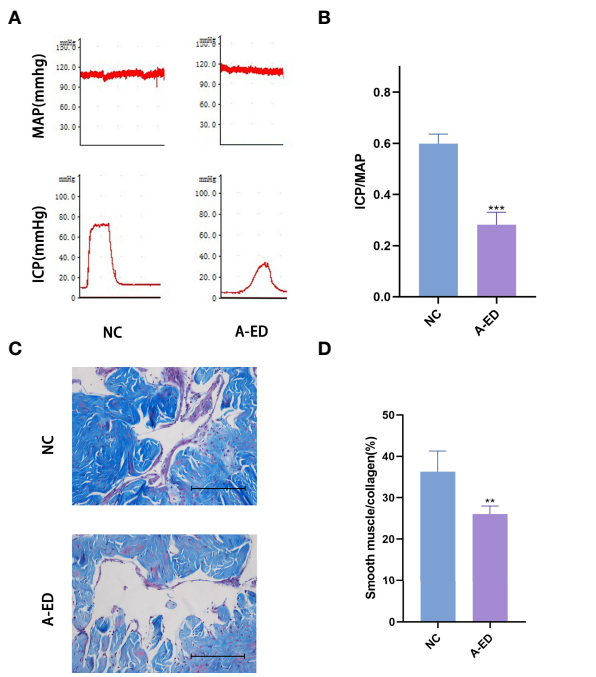

FIGURE 1 | Evaluation of Erectile Dysfunction **(A)**: Mean arterial pressure (MAP) and intracavernous pressure (ICP) of rats in the A-ED group and NC group. **(B)**: Bar plot of the ICP/MAP of A-ED group and NC group. ***p < 0.001. **(C)**: Masson-trichrome (Masson) staining showing the fibrosis in the A-ED and NC groups. **(D)** Bar plot of the ratio of smooth muscle to collagen in the A-ED and NC groups. **p < 0.01.

Differentially expressed mRNAs and lncRNAs were analyzed using volcano plots (**Figures 3A, B**). Upregulated and downregulated mRNAs and lncRNAs, when compared to the geriatric NC group, were shown using orange and blue dots respectively. Ultimately, a total of 69, 364 and 29 differential expressed lncRNAs (49 up-regulated, 20 down-regulated), mRNAs (143 up-regulated, 221 down-regulated), and miRNAs (18 down-regulated, 11 up-regulated) respectively, were identified (**Figures 3C–E**).

Validation of qPCR

Using an inclusion criteria for lncRNAs of 1<count number<120, fold change > 2, $p< 0.05$, seven lncRNAs were identified and used to validate RNA-seq results in seven pairs of geriatric ED and NC samples (MSTRG.3646.1, ENSRNOT00000085383, ENSRNOT00000093493, ENSRNOT00000081965, ENSRNOT00000029245, ENSRNOT00000090050, ENSRNOT00000076482, ENSRNOT00000085383). According to qPCR results, ENSRNOT00000076482(p=0.0175), ENSRNOT00000090050(p=0.007) and ENSRNOT00000029245 (p=0.0006) were highly expressed in the ED group, corroborating sequencing results (**Figure 4**). However, expression levels of the other four lncRNAs did not statistical differ between groups.

GO and KEGG Analysis

To further explore the specific function of these lncRNAs, related mRNAs were used to carry out GO and KEGG enrichment analyses and the top 20 results of enrichment analysis (including KEGG and GO) according to p values, are shown in **Figure 5**. In doing KEGG analyses, we identified pathways for PI3K-AKT & Jak-STAT signaling, terpenoid backbone biosynthesis, synthesis and degradation of ketone bodies, EGFR tyrosine kinase inhibitor resistance, fluid shear stress and atherosclerosis.

From GO enrichment analyses, cellular component (CC) annotations showed that the following components played key roles: coated pit (GO:0005905), striated muscle myosin thick filament (GO:0005863), dehydrodolichyl diphosphate synthase complex (GO:1904423), muscle myosin complex (GO:0005859), and phosphatidylinositol 3-kinase complex, class IB (GO:0005944). Corresponding annotations of molecular functions included translation initiation factor binding (GO:0031369), hydroxymethylglutaryl-CoA synthase activity (GO:0004421), and interleukin-6 receptor activity (GO:0004915).

Moreover, from the annotations of biological processes, the following were enriched (**Figure 5**): response to purine-containing compound (GO:0014074), regulation of long term synaptic depression (GO:1900452), muscle cell cellular homeostasis (GO:0046716), regulation of platelet-derived growth factor receptor signaling pathway (GO:0010640), and organophosphate biosynthetic process (GO:0090407).

Construction of lncRNA-miRNA-mRNA Network

From the results of RT-qPCR and RNA-seq, three verified lncRNAs, 29 differentially expressed miRNAs, and 364 differentially expressed mRNAs were selected, and the regulatory network was constructed according to its

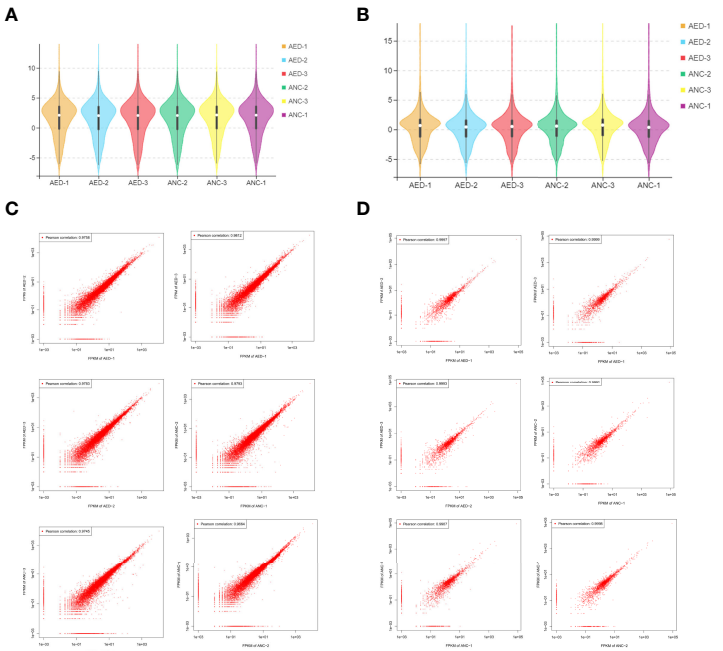

FIGURE 2 | Data distribution and quality assessment. **(A)** A violin diagram of the FPKM value of all mRNAs within each sample. **(B)** A violin diagram of the FPKM value of all lncRNAs within each sample. **(C)** Scatter plot of correlation between mRNAs expression levels in two different samples. **(D)** Scatter plot of correlation between lncRNA expression levels of two different samples.

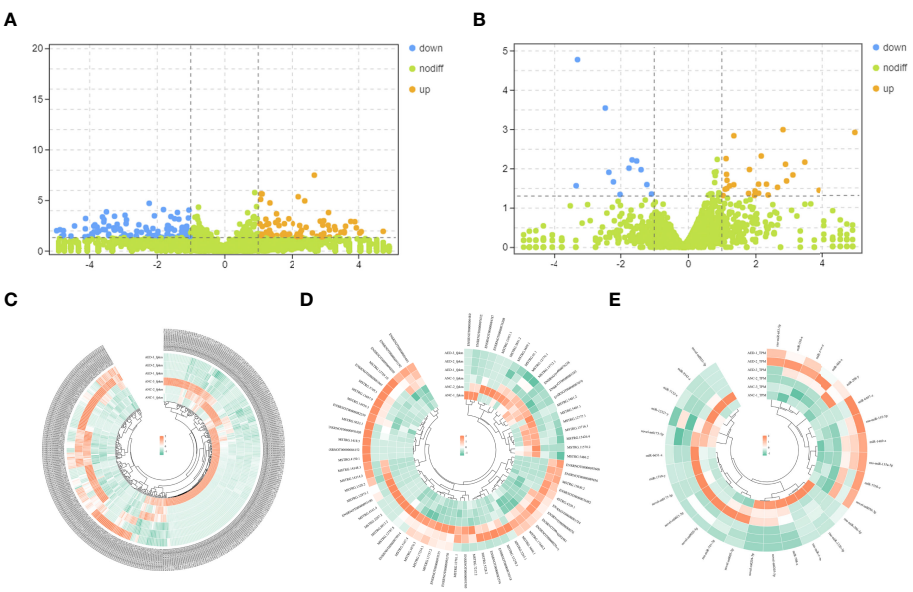

FIGURE 3 | **(A)** Volcano plots of differentially expressed mRNAs. The abscissa represents the log of the multiple of the difference between the two groups, and the ordinate represents the negative Log10 value of the FDR of the difference between the two groups. **(B)** Volcano plots of differentially expressed lncRNAs. The abscissa represents the log of the multiple of the difference between the two groups, and the ordinate represents the negative Log10 value of the FDR of the difference between the two groups. **(C–E)** Heatmaps demonstrate the distribution of differentially expressed lncRNAs, miRNAs, and mRNAs.

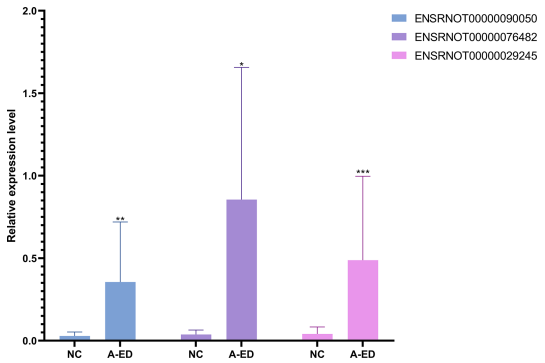

FIGURE 4 | Bar cartoon of qPCR shows the expression of 3 lncRNAs between the A-ED and NC groups. *P < 0.05, **p < 0.01, ***p < 0.001.

hypothesis. **Figure 6** illustrates the lncRNA-miRNA-mRNA regulatory network of geriatric ED rats. In our network, the up-regulated lncRNA ENSRNOT00000029245 upregulated the expression of 23 mRNAs (Tspan18, Mtss1, Szrd1, Pik3cg, NEWGENE_619861, Fgf1, Btrc, B2m, Ggta1, kdm4a, Fxyd7, Naf1, Vps9d1, Ppm1l, Bcl2l1, RGD1560281, Scamp1, Sele, Nrcam, Hsp90b1, Cmpk1, Fam20b, Hiatl3) by miR484x axis. Similarly, the lncRNA ENSRNOT00000029245 regulated the 14 mRNAs (Sorcs3, LOC100910882, Morf4l2, Ptprd, Hmgcs1, Lox, Gpm6a, Trim32, Tnfrsf11b, Phf14, Ncapg2, Cyyr1, Reep3, Nus1) by rno-miR-653-5p axis. Interestingly, according to our network, the mRNA Il6r was upregulated by both the miR484x axis and the rno-miR-653-5p axis.

Validation in Cells

The RAOEC cells were identified by staining with anti-CD31 antibody (red), anti-vWF antibody (green), and cell nuclei were identified using DAPI (blue) (**Figure 7A**). The sa-β-Gal staining demonstrated that the RAOEC cells were induced senescence at 100μM H_2O_2 (**Figure 7B**). Interestingly, the expression of the three lncRNAs (ENSRNOT00000029245, ENSRNOT00000076482, and ENSRNOT00000090050) between cell lines showed that only ENSRNOT00000029245 had a statistical significance (**Figure 7C**).

DISCUSSION

As the global population of the elderly rises, so does erectile dysfunction, a disease closely related to aging. Physiologically, an erection occurs when, NO, released from the endothelium and parasympathetic nerve terminals, enhances cGMP, thus promoting the loss of intracellular calcium and resulting in the relaxation of smooth muscles. The venous return is then blocked, triggering an erection.

Although PDE5Is are currently recommended as first-line treatments, they only relieve symptoms without curing, and the effective PDE5 dose increases with age (26). This, therefore, necessitates new insights into the molecular biology of age-related erectile dysfunction for the discovery of novel drug targets and therapies.

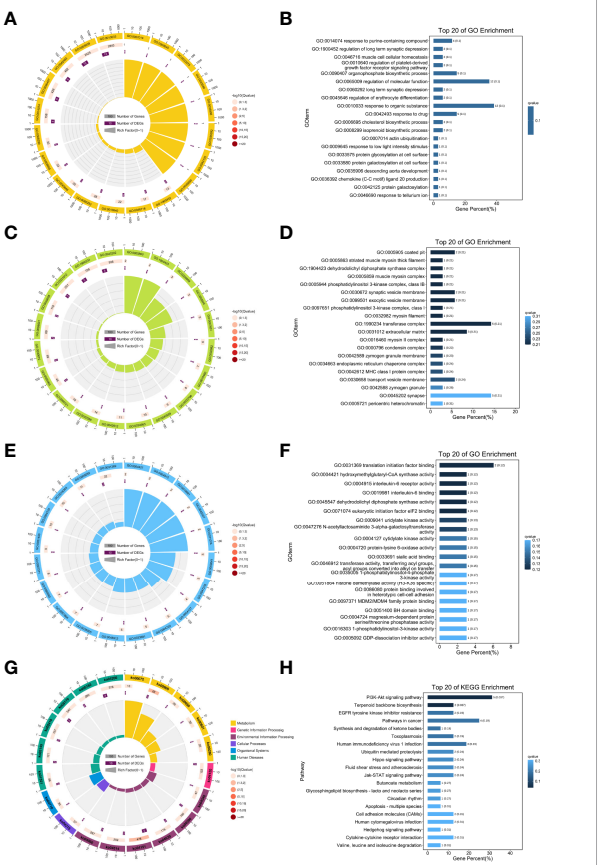

FIGURE 5 | Results of the GO and KEGG enrichment. Top 20 enrichment results **(A, B)** biological processes; **(C, D)** cellular component; **(E, F)** molecular function; **(G, H)** KEGG pathways enrichment.

In this study, we comparatively analyzed transcriptomes based on a $p< 0.05$, logFC>2 criteria, to identify differentially expressed RNAs. A total of 69, 364, and 29 differentially expressed lncRNAs, mRNAs, and miRNAs respectively, were identified from comparisons of transcriptomes of geriatric ED and NC rats. Using the criteria of 1<count number<120, seven lncRNAs were identified and validated by qPCR. Furthermore, three lncRNAs were differentially expressed between the two groups. However, only lncRNA ENSRNOT00000029245 was used to successfully construct a lncRNA-miRNA-mRNA regulatory network.

Moreover, we conducted GO and KEGG enrichment analysis based on lncRNA ENSRNOT00000029245 related mRNAs. The results of these enrichment analyses showed that in terms of GO enrichment, coated pit, striated muscle myosin thick filament, dehydrodolichyl diphosphate synthase complex, muscle myosin complex, phosphatidylinositol 3-kinase complex, and translation initiation factor binding were enriched. The results of the KEGG pathway analysis indicated that PI3K-AKT and Jak-STAT signaling, terpenoid backbone biosynthesis, synthesis and

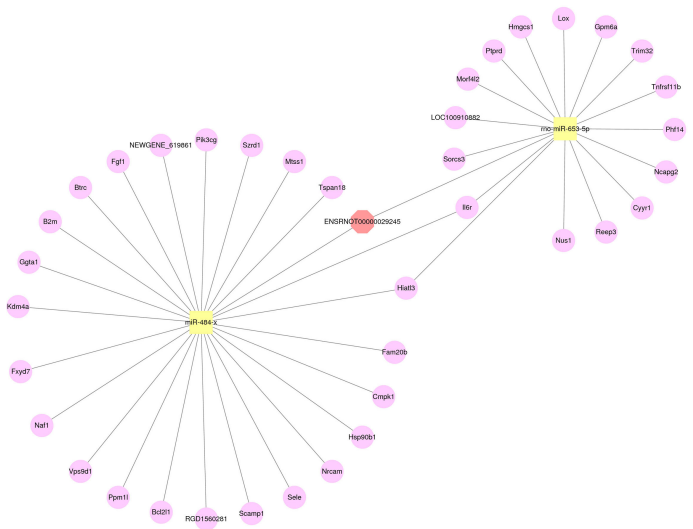

FIGURE 6 | The network of lncRNA-miRNA-mRNA. Red octagons represent up-regulated lncRNAs, yellow squares represent up-regulated miRNAs, and pink circles represent up-regulated mRNAs.

degradation of ketone bodies, EGFR tyrosine kinase inhibitor resistance, fluid shear stress, and atherosclerosis pathways were enriched. The PI3K-AKT signaling pathway plays a critical role in maintaining vascular endothelial function (27). It also regulates the opening of the endothelial tight junction, as well as the permeability and mobility of endothelial cells (28). This suggests that aging leads to mutations in the PI3K pathway, which in turn leads to erectile dysfunction by affecting endothelial cell function.

ceRNA crosstalk has possible roles in numerous diseases, as lncRNAs regulate diseases through ceRNA (29, 30). Using both an exclusion criteria and qPCR results, we elected the differentially expressed lncRNA ENSRNOT00000029245 to build a lncRNA-miRNA-mRNA regulate network that could identify microregulatory processes in age-related ED.

Phosphatidylinositol 3-kinase (PI3K), an important component in the PI3K-AKT pathway, is a vascular protective factor with anti-apoptotic functions (31). Yu et al. found that PI3Kγ in vascular smooth muscle cells(VSMC) was upregulated after vascular injury, and PI3Kγ could promote proliferation and migration of VSMC (32). Thus, PI3Ks possibly contribute in large part to ED. In our study, ENSRNOT00000029245 was validated by qPCR as highly expressed in ED rats, and related to Pik3cg. In our regulatory network, ENSRNOT00000029245

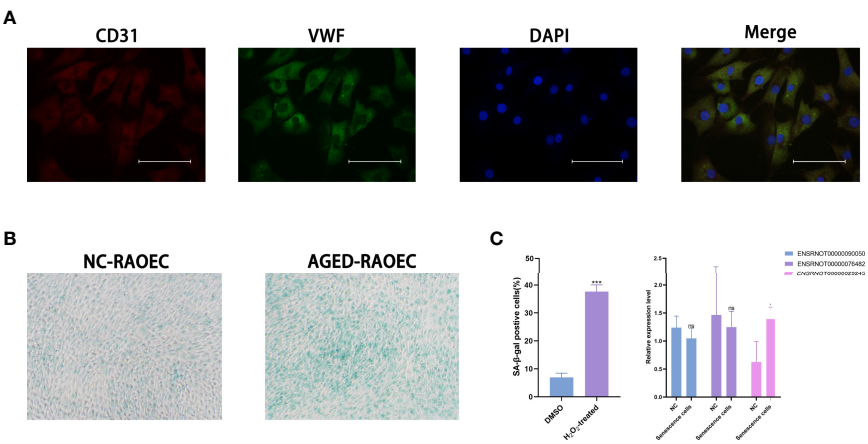

FIGURE 7 | Cell lines validations. **(A)** Double-immunofluorescent staining for biomarkers CD31 and vWF. **(B)** β-Galactosidase staining for RAOEC cell in the DMSO and the H_2O_2 treated group. **(C)** Relative expression of three lncRNAs (ENSRNOT00000090050, ENSRNOT00000076482, and ENSRNOT00000029245) in Senescence cells and NC cells. *P < 0.05. Scale bars = 25 μm.

regulates Pik3cg by modulating miR484-x. High expression of miR-484 downregulates eNOS transcripts, thus affecting homeostasis of endothelial function (33).

ED is accompanied by irreversible death of smooth muscle and endothelial cells (2). BCL2 family proteins are important regulators of apoptosis. Our network indicates that LncRNA ENSRNOT00000029245 may be associated with apoptosis in either cavernosal endothelium or smooth muscle cells *via* regulating miR-484-x-mediated Bcl2l1 expression. Moreover, tripartite motif-containing 32 (trim32), is up-regulated when cells undergo oxidative stress, promote ROS generation, and induce apoptosis (34). From our findings, trim32 was upregulated *via* the ENSRNOT00000029245-rno-miR-653-5p axis. This supported the hypothesis that upregulated ENSRNOT00000029245 promotes apoptosis of corporeal smooth muscle and endothelial cells, thus inducing ED in geriatric rats.

Our network findings suggest that ENSRNOT00000029245 targets miRNAs and mRNAs that affect either fibrosis or apoptosis in the corpus cavernosum and this ultimately results in erectile function.

However, our study still has some weaknesses. First, our model was based on rats and, despite lncRNAs being conserved between humans and rats, it thus does not provide direct evidence for the feasibility of our ceRNA network for humans. Ideally, this network should have been constructed from comparisons of cavernous tissues of elderly-ED patients to that of elderly healthy volunteers. Second, we constructed the ceRNA network of lncRNA without considering crosstalk from pseudogenes and circular RNAs. Lastly, we did not validate the proposed regulatory effect of this lncRNA on erectile function *in vivo*.

CONCLUSION

In this study, we identified 69, 29, and 364 differentially expressed lncRNAs, miRNAs, and mRNAs respectively, by comparing transcriptomes from A-ED rats to those from NC rats. Three lncRNAs (ENSRNOT00000029245, ENSRNOT00000076482, and ENSRNOT00000090050) were validated by qPCR, as highly expressed in the A-ED group. The results of GO enrichment and KEGG pathway analyses showed that A-ED may be associated with PI3K-AKT signaling, fluid shear stress, and atherosclerosis pathways. Moreover, the constructed network showed that ENSRNOT00000029245 possibly contributes to either fibrosis or apoptosis in the corpus cavernosum *via* either a rno-miR-653-5p-trim32 axis or by regulating miR-484-x-mediated Bcl2l1 expression. Therefore, it is worthwhile to investigate molecular mechanisms through which lncRNA ENSRNOT00000029245 regulates age-related ED.

DATA AVAILABILITY STATEMENT

The RNA-Seq data presented in the study are deposited in the SRA databank (www.ncbi.nlm.nih.gov/sra) and is accessible via the PRJNA848782 SRA (Bioproject) accession number.

ETHICS STATEMENT

The animal study was reviewed and approved by The Animal Care and Use Committee of the First Affiliated Hospital of Nanjing Medical University.

AUTHOR CONTRIBUTIONS

NS and XM designed this work. XM, XZ (1st author), LY, and RC wrote the manuscript. QZ, XiZ, and JL performed the bioinformatics analysis. XZ (7th author), XR, and TZ performed the data review. All authors have read and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.887486/full#supplementary-material

Supplementary Figure 1 | The bar plot of qPCR of these 7 lncRNAs between A-ED and NC group (*p < 0.05, **p < 0.01, ***p < 0.001).

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Molecular pathogenesis and treatment of cavernous nerve injury-induced erectile dysfunction: A narrative review

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Introduction: Erectile dysfunction (ED) is a common complication after radical prostatectomy (RP), and it seriously affects the quality of life in patients and their partners. The primary trigger of postoperative ED is surgical injury to the cavernous nerves that control penile erection and run along the anterolateral aspect of the prostate. Despite the introduction and ongoing innovation of nerve-sparing techniques, a significant number of patients still suffer from moderate cavernous nerve injury (CNI), which is thought to be transient and reversible. Therefore, early postoperative penile rehabilitation therapy may salvage patients' erectile function by promoting cavernous nerve regeneration and preventing penile structural alterations.

Aims: To present a comprehensive overview of the current molecular pathogenesis of CNI-induced ED, as well as novel therapeutic strategies and their potential mechanisms.

Methods: A literature search was performed using PubMed. Search terms included *erectile dysfunction*, *cavernous nerve injury*, *pathogenesis*, *pathway*, and *treatment*.

Results: The NOS/NO pathway, oxidative stress-related pathway, RhoA/ROCK pathway, transforming growth factor-β (TGF-β), sonic hedgehog (Shh), and hydrogen sulfide (H_2S) are involved in the molecular pathogenesis of CNI-induced ED. Multiple neurotrophins, including brain-derived nerve growth factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), and neurturin (NTN), were found to promote cavernous nerve regeneration. Emerging therapeutic approaches can be roughly summarized into four categories, namely small molecule and drug, stem cell-based therapy (SCT), micro-energy therapy and platelet-rich plasma (PRP) therapy.

Conclusion: These pathways collectively lead to the irreversible damage to the penile structure after CNI. The combined early rehabilitation strategies of promoting upstream nerve regeneration and recovering abnormal molecular signals of downstream penis are presumed to save patients' erectile function after RP. In future studies, the cross-talk between these molecular pathways

needs to be further clarified, and the questions of how denervation injury induces the molecular alterations in the penis also need to be addressed.

KEYWORDS

erectile dysfunction, cavernous nerve injury, signaling pathway, pathogenesis, nerve regeneration

1 Introduction

Neurogenic erectile dysfunction is caused by insufficient nerve signal transmission to the corpus cavernosum. The main etiology of neurogenic ED can be classified as central lesions (e.g., multiple sclerosis, Parkinson's disease, spinal cord injury, *etc.*) and peripheral lesions (e.g., cavernous nerve injury caused by pelvic surgery or pelvic fracture) (Thomas and Konstantinidis, 2021), among which, cavernous nerve injury (CNI) caused by radical pelvic surgery (especially radical prostatectomy) as the main cause of iatrogenic ED has been recognized as a point of concern for clinicians. Several high-quality studies have exhibited that up to 80% of patients develop ED after radical prostatectomy (RP) (Emanu et al., 2016). Even with meticulous nerve-sparing radical prostatectomy (NSRP) performed by skilled surgeons, it is still inevitable that many patients experience declines in erectile function after surgery. A study by Tal and colleagues in 2009 showed that the incidence of venous leakage at 6 months after bilateral NSRP was 7%, and at 18 months, the recovery rate of erectile function without drug assistance was 49% (Tal et al., 2009). With continuous innovations in prostatic procedures and ever-growing knowledge of prostatic anatomy, especially the application of robotic-assisted technology, which enhances the accuracy of surgery, it is possible to improve the preservation of periprostatic neurovascular bundles (NVBs). Nonetheless, several meta-analyses have suggested that whether robotic procedures can improve postoperative erectile function is still debatable (Ficarra et al., 2012; Du et al., 2018; Wang et al., 2018). Currently, phosphodiesterase type 5 inhibitor (PDE5I) therapy is the first-line treatment for erectile dysfunction. A meta-analysis performed by Chen et al. (2015) presented that the overall effective rate of sildenafil 50 mg is up to 47% against placebo. However, PDE5I therapy is not quite satisfactory for the recovery of erectile function after RP. Meta-analyses performed by Limoncin et al. (2017) and Liu et al. (2017) showed that PDE5Is have no significant effect on the amelioration of postoperative spontaneous (no drug-assisted) erectile function and can merely improve the rate of drug-assisted EF recovery by 10% or more as compared to placebo. Due to the fact that there is no surgical technique to completely avoid cavernous nerve injury and the curative effect of penile rehabilitation therapy is still uncertain, it has become an important issue to clarify the molecular pathogenesis of CNI-related ED and to explore new treatments for the restoration of postoperative erectile function. This review presents an overview of the research progress in the molecular pathogenesis, molecular signaling pathways, and novel therapies of CNI-related ED.

2 Neuropathology and animal models of cavernous nerve injury

According to the Seddon classification, peripheral nerve injury is stratified into three degrees: neurapraxia, axonotmesis, and neurotmesis, whose severity increases in turn, as well as recovery time (Kaya and Sarikcioglu, 2015). Neurapraxia is primarily a demyelinating injury, which means the connections of axons remain mostly unimpaired and Wallerian degeneration hardly occurs. Thus, the injury site can be remyelinated and repaired by Schwann cells, and the conduction of the nerve will soon be well restored. In terms of axonotmesis, due to the interruption of axons, Wallerian degeneration occurs on the segment distal from the site of injury, and only the surrounding supporting connective tissues remain partially or fully retained. Consequently, the recovery time of nerve conduction is prolonged, depending on the capacity of axonal regeneration and the integrity of surrounding supporting tissues after injury (Robinson, 2018). The cavernous nerve which originates from the major pelvic ganglion (MPG), contains both parasympathetic (nitrergic) nerves and sympathetic nerves, mediating the relaxation and contraction of cavernous smooth muscle respectively, together regulating the erectile function of penile tissue (Dean and Lue, 2005). Hsieh et al. (2003) have proven that the ratio of parasympathetic nerves to sympathetic nerves decreased after CNI, and the sympathetic nerve components showed a stronger ability to regenerate after injury. Hence, they proposed a hypothesis that the imbalance of these two nerve components may be responsible for RP-related ED. NSRP strives to preserve the continuity of the cavernous nerve as much as possible, but during the operation, the neurovascular bundles are inevitably affected by traction, crushing, and/or thermal injury, resulting in neurapraxia or mild axonotmesis. Although the nerve remains intact in appearance, its transmission will be temporarily blocked, which is believed to cause structural changes in penile tissue (Fode et al., 2013). During this transmission-blocking period, the penile tissue is in a persistent state of ischemia and hypoxia, which may lead to smooth muscle apoptosis and tissue fibrosis, which subsequently leads to venous leakage. Thus, it is convincing that this kind of structural changes may be an essential component for long-

term ED after NSRP (Fode et al., 2013). However, the molecular mechanism behind this pathophysiological process has not been elucidated, leading to the inaccurate effect of current postoperative penile rehabilitation.

At present, the animal model of CNI takes rat as the main carrier, a recent consensus statement (Weyne et al., 2020) recommended several standardized guidelines for the construction of CNI models, including but not limited to: I, bilateral rather than unilateral CNI should be used as the standard model; II, important parameters such as injury mode (e.g., crush, transection, heat. Crush injury is the most common method to simulate nerve injury caused by NSRP), used instrument and injury duration should be recorded in detail; III, priority should be given to intracavernous pressure (ICP) rather than corpus spongiosum pressure (CSP) as an index to evaluate erectile function. In conclusion, the cavernous nerve crush rat model is recommended for simulating the condition of moderate CNI in post-RP patients.

3 Molecular pathogenesis of cavernous nerve injury-erectile dysfunction

3.1 Nitric oxide synthase/nitric oxide pathway

Nitric oxide (NO), a key molecule in mediating penile erection, is catalyzed from L-arginine by nitric oxide synthase (NOS). The NOS/NO signaling pathway, which is currently the most important and comprehensive understanding pathway of physiological erection, also plays a crucial role in the pathogenesis of CNI-ED. Once sexual signals are transmitted to the corpus cavernosum *via* cavernous nerve, NO is generated in nitrergic nerve terminals and endothelial cells of the cavernosa under the activation of nNOS and eNOS, respectively. Synthetic NO then diffuses into corpora cavernosum smooth muscle cells (CCSMCs) to bind and stimulate soluble guanylate cyclase (sGC), which converts GTP into the second messenger cGMP. cGMP activates cGMP-specific protein kinase (PKG), which phosphorylates and regulates a variety of ion channels: I, it inhibits the activity of transmembrane L-type calcium channels, consequently preventing extracellular calcium influx; II, it promotes the transfer of cytoplasmic calcium into the endoplasmic reticulum, together resulting in a decline in cytoplasmic calcium concentration; III, it inhibits calcium-activated chloride channels (CaCCs), causing a reduction of in chloride outflow. IV, it activates transmembrane large conductance Ca^{2+}-activated K^+ channel (BKCa) and ATP-dependent K^+ channel (KATP), promoting intracellular potassium outflow and generating intracellular hyperpolarization potential, resulting in the suppression of calcium channel activation and further reduction of cytoplasmic Ca^{2+} concentration. The reduction in cytoplasmic Ca^{2+} ultimately induces dephosphorylation of myosin light chain (MLC), causing relaxation of CCSMCs, vasocongestion of penis, and erection. PDE type 5 (PDE5), which catalyzes and deactivates cGMP into 5′-GMP, is the most active of the thirteen PDEs found in cavernosal tissue and its inhibitor is currently the most effective drug for the treatment of ED (Kuthe et al., 2001).

It has been demonstrated that the expression and activity of nNOS protein decrease in the MPG and penis of CNI rats, resulting in the decline of nerve-derived NO, which in turn affects the relaxation of corpus cavernosum smooth muscle and erectile function (Karakus et al., 2017). The phosphorylation of nNOS is one of the main mechanisms for regulating the bioactivity of nNOS, which can be mediated by several protein kinases: protein kinase A (PKA), protein kinase B [Akt (or PKB)], AMP-activated protein kinase, and Ca2+/calmodulin-dependent protein kinase II (Murphy et al., 2009; Song et al., 2012). There is evidence that phosphorylation of the nNOS Ser^{1412} site activates nNOS, while the phosphorylation of Ser^{847} inhibits its activity (Rameau et al., 2007). Hurt et al. have revealed the important role of PKA-mediated Ser^{1412} phosphorylation of nNOS in physiological erection (Hurt et al., 2012). Their subsequent study further indicated the favorable regulatory effect of PKA activator colforsin on nNOS after CNI (Karakus et al., 2017). The results showed that the phosphorylation of Ser^{1412} and Ser^{847} of nNOS in the MPG and penis were both significantly up-regulated after CNI, suggesting that hyperphosphorylation of Ser^{1412} may conversely lead to inactivation of nNOS, which seems inconsistent with previous study. Furthermore, the level of nNOS uncoupling and protein inhibitor of nNOS (PIN) binding to nNOS were also upregulated. nNOS has the catalytic activity to generate NO when it is in the dimer state. In contrast, when it is uncoupled as a monomer, its activity for the formation of NO is lost. Instead, the ability to catalyze the production of reactive oxygen species (ROS) is significantly elevated, which is supported by the upregulation of H_2O_2 and total ROS in the penis. It has been elucidated that ROS not only contribute to the reduction of NO bioavailability (Jones et al., 2002), but also lead to structural impairment of penile tissue (Lagoda et al., 2007). The main causes of nNOS uncoupling were considered to be the phosphorylation of Ser^{847} of nNOS and the increase of PIN. Moreover, the study also identified that NADPH oxidase subunit gp^{91}phoxin was upregulated in the MPG after CNI, indicating that NADPH oxidase may be another source of oxidative stress in the MPG. In summary, the authors proposed a hypothesis that the inactivation of nNOS and the increase of oxidative stress collectively leads to the occurrence of neurogenic ED. The PKA activator colforsin reversed these molecular changes, namely reduced the hyperphosphorylation of Ser^{1412} and Ser^{847}, prevented the uncoupling of nNOS and diminished the level

of oxidative stress, thus improving erectile function. However, the potential mechanism of Colforsin reversing these molecular changes remains to be further investigated.

In addition, nNOS could indirectly enhance the activity of eNOS by increasing penile blood flow and shear stress, whose molecular mechanism is to activate the phosphatidylinositol 3-kinase (PI3K)/Akt pathway and consequently phosphorylate Ser^{1177} of eNOS (Hurt et al., 2002; Hurt et al., 2012). Therefore, it is reasonable to assume that the downregulation of expression and activity of nNOS after CNI leads to a corresponding reduction of eNOS-dependent NO synthesis, together resulting in persistent flaccidity and low oxygen supply in the penis. Some scholars presume that long-term hypoxia may lead to apoptosis and fibrosis of penile smooth muscle cells, damaging the vital mechanism of venous occlusion (Fode et al., 2013). It has been observed that hypoxia could induce the expression of transforming growth factor-β1 (TGF-β1) and inhibit the synthesis of prostaglandin E in cultured CCSMCs (Moreland, 1998; Leungwattanakij et al., 2003). TGF-β1 is recognized to induce collagen synthesis, while prostaglandin E may inhibit it. Nevertheless, the specific molecular mechanism of penile structural changes caused by long-term hypoxia remains to be further clarified.

A recent study (Musicki et al., 2018) also revealed that the S-nitrosylation modification of eNOS and its downstream signaling molecule sGC in the penis mediates the occurrence of CNI-ED. S-nitrosylation refers to the covalent modification of protein cysteine residues by NO to form an S-nitrosothiol (SNO) (Lima et al., 2010), and the effect of NO on protein S-nitrosylation is independent of the cGMP/PKG pathway. S-nitrosylation is involved in the post-translational modification of many proteins, including NOS and sGC itself in the classical NOS/NO pathway. This study indicated that the S-nitrosylation modification of eNOS and sGC inhibits their catalytic activity, while N-acetyl-cysteine (NAC, a de-S-nitrosylation reagent) can preserve the effect of the NO/cGMP signaling pathway and protect erectile function (Musicki et al., 2018).

3.2 Oxidative stress-related pathway

Oxidative stress is a state of imbalance between oxidation and antioxidation, which is considered to be an important factor leading to aging and disease. Wang et al. (2019); (Wang et al., 2015); found that the levels of glutathione peroxidase (GPX) and 3-nitrotyrosine (markers of oxidative stress) in the penis of CNI rats were significantly increased, and suggested that oxidative stress may be an important mechanism of CNI-ED. In addition, oxidative stress has also been shown to be involved in other types of ED, such as hypertension-related, diabetic, and radiation-induced ED (Jin et al., 2008; Suresh and Prakash, 2011; Kimura et al., 2012). Free radicals in the human body include ROS and reactive nitrogen species (RNS). Although the source of free radicals in penis after CNI has not been fully understood, according to the evidence of other types of ED, it is reasonable to speculate that NADPH oxidase (NOX) is activated after CNI and plays an important role in upregulating the level of oxidative stress (Jin et al., 2008; Kimura et al., 2012). NOX catalyzes NADPH to form superoxide anion (O^{2-}), which reacts with NO to form peroxynitrite (ONOO-), thus reducing the content of NO (Agarwal et al., 2006). In addition, uncoupled nNOS has been elucidated to be another source of ROS after CNI (Karakus et al., 2017). Besides reducing the content of NO, oxidative stress may also induce corpus cavernosum fibrosis, promote apoptosis of smooth muscle cells, and lead to endothelial dysfunction (Kimura et al., 2012). Furthermore, it has been shown that oxidative stress can also inhibit cavernous nerve regeneration and induce apoptosis of MPG neurons after CNI (Zhao et al., 2016). Therefore, reducing the level of oxidative stress is indeed an alternative and efficient therapy to improve erectile function after RP.

3.3 Transforming growth factor-β pathway

Many studies have shown that the expression of TGF-β increases significantly after CNI, which is associated with cavernous fibrosis (Leungwattanakij et al., 2003; Hu et al., 2004; Shin et al., 2011). After binding to type II receptor (TGF-βRII), TGF-β recruits the type I receptor (TGF-βRI) and bridges it with the type II receptor to form a receptor complex. There is a highly conserved near-membrane domain (also known as the GS domain) rich in Gly and Ser on TGF-βRI. Several Ser and Thr residues in the GS domain are phosphorylated by type II receptors to activate TGF-βRI. Activated TGF-βRI then recruits and phosphorylates the downstream signaling molecules SMAD family members SMAD2 and/or SMAD3, which subsequently combine with SMAD4 to form a heterotrimeric complex. The SMAD complex is subsequently transported into the nucleus to regulate the transcription of TGF-β target genes, thus inducing collagen formation and fibrosis-related changes (Roberts and Derynck, 2001; Schmierer and Hill, 2007; Shin et al., 2011). In addition, SMAD7 has also been proven to be involved in the above pathway, which inhibits the phosphorylation of SMAD2 and SMAD3 mediated by TGF-βRI, thereby inhibiting downstream signal transmission. Song et al. (2014) used adenovirus to transfect the SMAD7 gene into the corpus cavernosum of CNI mice and successfully restored erectile function *via* hindering penile fibrosis, inhibiting endothelial cell apoptosis and enhancing the phosphorylation of Ser^{1177} of eNOS. Previous studies have elucidated that the TGF-β/SMAD pathway mediates apoptosis

of vascular endothelial cells (Lu et al., 2009) and smooth muscle cells (Redondo et al., 2005) in other organs, yet the role of this pathway in penile tissue apoptosis after CNI remains uncertain. Moreover, a study by Miyajima et al. (2000) demonstrated that there is a correlation between TGF-β and the expression and activity of NOS in the kidney with unilateral ureteral obstruction, so whether TGF-β after CNI affects the activity of eNOS and its molecular mechanism are also worthy of further investigation.

TGF-β may also induce CNI-related ED *via* non-SMAD signaling pathways, such as the RhoA/ROCK, RAS/MEK/ERK, and PI3K pathways (Derynck and Zhang, 2003). Hannan et al. (2014) found that the expression of histone deacetylase (HDAC) family members HDAC3 and HDAC4 is up-regulated in the penis of CNI rats, and is involved in the induction of penile fibrosis. Intraperitoneal injection of the HDAC inhibitor valproic acid (VPA) reverses fibrosis and improves erectile function. HDAC regulates gene transcription *via* catalyzing the deacetylation of acetyl-L-lysine side chains in histones (Lombardi et al., 2011). Additionally, many transcriptional factors and signaling proteins and other non-histone proteins also act as targets of HDAC to regulate a variety of biological functions (Glozak et al., 2005). There is evidence (Barter et al., 2010) that HDAC is necessary for TGF-β to activate ERK and PI3K pathways and to induce subsequent expression of fibrogenic genes. The above studies not only suggest that HDAC is an effective molecular target for reversing penile fibrosis induced by TGF-β after CNI, but also imply that the SMAD-independent pathways of TGF-β may be involved in the profibrotic cascade. Furthermore, the studies by Cho et al. (2011); (Song et al., 2015) indicated that the sphingosine-1-phosphate (S1P) and RhoA/ROCK1 signals may mediate cavernous fibrosis *via* cooperating with TGF-β after CNI, which also supports the above hypothesis. In conclusion, it is significant to clarify the driving role of these TGF-β atypical pathways in CNI-ED, as relevant studies may reveal the complex cross-talk between these pathways and provide novel molecular targets for the selection of specific drugs.

3.4 RhoA/ROCK pathway

Rho, a small monomer of the G protein Ras superfamily, is a 20–30 KD GTP binding protein with GTP enzyme activity. Rho-associated kinase (ROCK) belongs to the serine/threonine kinase family and includes two isoforms, ROCK1 and ROCK2. The structure of ROCK consists of three parts: an N-terminal serine/threonine kinase domain, a pleckstrin homology domain at the carboxyl terminus, and a helix domain containing the Rho-binding domain (Loirand, 2015). RhoA/ROCK exists in many tissues throughout the human body and participates in regulating a variety of physiological functions, including but not limited to, cell contraction, migration, proliferation, and adhesion. In Chitaley et al. (2001) detected the expression of endogenous ROCK in corpus cavernosum for the first time and revealed the effect of RhoA/ROCK pathway on penile erection. In the process of mediating the contraction of CCSMCs, ligands such as endothelin-1 (ET-1), angiotensin II (Ang II) and norepinephrine are released from endothelial cells and cavernous nerve terminals above all. Subsequently, ligands bind to the G protein-coupled receptor (GPCR) of smooth muscle cells to activate guanine exchange factor (GEF), which converts inactive RhoA-GDP into active RhoA-GTP. RhoA-GTP then detaches from the RhoA-GDP dissociation inhibitor and translocates to the cell membrane, where it binds to ROCK and leads to the consequent autophosphorylation and activation of ROCK. Activated ROCK then facilitates the phosphorylation of myosin light chain phosphatase (MLCP). Unphosphorylated MLCP can dephosphorylate myosin light chain (MLC) and promote the release of myosin from actin and the relaxation of smooth muscle. Phosphorylated MLCP acts conversely, resulting in contraction of smooth muscle (Sopko et al., 2014). Previous studies (Gratzke et al., 2010; Sopko et al., 2014) suggested that ROCK2 is the primary isoform up-regulating in the penis of CNI-ED rats, while ROCK1 is overexpressed in diabetic ED model, which yet remains uncertain and requires further elucidation.

In addition to mediating the contraction of CCSMCs, it has also been revealed that the activation of the RhoA/ROCK pathway in penile tissue of CNI rats leads to the induction of corporal apoptosis and fibrosis (Cho et al., 2011; Hannan et al., 2013; Cho et al., 2015). The TGF-β/S1P/RhoA/ROCK1/LIMK2/cofilin pathway has been indicated to mediate penile fibrosis after CNI (Cho et al., 2011; Song et al., 2015), while the RhoA/ROCK/Akt/Bad/Bax/caspase-3 pathway may be involved in cavernous smooth muscle apoptosis (Cho et al., 2015). In addition, Hannan et al. (2016) showed that RhoA/ROCK is also activated in the MPG after CNI and induces the caspase-3-dependent apoptosis of nitrergic neurons. The application of ROCK inhibitor Y-27632 promotes the outgrowth of axons of cultured MPGs *in vitro*.

The RhoA/ROCK pathway regulates the tension of penile smooth muscle in an NO-independent manner, but several studies have suggested that there may be a wide correlation between the RhoA/ROCK pathway and NO pathway. Up-regulation of RhoA/ROCK can inhibit the expression and activity of eNOS in penis (Hannan et al., 2013). The underlying mechanism of low expression of eNOS may be attributed to the fact that RhoA/ROCK reduces the stability of NOS3 mRNA, which encodes eNOS (Laufs and Liao, 1998). Meanwhile, ROCK reduces the activity of eNOS by directly phosphorylating Thr^{495} of eNOS or indirectly phosphorylating

and deactivating the upstream regulatory molecule PKB of eNOS (Ming et al., 2002; Sugimoto et al., 2007). In addition, intraperitoneal injection of ROCK inhibitor Y-27632 into CNI rats could prevent the uncoupling of nNOS in the MPG, increase the expression and activity of nNOS in the MPG and partially in cavernous tissue (Hannan et al., 2013; Hannan et al., 2016), which may be related to the neuroprotective effect of Y-27632 on the MPG/CN. Moreover, PKG can prevent RhoA-GTP binding to ROCK by phosphorylating it, thus inhibiting the activity of ROCK (Sauzeau et al., 2000).

3.5 Sonic hedgehog pathway

Sonic hedgehog (Shh) protein is a secretory protein that belongs to the Hedgehog (Hh) family, along with Indian Hedgehog (Ihh) and Desert Hedgehog (Dhh). The evolutionarily conserved Hh pathway is required for proper embryonic development and plays an important role in adult tissue maintenance, renewal, and regeneration (Beachy et al., 2010). There is evidence showing that the expression of Shh is essential for penile embryonic development, postnatal differentiation, and maintenance of adult penile tissue integrity (Podlasek et al., 2005). Shh protein is mostly abundant in the smooth muscle of cavernous sinus, as well as the MPG and cavernous nerve that innervate the corpus cavernosum (Podlasek, 2009). The Shh pathway has been thoroughly explored in embryonic development and some other organs, but there are few reports on it in adult penis and MPG.

3.5.1 Typical sonic hedgehog pathway

The Patched 1 (PTCH1)-Smoothened (SMO)-suppressor of fused (SUFU)-GLI axis is the core component of the typical signaling pathway of Shh (Ingham et al., 2011). The Shh protein initiates signal transduction by binding to the typical receptors PTCH1 and coreceptors growth arrest-specific 1 (GAS1), CAM-related/downregulated by oncogenes (CDO), brother of CDO (BOC) and low-density lipoprotein receptor-related protein 2 (LRP2). PTCH1 is concentrated in and around the primary cilia. When Shh does not bind to PTCH1, PTCH1 inhibits the activity of SMO. Without activated SMO, the GLI proteins (GLI2 and GLI3) that bind to SUFU are first phosphorylated by PKA, and then further phosphorylated by glycogen synthase kinase 3 (GSK3) and casein kinase 1 (CK1). Phosphorylated GLIs are hydrolyzed and cleaved to generate the transcriptional repressor forms GLIRs. Once Shh binds to PTCH1, PTCH1 leaves the primary cilia, releasing the inhibition on SMO. SMO is subsequently phosphorylated by G protein-coupled receptor kinase 2 (GPRK2) and CK1, enters the primary cilia together with β-arrestin and the microtubule motor KIF3A, and enriches in the cilia with the assistance of Ellis-van Creveld syndrome protein (EVC) and EVC2. Enriched SMO induces the separation of GLI from SUFU, allowing GLI to bypass the proteolytic processing and transport into the nucleus in its full-length activated form, which acts as a transcriptional activator to regulate the transcription of target genes (Podlasek, 2009; Ingham et al., 2011; Briscoe and Therond, 2013). The target genes of Shh pathway include vascular endothelial growth factor (VEGF), NOS, BMP-4 and Hoxd-13 (Podlasek et al., 2005; Podlasek et al., 2007; Bond et al., 2013a), all of which are linked to the development of ED. GLI, as a major transcriptional regulator in the Shh pathway, has the capacity to control transcription in both directions. In the absence of Shh signaling, GLI3 acts as the primary transcriptional suppressor. Once Shh ligand is present, GLI2 acts as the main activator, triggering the expression of GLI1 and other Shh target genes. GLI1, as another powerful transcriptional activator, further enhances the expression of target genes (Hui and Angers, 2011).

3.5.2 Atypical sonic hedgehog pathway

The term “atypical Shh pathway” is not well defined. At present, it may be classified into two categories: one is mediated by PTCH1, but independent of its inhibition on SMO. PTCH1 has been shown to trigger apoptosis through the DRAL-caspase-9 complex in the absence of Shh (Guerrero and Ruizi Altaba, 2003; Thibert et al., 2003; Mille et al., 2009). The other is mediated by SMO but independent of GLI signaling. For example, Shh induces axonal growth through SMO-mediated activation of Src family kinases (SFKs) (Yam et al., 2009); Shh promotes fibroblast migration by activating small Rho GTP enzymes Rac1 and RhoA through SMO (Polizio et al., 2011). In addition, the GLI-mediated pathway independent of SMO regulation is also considered as one of the atypical Shh pathways (Teperino et al., 2014).

Podlasek et al. (2007); (Bond et al., 2008) observed that the Shh protein in penile tissue of CNI Sprague Dawley rats was significantly decreased, resulting in abundant apoptosis of CCMSCs. Intracavernous injection of Affi-Gel beads containing Shh inhibitor into normal rats increased the apoptosis of CCMSCs by 12-fold as compared with the control group. In contrast, intracavernous injection of Shh protein could prevent apoptosis of corpus cavernosum cells. Thereafter, more research was conducted to better understand the involvement of the Shh pathway in MPG/CN. The results showed that Shh protein is vital to maintain the integrity of the CN and that inhibition of Shh signaling in the MPG leads to CN demyelination and axonal degeneration. Conversely, the application of Shh protein to the damaged CN nerve can promote CN regeneration, inhibit penile cell apoptosis and eventually improve erectile function (Angeloni et al., 2011; Dobbs et al., 2018). The molecular mechanism by which Shh promotes CN regeneration is not entirely understood, although part of it is related to the upregulation of brain-derived neurotrophic factor (BDNF), which has been determined to have a neuroprotective effect on nNOS positive neurons

(Bond et al., 2013b). Furthermore, it has also been proven that BDNF promotes axonal growth by activating the JAK/STAT pathway after CN injury (Lin et al., 2010). Shh protein is abundantly expressed in MPG neurons and may have a paracrine effect on satellite glial cells surrounding the MPG (Angeloni et al., 2013). The communication between neurons and glial cells carried out by the Shh signaling pathway may play an important role in neuroprotection and regeneration, and further studies should be conducted to determine whether Shh directly mediates above communication or indirectly mediates its downstream target molecules. Shh is also abundant in Schwann cells of CN, which seems to be necessary for maintaining the integrity of CN and CN regeneration (Bond et al., 2008; Angeloni et al., 2011). Moreover, regarding the mechanism of MPG/CN regulating Shh protein in the penis, the team (Bond et al., 2008; Angeloni et al., 2009) concluded that Shh cannot be transported anterograde from the MPG to the corpus cavernosum through the CN; CN impulses affect the expression of Shh protein in the penis; and some nutritional factors produced in the MPG are delivered by the CN to the corpus cavernosum to control its internal Shh signal. Hedgehog-interacting protein, one of the target proteins of the Shh pathway, has been shown to play such a role, as well as maintain the integrity of the CN.

A recent study (Choe et al., 2016) has shown that the Shh pathway is also implicated in the fibrosis of cavernous tissue following CN injury, and the decrease of Shh protein in the penis leads to a rise in collagen. There is evidence of a relationship between Shh pathway and TGF-β pathway in other diseases (Hu et al., 2015). Nevertheless, the specific mechanism by which Shh promotes fibrosis in penile tissue remains to be further elucidated. In addition, a study (Dobbs et al., 2019) further showed that the Shh pathway can induce the expression of ROCK1 in MPG/CN, suggesting that there may be cross-talk between the Shh pathway and the Rho/ROCK pathway in the pathogenesis of CNI-related ED.

3.6 Endogenous hydrogen sulfide pathway

Endogenous hydrogen sulfide (H_2S) is produced from L-type cysteine (L-Cys) or homocysteine under the catalysis of cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE) or 3-mercaptopyruvate sulfurtransferase (3-MST). As another gas transmitter, H_2S may play a regulatory role similar to that of NO. Srilatha et al. (2006) first discovered the crucial role of endogenous H_2S in penile erectile function in 2006, subsequently, Roberta et al. (D'Emmanuele Di Villa Bianca et al., 2009) confirmed that both cavernous nerve and cavernous smooth muscle cells express it. H_2S in cavernous smooth muscle cells can activate BKCa and KATP ion channels, produce intracellular hyperpolarization potential and promote relaxation, along with its ability to inhibit the expression and activity of NADPH oxidase and reduce the level of oxidative stress. The adenylyl cyclase (AC)/cAMP/PKA cascade may mediate the above H_2S-induced molecular changes. Meanwhile, H_2S can directly inhibit the activity of PDE5 and mitigate the degradation of cGMP (Liaw et al., 2011; D'Emmanuele Di Villa Bianca et al., 2011). In addition, H_2S may also act as an important endogenous regulator of cell proliferation and apoptosis (Kanagy et al., 2017). Although abundant research has revealed the mechanism of H_2S in penile erection, little is known about the role of H_2S in the pathogenesis of neurogenic ED. Recently, Zeng et al. (Qinyu et al., 2021) have revealed for the first time that the decrease of H_2S concentration in penile tissue is related to the occurrence of BCNI-ED, and exogenous H_2S inhibits the phenotypic transformation of CCSMCs and improves the erectile function of BCNI rats by inhibiting the RhoA/ROCK1 pathway and consequently affecting its downstream factors CDK2, Cyclin E1 and $P27^{kip1}$. The concept of phenotypic transformation of CCSMCs originated from that of vascular smooth muscle cells (VSMCs). It is believed that during the development of vascular pathologies such as intimal hyperplasia, vascular stenosis and atherosclerosis, VSMCs are no longer in a resting state, and the expression of contractile proteins is down-regulated, moreover, the ability to proliferate, migrate and produce extracellular matrix proteins is elevated (Beamish et al., 2010). These processes are defined as the phenotypic transformation of VSMCs, that is, the shift of "contractile" VSMCs to "synthetic" or "proliferative" fibroblast-like VSMCs. A study by Yang et al. (2014) first demonstrated that there is phenotypic transformation of CCSMCs in CNI rats and is associated with penile fibrosis. Subsequent *in vitro* experiments (Lv et al., 2014; Yan et al., 2017) showed that hypoxia could induce the phenotypic transformation, which may be mediated by the platelet derived growth factor (PDGF)/PDGFR/STAT3 signaling pathway. In addition, PDGF also activates the RhoA/ROCK pathway through PDGF receptor to promote the phenotypic transformation of VSMCs and CCSMCs (Tang et al., 2018; Qinyu et al., 2021). H_2S, as a new therapeutic molecular target in CNI-ED, has been explored to a limited degree. However, how it inhibits the RhoA/ROCK pathway and whether there are alternative H_2S-dependent signaling pathways involved in CNI-ED still need further elucidation.

4 Neurotrophin-related neuroprotective and neuroregenerative pathways

Accumulating evidence has indicated that the temporary blockade of nerve conduction caused by cavernous nerve injury leads to structural changes in penile erectile tissue, which are often difficult to reverse, resulting in long-term and

difficultly recovered erectile dysfunction. Therefore, fundamentally promoting the regeneration of injured nerves, specifically nitrergic nerves, and shortening the duration of penile denervation after RP will bring a tremendous therapeutic effect for the restoration of postoperative erectile function. In addition to the above-mentioned SHH and RhoA/ROCK pathways, which may be involved in the processes of cavernous nerve protection and regeneration, neurotrophins, immunophilins, erythropoietin and neuregulins et al. (Campbell and Burnett, 2017) have also been suggested to be alternative targets. Here, we focus on the review of neurotrophins and their possible pathways.

Neurotrophins are a family of proteins that support the survival, development and normal function of neurons. At present, neurotrophins which have been proved to recover erectile function after CNI mainly comprise brain-derived nerve growth factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), neurturin (NTN), growth differentiation factor-5 (GDF-5) and VEGF (Bella et al., 2009; Campbell and Burnett, 2017). The underlying pathways of above neurotrophins promoting cavernous nerve regeneration are mostly unclear. A study by Bella et al. (2006) has shown that BDNF stimulates axonal growth of rat MPGs cultured *in vitro* mainly through the JAK/STAT pathway, while the MEK/ERK and PI3K/Akt pathways activated by tropomyosin-related kinase B (TrkB) and pan-neurotrophin 75 (p75) receptors may only act as auxiliary pathways. It is currently unclear how BDNF activates the JAK/STAT pathway, and a possible mechanism is indirect activation through Schwann cells. GDNF and NTN both belong to the GDNF family, and are similar in physiological function and downstream signaling pathway. GDNF and NTN bind to their receptor GFR-α and activate the Ret receptor tyrosine kinase, thus initiating downstream intracellular signaling pathways, including the PI3K, MAPK and Src family kinase pathways. Although the signaling pathways of GDNF family in cavernous nerve regeneration have not been completely clarified, Wanigasekara and Keast (2005) have proven that NTN initiates axonal growth of parasympathetic neurons of the MPG through the PI3K pathway and regulates microtubule assembly through the MAPK and Src kinase pathways. More interestingly, compared with parasympathetic neurons, the stimulating effect of NTN on axonal growth of sympathetic neurons in MPG is significantly weaker, although the underlying mechanism is yet unknown, the inconsistent effects on promoting axonal regeneration seem to be more helpful in normalizing the imbalance between sympathetic and parasympathetic innervation after CNI, therefore, are more conducive to the recovery of erectile function. GDF-5 is a member of the bone morphogenetic protein (BMP)/TGF-β superfamily, the possible candidate pathways of which for its neuroprotective effect include Smad and p38 mitogen-activated protein kinase (MAPK) pathways (Bella et al., 2009). In addition to the neuroprotective effect, Fandel et al. (Fandel et al., 2008) found that intracavernous injection of GDF-5 can significantly reduce the level of TGF-β mRNA in the penis after CNI in a dose-dependent manner, which may be attributed to the direct competition between GDF5 and TGF-β for Smad pathway and inhibition of TGF-β-induced self-expression. This additional effect also suggests that NTN may improve erectile function after RP through multiple-site protective effects and increase the possibility of clinical translation. With regard to VEGF, Chen et al. (2005) have demonstrated that intracavernous injection of VEGF could enhance cavernous nerve regeneration, and co-administration with BDNF could further improve the curative effect. Furthermore, another study (Yang et al., 2020) showed that transplantation of adipose stem cells (ADSCs) coexpressing VEGF and GDNF around the MPG could rapidly repair injured cavernous nerves. VEGF not only promotes angiogenesis and vascular permeability, but also has neurotrophic activity and stimulates axonal growth through flk-1 receptors (Zhang et al., 2010). Possible pathways of VEGF-mediated cavernous nerve regeneration include the Ras/Raf, PI3K/Akt or JAK/STAT pathways, yet, require further investigation.

In addition to the above-mentioned neurotrophic factors, some new molecules conducive to cavernous nerve regeneration have been found in recent years, such as galanin, insulin-like growth factor-1 (IGF-1) and LM11A-31 (Weyne et al., 2018; Haney et al., 2019; Yin et al., 2021), which exhibit significant effects on the restoration of postoperative erectile function in rat models, and need more studies to clarify their potential pathways.

In conclusion, the molecular mechanism of CNI-ED is showed in Figure 1. After cavernous nerve injury, the penile tissue loses the control of nitrergic nerve. The molecular substance such as NO and H2S secretion from the nerve declines which leads to the persistent relaxion of CCSMCs and ED. The persistent flaccidity induces hypoxia of penile tissue. The process will increase the activation of TGF-β pathway and cause penile tissue fibrosis. RhoA/ROCK pathway also participates in penile fibrosis by interacting with TGF-β pathway through S1P molecule. Ligands such as ET-1, Ang II and norepinephrine released from endothelial cells and cavernous nerve terminals bind to the receptors on CCSMCs, corpora cavernosum endothelial cells and fibroblast and activate RhoA/ROCK pathway. The up-regulation of RhoA/ROCK pathway can inhibit the expression and activity of eNOS in endothelial cells. RhoA/ROCK pathway also mediates the contraction of CCSMCs by facilitating the phosphorylation of MLCP and induces the apoptosis of CCSMCs by activating the Akt/Bad/Bax/caspase-3 pathway. The upregulation of Shh pathway also accelerates the apoptosis of CCSMCs. The injury of cavernous nerve and the state of hypoxia cause oxidative stress which produces much free radicals. It also contributes to tissue fibrosis, apoptosis of CCSMCs. These changes of penile tissue also produce oxidative stress conversely. The interaction between

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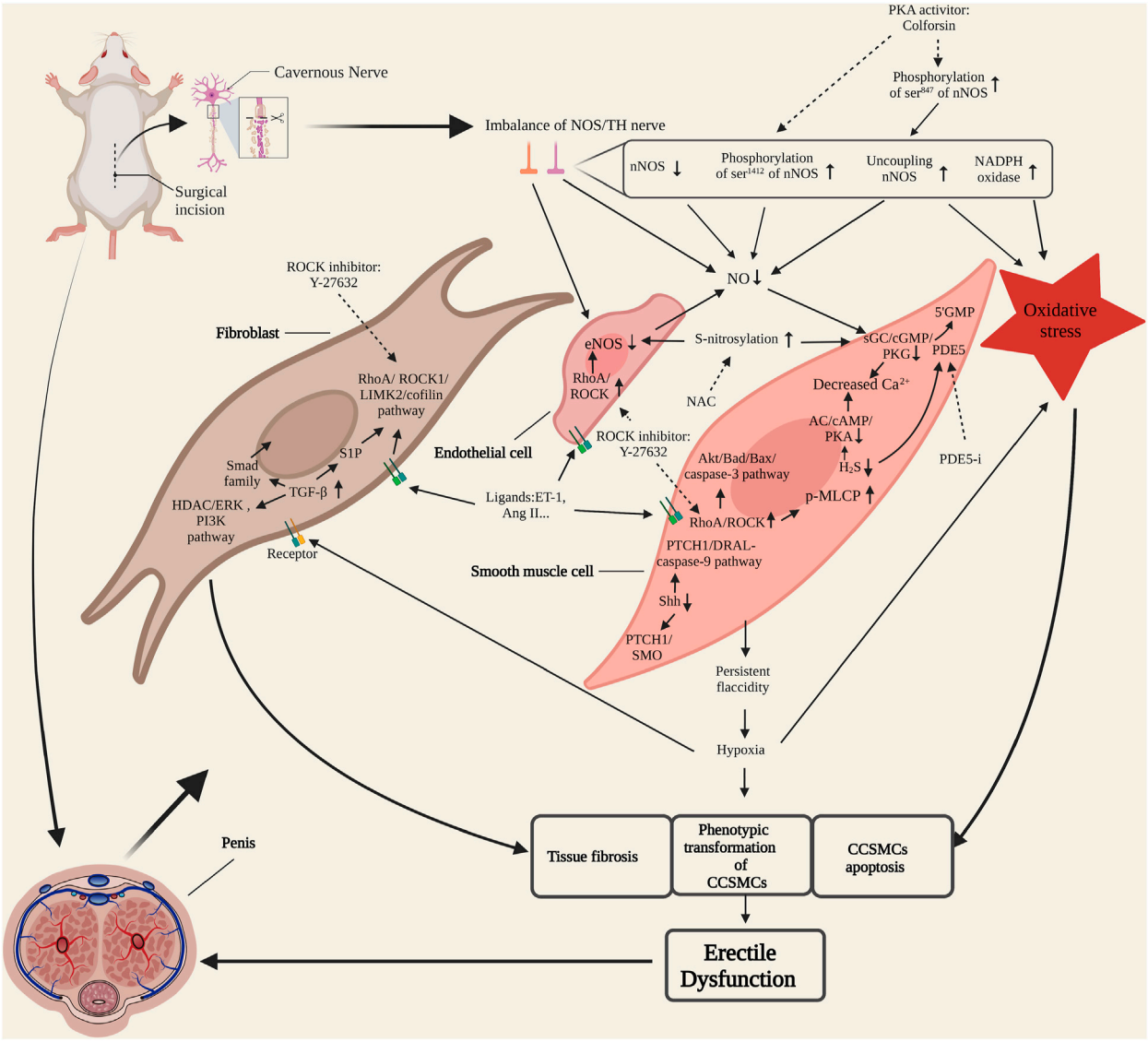

FIGURE 1
Molecular mechanism of cavernous nerve injury-erectile dysfunction (CNI-ED). NOS/TH nerve, nitrergic and sympathetic nerve. CCSMCs, corpora cavernosum smooth muscle cells; NOS, nitric oxide synthase; PKA, protein kinase A; NO, nitric oxide; sGC, soluble guanylate cyclase; PKG: cGMP-specific protein kinase; PDE5, phosphodiesterase type 5; PDE5-i, phosphodiesterase type 5 inhibitor; AC, adenylate cyclase; NAC, N-acetyl-cysteine; ET-1, endothelin; Ang II, angiotensin II; TGF-β, transforming growth factor-β; S1P, sphingosine-1-phosphate. Dotted arrow represents inhibition.

these molecules and pathways contributes to the occurrence of ED together.

5 Latest therapeutic strategies of cavernous nerve injury-related erectile dysfunction

Cavernous nerve injury causes an imbalance between nitrergic and sympathetic innervation of the penis, followed by long-term hypoxia, oxidative stress, tissue apoptosis and fibrosis (Figure 1). Therefore, therapeutic strategies should focus on cavernous nerve protection and regeneration, improving penile blood supply, reducing oxidative stress and normalizing recognized molecular signaling pathways. At present, exploration of CNI-ED treatments mainly stays in the stage of preclinical trials, but the results are encouraging, and with people paying constant attention to translational medicine, it is believed that there will be more therapeutic methods to enter the clinical research in the future. Emerging therapeutic

TABLE 1 Novel therapeutic strategies of CNI-Related ED.

Therapeutic strategies	Method and location of administration	Duration of treatment	Curative effect	Potential mechanism	Representative study
Shh protein	Direct delivery into penis or cavernous nerve *via* peptide amphiphile (PA) nanofiber hydrogels vehicle	9 days (penis); 6 weeks (CN)	Improving erectile function of CNI rats	suppressing both caspase 9 and 8 apoptotic mechanisms; promoting CN regeneration	Martin S, 2021 Martin et al. (2021); Angeloni NL, 2011 Angeloni et al. (2011)
Icariside II flavonoid derivative	Dissolved in PEG 400 and administered intragastrically	3 weeks	Improving erectile function of CNI rats	Activating the proliferation and differentiation of penile endogenous stem cells (SCs) via up-regulation of Wnt/β-catenin signaling pathway	Gu SJ, 2021 Gu et al. (2021)
Combination of JNK inhibitor and LIMK2 inhibitor	Intraperitoneal injection	5 weeks	Restoring the cavernous veno-occlusive function (CVOF)	Inhibiting apoptosis and fibrosis of corpus cavernosum to restore CVOF	Cho MC, 2021 Cho et al. (2021)
fidgetin-like 2(FL2)-siRNA	Direct injection into MPG and CN immediately after CNI *via* nanoparticle encapsulation	4 weeks	Improving erectile function of CNI rats	Inhibiting the FL2-mediated severing of dynamic microtubules in distal axon shaft and growth cone to promote CN regeneration	Baker L, 2021 Baker et al. (2021)
proNGF neutralizing antibody	Intracavernous injection	2 weeks	Improving erectile function of CNI mice	Regulating the production of neurotrophic and angiogenic factors in penis	Chung DY, 2021 Chung et al. (2021)
PKA agonist colforsin	Intraperitoneal injection	3 days	Improving erectile function of CNI rats	Enhancing bioactivity of nNOS and reducing oxidative stress	Karakus S, 2017 Karakus et al. (2017)
TrkA-mAb	Intraoperative injection into MPG and postoperative intracavernous injection	6 weeks	Improving erectile function and sexual behavior of CNI rats	Suppressing sympathetic nerve regeneration and facilitating parasympathetic nerve regeneration through blockade of NGF/TrkA pathway	Lin G, 2015 Lin et al. (2015)
Rho-Kinase Inhibitor fasudil	Oral administration	4 weeks	Improving erectile function and CVOF of CNI rats	Inhibiting Akt/Bad/Bax/caspase-3 and LIMK2/cofilin pathways to reduce cavernous apoptosis and fibrosis	Cho MC, 2015 Cho et al. (2015)
VEGF and BDNF combined gene therapy	Transfected rat adipose stem cells (ADSCs) with lentivirus, and then transplanted ADSCs around MPG	2 weeks	Improving erectile function of CNI rats	Collaboratively promoting CN regeneration	Yang W, 2020 Yang et al. (2020)
Induced pluripotent stem cell–derived mesenchymal stem cells (iMSC)	Intracavernous injection	4 weeks	Improving erectile function of CNI rats	Promoting angiogenesis, neurogenesis, anti-apoptosis and anti-oxidative stress *via* paracrine factors such as VEGF, IGF1, NGF	Chen Z, 2019 Chen et al. (2019)
MSC-derived exosomes	Intracavernous injection	4 weeks	Improving erectile function of CNI rats	Inhibiting apoptosis of CCSMCs	Ouyang X, 2018 Ouyang et al. (2018)
LI-ESWT	placed on the suprapubic region and oriented towards the penis	4 weeks	Improving erectile function of pelvic neurovascular injury rats	Preserving neuronal and vascular integrity via induction of VEGF-release and anti-apoptosis	Wang HS, 2019 Wang et al. (2019)
LI-ESWT (in human)	applied to the root of penis, the shaft, and at a few millimeters proximal to the glans	6 weeks	Improving patients' IIEF-5 scores at 1 month and 1 year after treatment	Same as above	Frey A, 2016 Frey et al. (2016)
Optimized PRP	Intracavernous injection	4 weeks	Improving erectile function of CNI rats	Promoting CN regeneration through a variety of growth factors (such as PDGF, VEGF, etc.) released by platelets	Wu YN, 2016 Wu et al. (2016)

approaches can be roughly summarized into four categories, namely small molecule and drug, stem cell-based therapy (SCT), micro-energy therapy and platelet-rich plasma (PRP) therapy. Among small molecules, Shh protein, which protects injured nerves and preserves the anatomical integrity of cavernous tissue *via* multiple pathways, has been deeply studied. Another small molecule tyrosine kinase receptor type 1 monoclonal antibody (TrkA-mAb) provides us with an interesting perspective, that is, the utilization of neutralizing antibody to balance the regenerative activity of sympathetic and parasympathetic nerves. Low-intensity extracorporeal shock wave therapy (Li-ESWT) is the first proposed micro-energy therapy for CNI-ED, and its efficacy in vasculogenic ED has received abundant support. Recently, studies of its effect on neurogenic ED are gratifying, indicating that micro-energy therapy will be another considerable choose for neurogenic ED patients. As for PRP, it promotes cavernous nerve regeneration through a variety of growth factors (such as PDGF, VEGF, *etc.*) and cytokines (such as CXCL5) released by platelets (Scott et al., 2019; Wu et al., 2021). There have been several animal studies supporting the therapeutic effect of PRP on CNI-ED to date. Novel and promising therapeutic strategies and their related representative research are specifically introduced in Table 1.

6 Future perspectives

Each of these therapeutic categories has advantages and drawbacks, and additional optimization is required to obtain the ultimate clinical application. In terms of small molecules and drugs, oral and intravenous administrations offer the benefits of convenience and high acceptability, however, since medicine is dispersed throughout the body, a higher dose is inevitable to attain the effective concentration locally, necessitating more toxicity and efficacy tests. ICI and intraoperative para-cavernous nerve injection are now more broadly utilized and can successfully enhance local drug concentrations while reducing adverse effects. It is worth noting, though, that there is considerable blood flowing through the corpus cavernosum, and traditionally, blood flow is temporarily restricted *via* ligation of the penile base to lengthen the drug's action period, but the benefit is limited. Therefore, a sustained release drug delivery system (SRDDS) should be developed in the future, which will substantially minimize the number of ICIs as well as adverse effects. Similarly, a SRDDS is also required for para-CN injection, as it can only be conducted once during surgery. As for SCT, accumulating animal experiments have proven its therapeutic effect on CNI-ED, and several human ED studies have not reported serious complications (Raheem et al., 2021). Nevertheless, its long-term risk and optimal dosing remain unknown, coupled with the difficulty of obtaining autologous stem cells, low expansion ability and high cost limit its application. With the advancement of exosome and iMSC research, and existing studies which suggested that stem cells exert their effects primarily *via* paracrine mechanisms, iMSC-derived exosomes may overcome the limitations mentioned above and are expected to be widely applied in the clinic in the future. In terms of micro-energy therapy, it includes Li-ESWT, low-intensity pulsed ultrasound (LIPUS) and micro-energy acoustic pulse (MAP). The therapeutic effect of Li-ESWT on CNI-ED is well-documented, coupled with its non-invasive and easy-to-perform features, it is likely to be first approved for clinical usage. Efforts should be made in the future to clarify its potential mechanism, optimize the treatment protocol, and monitor the delayed adverse effects that possibly exist. LIPUS and MAP have recently been proven to promote CN regeneration, thus it is hypothesized that they can improve postoperative erectile function, but further evidence is needed (Chiang and Yang, 2019; Peng et al., 2020). PRP is relatively easy to prepare and has shown no clear side effects in limited clinical studies. However, placebo-controlled, multicenter studies that can confirm the efficacy of PRP in CNI-ED remain to be conducted, and the optimal injection dosage and duration of treatment have yet to be determined (Epifanova et al., 2020). In summary, the treatment methods for CNI-ED will be more diversified in the future, and the strategies of combination therapy may further benefit ED patients after RP.

7 Conclusion

With deepening research on the pathogenesis of CNI-ED, multiple molecules in the penis and their related pathways have been proven to be involved. Together, these pathways eventually lead to the irreversible damage to the penile structure after cavernous nerve injury, coupled with the wide correlations and interactions of these pathways, which makes the interpretation of the molecular mechanism of CNI-ED more complex. At present, in addition to the progress of molecular signals in the penis, encouraging results have also been achieved in the research on neurological pathways and treatments for nerve regeneration. The combined early rehabilitation strategies of promoting upstream nerve regeneration and recovering abnormal molecular signals of the downstream penis are presumable to save patients' erectile function after RP. In future studies, the cross-talk among these molecular pathways needs to be further clarified, and the questions of how denervation injury induces the molecular alterations in the penis need to be addressed as well. Despite the fact that there are still numerous issues and obstacles in this field, recent

findings indicate that the future is bright and patients are expected to no longer suffer from ED after RP.

Author contributions

Conception and design: GS and PH; Data curation and methodology: GS, PH, and JS; Writing of the manuscript: GS and PH; Review of the manuscript: YR and JL; Study supervision: YR and JL.

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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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Correlation analysis of carotid artery intima-media thickness, serum 25(OH)D and men with erectile dysfunction

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Our goal is to investigate the connection between serum 25(OH)D and carotid artery intima-media thickness (CIMT) in men with erectile dysfunction (ED).Serum 25(OH)D and CIMT were measured in 124 participants with erectile dysfunction and 39 healthy controls. The relationship between them and different patient-related parameters and disease-related parameters was studied. Compared with the control group and mild ED group, the level of serum 25(OH)D in moderate ED group and severe ED group decreased significantly(P<0.05). The CIMT values of moderate ED group and severe ED group were higher than those of the control group(P<0.05). The CIMT value of severe ED group was significantly higher than that of mild ED group(P<0.05). IIEF-5 score was positively correlated with serum 25(OH)D level, but negatively correlated with CIMT value(P<0.05). After adjusting for the influence of confounding factors, The CIMT values, 25(OH)D and IIEF-5 score were substantially associated(P<0.05). The serum level of 25(OH)D and IIEF-5 score were positively correlated, while the CIMT values and IIEF-5 score were negatively correlated. The level of serum 25(OH)D should be analyzed in men with ED, especially in patients with vasculogenic ED, and supplementation is recommended for those who were with vitamin D deficiency.

KEYWORDS

vitamin D, erectile dysfunction, carotid artery intima-media thickness, CIMT, 25(OH)D

Introduction

Erectile dysfunction (ED), is defined as that inability to achieve or maintain an erection that is firm enough to engage in sexual activity. Impotence is another term that has been used occasionally but is now less common. Nowadays, ED has gradually become one of the important diseases perplexing men all over the world. In recent years, studies from the United States, Britain and Italy have all shown that the onset of ED is gradually getting younger (1). Physiological process of penile erection is a vascular response under neuromodulation. Generally, sexual stimulation signals are transmitted to penile tissue, which will cause nerve terminals and endothelial cells to release bioactive factors such as nitric oxide (NO), and induce spongy smooth muscle relaxation, congestion and swelling of spongy smooth muscle. At the same time, the erectile penis compresses the vein and prevents blood flowing back from the corpora cavernous (2). Finally, the penis reaches and maintains sufficient rigidity to facilitate sexual intercourse. Therefore, when there is an obstacle in the process of blood entering the penis, it can give rise to the erectile function.

Carotid intima-media thickness (CIMT), which is thought to be an accurate predictor of systemic atherosclerosis and an objective measure of early atherosclerosis, is intimately associated with the development of cardiovascular disease (3). Sibal have demonstrated the role of CIMT in predicting cardiovascular disease (CVD) (4). At present, studies have shown that vascular diseases such as coronary heart disease, atherosclerosis and other vascular lesions are closely related to ED (5). Yao found that ED may be the earliest clinical manifestation when vascular lesions occur (6). In other words, the severity of ED may be associated with the risk of CVD, which seems to suggest that the severity of ED may also be associated with CIMT.

Vitamin D has mostly been recognized for its function in controlling calcium homeostasis and bone metabolism as a type of fat-soluble steroid hormone. The predominant form of vitamin D found in human body is 25-hydroxyvitamin D(25 (OH)D). Numerous studies have pointed out that low serum vitamin D level is closely related to cardiovascular system, erectile function, and endothelial function (7–9). Monteiro proposed that vitamin D deficiency was associated with atherosclerosis (10). The relevance of vitamin D deficiency (defined as serum 25(OH)D level< 20 ng/ml) and vitamin D insufficient (defined as serum 25(OH)D level< 25 ng/ml) to public health is indisputable, although there is still controversy about optimal vitamin D status. Maintenance of normal serum vitamin D levels is the constituting principal focus of public health strategies.

However, it is still unclear how serum 25(OH)D levels, CIMT values, and erectile function are related. In this study, we wanted to investigate the connections between the three.

Materials and methods

Patients

This study was conducted on 163 Chinese Han males who participated in physical examination in the Affiliated Hospital of Guizhou Medical University from October 2021 to January 2022. The average age of all subjects was 30-60years(45.41 ± 7.44). All participants denied the use of Phosphodiesterase type-5 inhibitor(PDE5i) and vitamin D supplements. And all of them were subjected to detailed history taking, including smoking history (smoking ≥ 1 cigarette/D, time ≥ 6 months) and drinking history (alcohol intake > 25g/D), clinical examination, measurement of systolic blood pressure (SBP) and diastolic blood pressure (DBP), and determination of Body mass index (BMI). All participants were divided into four groups according to the IIEF-5 score, namely control group (IIEF>21), mild ED group (12≤IIEF ≤ 21), moderate ED group (8≤IIEF ≤ 11), severe ED group (0<IIEF ≤ 7).

Exclusion criteria included structural deformities of the penis like hypospadias. Medications-inducing ED such as antidepressants, antihypertensive, antiandrogens, neurogenic and psychogenic illnesses as well as abnormal serum testosterone levels were considered exclusion criteria. Patients who were underweight, those with type II diabetes, and those with illnesses known to influence 25(OH)D levels were also eliminated. Participants with a history of coronary artery disease, hypertension, hypogonadism, renal failure, autoimmune or inflammatory illnesses, or infection during the last six months were excluded. Similarly, all patients denied lower urinary tract symptoms(LUTS), prostatitis and prostatic hyperplasia in the past six months.

Laboratory measurements

5 ml of peripheral venous blood was taken from each subject on an empty stomach in the morning. Fasting blood glucose (FBG), triglyceride (TG), total cholesterol (TC),high density lipoprotein cholesterol (HDL-C),low density lipoprotein cholesterol (LDL-C) and other biochemical indicators,were uniformly detected by the chemical automatic analyzer of the laboratory of the hospital.Serum 25(OH)D concentration was detected by enzyme-linked immunoassay (ELISA) (purchased from Shanghai Xitang Biotechnology Co. Ltd.).

Evaluation of erectile dysfunction

The international index of erectile function-5(IIEF-5) score was used to evaluate the erectile function of the subjects. If the IIEF-5 score ranged from 22 to 25, the subjects were considered to have no ED. When IIEF-5 score <21, different degrees of ED was diagnosed. The severity of ED was classified according to IIEF-5 score, namely severe (0-7 points), moderate (8-14 points) and mild (15-21 points) (11).

Measurement of CIMT

CIMT was measured for all participants. CIMT examination was performed by doctor from ultrasound department in a supine position with the head slightly overstretched and rotated to the contralateral side. In accordance with the Vascular Ultrasound Guidelines, CIMT measurements were automatically obtained using an ultrasonic system with a 5~13 MHz linear phased array probes in B-mode pulsed doppler mode and colour mode. It measures the distance between two echogenic lines separated by the echo gap of the distal segment wall of the common carotid artery. CIMT was expressed as the mean the measurement of intima-media thickness of the left and right carotid arteries. Be careful to keep the frequency level the same for each patient.

Statistical analysis

Data were analyzed by SPSS 26.0 statistical software. Categorical variables were described in numbers and percentages and compared with chi-square test.Kolmogorov-Smirnov test was used to verify the normality of the distribution of quantitative variables. The quantitative data of normal distribution were described by mean ± standard deviation and compared by one-way ANOVA, whereas the quantitative data of abnormal distribution were described by median (quartile range) and compared by non-parametric Kruskal-Wallis test. Spearman correlation was used to analyze the influencing factors of IIEF-5 score. Multiple linear regression was used to eliminate the influence of confounding factors and independently analyze the influencing factors associated with IIEF-5 score. Results were considered significant at $P<0.05$.

Results

Clinical data and laboratory parameters

According to the IIEF-5 score, 163 participants were divided into 4 groups, including 29 in the control group, 44 in the mild ED group, 51 in the moderate ED group, and 39 in the severe ED group.There is no significant difference in age, BMI, SBP, DBP, glucose, serum creatinine(Cr), Cholesterol, HDL, LDL, smoking and drinking among the four groups($P>0.05$). The level of serum 25(OH)D in moderate and severe ED groups was lower than that in control group($P<0.05$) (Figure 1). CIMT values in moderate and severe ED groups were significantly higher compared with control group($P<0.05$) (Figure 2). Futhermore, CIMT value in severe ED group was significantly higher than that in mild ED group($P<0.05$) (Table 1).

Correlation analysis between IIEF-5 score and different indicators

As a result of Spearman correlation analysis, IIEF-5 score was positively correlated with serum 25(OH)D levels, but negatively correlated with CIMT values and triglyceride ($P<0.05$) (Table 2).

Correlation analysis between IIEF-5 score, serum 25(OH)D level and CIMT values

Taking the IIEF-5 score as the dependent variable and 25 (OH)D, CIMT, and triglyceride as the independent variables, the results of multiple linear regression analysis showed that after adjusting for the confounding factors of triglyceride, IIEF-5 score was significantly correlated with the CIMT and serum 25 (OH)D($P<0.05$) (Table 3 and Figures 3–5).

Discussion

The incidence of ED increases with age. Although ED is not life-threatening, it can negatively impact the quality of life of the patients and their partners, especially in young men. Erectile function relays on complex interplay of vessels and nerves. The cavernosal branches of the internal pudendal artery supply the majority of the blood to the penis, while a network of tiny, easily compressed venules is responsible for venous outflow. Parasympathetic activity from the spinal cord's sacral segments causes a cascade of processes to start when arousal happens, releasing nitric oxide and raising the level of cyclic guanosine monophosphate(cGMP) inside the cell. Increased cGMP results in relaxation of vascular smooth muscle and increases blood into the corpora cavernosa. Increased pressure in the corpus cavernosum results from the rapid entry of blood compressing the venule network and decreasing venous outflow, eventually resulting in an erection. Therefore, any condition that harms the neuronal or vascular circuits that support erections can lead to erectile dysfunction.

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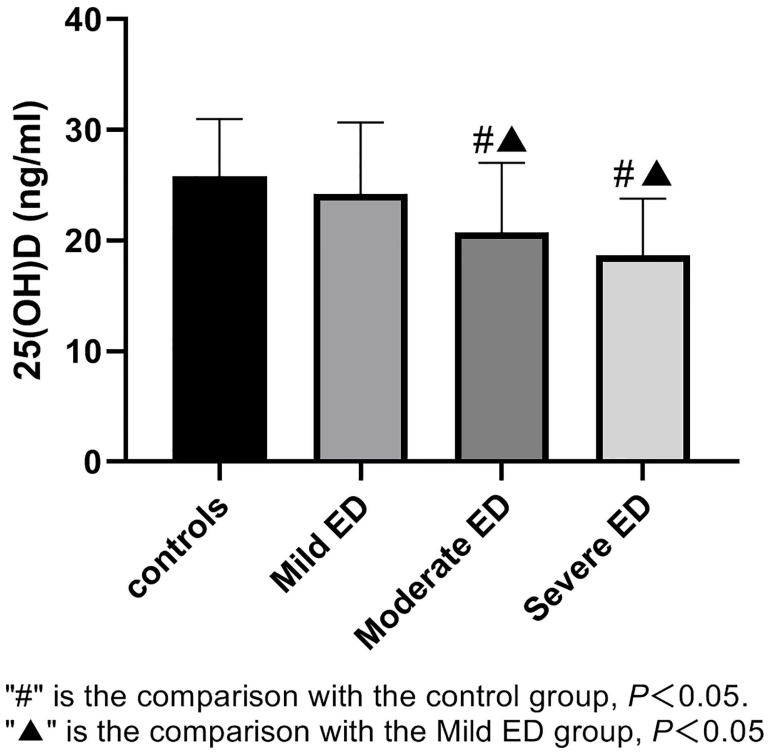

FIGURE 1
Serum 25(OH)D levels in healthy controls, mild ED, moderate ED, Severe ED. "#" is the comparison with the control group, P<0.05. "▲" is the comparison with the Mild ED group, P<0.05.

TABLE 1 Clinical and laboratory features of participants.

	Controls (n=39)	Mild ED (n=51)	Moderate ED (n=44)	Severe ED (n=29)
Age	45.26 ± 6.96	45.78 ± 8.18	45.16 ± 6.74	45.34 ± 8.05
BMI	23.46 ± 2.88	23.62 ± 3.15	23.19 ± 2.27	23.81 ± 3.14
SBP(mmHg)	126.38 ± 12.39	127.12 ± 14.41	124.75 ± 11.36	128.83 ± 13.50
DBP(mmHg)	71.10 ± 7.50	71.92 ± 9.53	70.66 ± 6.44	72.07 ± 7.97
FBG(mmol/L)	4.96(4.66-5.12)	5.12(4.60-5.77)	5.19(4.47-5.67)	5.15(4.83-6.08)
Cr(umol/L)	74.56 ± 12.81	74.00 ± 11.65	75.23 ± 12.86	74.69 ± 10.30
UA(umol/L)	329.23 ± 59.57	320.43 ± 86.28	333.25 ± 71.66	317.52 ± 65.94
TG (mmol/L)	1.46(1.23-1.71)	1.58(1.29-1.89)	1.62(1.43-1.89)	1.68(1.26-2.55)
TC(mmol/L)	4.40 ± 0.58	4.37 ± 0.79	4.41 ± 0.72	4.39 ± 0.71
HDL-C(mmol/L)	1.19(1.04-1.32)	1.08(0.96-1.24)	1.16(0.99-1.27)	1.07(0.93-1.23)
LDL-C(mmol/L)	2.94(2.52-3.23)	2.97(2.39-3.33)	3.07(2.69-3.33)	3.05(2.66-3.56)
25(OH)D (ng/ml)	25.81 ± 5.18	24.18 ± 6.50	20.75 ± 6.27[ab]	18.64 ± 5.13[ab]
CIMT(mm)	0.70(0.60-0.80)	0.75(0.70-0.85)	0.80(0.70-0.90)[a]	0.90(0.78-1.03)[ab]
Smoking(%)	15(38.5%)	19(37.3%)	17(38.6%)	11(37.9%)
Drinking(%)	10(25.6%)	14(27.5%)	12(27.3%)	7(24.1%)

"a" is the comparison with the contorl group,P<0.05; "b" is the comparison with the mild ED group,P<0.05.

TABLE 2 Correlation analysis between IIEF-5 score and different indicators.

Variable	R	P
CIMT(mm)	-0.412	0.000
25(OH)D (ng/ml)	0.430	0.000
Age	0.019	0.814
BMI	-0.009	0.907
SBP(mmHg)	-0.015	0.846
DBP(mmHg)	0.010	0.899
FBG(mmol/L)	-0.080	0.310
Cr(umol/L)	0.005	0.951
UA(umol/L)	0.007	0.927
TG(mmol/L)	-.0199	0.011
TC(mmol/L)	0.010	0.897
HDL-C(mmol/L)	0.134	0.089
LDL-C(mmol/L)	-0.093	0.236

Vitamin D and erectile dysfunction

According to the World Health Organization (WHO), when 25(OH)D concentrations are above 25ng/ml, it is considered normal. Vitamin D deficiency is defined as 25 (OH)D concentrations below 20 ng/ml and vitamin D insufficiency as 25(OH)D concentrations between 20 and 25 ng/ml. Vitamin D deficiency to public health is indisputable. In this study, we discovered that participants with moderate and severe ED had lower levels of 25(OH)D, respectively, while 25 (OH)D level was basically normal in participants with mild ED, implying that vitamin D deficiency may exist in ED patients, especially in patients with severe ED. Moreover, the 25(OH)D level and IIEF-5 score had a positive connection. Vitamin D deficiency and vitamin D insufficiency were currently considered to exist worldwide (12). Both Caretta's (13) and Farag's (14) studies found that the serum 25(OH)D level in ED patients was relatively low. According to Culha (15), there is a positive correlation between 25(OH)D levels and erectile function, which is consistent with our findings. However, in a meta-analysis (16), it was found that 25(OH)D levels did not show any significant difference between patients with and without ED. Similarly, the relationship between vitamin D levels and ED risk was not strongly confirmed in another meta-analysis (17). These conflicting results suggest that further well-designed studies with larger sample size included and outcome measures are needed in the future. Kim (8)

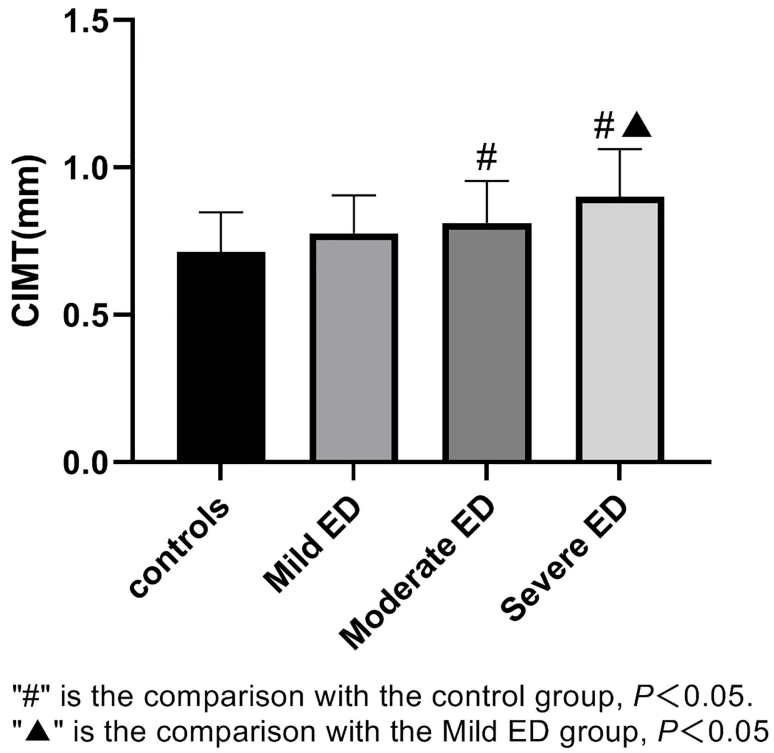

FIGURE 2
CIMT values in healthy controls, mild ED, moderate ED, Severe ED. "#" is the comparison with the control group, P<0.05. "▲" is the comparison with the Mild ED group, P<0.05.

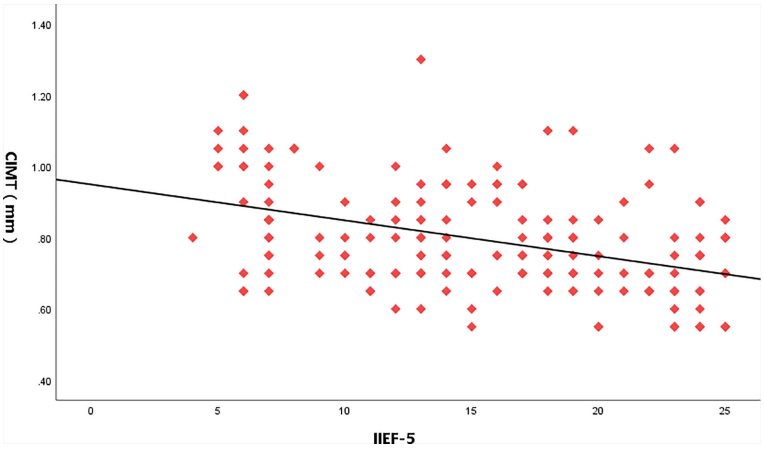

FIGURE 3
Relationship between IIEF-5score and CIMT values.

reported that vitamin D was closely related to endothelial cell function, and mainly acted on endothelial cells in two main ways. First, vitamin D may control nitric oxide synthase (eNOS) expression and activity in endothelial cells, which could have an impact on how much NO is produced and released by endothelial cells; Second, vitamin D may mitigate damage of oxidative stress to endothelial cell function by regulating the antioxidant capacity of endothelial cells. The hypothesis of systemic endothelial dysfunction in patients with ED has been tested in several human studies, among which ED is the first clinical manifestation (17). Therefore, we speculated that vitamin D may affect the relaxation of vascular smooth muscle in cavernous tissue by regulating endothelial function, thereby affecting the erectile function eventually.

Carotid artery intima-media thickness and erectile dysfunction

Endothelial and vascular smooth muscle cell dysfunction in the cavernous tissue is two of the primary causes of ED. When the function of vascular endothelial cells is impaired, the release of NO from endothelial cells is reduced (8), resulting in smooth muscle relaxation disorder and erectile dysfunction. At the same time, after the destruction of vascular endothelium, the lipid deposition between vascular walls increases, and after a series of reactions such as the impaired plasma coagulation and fibrinolysis mechanism, the increasing of growth factors, chemokines and so on, eventually leading to atherosclerosis (18). Thickening of CIMT, a well-known

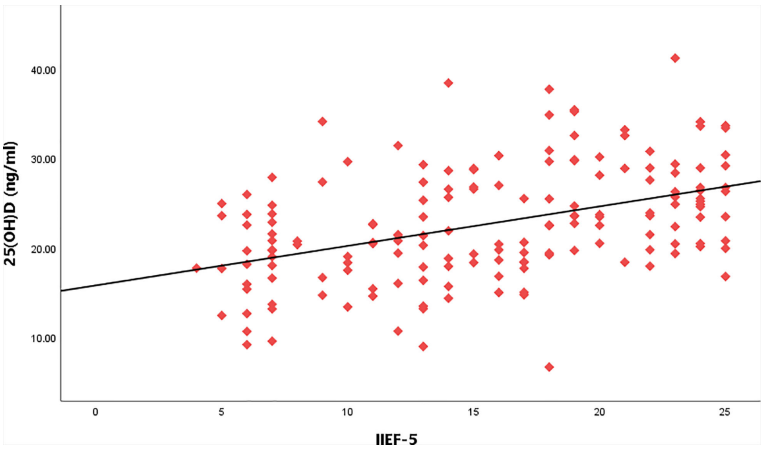

FIGURE 4
Relationship between IIEF-5score and serum 25(OH)D level.

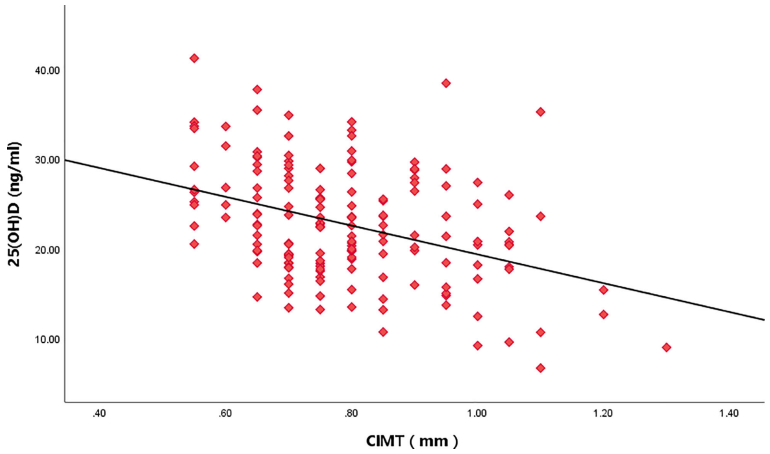

FIGURE 5
Relationship between serum 25(OH)D level and CIMT.

marker of atherosclerosis, occurs almost exclusively in arterial vessels (19), it is also an early change in atherosclerosis. Our study found that CIMT values in patients with ED were higher than those in control group, and we speculated that men with thickened CIMT may be at greater risk for ED. Mulla (20) reported in his study that CIMT was negatively correlated with IIEF-5 score, in other words, the thickness of CIMT in patients with ED was significantly thicker than that of healthy men, which was consistent with the results of our study. We suspected that the vessels in penile cavernous tissue of ED patients may also have lesions along with the vascular system of the whole body. Furthermore, CIMT can be used to predict the severity of ED and how well patients with vasculogenic ED respond to phosphodiesterases, according to additional research that has supported these findings (21). According to the findings, patients with elevated CIMT responded to tadalafil less favorably than those with normal CIMT. Tadalafil's poor efficacy in patients with elevated CIMT (>0.67 mm) is most likely caused by endothelial dysfunction and structural abnormalities in artery walls that block the nitrite oxide route. Therefore, in ED patients with thickened CIMT, physicians should be warned that they may not respond well to phosphodiesterase 5 inhibitor(PDE5-I), and other treatment options such as penile sponge injection should be considered in these patients.

Vitamin D and carotid artery intima-media thickness

In this study, comparing the moderate and severe ED groups to the control and mild ED groups, we discovered statistically significant drops in serum 25(OH)D levels, and we discovered a notably greater level of CIMT in the groups with moderate and severe ED. Serum 25(OH)D levels appeared to be inversely linked with CIMT. Similarly, Van (22) demonstrated that increased CIMT and low vitamin D levels were related. In 2017, A meta-analysis of 21 studies (23) revealed that patients with vitamin D insufficiency also had significantly greater CIMT and carotid plaque prevalence. A meta-analysis of an additional 11 trials (18) revealed that serum vitamin D levels were a preventative measure against carotid plaque. Uncertainty exists regarding the processes underlying the link between serum vitamin D and CIMT. However, researches (24, 25) has shown that a lack of vitamin D increases the risk of cardiovascular disease and stiffens the arteries. This might be because vitamin D helps to activate the renin-angiotensin system.

TABLE 3 Correlation analysis between IIEF-5 score,serum 25(OH)D level and CIMT values.

Variable	B	Std.Error	β	t	95% CI	P
(constant)	17.983	3.387	–	3.776	0.558~1.418	0.000
25(OH)D(ng/ml)	0.309	0.071	0.319	4.325	0.168~0.450	0.000
CIMT(mm)	-11.889	3.018	-0.290	-3.939	-17.85~-5.928	0.000

Combined with these results, we speculated that there may be a potential correlation between serum vitamin D level, CIMT and erectile function. These results of this study preliminarily verified our conjectures that erectile function was positively correlated with serum 25(OH)D level, and negatively correlated with CIMT, while serum 25(OH)D level was negatively correlated with CIMT. At this point, we wondered if vitamin D might cause vascular disease and ED. In Kim's research, he reported that endothelial cells played a key role in regulating vascular homeostasis and hemodynamics, especially in vasodilation. Endothelial cells cause vascular smooth muscle relaxation mainly through production of eNOS and release of NO. The mechanism of penile erection happens to be the release of NO by non-adrenergic noncholinergic (NANC) nerve fibres. Following signaling pathways result in elevated cGMP levels, decreased intracellular Ca2+ levels, and relaxation of the smooth muscle cell (26, 27). Men finally have an erection as a result of the smooth muscle cells relaxing allowing blood to enter the lacunar gaps in the corpora cavernosa and compressing the unilateral inferior veins and restricting the vein's outflow. Therefore, vitamin D may affect erectile function through its interaction with endothelial cells of corpora cavernosa, which will guide the direction of our further research.

It is worth noting that men with a reduced IIEF-5 score alone cannot be diagnosed as vasculogenic ED, it is equally important that psychogenic ED and endocrine ED should be excluded. Most of the participants in this study were men who came to hospital for routine physical examinations. Because of various reasons, the majority of participants refused further examinations to exclude organic ED, which is also the deficiency of this study and will be improved in the follow-up studies of our research group. Secondly, the blood collection period in this study was autumn and winter, so, factors, such as sunshine, season, climate, region may affect the fluctuation of serum 25(OH)D levels, which is planned to be further improved in the subsequent series of studies.

Conclusion

In conclusion, this study found that erectile function was positively correlated with serum level of vitamin D, and negatively correlated with CIMT. The mechanism of vitamin D influencing on erectile function was still unknown, and whether CIMT can predict severity of erectile function, all these questions need to be further confirmed. Lower level of vitamin D may increase the risk of morbidity of ED by regulating endothelial function. Serum vitamin D level should be analyzed in men with ED, especially in patients with vasculogenic ED, and supplementation is recommended for those who were with vitamin D deficiency.

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 The Ethics Committee of the Affiliated Hospital of Guizhou Medical University.

Author contributions

J-HZ came up with the idea for the study, helped with its planning and data gathering, carried out the statistical analysis, and wrote the manuscript. The project was created by C-HH and K-FT, who also contributed with its planning, design, statistical analysis, and manuscript writing. Techniques were offered as support by C-YW and Z-DS. WL, A-NZ, B-ZJ, and Y-WL assisted gather the data and with the statistical analysis. 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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The role of microRNAs in erectile dysfunction: From pathogenesis to therapeutic potential

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Erectile dysfunction (ED) is a common male sexual dysfunction disease, and it was predicted that the number of ED patients worldwide will reach 322 million by 2025. However, the pathogenesis of ED is complex and the current treatment options are still limited, so it is urgent to explore new treatment strategies. Recent studies have shown that microRNAs (miRNAs) play an important role in ED, and these single-stranded non-coding small RNA molecules are involved in key pathophysiological processes in the occurrence and development of ED. Therefore, miRNAs have remarkable potential as therapeutic targets in ED. Here, this review introduces the physiological basis of erectile function and the pathophysiological changes in ED and summarizes the current knowledge on the expression, biological functions, and molecular mechanisms of miRNAs in ED, especially the potential of miRNA-targeted therapies to improve ED. This review will provide a comprehensive view of the role of miRNAs in the pathogenesis of ED and the potential value of miRNAs in the treatment of ED.

KEYWORDS

erectile dysfunction, microRNA, expression, pathogenesis, therapy

Introduction

Erectile dysfunction (ED) is a term established by the 1993 NIH consensus conference on impotence, which refers to the persistent or recurrent inability to achieve or maintain sufficient penile erection to complete a satisfactory sexual life (1). As a common male sexual dysfunction disorder, ED has been shown to have a severe negative impact on an individual's life, health, and well-being (2). Epidemiological investigation indicated that the prevalence of ED increases with age. It was reported that about 50% of men aged 40-70 years suffer from ED, while the prevalence of ED can rise to

70% in men over 70 years of age (3, 4).. In the past, ED was often considered to be mainly caused by psychological factors. However, current evidence showed that in addition to psychological factors, age, cardiovascular disease, diabetes, history of pelvic surgery, spinal cord injury, obesity, etc. were all crucial risk factors for ED (5–11). At present, the therapeutic methods of ED mainly included oral drugs, physical therapy, injection of active drugs in the cavernosal body, intraurethral alprostadil injection, and surgical treatment. Among them, oral phosphodiesterase type 5 inhibitor (PDE5i) is the most commonly used first-line treatment of ED in clinical practice (12). However, there are still a large number of ED patients with poor or no response to PDE5i. At the same time, the current therapeutic methods for ED cannot adequately meet the needs of the patients for sexual life (13). Therefore, the above problems prompt researchers to constantly explore and find novel methods for the treatment of ED.

MicroRNA (miRNA) is a single-stranded non-coding RNA molecule with a length of about 22 nucleotides (14, 15). Since lin-4 was reported as the first miRNA in 1993 (16), intensive research on miRNA has been conducted for more than two decades, which has led to a more detailed understanding of the epigenetic regulation process. In the nucleus, most miRNA genes are transcribed by RNA polymerase II (Pol II) to generate primary miRNAs (pri-miRNAs), which will be cleaved into precursor miRNA (pre-miRNA) by the Drosha, an endonuclease that binds to the double-stranded RNA-binding protein DGCR8/Pasha. Subsequently, pre-miRNAs are transported out of the nucleus and further cleaved into small double-stranded RNAs in the cytoplasm by Dicer, an endonuclease that binds to the double-stranded RNA-binding protein TRBP/Loquacious. Finally, one strand of the double-stranded RNA is loaded into the Argonaute protein to form the RNA-induced silencing complex (RISC), while the other strand is degraded (17). At present, miRNA has been verified to inhibit messenger RNA (mRNA) translation mainly by binding their complementary 3'-untranslated region (3' UTR), thereby regulating gene expression at the post-transcriptional level (18, 19) (Figure 1). A series of studies have shown that miRNAs are involved in the occurrence and progression of various diseases, such as cardiovascular disease (20), diabetes (21), neurological dysfunction (22), metabolic syndrome (23) et al. Notably, miRNAs also play an important role in male sexual dysfunction (24) and reproductive dysfunction (25), and may serve as biomarkers and novel therapeutic targets for these diseases.

In recent years, great progress has been made in the treatment of ED worldwide. In addition to the improvement of existing treatment methods, stem cell therapy and gene therapy were considered to be promising forms of ED treatments (26, 27). As one of the key molecules for post-transcriptional regulation, miRNAs have been shown to serve as targets for stem cell and gene therapy for the treatment of ED (24, 28–30). However, the development of these therapeutic approaches still relies on in-depth studies on the pathogenesis of ED, continuous exploration of the miRNA mechanisms, and the identification of proper vectors for intervening miRNAs.

In this regard, this review will summarize the pathophysiology of ED, as well as a variety of miRNAs involved in ED-related pathophysiological processes, and discuss the potential roles of these miRNAs as biomarkers in the diagnosis and treatment of ED.

The physiology of penile erection and the pathophysiology of ED

The physiology of penile erection

Normally, the contraction of the corpus cavernosum smooth muscle maintains the penis in a flaccid state. When sexual stimulation occurs, smooth muscle relaxation leads to local venous compression and blood reflux obstruction, resulting in erection (4). Therefore, the relaxation and contraction of corpus cavernosum smooth muscle are crucial for the regulation of penile erection. Current studies have indicated that multiple signaling pathways were involved in penile erection, among which the NO/cGMP pathway which mediates smooth muscle relaxation and the RhoA/ROCK pathway which mediates smooth muscle contraction were the most critical (12, 31, 32). In response to nitric oxide (NO), guanylate cyclase (GC) converts guanosine triphosphate (GTP) to cyclic guanylate phosphate (cGMP) and activates cGMP kinase in corpus cavernosum smooth muscle cells during its relaxation, which leads to the reduction of intracytoplasmic calcium ions, dephosphorylation of myosin light chain (MLC), and finally to smooth muscle relaxation and penile erection (33, 34) (Figure 2A). In contrast, in the process of smooth muscle contraction, under the action of substances such as norepinephrine and endothelin, the concentration of calcium ions in the cytoplasm of smooth muscle cells raises, and the GDP-binding Ras superfamily member RhoA is converted from RhoA-GDP to RhoA-GTP. Meanwhile, increased Ca^{2+} concentration also activates Rho-related protein kinase (ROCK), which expands the sensitivity of MLC to calcium ions, maintains MLC phosphorylation, and eventually promotes smooth muscle contraction and penile weakness (35–37) (Figure 2B).

The pathophysiology of ED

Penile erection is a hemodynamic process accomplished by the corpus cavernosum under the joint action and mutual coordination of neurological, endocrine, and psychological factors, and is also influenced by multiple factors such as

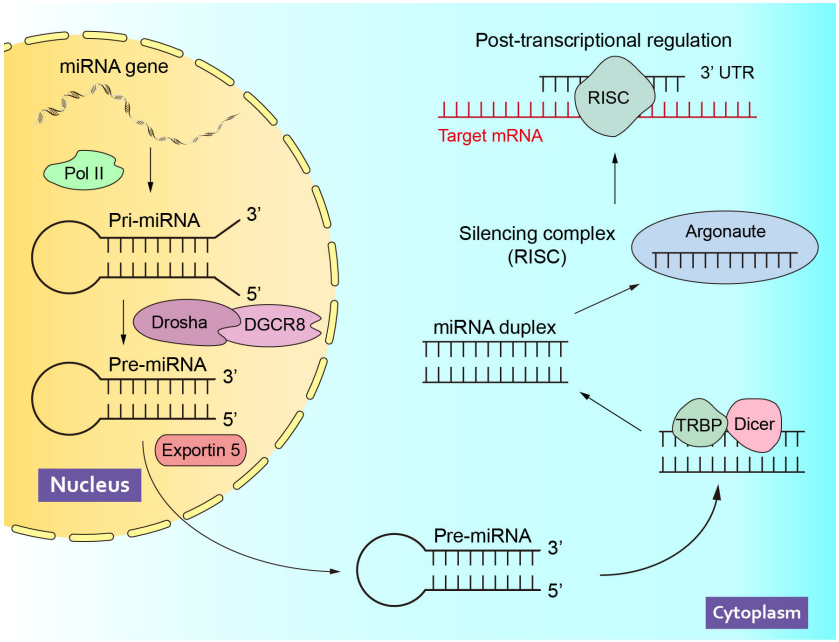

FIGURE 1
The Biogenesis and Function of miRNAs. Pol II, polymerase II; pri-miRNA, primary miRNAs; pre-miRNA, precursor miRNA; RISC, RNA-induced silencing complex; mRNA, messenger RNA; 3' UTR, 3'-untranslated region.

drugs, age, lifestyle habits, and systemic diseases (38). Abnormalities in one or more of these factors can lead to ED. ED can be divided into psychological ED, organic ED, or mixed psychological and organic ED, of which mixed ED is the most common (39).

Age is the most common risk factor in ED, the prevalence and the severity of ED increases with age, and age-related ED is one of the main types of ED being studied (40, 41). On the one hand, for men in the aging process, hypogonadism is one of the important reasons that may cause ED; on the other hand, the penis itself in the aging process of tissue and morphological changes also play a pivotal role in the occurrence of ED (41). Studies have shown that the aging of smooth muscle cells and endothelial cells in the penis can cause oxidative stress, which in turn causes an inflammatory reaction. Over time, the inflammation can cause cumulative damage to the penis, including disruption of vascular endothelial integrity and reduction in penile smooth muscle content (42, 43). It is also worth noting that most studies have demonstrated that aged-related ED has many commonalities in pathology with essential hypertension. Therefore, almost all risk factors that can cause primary hypertension can increase the incidence of ED, and likewise, ED can serve as an antecedent predictor of early cardiovascular events (44, 45).

Diabetes is the second most common risk factor for ED, with more than 50% of diabetics having ED as a complication and approximately 12% of patients with diabetes having ED as a first symptom (46, 47). As a complication of diabetes, the pathogenesis of diabetic ED is more complex and the extent of the disease is more severe (48). Studies have revealed that in the early stages of diabetes, vascular disorders and endothelial dysfunction occurred in the penis due to the effects of hyperglycemia, resulting in a decrease in NO production that would be produced by endothelial cells, as well as abnormal activation of the RhoA/ROCK pathway. These changes cause an imbalance in the diastolic and contractile functions of smooth muscle within the penis, causing diabetic ED. As the disease progresses, under the stimulation of long-term hyperglycemia and the accumulation of advanced glycation end products (AGEs), a large number of reactive oxygen species and pro-fibrotic factors were produced in the penile corpus cavernosum, which exacerbate smooth muscle apoptosis and eventually lead to structural changes such as fibrosis in the penis, thus further aggravating diabetes (49–52). In addition, diabetes caused by hypogonadism, neuropathy, and the patient's psychological burden was also closely related to the development of ED (53–55).

Neurogenic ED is also the more commonly studied type of ED currently. Neurological ED is often associated with a variety of neurological disorders, such as multiple sclerosis, Parkinson's disease, epilepsy, spinal cord injury, etc (56). Additionally, patients who have undergone radical pelvic surgery (e.g., radical cystectomy, radical prostatectomy) are at particularly high risk of neurogenic ED, mainly due to intraoperative damage

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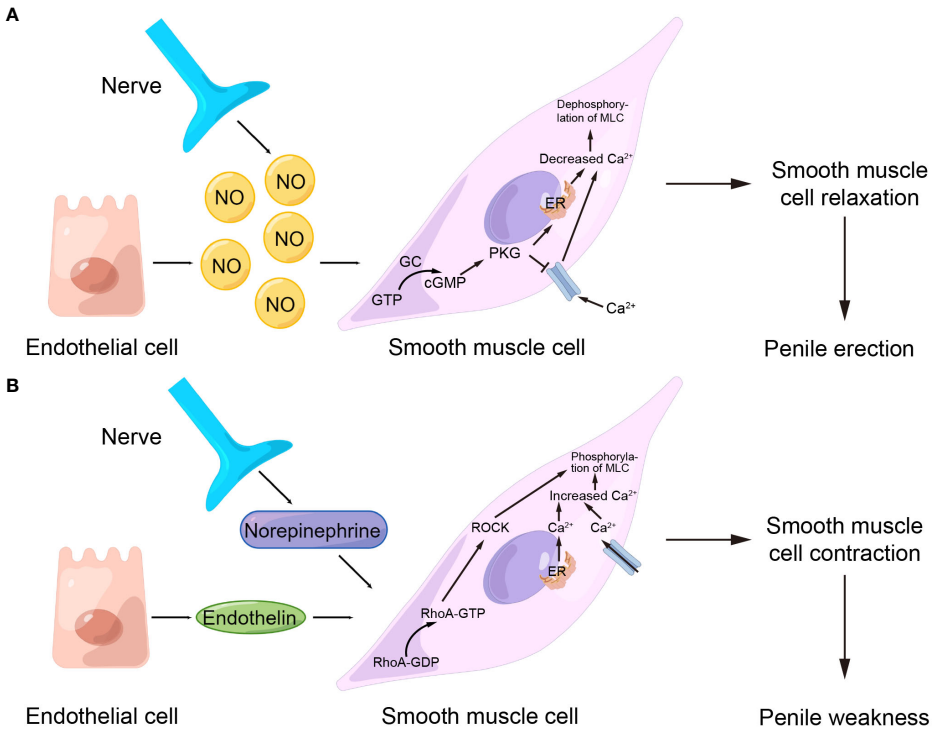

FIGURE 2
Important pathways that regulate penile contraction and relaxation. **(A)** NO/cGMP pathway. **(B)** RhoA/ROCK pathway. NO, nitric oxide; GTP, guanosine triphosphate; cGMP, cyclic guanylate phosphate; GC, guanylate cyclase; MLC, myosin light chain; ER, endoplasmic reticulum; ROCK, Rho-related protein kinase; GDP, guanosine diphosphate.

to the cavernous nerves (57). Most studies have shown that in neurogenic ED, there was substantial apoptosis of smooth muscle and vascular endothelial cells in the penile corpus cavernosum with excessive collagen deposition, causing penile fibrosis (58–60).

As with neurogenic ED, research on obesity-related ED has increased in recent years. Because as known, obesity is a major global public health problem, and obesity is also associated with a high prevalence of ED. Studies have suggested that the main pathophysiological processes in obesity-related ED include oxidative stress (61, 62), inflammatory response (63, 64), and the resulting insulin (65, 66) and leptin resistance (67).

Epidemiological studies have revealed the common prevalence of psychological factors in the etiology of erectile dysfunction (4). In particular, depressive symptoms, pessimistic attitude, emotional stress, anxiety, etc. were closely related to psychogenic ED (68). Currently, it is believed that these negative emotional states may affect male sexual arousal through cognitive factors. Functional magnetic resonance imaging (fMRI) indicated that the medial preoptic area, amygdala, basal ganglia-thalamic-cortical circuit, and salience network were involved in chronic mild stress-mediated sexual arousal and erectile dysfunction in rats (69). In addition, dopamine D2 receptors in the basolateral amygdala and nucleus accumbens modulate erectile function in a rat model of chronic mild stress (70, 71). At the spinal level, acute severe stress significantly affected the spinal gastrin-releasing peptide (GRP) system, reducing GRP expression and androgen receptor (AR) response to circulating testosterone, resulting in suppressing the penile reflex (72). The dysfunction of the dopaminergic system is also an important cause of ED in depression model rats. The expression of dopamine receptor D2 and transporter solute carrier family 6 member 3 (SLC6A3) in the penis was decreased, and the catechol-O-methyltransferase (COMT) was increased in the depression model rats (73). Meanwhile, negative emotions led to the overactivation of adrenergic fibres, which release norepinephrine to act on α-receptors of the smooth muscle of the corpus cavernosum, activated RhoA/ROCK pathway to cause smooth muscle contraction, and inhibited penile erection (4).

Mixed ED is the most common etiological pattern in patients in clinical practice. In reality, there is a bidirectional relationship between organic ED and emotional disturbances, that is, organic ED itself can cause or aggravate depressive emotions, while moderate or severe depression can cause organic ED, which leads to the complexity of the

pathophysiological process of mixed ED (74). In recent years, it has been found that the expression of endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS) in the cavernosum of the chronic mild stress rat model and the levels of TNF-α, IL-1, and IL-6 in the corpus cavernosum of rats were increased, accompanied with decreased testosterone (75). Anyhow, compared with organic ED, the pathophysiology of psychogenic ED and mixed ED is still insufficient, and a large number of basic studies are needed to clarify the pathogenesis.

miRNA expression in ED

This review provides a comprehensive overview of studies with the keywords ("microRNA" or "miRNA") and ("erectile dysfunction") published in the past 11 years (January 2011 to August 2022) using the Web of Science database in English. Fifty-six articles were identified based on the above search strategy. After excluding reviews and the articles not relevant to this review, a total of 44 articles were included in the subsequent review. Compared to other diseases, miRNA research in ED started relatively late, but the number of studies is increasing annually, indicating that miRNAs and their role in the development of ED are receiving more and more attention.

Most studies have now demonstrated that miRNA expression is abnormally up-or down-regulated in various diseases compared to normal conditions. Since a single miRNA can target hundreds of mRNAs, such altered miRNA expression is an influential factor affecting both disease initiation and progression (76, 77). With the development of bioinformatics and sequencing technologies, more and more aberrantly expressed miRNAs have been identified in various types of ED. Our review summarized these aberrantly expressed miRNAs in Table 1, which contained miRNAs that were up- and down-regulated in different ED types. These miRNAs have been shown in several studies to be involved in the development of ED with altered expression and may also serve as diagnostic markers for ED. Table 2 summarizes the roles of some miRNAs in different ED.

As shown in Table 1, in aged-related ED, Pan et al. identified four upregulated miRNAs (miR-200a, miR-1, miR-203, and miR-206) by microarray analysis of miRNA expression and validation by quantitative real-time polymerase chain reaction (qRT-PCR) techniques on penile tissue from 18-month-old ED rats and 3-month-old young rats (78, 84). All of the abnormally expressed miRNAs were associated with endothelial dysfunction and were essential factors affecting erectile function in aged rats.

In diabetic ED, related studies from multiple research groups validated a total of six up-regulated miRNAs and five down-regulated miRNAs. Specifically, among the upregulated miRNAs, Jiang et al. found upregulated expression of miR-93, miR-320, and miR-16 in the serum of patients suffering from diabetic ED by qRT-PCR, suggesting that these miRNAs might be useful for the early diagnosis of diabetic ED (87). Using a rat model of diabetic ED, Wen et al. demonstrated that miR-205 expression was upregulated in penile tissue by qRT-PCR and could inhibit the androgen receptor (AR) (90). In addition, Pan et al. screened 21 differentially expressed miRNAs (fold change ≥ 3) in penile tissues of diabetic rats by GeneChip array techniques (Affymetrix miRNA 4.0 Array) and applied qRT-PCR to confirm that miR-18a and miR-206 were upregulated in penile tissues and inhibited insulin-like growth factor (IGF-1), while miR-122 and miR-133 were downregulated in penile tissues of diabetic ED rats (79). Besides, among other down-regulated miRNAs, Wen et al. verified that miR-141 was down-regulated in penile tissues of rats with diabetic ED by qRT-PCR and demonstrated that miR-141 had a protective effect on the smooth muscle in the corpus cavernosum (88). Also, miR-141 was proved to have a suppressive effect on the RhoA/ROCK pathway and thus decreased miR-141 expression was associated with the development of diabetic ED (91). Huo et al. comfirmed that miR-874-3p and miR-21-5p expression were downregulated in diabetic ED rats by qRT-PCR and correlated with apoptosis of smooth muscle cells (85, 86). Currently, Kang et al. identified that miR-6321 and miR-122-5p were up-regulated, while miR-1298 was down-regulated in diabetic ED rats with miRNA sequencing (80).

In neurogenic ED, Liu et al. identified 124 aberrantly expressed miRNAs using RNA-seq in a rat model of bilateral cavernous nerve injury and subsequently verified four of them, miR-101a, miR-138, miR-338, and miR-142, as up-regulated miRNAs by qRT-PCR (81). Zheng et al. also reported the upregulation of miR-33 using the same model and further investigated that the upregulation of miR-33 suppressed neurotrophic factors involved in the ED recovery process (89).

In obesity-related ED, Barbery et al. applied the quantifiable miRNA profiling technique (NanoString) to the penile corpus cavernosum of mice on a high-fat diet (HFD) and identified 5 up-regulated miRNAs and 65 down-regulated miRNAs (fold change ≥ 2). However, in the qRT-PCR validation, they found only miR-1937c and miR-151-5p significantly upregulated in HFD mice, whereas the other three (miR-720, miR-1937a, miR-205) identified by microarray trended in the correct direction. Among the down-regulated miRNAs, they only verified the statistically different expression of miR-153 and miR-425 by qRT-PCR, and a large number of down-regulated miRNAs screened by microarray were not verified (82). Notably, as described above, miR-205 has also been reported to be upregulated in diabetic ED (90). Bai et al. examined the miRNA expression profile of obese rat spongy tissue by miRNA microarray analysis and found 68 differentially expressed miRNAs (fold change ≥ 1.5), and applied PCR to verify that miR-328a were upregulated in obese rats (83).

TABLE 1 miRNAs were screened by microarray analysis or sequencing.

Author (Year, reference)	ED type	Specimen	Upregulated miRNA	Downregulated miRNA	Screening threshold
Pan et al. (78)	Age-related ED	Penis (rat)	miR-1, miR-29b, miR-183, miR-199a, miR-200a, miR-200b, miR-200c, miR-203, miR-205, miR-206	miR-98, miR-125a, miR-127, miR-133a, miR-148, miR-196b, miR-322, miR-324, miR-328, miR-351, miR-379, miR-434, miR-494, miR-532, miR-541, miR-674	Fold change > 2
Pan et al. (79)	Diabetic ED	Penis (mouse)	miR-18a, miR-190a, miR-206, miR-210, miR-215, miR-290b, miR-542	miR-10a, miR-122, miR-133a, miR-224, miR-299b, miR-301a, miR-338, miR-379, miR-410, miR-451b, miR-466, miR-485, miR-96, miR-99a	Fold change ≥ 3
Kang et al. (80)	Diabetic ED	Penis (rat)	miR-1298, miR-337-3p, miR-134-5p, miR-503-5p, miR-148a-5p	miR-202-5p, miR-122-5p, miR-1b, miR-204-5p, miR-145-3p, miR-6321, miR-743a-3p, miR-463-5p, miR-211-5p, miR-741-3p, miR-881-3p, miR-743b-5p, miR-871-5p, miR-743b-3p, miR-471-5p	Fold change > 2
Liu et al. (81)	Neurogenic ED	Penis (rat)	miR-451, miR-144, miR-3065, miR-338, miR-872, miR-138-2, miR-142, miR-324, miR-425, miR-181b-1, let-7i, miR-103-1, miR-93, miR-101a, miR-101b, miR-21, miR-674, miR-10a, miR-139, miR-138-1, miR-29b-2	–	Fold change > 2
Barbery et al. (82)	Obesity-related ED	Penis (mouse)	miR-151-5p, miR-1937c, miR-720, miR-1937a, miR-205	miR-550, miR425, miR-134, miR-153, and miR-26b and 60 other miRNAs (no detail mentioned)	Fold change > 2
Bai et al. (83)	Obesity-related ED	Penis (rat)	miR-200a-3p, miR-370-3p, miR-26b-5p, miR-215, miR-134-5p, miR-10b-5p, miR-142-3p, miR-96-5p, miR-541-5p, miR-92a-3p, miR-150-5p, miR-206-3p, miR-210-3p, miR-340-3p, miR-218a-5p, miR-376b-3p, miR-342-3p, miR-203a-3p, miR-194-5p, miR-22-5p, miR-23a-3p, miR-101b-3p, miR-298-5p, miR-33-5p, miR-301a-3p, miR-450a-5p, miR-540-3p, miR-128-3p, miR-137-3p, miR-183-5p, miR-1-5p, miR-328a-3p, miR-100-5p, miR-25-3p, miR-122-5p, miR-103-3p, miR-323-3p, miR-30a-5p, let-7d-3p, miR-365-3p, miR-19a-3p, miR-539-5p, miR-297, miR-487b-3p, miR-32-5p, miR-292-3p, miR-349, miR-30a-3p	miR-127-3p, miR-223-3p, miR-409a-5p, miR-142-5p, miR-124-3p, miR-99b-5p, miR-207, miR-382-5p, miR-24-1-5p, miR-34b-5p, let-7b-5p, miR-361-5p, miR-341, miR-483-3p, miR-18a-5p, let-7a-5p, miR-140-3p, miR-489-3p, miR-499-5p, miR-187-3p	Fold change≥1.5

ED, erectile dysfunction.

Biological functions and molecular mechanisms of miRNA in ED

Apoptosis

Apoptosis is an important biological process in the development of ED. Under various stimuli such as high glucose, reactive oxygen species, and inflammation, smooth muscle cells or endothelial cells in the penile corpus cavernosum undergo apoptosis, which affects erection (92, 93). Multiple studies have now suggested that miRNAs play a bidirectional role in the modulation of apoptosis in ED. On the one hand, miRNAs can promote apoptosis in some pathological situations. For example, Wen et al. demonstrated that miR-205 can inhibit the androgen receptor, thus causing smooth muscle apoptosis in the penile corpus cavernosum of diabetic ED rats (90). Another example is that Liu et al. predicted that four miRNAs, miR-101a, miR-138, miR-338, and miR-142, might be involved in apoptosis in neurogenic ED by gene ontology analysis and the Kyoto Encyclopedia of Genes and Genomes pathway analysis of the target genes (81). Among them, miR-101a and miR-338 can directly regulate genes related to vascular smooth muscle contraction, while miR-142 and miR-138 can regulate apoptosis through calcium signaling pathway. Recently, Zhou et al. applied comprehensive transcriptome analysis to identify that miR-484-x and miR-653-5p were regulated by lncRNA ENSRNOT00000029245 to promote apoptosis of cavernosal smooth muscle, endothelial cells and ED in geriatric rats (94). On the other hand, miRNAs can also inhibit apoptosis by targeting the expression of specific genes to inhibit apoptosis, thus providing an ameliorative effect on ED. For instance, the Nupr1/Chop pathway is an endoplasmic reticulum stress pathway that mediates apoptosis. Huo et al. found that miR-874-3p can inhibit Nupr1 and thereby downregulate the Nupr1/Chop pathway in diabetic ED, exerting an anti-apoptotic effect (85, 95). NGFRAP1 is a nerve growth factor receptor-associated protein that interacts with the neurotrophic factor receptor p75 and mediates apoptosis. Wen et al. found that miR-141 inhibited NGFRAP1 in diabetic ED, resulting in reduced apoptosis (88). PDCD4 has various functions such as cell growth inhibition and apoptosis induction and

TABLE 2 The expression and function of differentially expressed miRNAs in various EDs.

ED Types	miRNA	Specimen	Differential Expression	Identification method	Function	Reference
Age-related ED	miR–200a	penis (rat)	up	Microarray qRT-PCR	attenuate endothelial function via SIRT1 inhibition	(78, 84)
	miR-1	penis (rat)	up	Microarray qRT-PCR	contribute to apoptosis, fibrosis, and endothelium dysfunction (bioinformatic analysis)	(78)
	miR-203	penis (rat)	up	Microarray qRT-PCR	contribute to apoptosis, fibrosis, and endothelium dysfunction (bioinformatic analysis)	(78)
	miR-206	penis (rat)	up	Microarray qRT-PCR	contribute to apoptosis, fibrosis, and endothelium dysfunction (bioinformatic analysis)	(78)
Diabetic ED	miR-874-3p	penis (rat)	down	qRT-PCR	binds to Nupr1 and inhibits Nupr1/Chop-mediated pathway	(85)
	miR-205	penis (rat)	up	qRT-PCR	contribute to the pathogenesis of diabetic ED via down-regulation of androgen receptor expressions	(73)
	miR-21-5p	penis (rat)	down	qRT-PCR	suppressed PDCD4 expression and ED in rats with DM	(86)
	miR-93	blood (human)	up	qRT-PCR	prospective markers	(87)
	miR-320	blood (human)	up	qRT-PCR	prospective markers	(87)
	miR-16	blood (human)	up	qRT-PCR	prospective markers	(87)
	miR-141	penis (rat)	down	qRT-PCR	miR-141 binds to NGFRAP1 and restores the erectile function via downregulation of NGF/p75NTR signaling	(88)
	miR-18a	penis (mouse)	up	Microarray qRT-PCR	contribute to apoptosis, fibrosis, eNOS/cGMP/PKG, and vascular smooth muscle contraction processes (bioinformatic analysis)	(79)
	miR-206	penis (mouse)	up	Microarray qRT-PCR	contribute to apoptosis, fibrosis, eNOS/cGMP/PKG, and vascular smooth muscle contraction processes (bioinformatic analysis)	(79)
	miR-122	penis (mouse)	down	Microarray qRT-PCR	contribute to apoptosis, fibrosis, eNOS/cGMP/PKG, and vascular smooth muscle contraction processes (bioinformatic analysis)	(79)
	miR-133	penis (mouse)	down	Microarray qRT-PCR	contribute to apoptosis, fibrosis, eNOS/cGMP/PKG, and vascular smooth muscle contraction processes (bioinformatic analysis)	(79)
	miR-6321	penis (rat)	up	miRNA Sequencing	contribute to cellular response to growth factor stimulus angiogenesis, positive regulation of apoptotic process (bioinformatic analysis)	(80)
	miR-122-5p	penis (rat)	up	miRNA Sequencing	contribute to cellular response to growth factor stimulus angiogenesis, positive regulation of apoptotic process (bioinformatic analysis)	(80)
	miR-1298	penis (rat)	down	miRNA Sequencing	miR-1298/B4GalT1 axis might exert function in stem cell therapy for ED	(80)
Neurogenic ED	miR-33	penis (rat)	up	qRT-PCR	preserved the erectile function of BCNI rats through regulating miR-33/GDNF axis	(89)
	miR-101a	penis (rat)	up	miRNA Sequencing qRT-PCR	regulate the processes of cell proliferation, differentiation, apoptosis, inflammation, and fibrosis (bioinformatic analysis)	(81)
	miR-138	penis (rat)	up	miRNA Sequencing qRT-PCR	regulate the processes of cell proliferation, differentiation, apoptosis, inflammation, and fibrosis (bioinformatic analysis)	(81)
	miR-338	penis (rat)	up	miRNA Sequencing qRT-PCR	regulate the processes of cell proliferation, differentiation, apoptosis, inflammation, and fibrosis (bioinformatic analysis)	(81)
	miR-142	penis (rat)	up	miRNA Sequencing qRT-PCR	regulate the processes of cell proliferation, differentiation, apoptosis, inflammation, and fibrosis (bioinformatic analysis)	(81)
Obesity-related ED	miR-720	penis (mouse)	up	Microarray	no validation	(82)

(Continued)

TABLE 2 Continued

ED Types	miRNA	Specimen	Differential Expression	Identification method	Function	Reference
	miR-1937a	penis (mouse)	up	Microarray	no validation	(82)
	miR-1937c	penis (mouse)	up	Microarray qRT-PCR	no validation	(82)
	miR-205	penis (mouse)	up	Microarray	no validation	(82)
	miR-151-5p	penis (mouse)	up	Microarray qRT-PCR	no validation	(82)
	miR-550	penis (mouse)	down	Microarray	no validation	(82)
	miR-425	penis (mouse)	down	Microarray qRT-PCR	no validation	(82)
	miR-134	penis (mouse)	down	Microarray	no validation	(82)
	miR-153	penis (mouse)	down	Microarray qRT-PCR	no validation	(82)
	miR-26b	penis (mouse)	down	Microarray	no validation	(82)
	miR-328a	penis (rat)	up	Microarray qRT-PCR	decrease the signaling mediator HMOX1/HO-1 of erectile function expression	(83)

ED, erectile dysfunction; qRT-PCR, quantitative real-time polymerase chain reaction.

is an essential regulator mediating apoptosis in vascular smooth muscle cells (96). Huo et al. found that miR-21-5p could inhibit the expression of PDCD4 in diabetic ED rats and reduce smooth muscle apoptosis (86).

Fibrosis

In ED, fibrosis is an important pathological change in the penile corpus cavernosum, which is mainly characterized by the massive proliferation of pro-fibrotic factors and deposition of collagen (97). It has been reported that miR-101a, miR-138, miR-338 and miR-142 may be involved in ED fibrosis through TGF-β signaling pathway and Wnt signaling pathway (81). In contrast, miR-145, miR-let7b, and miR-let7c were validated to play an anti-fibrotic role in ED (98, 99). miR-145 has been associated with the TGF-β receptor subsequent pathways. As an important pro-fibrotic cytokine, TGF-β has been described in detail in numerous studies as a major factor involved in penile corpus cavernosum fibrosis, and which induces fibrosis through both SMAD and non-SMAD signaling pathways (100). Liu et al. revealed that miR-145 inhibits the TGF-β receptor, which in turn reduced collagen deposition by inhibiting the SMAD2 signaling pathway (99). In another study, Zhu et al. found that miR-let7b and miR-let7c in exosomes derived from adipose stem cells could play an anti-apoptotic role in diabetic ED and the exosomes encapsulating miR-let7b and miR-let7c were critical for reducing collagen deposition in penile tissue (98).

Angiogenesis

In recent years, there has been a new understanding of the role of the vascular endothelium in erection and the impact of vascular dysfunction on ED. Damage to the vascular endothelium can lead to vascular dysfunction, which in turn can cause ED. In contrast, the use of vascular growth factors to promote revascularization can effectively improve ED (101, 102). RNA sequencing data from Ouyang et al. showed that miR-21-5p, let-7 family, miR-10 family, miR-30 family, and miR-148a-3p from human urinary-derived stem cell exosomes can promote angiogenesis in diabetic ED (103). Meanwhile, miR-126, miR-130a, and miR-132 from adipose stem cell exosomes were also revealed to promote angiogenesis in diabetic ED (98). In a recent study, Zou et al. also investigated the pro-angiogenic effects of miR-126 in the ED, showing that miR-126 regulated a variety of transcription factors that regulate cell growth, such as IRS1 and KLF10, thereby promoting angiogenesis and improving erectile function (104). Alternatively, vascular endothelial growth factor (VEGF) is a well-known pro-angiogenic substance that stimulates the growth and proliferation of endothelial cells and has an important regulatory role in angiogenesis (105). Wang et al. proposed in a rat model of neurogenic ED that miR-200a inhibits VEGF and is an essential target in the development of ED (106).

NO/cGMP pathway

The NO/cGMP pathway is currently one of the most well-studied and important pathways in ED. As described in the previous section on erectile physiology, normal levels of NO/

cGMP pathway-related molecules directly affect erectile function. Currently, a variety of miRNAs reported in relevant studies all negatively regulated the NO/cGMP pathway, including miR-328 (102, 107), miR-200a (78, 84), miR-1 (78), miR-203 (78), miR-206 (78, 79), miR-18a (79), miR-155 (108), and miR-146a (109). In particular, miR-328 has been shown to inhibit the NO/cGMP pathway by studies from different groups. Bai et al. suggested that miR-328 could reduce cGMP levels by inhibiting HO-1 in an obese ED rat model (83), while Li et al. found that the miRNA could directly target and inhibit eNOS by using a diabetic ED rat model (107). Pan et al. first found that miR-200a, miR-1, miR-203, and miR-206 could regulate the NO/cGMP pathway in senile ED by bioinformatics analysis (78), and then further demonstrated that miR-200a could inhibit SIRT1, which in turn affected eNOS and cGMP and reduced endothelial function (84). In another study by pan et al., they also elaborated on the possible regulatory role of miR-206 as well as miR-18a on the NO/cGMP pathway in diabetic ED (79). In addition, Rocha et al. showed that miR-155 inhibits eNOS, and that exercise, diet control, and atorvastatin treatment can reduce miR-155 expression, thereby improving endothelial dysfunction (108). Ding et al. found that miR-146a could directly target nNOS inhibition, while inhibition of miR-146a significantly increased the level of nNOS (109).

Other biological functions and molecular mechanisms

In addition, miRNAs also have regulatory effects on AGEs, neurotrophic factors, and other biological factors involved in the pathophysiology of ED. For example, studies have reported that inhibition of miR-328 reduces AGEs in diabetic ED (107), while miR-33 inhibits GDNF, a neurotrophic factor involved in the neurological ED response process (89). However, at present, the number of reports on the regulatory effects of miRNA on these biological factors in ED is relatively less, which needs to be further confirmed by more studies in the future. We summarized the biological functions and molecular mechanisms of these above miRNAs in the ED in Figure 3.

Therapeutic potentials of miRNA in ED

miRNAs play an influential role in all stages of ED and thus can be used as potential targets for ED therapeutic interventions, which opens a new window for the development of ED therapeutics. Here, this review will discuss various miRNA-based therapeutic strategies for ED based on the available relevant literature.

First, a variety of miRNAs have been summarized and described in detail in the above review as having protective effects on erectile function and improving effects on ED. However, the expression of some of these miRNAs was usually down-regulated in ED, so targeted supplementation of these under-represented miRNAs may be an effective ED treatment option. Currently, the more studied miRNA supplementation methods in the ED mainly included the delivery of corresponding miRNAs *via* stem cells or exosomes (Figure 4A). Among them, the main idea of using stem cells to deliver miRNA is to first transfect viruses overexpressing specific miRNAs into stem cells and subsequently transplant the stem cells into the penile corpus cavernosum to increase the local content of specific miRNAs in the penis through paracrine secretion of stem cells. For example, Zou et al. transplanted miR-126 over-expressing lentivirus muscle stem cells into the penile corpus cavernosum of ED rats and found that the erectile function of the rats was significantly improved after transplantation (104). Alternatively, an increasing number of studies in recent years have shown that increasing miRNA content through direct injection of stem cell-secreted exosomes can also be effective in improving ED (86, 98, 103, 110). As non-viral and non-cellular substances, exosomes have the advantages of high safety and low immunogenicity, and can directly deliver the carried miRNAs into cells. Therefore, exosomes may be the main carriers for delivering miRNAs in the future (111, 112).

Secondly, miRNA mimic or miRNA agomir is also a common current regulatory tool to target insufficient levels of specific miRNAs (Figure 4B). miRNA mimic is a chemically synthesized double-stranded RNA molecule that mimics the function of an endogenous miRNA, which can mimic miRNAs that are downregulated due to disease (113). Wen et al. demonstrated that miR-141 mimic can effectively compensate for the deficiency of miR-141 in diabetic ED rats, increase the pressure in the penile corpus cavernosum of rats and improve erectile function (88). miRNA agomir is a special chemical modified miRNA agonist that acts similarly to the miRNA mimic. Experimental animal studies by Huo et al. have demonstrated that supplementation with miR-874-3p agomir can effectively improve ED (85). In contrast to miRNA mimic or miRNA agomir, miRNA inhibitor or miRNA antagomir can selectively inhibit miRNA (114, 115). Currently, both miRNA inhibitor and miRNA antagomir have been applied in ED therapeutic studies to reduce miRNAs that are abnormally highly expressed in the ED to delay or prevent the progression of ED. Wen et al. reported that the miR-205 inhibitor reduced the inhibitory effect of miR-205 on androgen receptors in diabetic ED rats to improve erectile function (90). Li et al. used miR-328 antagomir to antagonize the inhibitory effect of miR-328 on eNOS in diabetic ED rats, thereby increasing the content of cGMP in rat penile tissue (107).

In addition, there are biomolecules or related drugs that target miRNAs that have the potential to be novel approaches for the treatment of ED. Recent studies have shown that long non-

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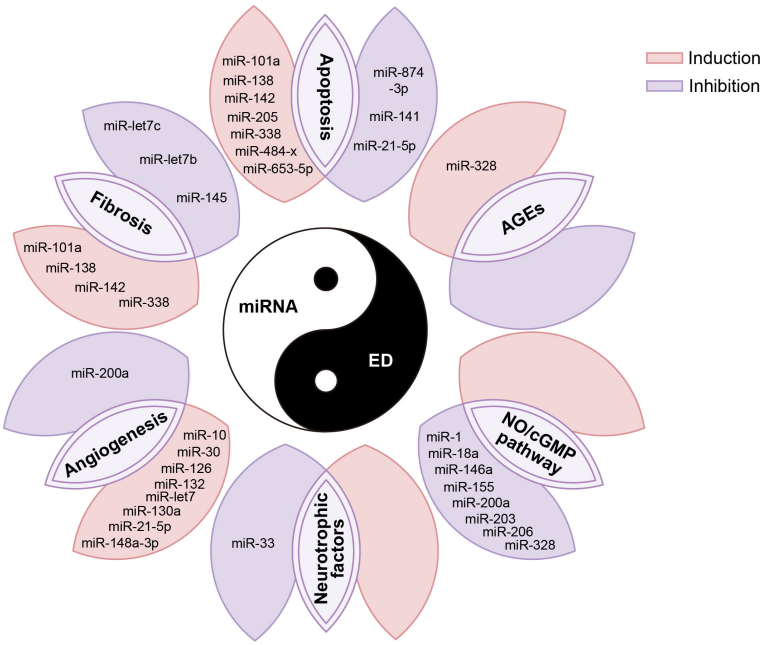

FIGURE 3
miRNAs are involved in various pathophysiological processes in the occurrence and development of ED. AGE, advanced glycation end products; NO, nitric oxide; cGMP, cyclic guanylate phosphate.

coding RNAs can act as competitive endogenous RNA that binds to miRNA interactions and acts as an inhibitor of miRNA (116, 117). Wang et al. used lncRNA-MIAT, which reduced the ability of miR-200a to interfere with mRNA-encoded proteins of target genes through its targeted competitive adsorption on miR-200a, thus promoting the differentiation of bone marrow stem cells to endothelial cells and acting as a therapeutic agent for ED (106). Icariin II is a flavonoid isolated from the traditional Chinese medicine Epimedium, which has been shown to have a therapeutic effect on ED (118). In recent studies, Icariin II has been shown to inhibit specific miRNAs in neurogenic ED, thereby promoting the differentiation of adipose stem cells to Schwann cells to improve neurogenic ED (89, 119).

Discussion

In recent years, studies on the involvement of miRNAs in disease development, diagnosis, and treatment were growing rapidly, and the understanding of the potential role of miRNAs in ED had increased significantly. miRNA aberrant expression was closely associated with ED, and miRNAs played an important role in key biological processes such as penile corpus cavernosum fibrosis, apoptosis, angiogenesis, and NO release, which highlighted its potential as a therapeutic target in ED. Increasing down-regulated miRNAs that promote erectile function and decreasing up-regulated miRNAs that inhibit erectile function through various approaches have great value and promise for the development of new ED therapeutic agents.

Despite the vigorous development of miRNA-related studies in ED, there are still some limitations in existing studies. We are delighted to see that we can screen out a large number of miRNAs by microarray and miRNA sequencing that may play an important role in the pathogenesis of ED. Some molecules such as miR-200a and miR-122 are functional in the ED by different research groups. However, the vast majority of molecules screened by microarray or sequencing were not validated by qRT-PCR, making them less credible. In addition, the fold change for identifying differentially expressed miRNAs varied between studies, making it difficult to compare these studies. Sequencing studies of human blood samples to provide a comprehensive view of miRNAs in ED patients are still lacking. It should not be ignored that the proportion of psychogenic ED and mixed ED in clinical patients is gradually increasing, but so far, there is no relevant report on miRNA and non-organic ED.

In the therapeutic field, although a large number of animal experiments involving miRNAs for the treatment of ED have been conducted in recent years, none of the miRNAs have entered clinical trials so far. One of the biggest challenges in developing miRNA-based therapies is identifying the best miRNA targets for each ED type. However, existing basic research is sketchy in screening miRNA targets and lacks strong experimental validation. miRNA is a double-edged sword. On the one hand, it can act on multiple target genes,

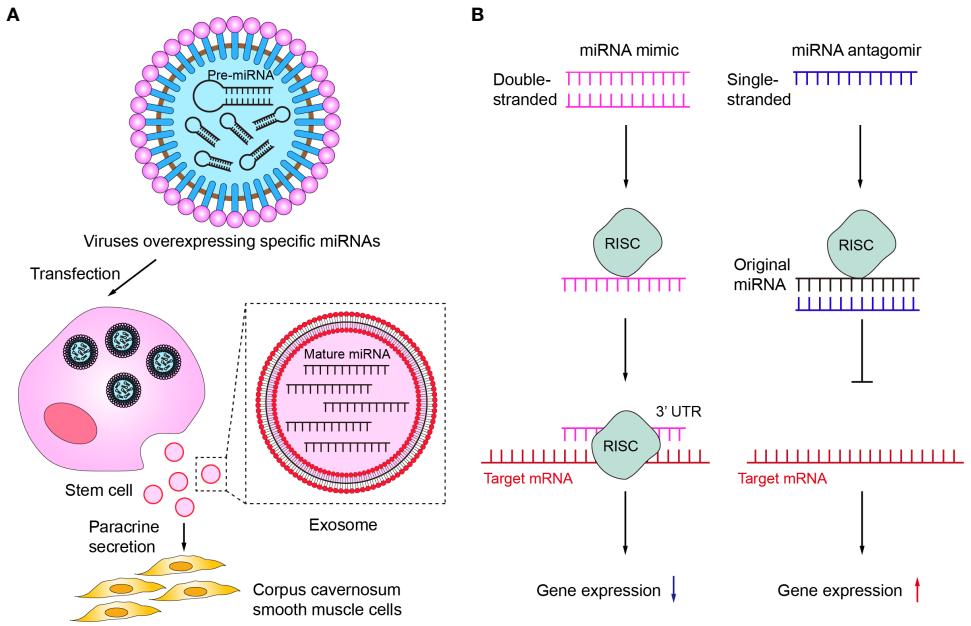

FIGURE 4
Therapeutic potentials of miRNA in ED. **(A)** Delivery of corresponding miRNAs *via* stem cells or exosomes. **(B)** The mechanisms of miRNA mimic and miRNA antagomir. RISC, RNA-induced silencing complex.

and on the other hand, it may act on pathways unrelated to ED or even opposite to the main target genes at the same time, which makes the final therapeutic effect of miRNA uncontrollable. Therefore, the mapping of miRNA target genes through rigorous, high-quality genomic and proteomic studies is a key step to achieving the clinical translation of miRNA. In addition, endothelial cells, smooth muscle cells, and immune cells constitute a complex microenvironment during the occurrence and development of ED, leading to the heterogeneity of miRNA expression. How to target miRNA to specific cells, effectively reduce the off-target rate, and minimize the toxic effects of miRNA on cells are also issues that need to be addressed. All in all, although results have been achieved at some magnitude and definite progress has been made in this field, several hurdles remain to be overcome before miRNAs can be formally used as therapeutic targets. Future studies should identify the target gene profiles of miRNAs and develop safe and efficient drug delivery platforms, to make miRNA therapy a clinical reality with bright prospects.

Conclusion

In conclusion, the advances in the miRNA field so far continue to bring new surprises, but there is still a long way to go. ED remains a major problem affecting men's health. Fortunately, new research on the pathogenesis of miRNAs in ED offers hope for miRNA-based therapeutic strategies for ED.

Author contributions

Conceptualization: JS. Methodology: JS, KL. Investigation: JS, TS. Formal analysis: JS, KL. Visualization: WX, JW. Funding acquisition: JL. Supervision: JL. Writing – original draft: JS, JW. Writing – review & editing: JL. All authors have made substantial contributions to the study and have read and approved the final 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.

Publisher's note

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Comparison of characteristics between Chinese diabetes mellitus-induced erectile dysfunction populations and non-diabetes mellitus-induced erectile dysfunction populations: A cross-sectional study

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Background: Erectile dysfunction (ED) is a common disease in adult men, and diabetes is an independent risk factor for ED. However, there are few reports on the distinction between diabetes mellitus-induced erectile dysfunction (DMED) and non-DMED features, as well as ED features of varying severity in the two groups.

Methods: A total of 365 ED patients treated at two clinics in China from 2019 to 2022 were included. Questionnaires of the International Index of Erectile Function (IIEF-5), Erectile Hardness Score (EHS), Premature Ejaculation Diagnostic Tool (PEDT), Patient Health Questionnaire-9 (PHQ-9), and Generalized Anxiety Disorder-7 (GAD-7) were administered to the patients. They were divided into three groups according to the IIEF-5 score: 5-7 for severe ED, 8-11 for moderate ED, and 12-21 for mild ED. In addition, the patient's age, weight, height, fasting blood glucose (FBG), total cholesterol (TC), triglycerides (TG), follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), total testosterone (TT) and other indicators were also collected. Statistical analysis was performed using SPSS 26, comparing all parameters between groups.

Results: Age (P<0.001), height (P=0.009), body mass index (BMI) (P=0.002), PEDT (P<0.001), FBG (P<0.001), FSH (P<0.001), TG (P<0.001), TT (P<0.001) and

triglyceride-glucose index (TyG) (P<0.001) were significantly different between diabetic ED and nondiabetic ED subjects. The trend test in the nondiabetic ED population found a negative correlation between the IIEF-5 score and PHQ-9 (P for trend=0.15). Multivariate ordinal logistic regression in the diabetic ED population showed that elevated LH OR=11.37 (95% CI: 0.966, 3.897) and elevated PRL OR=4.10 (95% CI: 0.410, 2.411) were associated with an increased risk of more severe ED.

Conclusions: The aetiology, demographic parameters, degree of premature ejaculation, and related biochemical tests were significantly different between the DMED and non-DMED populations.

KEYWORDS

erectile dysfunction, diabetes mellitus, influence factors, cross-sectional study, clinical study

Introduction

Erectile dysfunction (ED) is a common disease in adult men, and diabetes is one of the clear independent risk factors for ED (1). Diabetes mellitus erectile dysfunction (DMED) patients tend to have more severe symptoms, a worse curative effect, a greater impact on their quality of life, and a serious impact on their marital relationship and even their social harmony (2, 3). The pathogenesis of DMED is extremely complex, and studies have shown that damage to smooth muscle cells in the cavernous sinus and endothelial cell dysfunction caused by hyperglycaemia are its core mechanisms (4, 5). Hyperglycaemia can affect the normal function of mitochondria in diabetic patients and stimulate oxidative stress. Excessive oxidative stress can lead to damage and dysfunction of the smooth muscle cells and vascular endothelial cells in the corpus cavernosum, which in turn affects erectile function (5–7). Phosphodiesterase type 5 inhibitors (PDE-5Is) are currently the first-line regimen for ED treatment (6), but clinically, it has been found that they are less effective in DMED patients (7, 8).

In fact, compared with non-DMED patients, DMED patients not only have a higher incidence of ED (twice the rate of non-DMED) but are also characterized by worse treatment effects and more severe erectile dysfunction (8–11). Therefore, clarifying the difference between the two is crucial for improving ED diagnosis and treatment. However, differences in the characteristics of DMED and non-DMED, as well as differences in ED-related characteristics of different severities in diabetic patients, have rarely been reported (12).

Therefore, this study intended to explore the characteristics and differences between the DMED and the non-DMED populations among Chinese male patients attending an andrology outpatient clinic, which is of great significance for improving ED diagnosis and treatment (13–15).

Materials and methods

Participants

This study was designed as a cross-sectional study and was approved by the local ethics committees. All patients gave informed consent according to the Institutional Review Board guidelines and the Declaration of Helsinki. All protocols were approved by the institutional review boards of Xiangya Hospital of Central South University (No. 201904092) and the Fifth Affiliated Hospital of Sun Yat-Sen University (No. 2019-K231).

The consecutive enrolment method was used to recruit patients who visited the andrology outpatient clinics of Xiangya Hospital of Central South University and the Fifth Affiliated Hospital of Sun Yat-sen University from 2019 to 2022. Informed consent was obtained, and interviews could be interrupted or withdrawn from at any time. Inclusion criteria for the diabetic ED group: 1. History of diabetes; 2. The IIEF-5 score suggests ED; 3. No other ED-related diseases; 4. Willingness to participate in this study. Inclusion criteria for the nondiabetic ED group: 1. No history of diabetes; 2. The IIEF-5 score suggests ED; 3. No other diseases; 4. Willingness to participate in this study. According to the above criteria, a total of 365 participants were included in this study, including 135 patients with diabetic ED (91 mild, 26 moderate and 18 severe) and 230 nondiabetic ED patients (146 mild, 60 moderate and 24 severe).

Measures

Classification of ED according to IIEF-5

All participants were administered the IIEF-5 questionnaire, underwent a physical examination and provided a medical history to diagnose ED. The patients were divided into three groups according to symptom severity. Patients with an IIEF-5 score of 21–25 points were included in the mild ED group, patients with an IIEF-5 score of 11–20 points were included in the moderate ED group, and patients with an IIEF-5 score of 5–10 points were included in the severe ED group.

Laboratory analysis

Fasting blood glucose (FBG), total cholesterol (TC), triglycerides (TG), total testosterone (TT), follicle-stimulating hormone (FSH), prolactin (PRL) and luteinizing hormone (LH) of all subjects were obtained from the hospital laboratory. Normal range: FBG: 3.9-6.1 mmol/L, TC: <5.18 mmol/L, TG: <1.7 mmol/L, TT: 8.64-29.0 mmol/L, FSH: 1.5-12.4 mmol/L, PRL: 4.04-15.02 mmol/L and LH: 1.7-8.6 mmol/L. The triglyceride-glucose index (TyG), developed by Simental-Mendía et al. (16), was calculated from the fasting serum glucose and triglyceride values.

Related scales

Sexual function was assessed by andrology-related scales, including the IIEF-5, EHS, and PEDT. The erection hardness was evaluated using the EHS: penis is larger but not hard (I), penis is hard but not hard enough for penetration (II), penis is hard enough for penetration but not completely hard (III), and penis is completely hard and fully rigid (IV). Premature ejaculation (PE) was diagnosed by the PEDT: PE (≥11), suspected PE (9–10), and non-PE (≤8).

The patients were also asked to complete the GAD-7, which was used to assess anxiety symptoms, as well as the PHQ-9, which was used to evaluate depressive symptoms.

Questionnaire validity

The Cronbach's alpha score was calculated as 0.78, showing adequate internal consistency. The test-retest correlation coefficients of each item were ≥0.77, indicating excellent stability over time (P < 0.001) (Table 1).

Statistical analysis

Data are expressed as the mean ± SD for normally distributed parameters and as the median (quartile) for nonnormally distributed parameters. When normal and nonnormal distributions were used, correlations were performed using Student's t test and the Wilcoxon rank-sum (Mann–Whitney U) test, respectively. Univariable and multivariable ordinal logistic regression analyses were used for multivariate analysis and continuous or categorical dependent variables, respectively. ANOVA was used to assess the differences in the clinical variables between the two populations. We used G-Power software to verify the sample size of the study. Under the assumption of an effect size of 0.5, α=0.5, and a test power of 0.95, the sample size needed to be greater than 42 to effectively verify the conclusion. All statistical analyses were performed using SPSS Statistics (IBM, version

TABLE 1 Test–retest correlation coefficients (R) and P values of IIEF-5, PEDT, PHQ-9, EHS and GAD-7.

	IIEF-5					PEDT				
Question	1	2	3	4	5	1	2	3	4	5
R	0.785	0.773	0.777	0.779	0.791	0.773	0.770	0.771	0.766	0.770
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	PHQ-9									**EHS**
Question	1	2	3	4	5	6	7	8	9	
R	0.771	0.768	0.772	0.767	0.775	0.766	0.773	0.771	0.776	0.781
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	GAD-7									
Question	1		2	3	4		5	6		7
R	0.767		0.769	0.771	0.771		0.769	0.766		0.771
P	<0.001		<0.001	<0.001	<0.001		<0.001	<0.001		<0.001

26.0; SPSS, Armonk, NY, USA) for Windows. P < 0.05 was considered to indicate statistical significance.

Results

Comparison of the characteristics of the two populations

Table 2 shows the results of the main sociodemographic and biochemical characteristics and related scales of the participants with DMED and non-DMED. Among them, 135 patients with DMED and 230 patients with non-DMED were included in this study. In our study, age (P<0.001), height (P=0.009), BMI (P=0.002), PEDT (P<0.001), FBG (P<0.001), FSH (P<0.001), TG (P < 0.001), TT (P < 0.001) and TyG (P < 0.001) were significantly different between the two groups.

Internal feature comparisons

EHS (P<0.001) and TC (P=0.022) were significantly different among the three groups with different degrees of DMED (Table 3.1). GAD7 (P=0.048) and FBG (P=0.032) were significantly different in mild and moderate cases. TT (P=0.031) was only significantly different between the moderate and severe groups.

EHS (P < 0.001) and PHQ9 (P = 0.011) were significantly different among the three groups with different degrees of non-DMED (Table 3.2). Subsequently, the correlation analysis of IIEF-5 and PHQ9 found a negative correlation between the two ($P_{for\ trend}$=0.15). PRL (P=0.016) was significantly different between patients with moderate and severe disease.

Logistic regression results for the two populations

Univariate and multivariate ordinal logistic regression analyses were performed on various factors that may affect the degree of ED in the DMED populations, and EHS (P<0.001) and total cholesterol were included as covariates (TT was normal in the DMED population and thus not included in the comparison).

Univariate ordinal logistic regression (Table 4.1) showed that major depression OR=7.32 (95% CI: 0.271, 3.710) was associated with an increased risk of more severe ED. Multiple ordinal logistic regression showed that increased LH OR=11.37 (95% CI: 0.966, 3.897) and PRL OR=4.10 (95% CI: 0.410, 2.411) were associated with an increased risk of more severe ED.

Univariate and multivariate ordinal logistic regression analyses were performed on various factors that may affect the degree of non-

TABLE 2 Comparison of the Characteristics between the Two Groups (DMED vs Non-DMED).

	DMED	Non-DMED	t	P
Age	**43.79 ± 8.91**	**33.48 ± 7.95**	**11.09**	**<0.001**
Height	**168.62 ± 5.79**	**170.30 ± 5.41**	**-2.76**	**0.006**
Weight	69.16 ± 8.41	67.78 ± 9.10	1.43	0.153
BMI	**24.32 ± 2.62**	**23.36 ± 2.89**	**3.20**	**0.002**
IIEF-5	13.56 ± 4.76	14.05 ± 4.71	-0.95	0.341
EHS	2.59 ± 0.77	2.56 ± 0.80	0.36	0.717
PEDT	**13.37 ± 4.92**	**17.76 ± 5.08**	**-7.98**	**<0.001**
PHQ9	6.46 ± 4.18	6.90 ± 4.98	-0.87	0.387
GAD7	6.32 ± 4.15	5.52 ± 4.55	1.61	0.098
FBG	**7.70 ± 2.10**	**5.17 ± 0.39**	**13.56**	**<0.001**
TC	4.78 ± 1.96	4.84 ± 1.06	-0.31	0.760
FSH	**5.29 ± 2.54**	**4.36 ± 2.10**	**3.63**	**<0.001**
TG	**2.31 ± 1.43**	**1.66 ± 0.93**	**4.60**	**<0.001**
PRL	13.32 ± 5.12	13.45 ± 8.75	-0.17	0.869
LH	5.28 ± 2.34	5.06 ± 2.01	0.90	0.368
TT	**14.38 ± 5.12**	**18.77 ± 6.65**	**-6.76**	**<0.001**
TyG	**7.76 ± 0.59**	**7.08 ± 0.52**	**10.63**	**<0.001**

TABLE 3.1 The Results of LSD Among Mild ED, Moderate ED and Severe ED in DMED Populations.

	Mild ED	Moderate ED	Severe ED	F	P
Age	43.55 ± 8.62	45.89 ± 10.69	41.94 ± 7.36	1.139	0.323
Height	168.57 ± 6.09	167.54 ± 4.17	170.44 ± 6.06	1.360	0.260
Weight	70.50 ± 9.03	69.37 ± 14.31	71.61 ± 10.91	0.251	0.779
BMI	24.79 ± 2.71	24.68 ± 4.58	24.59 ± 3.09	0.035	0.966
EHS	2.71 ± 0.62*	2.50 ± 0.95#	1.83 ± 0.99*#	10.55	**<0.001**
PEDT	13.40 ± 4.68	13.72 ± 5.14	12.78 ± 5.91	0.193	0.825
PHQ9	6.29 ± 3.67	6.08 ± 3.77	7.76 ± 6.42	0.993	0.374
GAD7	5.71 ± 3.49 #	7.60 ± 5.01#	7.18 ± 5.09	2.426	0.093
FBG	7.49 ± 1.65#	8.52 ± 3.31#	7.55 ± 2.10	2.402	0.095
TC	4.51 ± 1.22#	5.68 ± 3.31#	4.68 ± 1.91	3.912	**0.022**
FSH	5.40 ± 2.63	5.29 ± 3.01	4.95 ± 2.04	0.219	0.803
TG	2.36 ± 1.54	1.96 ± 1.15	2.43 ± 1.14	0.892	0.412
PRL	13.22 ± 4.96	12.63 ± 4.76	14.56 ± 5.20	0.836	0.436
LH	5.21 ± 2.27	5.45 ± 2.47	5.41 ± 2.62	0.137	0.872
TT	14.53 ± 5.27	15.46 ± 4.36 #	12.08 ± 4.95#	2.497	0.086
TyG	7.77 ± 0.57	7.66 ± 0.76	7.76 ± 0.59	0.688	0.500

#P<0.05, *P<0.001.

TABLE 3.2 The Results of LSD Among Mild ED, Moderate ED and Severe ED in Non-DMED Populations.

	Mild ED	Moderate ED	Severe ED	F	P
Age	33.32 ± 7.58	33.27 ± 7.94	35.00 ± 10.14	0.487	0.615
Height	170.27 ± 5.47	170.40 ± 4.80	170.22 ± 6.65	0.016	0.984
Weight	67.23 ± 9.05	69.95 ± 8.80	65.78 ± 9.53	2.464	0.087
BMI	23.18 ± 2.88	24.09 ± 2.85	22.69 ± 2.85	2.711	0.069
EHS	**2.83 ± 0.66**^{*1*2}	**2.22 ± 0.83**$^{*1\#}$	**1.78 ± 0.74**$^{*2\#}$	**30.442**	**<0.001**
PEDT	17.85 ± 4.89	17.36 ± 5.39	18.26 ± 5.65	0.320	0.727
PHQ9	**6.16 ± 4.37**$^{\#1}$	**8.05 ± 5.35** $^{\#2}$	**8.50 ± 6.60** $^{\#1\#2}$	**4.606**	**0.011**
GAD7	5.05 ± 4.14	6.32 ± 4.88	6.33 ± 5.74	2.104	0.124
FBG	5.18 ± 0.39	5.19 ± 0.34	5.09 ± 0.57	0.345	0.709
TC	4.82 ± 1.08	4.84 ± 0.85	4.94 ± 1.46	0.082	0.921
FSH	4.34 ± 2.23	4.38 ± 1.76	4.50 ± 2.10	0.046	0.955
TG	1.66 ± 0.92	1.69 ± 0.97	1.62 ± 0.91	0.032	0.968
PRL	13.47 ± 8.59	11.95 ± 6.18 $^{\#}$	17.82 ± 14.19 $^{\#}$	2.935	0.055
LH	5.11 ± 2.20	4.91 ± 1.47	5.15 ± 1.94	0.202	0.818
TT	19.19 ± 6.42	18.33 ± 7.59	16.86 ± 5.12	1.074	0.344
TyG	7.07 ± 0.52	7.09 ± 0.51	7.07 ± 0.61	0.019	0.981

#P<0.05, *P<0.001.

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TABLE 4.1 Univariable and multivariable ordinal logistic regression analysis among DMED Populations.

	Univariable OR (95% CI)	P	Multivariable OR (95% CI)	P
Age				
20-29	Ref		Ref	
30-39	2.83 (-1.268,3.350)	0.377	1.61 (-2.181,3.133)	0.726
40-49	1.29 (-2.095,2.609)	0.830	3.26 (-1.490,3.852)	0.386
50-59	2.33 (-1.495,3.185)	0.479	3.00 (-1.601,3.802)	0.425
>60	3.04 (-1.625,3.847)	0.426	1.86 (-2.759,3.994)	0.720
	P for trend	0.520	*P for trend*	0.899
PEDT				
Non-PE	Ref		Ref	
Suspected PE	0.659 (-1.589,0.725)	0.464	1.09 (-1.791,1.958)	0.930
PE	0.46 (-1.678,0.141)	0.098	0.35 (-2.464,0.384)	0.152
	P for trend	0.363	*P for trend*	0.499
PHQ-9				
No depression	Ref		Ref	
Mild depression	0.62 (-1.310,0.362)	0.267	0.56(-2.081,0.917)	0.446
Moderate depression	0.78 (-1.624,1.135)	0.728	0.15 (-4.014,0.200)	0.076
Severe depression	**7.32 (0.271,3.710)**	**0.023**	2.25 (-1.726,3.352)	0.530
	P for trend	0.072	*P for trend*	0.156
GAD-7				
No anxiety	Ref		Ref	
Mild anxiety	0.67 (-1.276,0.472)	0.367	0.80 (-1.723,1.281)	0.773
Moderate anxiety	2.45 (-0.145,1.936)	0.092	7.54 (-0.059,4.099)	0.057
Severe anxiety	3.46 (-0.941,3.424	0.265	10.36(-0.340,5.061)	0.087
	P for trend	0.250	*P for trend*	0.419
FBG				
Normal	Ref		Ref	
Elevated	0.41 (-2.174,0.389)	0.172	0.23 (-3.012,0.068)	0.061
	P for trend	0.187	*P for trend*	0.077
TG				
Normal	Ref		Ref	
Elevated	0.95 (-0.763,0.662)	0.889	0.64 (-1.440,0.563)	0.391
	P for trend	0.050	*P for trend*	0.050
FSH				
Normal	Ref		Ref	
Elevated	0.60 (-2.848,1.838)	0.673	1.53 (-3.811,4.664)	0.884
	P for trend	0.598	*P for trend*	0.883

(Continued)

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TABLE 4.1 Continued

	Univariable OR (95% CI)	P	Multivariable OR (95% CI)	P
LH				
Normal	Ref		Ref	
Elevated	1.69 (-0.498,1,552)	0.314	**11.37 (0.966,3.897)**	**0.001**
	P for trend	0.556	*P for trend*	0.816
PRL				
Normal	Ref		Ref	
Elevated	1.36 (-0.412,1.033)	0.399	**4.10 (0.410,2.411)**	**0.006**
	P for trend	0.867	*P for trend*	0.495

TABLE 4.2 Univariable and multivariable ordinal logistic regression analysis among Non-DMED Populations.

	Univariable OR (95% CI)	P	Multivariable OR (95% CI)	P
Age				
20-29	Ref		Ref	
30-39	0.81 (-0.821,0.395)	0.492	1.19 (-0.642,0.981)	0.682
>40	1.02 (-0.737,0.784)	0.951	2.01 (-0.364,1.758)	0.198
	P for trend	0.156	P for trend	0.948
PEDT				
Non-PE	Ref		Ref	
Suspected PE	0.61 (-1.676,1.539)	0.993	2.81 (-0.913,2.977)	0.298
PE	0.57 (-0.636,1.616)	0.395	0.84 (-1.688,1.333)	0.818
	P for trend	0.304	P for trend	0.599
GAD-7				
No anxiety	Ref		Ref	
Mild anxiety	0.81 (-0.802,0.374)	0.475	0.82 (-1.056,0.650)	0.641
Moderate anxiety	0.81 (-1. 080,0.666)	0.642	2.20 (-0.572,2.146)	0.257
Severe anxiety	0.26 (-2.473, -0.227)	**0.019**	0.28(-2.974,0.433)	0.144
	P for trend	0.266	P for trend	0.145
TG				
Normal	Ref		Ref	
Elevated	0.91 (-0.699,0.518)	0.771	0.51 (-1.445,0.089)	0.083
	P for trend	0.979	P for trend	0.864
TC				
Normal	Ref		Ref	
Elevated	0.87 (-0.773,0.486)	0.656	1.88 (-0.161,1.422)	0.118
	P for trend	0.438	P for trend	0.402
LH				

(Continued)

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TABLE 4.2 Continued

	Univariable OR (95% CI)	P	Multivariable OR (95% CI)	P
Normal	Ref		Ref	
Elevated	0.99 (-1.391,1.380)	0.995	2.42 (-0.846,2.615)	0.317
	P for trend	0.679	P for trend	0.971
PRL				
Normal	Ref		Ref	
Elevated	1.18 (-0.502,0.832)	0.628	1.15 (-0.664,0.953)	0.727
	P for trend	0.747	P for trend	0.792

DMED, and EHS (P<0.001) and PHQ9 (P=0.011) were included as covariates (FBG, FSH and TT were normal in the non-DMED populations and thus were not included in the comparison).

Univariate logistic regression (Table 4.2) showed that severe anxiety OR=0.26 (95% CI: -2.473, -0.227) was associated with an increased risk of more severe ED. However, this result was not observed in the multivariate logistic regression results.

Discussion

ED is one of the most common disorders in andrology clinics, with an estimated prevalence between 30% and 50% in the general population (17–20). As the number of people with diabetes worldwide is increasing, there is growing concern about erectile dysfunction in men with diabetes (19–21). At present, although there are many studies on DMED, comparisons of the characteristics of DMED and non-DMED populations, especially the differences in ED-related characteristics among diabetic patients with varying severity, are rarely reported. Therefore, this study fills this gap, which is of great significance for improving ED diagnosis and treatment.

This study compared the sociodemographic and biochemical characteristics and related scales of the DMED and non-DMED populations after assessing the validity of the questionnaire. The results showed that age (43.79 ± 8.91 vs. 33.48 ± 7.95, P<0.001), height (168.62 ± 5.79 vs. 170.30 ± 5.41, P=0.006), BMI (24.32 ± 2.62 vs. 23.36 ± 2.89, P=0.002), PEDT (13.374 ± 4.92 vs. 17.76 ± 5.08, P<0.001), FBG (7.70 ± 2.10 vs. 5.17 ± 0.39, P<0.001), FSH (5.29 ± 2.54 vs. 4.36 ± 2.10, P<0.001), TG (2.31 ± 1.43 vs. 1.66 ± 0.93, P < 0.001), TT (14.38 ± 5.12 vs. 18.77 ± 6.65, P < 0.001) and TyG (7.76 ± 0.59 vs. 7.08 ± 0.52, P < 0.001) were significantly different between the two groups. Table 3.2 indicates that the non-DMED population in this study mainly has psychogenic ED, and the regression analysis of the two populations suggests that the DMED populations mainly have organic ED. Thus, non-DMED patients were younger and had higher PEDT scores. In addition, studies have shown that diabetes not only causes increased FSH and atherosclerosis leading to increased TG but also leads to decreased serum TT levels and insulin resistance (higher TyG) (22, 23), which was also verified in this study. However, research on the underlying mechanism is rarely reported, which may be the direction of our future research (24).

In the internal comparison within the groups by symptom severity, EHS (P<0.001) and total cholesterol (P=0.022) were found to be significantly different in the three groups with different degrees of DMED. EHS (P<0.001) and PHQ9 (P=0.011) were significantly different in the three groups with different degrees of non-DMED, and the trend test found that the IIEF-5 score and PHQ9 were negatively correlated (P for trend=0.15), which suggests that psychogenic ED is predominant in non-DMED patients.

Finally, univariate and multivariate ordinal logistic regression analyses were performed on various factors that may affect the degree of ED among DMED and non-DMED populations, respectively. In the DMED population, univariate ordinal logistic regression showed that severe depression OR=7.32 (95% CI: 0.271, 3.710) was associated with an increased risk of severe ED. Multiple ordinal logistic regression showed that increased LH OR=11.37 (95% CI: 0.966, 3.897) and PRL OR=4.10 (95% CI: 0.410, 2.411) were associated with an increased risk of severe ED. In the non-DMED population, univariate logistic regression showed that severe anxiety OR=0.26 (95% CI: -2.473, -0.227) was associated with an increased risk of more severe ED, but no correlations were found in the multivariate logistic regression. A comparison of the two groups showed that increased LH and PRL were associated with an increased risk of more severe ED in the DMED population. This can be attributed to diabetes-induced metabolic syndrome leading to elevated LH and hyperprolactinemia (HyperPRL) causing ED, and the related mechanisms are currently being studied (25–31). Therefore, screening and appropriate interventions for men with erectile dysfunction is warranted (32). Our findings suggest that early

detection, early diagnosis and early treatment of diabetes are essential to prevent the occurrence of ED, and standardized treatment of diabetes is of great significance for improving erectile function.

This study has some merits and shortcomings. The main merit is this study was carried out in two top clinics in China, with highly professional staff, a sufficient number of patients and advanced equipment. Meanwhile, this study has the following limitations, which need to be resolved in future studies. First, objective measurements (night time penile erection monitoring equipment and Doppler ultrasound equipment) can evaluate ED more reliably than questionnaire surveys, but related studies have also confirmed the effectiveness of IIEF questionnaires for ED evaluation (33). The main basis of this study was using the IIEF-5 questionnaire, a physical examination and the medical history to diagnose ED cases and stratify them by severity. Second, this investigation was an observational cross-sectional study, and no longitudinal data were available for the interaction of diabetes and erectile function. Therefore, relevant studies, including longitudinal studies and treatments, are needed in the future to assess changes in erectile function during diabetes treatment and this may provide new insights into treatment strategies for ED.

In conclusion, there are significant differences in aetiology between diabetic ED and nondiabetic ED patients. Nondiabetic ED patients mainly have psychogenic ED, while diabetic ED patients mainly have organic ED. In addition, there are differences in demographic parameters such as age, height, weight, BMI, biochemical tests such as blood glucose, triglycerides, total testosterone, and PEDT. Therefore, individualized treatment according to the characteristics of the different populations is of great significance for improving erectile function.

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 Xiangya Hospital of Central South University (No. 201904092) and the Fifth Affiliated Hospital of Sun Yat-Sen University (No. 2019-K231). The patients/participants provided their written informed consent to participate in this study.

Author contributions

DL and YT designed the experiments. JP and LL contributed to the clinical data collection and assessment. JP, DL and YX analysed the results. JP, DL and YT wrote 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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Chronic liver diseases and erectile dysfunction

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Chronic liver diseases (CLDs) are characterized by progressive necrosis of hepatocytes, which leads to liver fibrosis and cirrhosis, and ultimately liver dysfunction. The statistics of 2020 shows that the number of patients with CLDs, including chronic hepatitis, fatty liver, and cirrhosis, may exceed 447 million in China. The liver is a crucial organ for the metabolism of various substances, including sex hormones and lipids. CLDs frequently result in abnormalities in the metabolism of sex hormones, glucose, and lipids, as well as mental and psychological illnesses, all of which are significant risk factors for erectile dysfunction (ED). It has been reported that the prevalence of ED in male patients with CLDs ranges from 24.6 to 85.0%. According to a survey of Caucasians, liver transplantation may improve the erectile function of CLDs patients with ED. This finding supports the link between CLDs and ED. In addition, ED is often a precursor to a variety of chronic diseases. Given this correlation and the significant prevalence of CLDs, it is important to evaluate the epidemiology, risk factors, etiology, and treatment outcomes of ED in male patients with CLDs, expecting to attract widespread attention.

KEYWORDS

chronic liver disease, erectile dysfunction, risk factors, testosterone, chronic hepatitis

1. Introduction

Erectile dysfunction (ED) in men is a common disease with a high incidence. Although more common in the middle-aged and elderly, ED can occur in mature men of any age, and its incidence increases with age (1). It was reported that about 23% of men aged 40–80 worldwide suffer from ED to varying degrees (2). In mainland China, the overall incidence of ED is ~ 49.69% (3). Additionally, the information currently available points to an increase in the prevalence of ED in men younger than 40 years of age (1). Therefore, ED is pervasive across all ethnic groups and is becoming a bigger concern globally (4). The etiology and pathogenesis of ED are extremely complicated. It is widely accepted that spiritual and psychological factors, as well as some clinical factors mostly related to neurological, vascular, hormonal levels, and some drugs, contribute to being the main causes of ED. Overall, diabetes mellitus, cardiovascular illness, and neurological disorders are the main risk factors for ED clinically.

Chronic liver diseases (CLDs) are also common in clinical practice. As of 2019, an estimated 1.69 billion people worldwide were affected by liver disease (5). According to 2020 data, the number of patients with CLDs, including cirrhosis, fatty liver, and chronic hepatitis, may exceed 447 million in China. The liver is crucial for the metabolism of many substances, including sex hormones and lipids. Disorders of sex hormone metabolism, glucose and lipid metabolism, as well as mental and psychological conditions, are all common adverse complications of CLDs and high-risk factors for ED. According to studies, the prevalence of ED in male patients with CLDs ranges from 24.6 to 85.0%, including 8.6–78.0% in patients with chronic hepatitis and 41.2–92.0% in patients with cirrhosis. These estimates may greatly understate the true occurrence because several CLD patients are focused on treating their "dangerous" liver illness and lack the time and energy to concentrate on this "slight" issue. Several surveys indicated that the erectile function of CLDs patients with ED might improve after liver transplantation. Significant differences were found in total testosterone, sex hormone-binding globulin, free androgen index, and the International Index of Erectile Function-5 (IIEF-5) scores pre- and post-operation (6, 7), which confirmed an association between CLDs and ED. The simplified IIEF-5 score is the most commonly used tool to assess the presence and severity of ED, mainly including erectile and orgasmal function, libido, sexual intercourse, and overall satisfaction, and is also the most reliable and objective tool (8). Based on the IIEF-5 score, the severity of ED is divided into three categories: a total score of 22–25 points is normal, 12–21 is mild, 8–11 is moderate, and 5–7 is severe. For the assessment of liver function, currently, the modified Child-Pugh score is mostly used to quantify the liver reserve function of patients, which categorizes liver function into three levels based on the total score: a total score of 5–6 points is clarified as degree A, 7–9 as degree B, 10 points or more as grade C.

Currently, the majority of attention to the risk factors of ED is concentrated on diabetes mellitus, cardiovascular disease, neurological disease, and psychological factors. Few studies have evaluated the prevalence and risk factors for ED in patients with CLDs, and the attention given by physicians to this symptom in the diagnosis and treatment of liver disease is still poor. Given this correlation and the significant prevalence of CLDs, it is important to evaluate the epidemiology, risk factors, etiology, and treatment outcomes of ED in male CLDs patients in order to garner public interest. Those underlying causes of ED are not the main content of this review, we will integrate the up-to-date evidence and concentrate on the correlations, pathogenesis, and treatment status between CLDs and ED. This paper will cite a substantial amount of evidence to illustrate this issue, and these evidences, which are gathered from various literatures, varies to some extent and is frequently not comparable, possibly as a result of the various etiologies of liver disease, the sample size, the survey and patient sampling methodologies, and the assessment tools employed in each study.

Abbreviations: CLDs, chronic liver diseases; ED, erectile dysfunction; NAFLD, Non-alcoholic fatty liver disease.

2. Epidemiology of ED in CLDs

CLDs is a general term for a group of diseases characterized by progressive necrosis of hepatocytes, which leads to liver dysfunction. Recent research has confirmed that a variety of liver diseases can result in ED, particularly for those suffering from advanced liver disease. Multifactorial diseases are highly related to the existence of ED, which is not just a comorbidity, but liver disease *per-se* also leads to the progression of ED.

2.1. Non-alcoholic fatty liver diseases

NAFLD refers to a group of diseases primarily characterized by macrovesicular steatosis of the liver that occurs in patients without excessive alcohol consumption (9). Currently, it is considered to be the most common CLDs worldwide, especially in the Western, with an incidence of nearly 25% (10). The actual prevalence of NAFLD may be higher because many remain asymptomatic in the early stages and there are no reliable non-invasive tests for screening. Its primary feature is fat deposition in hepatocytes, which can develop into fibrosis, cirrhosis, and even hepatocellular carcinoma. The incidence and mortality of hepatocellular carcinoma and cirrhosis brought on by NAFLD have kept increasing in recent years (11).

NAFLD has been regarded as the hepatic manifestation of systemic metabolic syndrome (12, 13). ED is a very important and common comorbidity of NAFLD, some patients even have abnormal semen parameters. The incidence of ED in patients with NAFLD ranges from 45 to 67% (14), which is much higher than the rate in the general population and 2.92 times higher than the rate in those without NAFLD. The degree of fat infiltration is closely related to ED. NAFLD is a significant independent factor associated with ED. The main reason may be that NAFLD and ED share common risk factors such as obesity, hypertension, and diabetes mellitus (15, 16). Numerous ED etiologies are present in 39.5% of NAFLD patients, and a significant portion of these patients have psychogenic ED. Therefore, careful evaluation of psychological status during diagnosis and treatment is important (14).

2.2. Cirrhosis

Cirrhosis is a chronic and progressive liver disease commonly seen in clinical, which is caused by long-term or repetitive impacts of one or more etiologies. It is

often accompanied by ascites, hepatic encephalopathy, and varicose hemorrhage.

A high prevalence of ED is shown in alcoholic hepatitis, which is one of the major etiologies of cirrhosis. At present, some scholars consider that there is a correlation between cirrhosis and ED, which can be explained by haemodynamic alteration, hypogonadism, hypotestosteronemia, unhealthy lifestyle, and a lower quality of life. Through meta-analysis, Yoo et al. (17) found that the prevalence of ED in cirrhotic patients was 79.08%, and in decompensated cirrhotic patients was 88.4%. After controlling for other conditions such as diabetes, alcoholism, severe cardiac conditions, etc., Paternostro et al. (18) discovered that among male cirrhotic patients, 55% were categorized as mild-to-moderate ED and 8.3% as moderate-to-severe ED, and that with the decline of liver function, the severity of ED increased and the IIEF-5 score decreased significantly. In cirrhotic patients, the increase in absolute level of hepatic venous pressure gradient is an independent predictor of ED, suggesting that ED is correlated with portal hypertension, and this may be related to hemodynamic alternation in the splanchnic circulation, which can directly impair physiological penile erection. In men with alcoholic cirrhosis, ED may be related to the direct toxic effects of ethanol and acetaldehyde on the gonads.

However, previous studies had shown conflicting results regarding the impact of cirrhosis on the prevalence and severity of ED. Some scholars compared the incidence of ED among alcoholics with or without cirrhosis and diabetes, found that there was no difference between the groups (19). According to some studies, no difference was found in the prevalence of ED between patients with chronic viral hepatitis and patients with established cirrhosis (20), only age and hypoalbuminemia were found to be independent factors for ED (18). The reasons for these disparities could be that these studies used different IIEF-5 cut-offs or that these patients had higher expectations of erectile function.

2.3. Chronic viral hepatitis

Chronic viral hepatitis is a common disease worldwide. Despite the promotion of vaccination, the landscape is still far from satisfactory, with more than 300 million people suffering from various kinds of hepatitis. According to the World Health Organization, an estimated 257 million people are living with chronic hepatitis B, more than 70 million with hepatitis C, 80% of whom develop chronic disease, and 62 million with chronic hepatitis D. As for hepatitis A, 1.4 million new cases are reported globally each year (21–24).

The total prevalence of ED in patients with chronic viral hepatitis ranges from 14 to 78% (20, 25), even after adjustment based on IIEF-5 scores $\leq$17, 40% of patients with chronic viral hepatitis had ED (26). According to the IIEF cut-off, ED was confirmed in 76.4% of hepatitis B patients, with 60.3% classified as severe. Those with diabetes mellitus had the highest incidence of ED (92.6%), followed by those who progressed to cirrhosis (85.7%) (27). Patients with HBV-related liver cirrhosis exhibited a higher prevalence and severity of ED than chronic hepatitis B patients (4, 28). In comparison to patients with HBV infection, those with HCV seem to be more susceptible to ED. This may be due to the distinct biological properties of these two viruses. HCV infection is more likely to cause inflammation of the vascular endothelium, which is one of the pathogenesis of ED (29, 30). Cryoglobulinemia may play an important role in this process. HCV infection is one of the causes of cryoglobulinemia; cryoglobulins depositing in the vascular endothelium can result in systemic vasculitis, primarily involving the small and medium arteries. HCV patients with cryoglobulinemia have a higher incidence of ED than those without cryoglobulinemia (31).

2.4. Alcoholic liver diseases

Alcoholic liver diseases are caused by long-term alcohol abuse and usually manifest as fatty liver at the initial stage and may develop into alcoholic hepatitis, liver fibrosis, and cirrhosis. Long-term alcohol abuse is generally defined as a history of drinking alcohol for more than 5 years, which is equivalent to ethanol $\geq$ 40 g/d for men and 20 g/d $\geq$ for women, or a history of heavy alcohol consumption within 2 weeks, which is equivalent to ethanol $>$ 80 g/d. There have been no specific reports retrieved on the association between alcoholic liver disorders and ED, and the points of view we discussed ut infra are all obtained from sporadic observations in other studies, and the available data are inadequately standardized and the cases are restricted.

Cornely et al. (19) observed that the prevalence and severity of ED were considerably higher in male cirrhotic patients with chronic alcohol abuse than in patients with more severe cirrhosis due to other etiologies. Another study found that the prevalence of sexual dysfunction (particularly ED and/or decreased libido) in alcohol-induced liver disease is comparable between cirrhotic and non-cirrhotic patients, leading to the conclusion that alcohol was the true cause of ED (32). However, the results from another study were completely different, Wang et al. (33) believed that the history of alcohol consumption had no effect on the prevalence of ED in patients with cirrhosis, but the levels of testosterone, estradiol, and PRL were different from those in the control group, suggesting that cirrhosis rather than alcohol was the real cause of sexual dysfunction.

3. Etiology

3.1. Overall health status

3.1.1. Age

For patients with CLDs, age often predicts a longer duration and more serious condition. In their study of 120 patients with Child-Pugh A cirrhosis, Maimone et al. discovered that the presence of ED was significantly associated with age, whether using univariate logistic regression analysis or multivariate analysis, and age was identified as an independent predictor of ED. The mean age of ED patients was significantly higher than that of non-ED patients, and the prevalence of ED gradually increased with age. Additionally, the severity of ED was positively correlated with age (34). In a Japanese study, 64 patients with chronic hepatitis and 53 patients with cirrhosis were studied with IIEF-5, and it was discovered that ∼50% of patients under the age of 50 developed ED, ∼79% of patients aged 50–59 years old had ED, and all patients over the age of 60 had ED (4).

3.1.2. Diabetes mellitus

Diabetes mellitus is a common comorbidity of CLDs. Persistent hyperglycemia can affect erectile function in multiple ways. First, it can negatively affect endothelial function, resulting in an imbalance between NO and endothelin-1, which causes relaxation disorder of smooth muscle cells (35); second, it can increase the levels of advanced glycosylation end products, which can interfere with the synthesis of protein polymerase chains and may directly affect DNA replication and transcription, ultimately leading to the atherosclerosis and stenosis of small and medium arteries, resulting in penile hypoperfusion and ED (36); third, the accumulation of advanced glycosylation end products can directly damage the structure and function of corpus cavernosum smooth muscle cells; fourth, a hyperglycemic state can damage the peripheral and autonomic nerves *via* the polyolol pathway, the protein kinase C pathway, and by competitively inhibiting inositol extraction by neural tissue (37, 38). Furthermore, insulin resistance can impair gonadal axis function. All of these factors could contribute to diabetes-related ED.

3.1.3. Hypertension

It is well established that hypertension can elevate the risk of ED. This could be due to hypertension itself or antihypertensive medications. In patients with chronic hepatitis B and cirrhosis, hypertension is an important independent factor for ED (18), and ED can also be used as an early marker of endothelial dysfunction in hypertension (39). Endothelial dysfunction and nitric oxide (NO) play important roles in the occurrence and development of ED in patients with hypertension (40, 41). Moreover, some antihypertensive medications, such as beta-blockers, aldosterone receptor antagonists, and thiazide diuretics, can also induce ED (42).

3.1.4. Mental state

Depression and anxiety are associated with impaired sexual function and satisfaction and have a high prevalence in patients with chronic viral hepatitis (43–45), and they have an independent negative effect on erectile function in these patients (26). The incidence of concomitant depression was also higher in patients with cirrhosis than in the general population (46). Depression appeared to have a stronger correlation with sexual dysfunction than testosterone levels (47). Mental disorders can contribute to the development of ED by reducing libido and physical activity (26). In addition, the adverse side effects, such as psychosocial problems caused by depression and antidepressants, may aggravate ED. Although the precise mechanism has not been clarified, it is proposed that depression and anxiety contribute to a vicious cycle that impairs the sexual relationship between patients and their partners, resulting in communication problems that further impede sexual functioning (48). Therefore, in patients with CLDs, active detection and intervention should be carried out to reduce the occurrence or severity of ED.

3.2. Etiology and severity of liver disease

The etiology and progression of liver disease contribute to the development of ED. Toda et al. (4) studied 117 subjects with viral hepatitis and idiopathic non-alcoholic liver disease, 53 of whom had cirrhosis (30 Child A, 17 Child B, and 6 Child C), and assessed their erectile function with the IIEF-5, finding that the incidence of ED increased as the Child-Pugh score worsened. However, in patients with Child-Turcotte-Pugh scores $\leq$10, their frequency and severity did not vary with the severity of liver disease.

Hepatitis viruses can directly damage the gonads and can also affect the occurrence of ED through a variety of complex mechanisms such as inflammation, oxidative stress, and apoptosis (25, 26). Chronic systemic inflammation with elevated C-reactive protein levels reduces the synthesis of NO in endothelial cells and ultimately leads to endothelial dysfunction, which may account for the relationship between ED and chronic hepatitis (49, 50). Compared with HBV, HCV infection had a more pronounced negative effect on erectile function; compared to the control group without infection, men with chronic HCV infection had significantly lower libido, erectile function, ejaculation, and overall satisfaction (51). Even after controlling for depression or other potentially confounding variables, the association between HCV and ED remains strong. This could be due to biological/virological factors or potential effects on the

hypothalamic-pituitary-gonadal axis (52). The prevalence of ED in cirrhotic patients is higher than in chronic hepatitis patients. The mechanism remains unclear, but gonadal dysfunction, sex hormone imbalance, and low albumin levels may be the major causes (27). Kim M et al. (28) also found that the incidence of ED was significantly higher in patients with HBV-related cirrhosis than in chronic hepatitis patients without cirrhosis, and this difference remained significant even after four patients with Child-Pugh grade B cirrhosis were excluded. Physiological disturbances caused by protein malnutrition in patients with decompensated CLDs may be associated with ED. Edema, ascites, hypoalbuminemia, pleural effusion, and deteriorating physical function are usually present in advanced liver diseases and can also reduce libido and lead to ED. Branched-chain amino acids can improve serum albumin, muscle metabolism, and prognosis in patients with CLDs (53). In cirrhotic patients treated with branched-chain amino acids, an improvement in erectile function was observed (54), suggesting a correlation between physiological dysfunction and ED. Portal hypertension, as an independent risk factor for erectile dysfunction, can impair penile erectile function by altering the hemodynamic status of the visceral circulation (18). In a study of cirrhotic patients with a wider range of liver failure, Phylonenco et al. identified minimal hepatic encephalopathy as a possible risk factor for the progression of ED (55).

Minimal hepatic encephalopathy is considered to be associated with a poor quality of life as well as some behavioral abnormalities such as depression and anxiety. In their study, Nardelli et al. (56) found that the prevalence of ED was significantly higher in patients with abnormal neurocognitive tests than in normal subjects, and that, in the subgroup of patients with Child-Pugh A cirrhosis ($n = 25$), 100% of patients with minimal hepatic encephalopathy ($n = 16$) developed ED. However, in multivariate analysis, it was not identified as an independent risk factor for ED. This could be due to the sample size restrictions, which make it challenging to distinguish the effects of the two variables, liver dysfunction and cognitive impairment (57). Huyghe et al. (58) used a questionnaire to survey patients with end-stage liver disease who were candidates for liver transplantation, using the IIEF-5 score to assess erectile function and the patient-baseline Treatment-Satisfaction Scale score to assess sexual satisfaction. Of the 98 candidates who completed the questionnaire, 28 (29%) were sexually inactive, while 52 (74%) of the 70 sexually active patients had ED. Approximately 50% of patients felt that their erectile function had deteriorated in the previous 6 months.

In addition, some rare diseases can also cause liver dysfunction and ED. Hemochromatosis is a chronic iron overload caused by a high-iron diet, massive blood transfusions, or systemic disease. Excess iron stored in substantial cells such as the liver, heart, and pancreas can lead to degeneration and diffuse fibrosis, as well as metabolic and functional abnormalities. It can also cause hypothalamic-pituitary-gonadal axis dysfunction, direct testicular damage, diabetes, and other conditions that can lead to sexual dysfunction (59, 60). Autoimmune hepatitis is a chronic and progressive autoimmune-mediated inflammatory disease of the liver. Approximately 34% of patients have no symptoms but abnormal liver function at the initial visit. Thirty percent have cirrhosis, and 8% have decompensated cirrhosis symptoms such as hematemesis and/or melena. It may be connected to other autoimmune conditions like diabetes, which can result in hormonal imbalance and disruption of the hypothalamic-pituitary-gonad axis, ultimately impairing sexual function (61).

4. Mechanisms of CLDs related ED

4.1. Gonadal dysfunction

The liver is the largest metabolic organ in the human body, and it is also the primary site of sex hormone metabolism. It is well known that CLDs patients exhibit significant feminization, including gynecomastia, fat redistribution, loss of body hair, and other symptoms, as a result of hypogonadism and excessive estrogen production (8). Up to 90% of patients with cirrhosis have lower testosterone levels (62), which correlate with disease severity, and these patients tend to lose their circadian rhythm in testosterone levels. In the compensatory stage of liver disease, sex hormones may not change noticeably, but as liver function deteriorates to a decompensated stage, patients may present with testicular atrophy and interstitial fibrosis, which reduce the synthesis and secretion of testosterone.

The precise mechanisms underlying the associations between gonadal dysfunction and CLDs remain poorly understood. Testicular atrophy is present in more than 50% of male patients with cirrhosis. It may manifest histological abnormalities such as atrophy of the testis germ epithelium, thickening of the tubule basement membrane, and fibrosis of the leydig. In addition to testicular atrophy, CLDs may cause gonadal dysfunction *via* a variety of mechanisms. As is well known, as liver disease progresses, the liver's inactivation effect on estrogen weakens, and the transformation of testosterone and estrone into estradiol increases, leading to an increase in serum estradiol level, which inhibits pituitary gonadotropin release and reduces androgen secretion by testicular interstitial cells (62). In patients with NAFLD, the hypotestosteronemia can be explained by the hypogonadal-obesity-adipocytokine hypothesis (63). An increase in visceral adipose tissue raises the activity of the aromatase enzyme, which can convert testosterone to estrogen and decrease testosterone levels. Low testosterone levels then enhance the activity of the lipoprotein lipase enzyme, which promotes triglyceride uptake into adipocytes and further increases visceral adiposity, resulting in a vicious cycle (64). In addition, pro-inflammatory adipocytokines released from adipose tissue, including tumor necrosis factor-α,

interleukin-1, and interleukin-6, have been shown to inhibit the pituitary axis, which in turn reduces testosterone levels (65). It had previously been demonstrated that ED caused by non-alcoholic steatohepatitis-induced testosterone deficiency was associated with increased TNF-α (66). The decreased activity of 17-β hydrogenase in the liver of CLDs patients reduces the conversion of androstenedione to testosterone, which is also one of the reasons for testosterone deficiency. However, what calls for special attention is that physiological decline should also be fully taken into account when analyzing testosterone levels in patients with CLDs, testosterone in men decays at a rate of 10% every decade after the age of 30 (67). ED may occur due to hormonal imbalances. Reduced testosterone levels can affect erectile function *via* multiple pathways, including increased apoptosis of smooth muscle cells and endothelial cells, resulting in decreased compliance and hemodynamic abnormalities of the corpus cavernosa; inhibition of the eNOS-No-cGMP pathway; and activation of the Rho A/Rho kinase pathway, resulting in decreased smooth muscle contraction and corpus cavernosum blood perfusion. In patients with cirrhosis, however, oral androgens may not increase serum testosterone levels but instead increase estrogen levels (8). Oral testosterone seemed to have no positive effect on sexual dysfunction in patients with cirrhosis, and androgen supplementation was only effective in cases of severe hypogonadism with testicular atrophy (51), implying that the main mechanism for ED may not be limited to total testosterone levels.

Although total testosterone levels are significantly lower in CLDs patients with ED, they were not significantly associated with the occurrence and severity of ED in multivariate analyses, raising the question of whether total or bioavailable testosterone is the more meaningful test method. Testosterone is mainly bound to albumin (50%), sex hormone-binding globulin (45%), and free testosterone (2%). Bioavailable testosterone includes albumin-bound testosterone and free testosterone. According to a meta-analysis, free testosterone levels were significantly lower in ED patients than in the non-ED group (17). Patients with hepatic insufficiency frequently have higher levels of estrogen and lower levels of testosterone, which can result in an increase in the production of sex hormone-binding globulin through a negative feedback loop. That then results in a higher binding between testosterone and sex hormone-binding globulin, further reducing bioactive free testosterone (62, 68). A hypothesis was proposed that low serum albumin could affect the ratio of free albumin to bound testosterone, thereby altering the testosterone response (51). In conclusion, free or bioactive testosterone may be a better marker of ED.

4.2. Drugs or medications

Drugs or medications can induce or aggravate ED in patients with CLDs.

4.2.1. Alcohol

Alcohol abuse is a well-known risk factor for the development of alcoholic cirrhosis (69), as well as a risk factor for ED in the general population (70). In alcoholic and non-alcoholic cirrhosis, the prevalence of ED is 70 and 25%, respectively. Alcohol can cause nutritional metabolism disorders and damage to multiple systems and organs, such as the gonads and nerves, altering the balance between linked and available steroid hormones, with the final effect being detrimental to normal sexual function. Martinez-Riera et al. (71) found that the levels of basal SHBG, androstenedione, estradiol, and prolactin in patients with alcoholic liver disease were higher than those in the control group, regardless of the presence of cirrhosis, and tended to increase the levels of FSH and LH. Abstaining from alcohol is effective for the recovery of gonadal dysfunction to a certain degree if these patients do not have testicular atrophy or a poor response of gonadotrophins to luteinizing hormone-releasing factor or clomiphene. A decreased responsiveness to androgens in alcohol addicts has not been verified because the androgen receptors may present some structural alterations limiting regular function. But Maimone et al. (34) observed no significant relationship between the prevalence or severity of ED and alcohol consumption. This study, however, cannot rule out the association between alcoholic cirrhosis and ED. This could be due to the relatively small sample size of patients with alcohol-related cirrhosis and heavy drinkers in this study, or to multiple coexisting factors that offset the potential difference in the prevalence of ED based on alcohol consumption.

4.2.2. Non-selective β receptor blockers

Non-selective β receptor blockers, such as propranolol and carvedilol, which are widely used to treat portal hypertension, have been shown to have a negative effect on erectile function (72), increasing ED by 2.32 times (18). The mechanism may be that non-selective β receptor blockers reduce adrenergic efflux by inhibiting angiotensin II, renin, and vasodilation (39).

4.2.3. Antidepressants

Some patients with CLDs are accompanied by varying degrees of depression, which *per-se* can lead to ED. Chronic use of antidepressants, no matter monoamine oxidase inhibitors, tricyclic drugs, or selective serotonin reuptake inhibitors (SSRIs), can cause further exacerbation of ED symptoms. The incidence of ED varies with different SSRIs. A multicenter survey conducted by the United States of 1,763 male depressed patients treated with SSRIs found that the incidence of sexual dysfunction ranged from 7 to 30%, with the highest incidence of paroxetine (34%) and the lowest incidence of fluoxetine (7%) (73). This adverse side effect could be caused by serotonin, dopamine, acetylcholine, prolactin, NO, or another transmitter (74).

4.2.4. Diuretics

Among diuretics, spironolactone has anti-androgenic properties due to its similar structure to sex hormones. Therefore, it can competitively inhibit dihydrotestosterone's binding to androgen receptors and enhance testosterone clearance, which in turn leads to decreased libido and ED.

4.2.5. Interferon

Interferon, a commonly used medicine for the treatment of viral hepatitis, can cause patients to be depressed and prone to decreased libido and sexual dysfunction (75, 76). This malfunction generally returns to normal following the course of treatment and is not a negative side effect of interferon *per-se* (77). But there are too few studies to clarify the issue or figure out whether the condition is related to interferon-induced depression or specific hormonal changes.

In addition, immunosuppressive therapy with steroid agents such as azathioprine, cyclosporine A, or tacrolimus can affect the hypothalamic-pituitary-gonadal axis by inhibiting androgen synthesis, leading to ED in liver transplant patients (78). In the treatment of CLDs, the incidence of ED is higher when the drugs aforementioned are used in combination.

4.3. Insulin resistance

IR is associated with hepatic fat deposition and is significantly increased in patients with NAFLD (79). The accumulation of lipid oxidation products in cells may interfere with the insulin signaling pathway *via* the phosphatidylinositol 32 kinase (PI32K), thereby affecting the transport function of glucose transporter 4, resulting in IR. IR can harm the vascular system through a variety of mechanisms, causing vascular dysfunction or structural destruction, such as atherosclerosis (80, 81), and eventually leading to ED (Figure 1). In the meantime, eNOS deficiency aggravates the early stage and accelerates the progression of NAFLD (82). This is a vicious circle, and it could be one of the mechanisms by which NAFLD leads to ED.

4.4. Anemia

Anemia is common in patients with decompensated CLDs, and ED is also associated with lower hemoglobin levels. Low serum hemoglobin levels may indicate advanced liver disease and severe portal hypertension. Anemia can exacerbate the cirrhosis-related hyperdynamic circulation (34), which is characterized by visceral vasodilation and peripheral vasoconstriction. This might impair penile circulation and lead to ED (83). Furthermore, anemia reduces the supply of oxygen to tissues, including the corpus cavernosum, potentially impairing erectile function (84).

In addition to the aforementioned mechanisms, oxidative stress, inflammatory response, apoptosis, and other factors are also involved in the occurrence of CLDs-related ED (85, 86). Therefore, the mechanism of ED caused by CLDs is very complex and differs from that of the general population in some aspects. These distinct causes must be fully considered. Unfortunately, there are few studies on its concrete mechanisms, such as signaling pathways, that have been retrieved so far. More in-depth studies are needed to uncover the distinct mechanisms by which CLDs lead to ED.

5. Pharmacotherapy

There are few reports on the treatment of CLDs-related ED, the improvement of ED symptoms in these patients is mostly found during treatment of the primary disease, such as liver transplantation, and so on. Evidence on the efficacy and safety of using specific drug therapies to treat ED in this population is scant at best (87). The few existing studies have focused on phosphodiesterase type 5 (PDE-5) inhibitors, which are recommended as first-line medicines for the treatment of ED in men. We assume that the following are the primary causes of this situation: (a) Patients with CLDs are eager to solve their liver problems first and rarely consider or feel embarrassed to mention their erectile dysfunction problems while visiting hepatologists; (b) both patients and hepatologists are concerned about the adverse side-effects of related drugs on liver disease, as well as the contraindications of their compatibility with drugs for liver disease.

PDE-5 inhibitors increase intracellular cGMP concentration by inhibiting the activity of PDE-5, thereby improving the relaxation function of corpus cavernosum smooth muscle cells, resulting in smooth muscle relaxation, increasing the blood flow of the intracavernous arteries, and finally causing the penile erection. Sildenafil has been used for the treatment of porto-pulmonary hypertension in liver transplant patients in order to ensure the success of transplantation (88). Several studies have demonstrated the safety and efficacy of sildenafil citrate in patients with liver cirrhosis and porto-pulmonary hypertension, reporting that it did not affect portal pressure or hepatic venous pressure gradient in patients with compensatory liver cirrhosis, although it resulted in lower arterial pressure. These studies had also shown that PDE-5 inhibitors could be safely used in Child's A and B cirrhosis, and they might be considered as a treatment option for CLDs patients (89, 90), but these studies had not documented the efficacy in patients with cirrhosis. Thakur et al. (87) conducted a prospective study in which 25 cirrhotic patients with Child-Pugh scores between 5 and 10 were enrolled. After administration of 10 mg of Tadalafil per day for 4 weeks, IIEF scores were significantly improved in all patients,

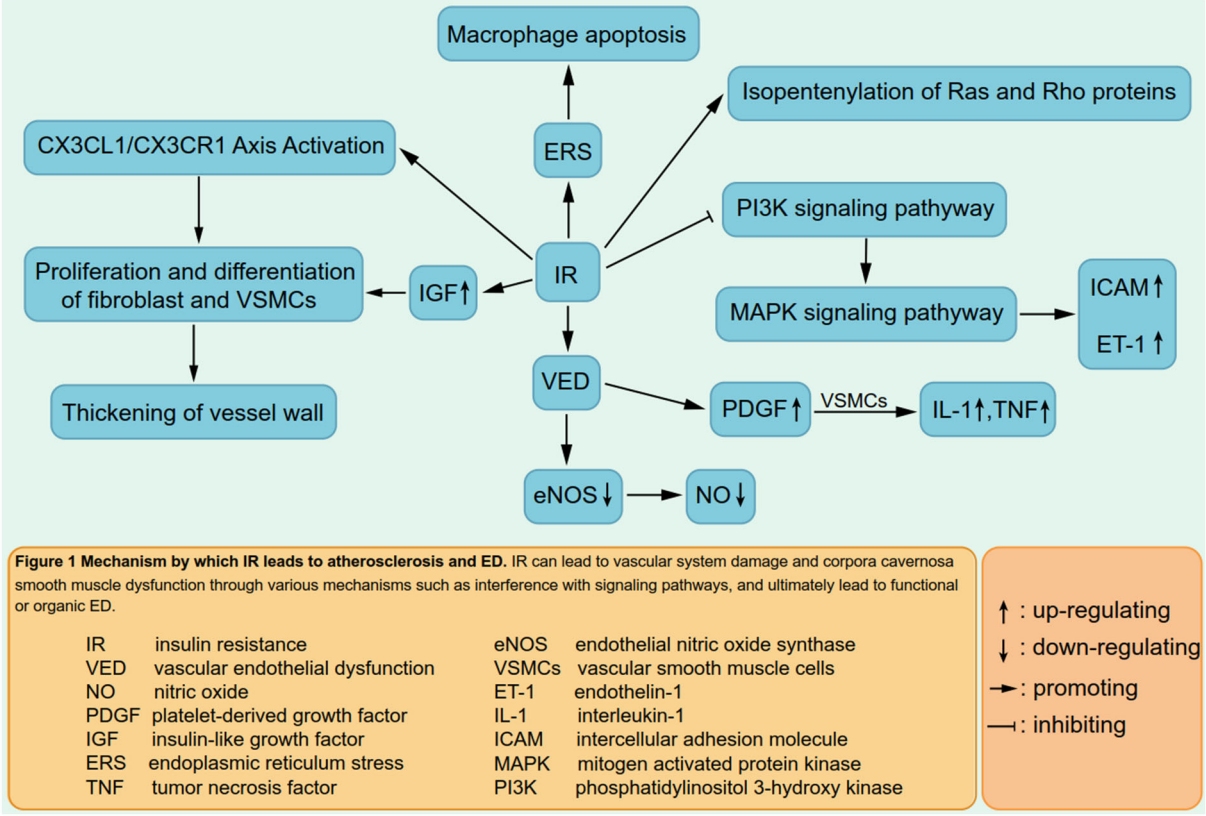

FIGURE 1
Mechanism by which IR leads to atherosclerosis and ED. IR can lead to vascular system demage and corpora cavernosa smooth muscle dysfunction through various mechanisms such as interference with signaling pathways, and ultimately lead to functional or organic ED.

and 44% of the patients had complete resolution of ED. Those with lower IIEF scores and more severe ED showed the most significant improvement. The area under the curve of tadalafil in subjects with mild and moderate hepatic impairment is similar to that in healthy subjects, and tadalafil is well tolerated with few significant side effects, so it can be safely used in these patients without dose adjustment, but there is no sufficient evidence of its effect in patients with severe hepatic impairment (27). This is mainly because, for safety reasons, these patients have generally been excluded from randomized clinical trials. A study of severe hepatic dysfunction has been retrieved, patients with ED in compensated CLD and advanced fibrosis were treated with tadalafil orally, 20 mg on alternate days. It was discovered that erectile function improved significantly in all patients after 12 weeks of treatment, with only 25% of patients still having ED at 6 months of follow-up (91). However, there were some problems with this study. First, the sample size was small and the dropout rate was high, only 23 of the 34 patients completed the 3-month follow-up and only 8 completed the 6-month follow-up; second, and most importantly, the study did not indicate how many patients with severe hepatic impairment completed the study. A decrease in liver stiffness measurement and fibrosis index based on 4 factor values were also observed in this study, possibly due to the antifibrotic effect of tadalafil (92).

PDE5-I can protect the liver from ischemia-reperfusion injury through a variety of signaling pathways, such as increasing intracellular cGMP levels, activating protein kinase C and protein kinase G (93), leading to the activation of cGMP-dependent protein kinase, which in turn induces vasodilation, anti-inflammatory and anti-proliferative effects, and reduces collagen synthesis (94, 95). Pretreatment with tadalafil can protect against thioacetamide-induced liver fibrosis in a dose-dependent manner and reduce biomarkers of inflammation and fibrosis (96, 97). Therefore, the use of PDE-5 inhibitors in patients with severe hepatic insufficiency is theoretically beneficial.

Through a comprehensive analysis of the limited literature, we believe that PDE-5 inhibitors can be safely used in patients with mild to moderate hepatic impairment, and due to the limited information on the clinical safety of this drug in patients with severe hepatic insufficiency, a lower starting dose of PDE-5 inhibitors should be considered. To date, it is unclear which PDE-5 inhibitor is more effective. Although two meta-analyses suggested that tadalafil and sildenafil were equally effective

(96, 97) and significantly better than vardenafil, another meta-analysis failed to show this significance (98). On account of its advantages in half-life and compliance, in particular, the pharmacokinetics of sildenafil citrate are affected by kidney and liver injury (91, 99), tadalafil may be more acceptable to clinicians and patients.

In the available literature, the most detailed protocol for the use of PDE-5 inhibitors according to the extent of liver function impairment comes from Neong and his colleagues (8). In order to improve the quality of life in the long term, larger studies and randomized trials are needed to further explore the efficacy, safety, and dosage of PDE-5 inhibitors in patients with CLDs. We also hope that other methods for treating ED, such as psychotherapy, combined androgen therapy, vacuum suction, functional electrical stimulation therapy, etc., will be applied to the study of CLDs-related ED so as to find more ways for these patients to improve their sexual satisfaction. In addition, the treatment of ED in patients with CLDs is challenging because it is often multifactorial and may require multidisciplinary involvement.

6. Conclusion

CLDs patients with ED are an overlooked group by both patients and doctors alike. At present, only a handful of related reports can be retrieved worldwide. Although findings vary from study to study and even conflict, most researchers believe that ED is common in patients with CLDs, irrespective of etiology, and that there is a correlation between CLDs and ED. Therefore, the erectile function and sexual satisfaction of CLDs patients should arouse the attention of clinicians. We strongly recommend a routine screen for ED in men with CLDs.

The mechanism by which liver damage affects penile erectile function is not fully understood. Through the analysis of literature results, we believe that this is multifactorial. Male patients with CLDs often have decreased testosterone levels and increased estrogen levels, as well as other sex hormone metabolism disorders. The resulting changes in penile tissue structure and the NO-cGMP pathway may be one of the mechanisms of ED in these patients. Age, liver function classification, hypohemoglobinemia, hypertension, diabetes mellitus, alcohol consumption, mental state, and certain therapeutic medicines may be risk factors for ED in patients with CLDs. While treating the primary disease, we should actively remove the risk factors to reduce the occurrence of ED.

There are few reports on the treatment of ED associated with CLDs. In addition to the treatment of the primary disease, short-term, low-dose PDE-5 inhibitors are safe and reliable. Whether combined androgen therapy can increase its efficacy is still controversial. We look forward to more relevant studies to provide data for reference.

Author contributions

The epidemiology, mechanisms, pathophysiology, and treatment of ED in CLDs patients are discussed in detail by all the authors. All the authors we list have made direct or indirect contributions to this work and approve its publication.

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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.

Publisher's note

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Advances in physical diagnosis and treatment of male erectile dysfunction

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Erectile dysfunction (ED) is the most common male sexual dysfunction by far and the prevalence is increasing year after year. As technology advances, a wide range of physical diagnosis tools and therapeutic approaches have been developed for ED. At present, typical diagnostic devices include erection basic parameter measuring instrument, erection hardness quantitative analysis system, hemodynamic testing equipment, nocturnal erection measuring instrument, nerve conduction testing equipment, *etc.* At present, the most commonly used treatment for ED is pharmacological therapy represented by phosphodiesterase five inhibitors (PDE5i). As a first-line drug in clinical, PDE5i has outstanding clinical effects, but there are still some problems that deserve the attention of researchers, such as cost issues and some side effects, like visual disturbances, indigestion, myalgia, and back pain, as well as some non-response rates. Some patients have to consider alternative treatments. Moreover, the efficacy in some angiogenic EDs (diabetes and cardiovascular disease) has not met expectations, so there is still a need to continuously develop new methods that can improve hemodynamics. While drug have now been shown to be effective in treating ED, they only control symptoms and do not restore function in most cases. The increasing prevalence of ED also makes us more motivated to find safer, more effective, and simpler treatments. The exploration of relevant mechanisms can also serve as a springboard for the development of more clinically meaningful physiotherapy approaches. Therefore, people are currently devoted to studying the effects of physical therapy and physical therapy combined with drug therapy on ED. We reviewed the diagnosis of ED and related physical therapy methods, and explored the pathogenesis of ED. In our opinion, these treatment methods could help many ED patients recover fully or partially from ED within the next few decades.

KEYWORDS

erectile dysfunction, physical diagnosis, physical treatment, pathophysiological mechanisms, pharmaco penile duplex ultrasonography, dual-energy CT Arteriography, penile cavernosography, low-intensity extracorporeal shock wave therapy

1 Introduction

Erectile dysfunction (ED) is defined as the inability of the penis to maintain or achieve sufficient erection hardness to satisfy satisfactory sexual performance and lasts for more than 3 months (Salonia et al., 2021), and easily be ignored by many clinical doctors. Statistic data indicates that the number of ED would rise to 322 million by 2025 globally (Ismail and El-Sakka, 2016). According to data, the prevalence of ED is 5.1% in men aged 29–30, 14.8% in men aged

40–59, 44% in men aged 60–69 (Calzo et al., 2021), and more than 50% of men over 70 are diagnosed with ED (Shamloul and Ghanem, 2013). This trend is consistent with the rise in life expectancy.

Many factors contribute to ED. Most current studies believe that ED is mainly caused by organic factors (neurogenic, vascular, diabetic, *etc.*), psychological factors (performance anxiety, stress, and mental disorders), iatrogenic factors (caused by surgical injury), and increasing age (aging) (Hellstrom et al., 2010; Chung et al., 2011; Wang, 2011; Shridharani and Brant, 2016).

At present, the clinical diagnosis methods of ED mainly include questionnaire surveys, psychological assessments, laboratory, and equipment examinations (Xiong et al., 2022). Some experts believe that clinicians should first conduct a comprehensive and targeted physical examination and questionnaire survey for patients with suspected ED (Zhang et al., 2014). The comprehensive questionnaire survey is the primary condition to help doctors diagnose ED and decide on the treatment plan. The most widely used questionnaire to evaluate male sexual function is the International Index of Erectile Function (IIEF)-5 (Vickers et al., 2020).

Currently, the treatment methods for ED mainly include lifestyle adjustment, psychotherapy, drug therapy, physical therapy, and surgical therapy (Liu et al., 2020a). However, the effect of lifestyle adjustment therapy is not obvious in the treatment of ED, and there is a lack of interventional studies (Yafi et al., 2016). The high cost of counseling and the uncertainty of efficacy of psychotherapy pose difficulties for most patients (Emanu et al., 2016). Because each person's anxiety factors are different, there are no standardized protocols for psychosomatic pharmacological treatment of ED (Melnik et al., 2011). The efficacy of drug therapy is currently positive, but some patients do not respond to the drug, such as patients with severe vascular ED, diabetic ED or neurogenic ED. Besides, some adverse effects also limit the application of drug therapy in ED patients (Brock et al., 2002). Few patients choose surgical therapy because of the high cost and risks. Taking prosthetic implants for example, it costs more than $20,000 and have a high risk of infection (Stephenson et al., 2005). Therefore, physical therapy has become the choice of more and more ED patients because the relatively certain efficacy and the acceptable cost. Given the increasing prevalence and low overall diagnosis rate of ED, we review the pathophysiological mechanism as well as the benefits and drawbacks of standard clinical diagnostic equipment and physical therapy device for ED in order to give physicians a better systematic understanding of the diagnosis and physical treatment of ED.

2 Pathophysiological mechanism

2.1 Organic ED

2.1.1 Neurogenic ED

The central regulation of erectile involves various primary afferents, spinal interneurons, sympathetic nerves, parasympathetic nerves, and so on (Agochukwu-Mmonu et al., 2020). Neurological disorders may lead to abnormalities in the endocrine system or the cardiovascular system, which can affect sexual function (Agochukwu-Mmonu et al., 2021). Approximately 10%–19% of the etiology of ED can be classified as neuropathy, which may be central, peripheral, or both (Del Popolo et al., 2020). The erection process requires stimulation of the hypothalamus received by the tactile, visual, and auditory sense organs followed by signals transmitted by neurons. This process may require an entire neural pathway (autonomic nervous system), so damage to any point in that neural pathway that disrupts the transmission of signals may result in ED (Giuliano, 2011). The study of Hicks et al. (Hicks et al., 2021) proved that peripheral neuropathy affected male erectile function to some extent, and they mentioned the highly overlapping relationship among peripheral neuropathy, ED, and cardiovascular diseases, which proved that peripheral neuropathy was a new risk factor of ED. Neuropathy can also be caused by being overweight, studies have shown that compared with normal weight, obesity in peripheral neuropathy (especially small nerve fibers lesions) is the more common form, Herman et al. (Herman et al., 2007) confirmed that corneal nerve fiber density and length are associated with the diagnosis of ED, but has no obvious relation with the severity of ED. Neurological injury due to trauma can also cause ED, depending on the degree of neurological injury and the integrity of the nerve. Trauma can cause damage to the cavernous nerve, the axonal density and conduction velocity of this nerve will be reduced, which leads to the occurrence of ED (Yin et al., 2013). Sympathetic and parasympathetic nerves are involved in the erection process of the penis, emanating from the lumbar spine and sacral root. Damage to these nerves blocks the conduction of corresponding nerve signals, directly leading to ED (Giglia and Stein, 2019). ED is involved in many surgical complications in clinical practice. For example, one of the most common complications after radical prostatectomy is ED, which is usually caused by intraoperative injury of the cavernous nerve (Lima et al., 2021). This nerve injury induces protein 1, Ninjurin-1, to participate in the neuroinflammatory response, which resulted in ED (Yin et al., 2013). Long-term postoperative complications of colorectal surgery due to autonomic nerve injury in the pelvis also include ED (Giglia and Stein, 2019). In a prospective study of 50 subjects by Hande Gokce, the incidence of ED after rectal cancer surgery was found to be about 10%–35%. The etiology of these ED patients is considered to be related to vascular nerve damage during the rectal surgery. (Gokce and Ozkan, 2019).

2.1.2 Diabetic ED

Diabetes mellitus is a metabolic disease with hyperglycemia caused by defective insulin secretion, defective insulin action, or both (Cloete, 2022) (Figure 1). Persistently high blood glucose levels can lead to nerve and blood vessel damage, cardio-cerebral circulatory complications, and even death (Faselis et al., 2020). Diabetes is considered as a major risk factor for ED, and the association between diabetes and the development of ED has been documented in animal models and humans since 1970 (Gur et al., 2014). Chronic hyperglycemia may lead to impaired nitric oxide (NO) synthesis and cycloguanosine monophosphate (cGMP) pathway, increased reactive free radical level, upregulated of RhoA/Rho kinase pathway, and damaged nerve function, which may be the mechanisms of ED in diabetic patients (Gurbuz et al., 2022). The persistent state of hyperglycemia will lead to an increase in advanced glycation end products (AGEs), which are the final products of amino non-enzymatic glycation of proteins, lipids, and nucleic acids in human tissues. Increased expression of AGEs in the corpus cavernosum of diabetic patients may lead to changes in tissue structure, such as thickened vascular walls, decreased elasticity, endothelial dysfunction, and atherosclerosis. This process can produce overloaded peroxynitrite, which can lead to oxidative damage to a number of

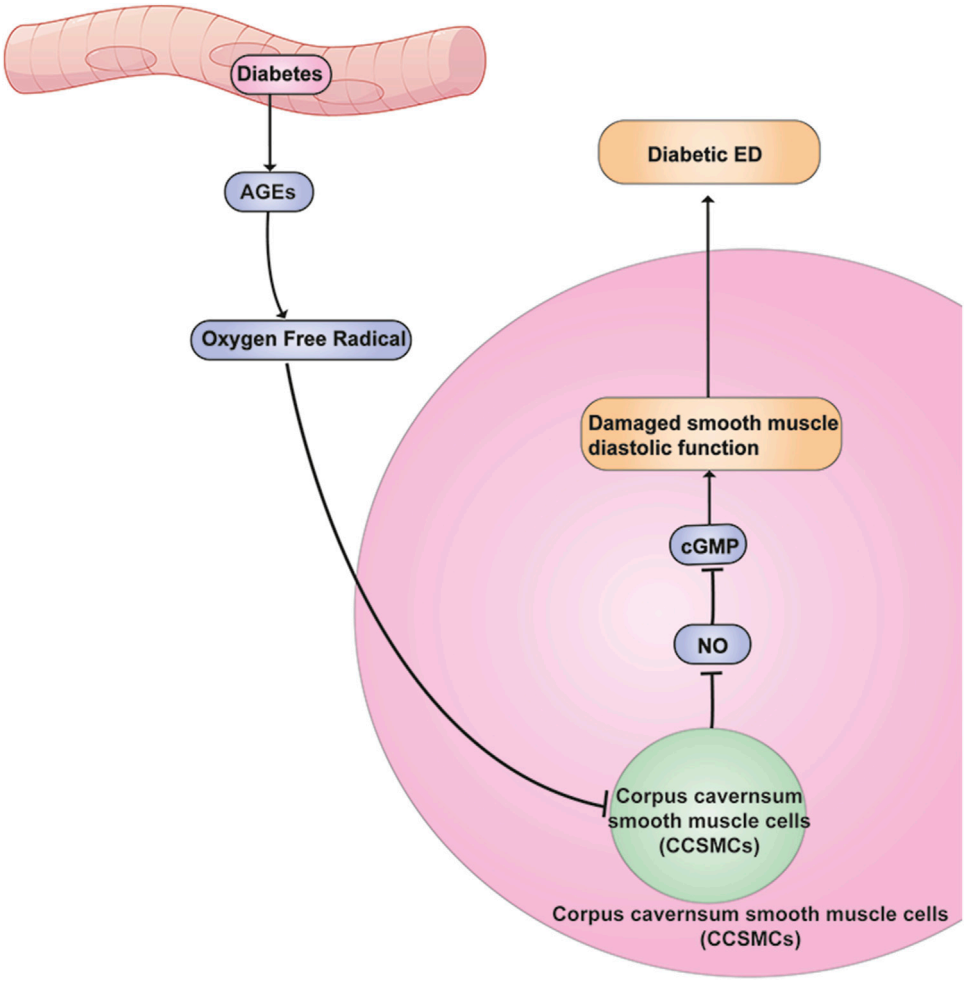

FIGURE 1
Mechanism of chronic hyperglycemia leading to ED. Elevated blood glucose leads to elevated AGEs, and their elevation leads to damage to cavernous smooth muscle cells affecting the diastolic function of this smooth muscle, which in turn leads to ED.

important biomolecules, resulting in ED (Trebaticky et al., 2019). The cGMP mainly induces the relaxation of cavernosal vascular smooth muscle through cGMP dependent protein kinase-1 (PKG-1) changing the levels of intracellular and extracellular calcium and potassium ions. Some researchers believe that the occurrence of ED in patients with diabetes is related to the reduction of cGMP and the impaired relaxation of cavernosal smooth muscle due to the oxygen free radicals produced by AGEs induced related cellular oxidative damage and the quenching of NO (Thorve et al., 2011). Nerve damage caused by chronic hyperglycemia can affect different sensory patterns as well as autonomic function (Sharma et al., 2020). Some sensory diagnostic devices can be used to evaluate sensory functions such as vibration perception, pressure perception, and heat perception, so as to improve diagnostic sensitivity in clinical practice (Freeman, 2014). Morning testosterone levels can also be used as an auxiliary diagnosis method of diabetic ED. Diabetes-associated ED patients often have low morning testosterone level (Gianatti and Grossmann, 2020). However, diabetes is not the unique etiology of low testosterone levels, therefore, it is critical to identify other diseases that can affect testosterone levels, such as endocrine disorders and urinary system diseases. (Onyeji and Clavijo, 2022).

2.1.3 Vasogenic ED

ED can be caused by a variety of vascular factors, such as atherosclerosis, arterial injury, and stenosis, penile venous fistula (Hoppe and Diehm, 2020; Sayadi et al., 2021; Wang et al., 2021). The role of monocyte/macrophage accumulation in vascular disease is not negligible (Davis and Gallagher, 2019; Miyata et al., 2021). Macrophages can stimulate plaque formation in blood vessels and play an important role in vascular injury (Marchio et al., 2019). Abnormal lipid metabolism and monocyte/macrophage interactions can also accelerate the formation of atherosclerotic plaques, which is closely related to the development of ED (Randrup et al., 2015). It has been shown that macrophages can affect endothelial function through macrophage-derived myeloperoxidase (MPO) - dependent ox-LDL (Mox-LDL) (Boudjeltia et al., 2013). MPO can promote impaired endothelial function and intravascular plaque instability, and Mox-LDL can stimulate macrophages to produce reactive oxygen species (ROS) and secrete cytokines to affect endothelial function (Calay et al., 2010). Endothelial damage may alter the state of blood flow within them, which in turn leads to ED (Salvio et al., 2021). Miner et al. (Miner et al., 2012) showed that vasogenic ED preceded coronary heart disease in younger ED patients. The pathology of arterial ED is

atherosclerosis of the internal pudendal artery, which prevents the corpus cavernosa from receiving sufficient blood flow to achieve erectile status (Pereira et al., 2013). Studies have shown that as vascular smooth muscle cells proliferate, collagen and fibrosis increase, leading to the thickening of the vessel wall and narrowing of the lumen. This made a decrease in blood flow to the penis, and resulted in ED (Hannan et al., 2011). The main diagnostic methods of vascular ED include color dual Doppler ultrasound, selective penile angiography, magnetic resonance imaging, and intravascular injection of vasoactive drugs (Ma et al., 2020).

2.2 Psychogenic ED

Psychological problems such as anxiety, stress, and mental disorders can significantly affect the development of ED (Tan et al., 2012), there is a higher risk of ED in people with psychosis who are at very high risk (Bourdeau et al., 2012). Various psychotropic drugs are widely used in young adults with psychosis-related ED. Some studies have shown that some antipsychotic drugs can affect the dopamine D2 receptor pathway, which in turn affects erectile function. (Reichenpfader et al., 2014; Chen et al., 2019). The study of Macdonald et al. verified that psychological problems such as low self-esteem, emotional retardation, and sleep disorders not only directly affected the sexual function of patients, but also showed that the severity of mental problems was positively correlated with the severity of ED (Macdonald et al., 2003). People who experience problems with erectile function are more likely to develop anxiety, which feeds back on them over time, leading to ED.

3 Physical diagnosis of ED

The incidence of ED is increasing over years, (Matz et al., 2019). Aging, smoking, and unhealthy lifestyle are all risk factors for ED. The diagnosis of ED is still in the process of being improved, for the purpose to achieve early detection and diagnosis, in order to draw up better treatment plans for ED patients. With the increasing demand for quality of life, sexuality must also be considered.

3.1 Basic parameters of penile erection

The basic parameters of penile erection, including the length and circumference of the penis and the temperature of the head during erection, can be easily obtained by a ruler. These parameters can be used as a diagnostic method for those who were suspected with ED, but should not be used alone as evidence for the diagnosis of ED.

3.2 Nocturnal penile tumescence (NPT)

NPT times are one of the reliable methods to distinguish psychological ED from organic ED. In clinical practice, many methods can be used to measure NPT, such as sleep laboratory testing, stamp tests, nocturnal electrobioimpedance volumetric assessment, and the Mercury strain (Zou et al., 2019). However, these methods have many obvious drawbacks such as being time-consuming, the inability to objectively analyze the causes and only arrive at yes and no results, and the possibility of other reasons affecting the test results during the test (Qin et al., 2018a). Until 1985, Bradley et al. invented Rigiscan, a portable device that measures penile circumference, axial and circumferential dilation rates, penile erection frequency, duration, and penile stiffness (Bradley et al., 1985). Zhang et al. found that the erection duration obtained by Rigiscan can be used to distinguish between arterial and venous ED, with a sensitivity of 81.4% and a specificity of 100% at the cutoff value of 12.5 min while predicting venous ED (Zhang et al., 2020). However, whether NPT times measured by Rigiscan are reliable in distinguishing psychological from organic ED remains debatable (Jannini et al., 2009). Some researchers believe that this method has many influencing factors, difficult to repeat, and increases the economic burden. Moreover, it can only make a simple distinction, and cannot identify the etiology of ED (Liu et al., 2020b). Wang et al. found that oral Phosphodiesterase inhibitor-5 (PDEi-5) concomitant with audiovisual stimulation, using Rigiscan to objectively assess penile swelling and tonicity, was a better way to differentiate between psychogenic and organic ED (Wang et al., 2018a).

3.3 Pharmaco penile duplex ultrasonography (PPDU)

Ultrasound assessment of ED was introduced in 1985 by Lue et al. (Lue et al., 1985), PPDU refers to color Doppler ultrasound combined with injection of intravascular active substances in the corpus cavernosum. The most used vasoactive agents are papaverine alone or a combination of papaverine, phentolamine, and prostaglandin 1 (PGE1) (Mutnuru et al., 2017). The advantage of PPDU is that it enables objective, minimally invasive assessment of hemodynamics at a relatively low cost (Golijanin et al., 2007). Blood flow to the penis can be assessed by color Doppler ultrasound, which can accurately assess venous leakage. Peak systolic velocity (PSV), end-diastolic velocity (EDV), and electrical resistance index (RI) are commonly used to evaluate arterial blood flow by ultrasonography (Jung et al., 2018). According to the standard operating procedures published by the International Society of Sexual Medicine in 2013, PSV is an accurate predictor of arterial disease in ED patients: PSV >30 cm/s and EDV<3 cm/s indicate normal arterial supply, while PSV<25 cm/s indicates arterial insufficiency. Venous occlusive dysfunction was defined as PSV >30 cm/s, EDV >6 cm/s, and RI <.6 (Sikka et al., 2013). Although PPDU can be used to identify arteriovenous ED, it has some limitations. It is complex, expensive, and censor-dependent; more importantly, the examination requires complete relaxation of the smooth muscle in order to truly reflect the vascular condition, and requires a relatively high volume of drug injection. Patients will inevitably experience tension and anxiety during the examination, which will lead to large errors in the examination results (Ma et al., 2020). It is these limitations that contribute to the high false positive rate of this inspection method (Caretta et al., 2019).

3.4 Selective internal pudendal arteriography (IPA) by digital subtraction angiography (DSA)

Selective IPA is a reliable and effective method for the diagnosis of arterial ED because it can show the morphological characteristics of the terminal branches of the internal pudendal artery (Wang et al.,

2019a). It can also help us to locate arterial lesions and assess the arterial blood supply by DSA technology (Wang et al., 2019a). However, this examination is an invasive examination, which may cause bleeding at the puncture site, pain to patients, and even arterial perforation, *etc.* Moreover, the addition of DSA technology will prolong the examination time and increase the patient's cost, making this technique rarely used in clinical practice.

3.5 Dual-energy CT arteriography (D-e CTA)

In recent years, dual-energy CT angiography has been more and more commonly used in the diagnosis and monitoring of male diseases (Rajiah, 2020). Dual-energy CT angiography is a novel, non-invasive and effective method to evaluate the penile arterial system, and shows high sensitivity and specificity in the diagnosis of arterial ED (Wang et al., 2022a). In 2001, Kalwanishi et al. used CTA and multi-slice CT for the diagnosis of ED and compared with DSA, proving their high accuracy and superior to DSA in the assessment of internal artery stenosis (Kawanishi et al., 2001). In addition, compared with DSA, CTA is relatively less invasive and cheaper. With more advanced imaging technology in the future, CTA can better surpass and replace DSA in the diagnosis of arterial ED.

3.6 Penile cavernosography

Penile cavernosography has been used to explore the venous system of the penile corpus cavernosum since the 1980s (Hsu et al., 2015). Up to now, penile cavernosography has been widely used to evaluate venous occlusive dysfunction in ED organic ED patients, and it has become the gold standard for the diagnosis of venous ED (Glina and Ghanem, 2013). Studies have shown that drug-induced cavernosography alone may lead to insufficient priapism, which is misdiagnosed as venous leakage. Dynamic continuous perfusion must be used to induce complete priapism in order to obtain more realistic detection results (Song et al., 2015). However, due to the influence of other veins, bones, or cavernous shadows, cavernosography cannot accurately display the target vein and accurately assess the leakage site (Xu et al., 2017a). Furthermore, because cavernosography is more invasive than PPDU, it is not recommended in some cases at which PPDU is sufficient to diagnose venous ED unless surgery or venous embolization is required. (Soylu et al., 2021).

3.7 320-Detector row dynamic volume CT (4D-CTA)

The 320-detector dynamic volume CT is composed of 320 detectors with a thickness of about .5 m and a width of about 16 cm along the *Z*-axis, and the gantry rotation time is 350 m (Hoe and Toh, 2009). Compared with DSA, 4D-CTA is less expensive and less time-consuming, involves no invasive procedures that could result in problems like thrombosis. (Gang et al., 2012; Biswas et al., 2015). 4D-CTA can instentlydisplay the flow of contrast agent in blood vessels following contrast agent injection t, and it can perform continuous volume scanning within a set time, which is used to estimate various conditions of hemodynamics and vessel morphology (Han et al., 2012; Biswas et al., 2015). Previously, 4D-CTA was frequently employed to identify myocardial ischemia and developing venous anomalies brought on by atherosclerotic heart disease (Dewey et al., 2009). In an article by Xu et al., they discovered that 4D-CTA was equally as accurate as CDDU in diagnosing arterial ED, with a specificity of 93.9% and an accuracy of 87.7%. (Xu et al., 2017b).

3.8 Discussion

The causes of ED are diverse, including arterial, venous, neurogenic, psychogenic, and medically induced injuries, which requires the availability of screening tools for each cause. Invasive tests such as PPDU and Penile cavernosography, even if they are less traumatic to the patient, still add to the patient's psychological burden and naturally have an impact on the diagnosis; other non-invasive tests are expensive and add to the patient's financial burden. For the above-mentioned examination methods, the author prefers dual-energy CT. First, it is a non-invasive examination method. This ensures that the patient can face the examination with a relatively calm state of mind and will not have much influence on the accuracy of the examination results. Secondly, dual-energy CT takes less time, shortens the patient's hospitalization period, and increases the patient's medical compliance. Finally, the accuracy and sensitivity of this test are high, which can accurately analyze the patient's lesion site and give clinicians more precise guidance on the direction of treatment. Therefore, we believe that the future development direction of diagnostic technology needs to March in the direction of convenient, affordable, and non-invasive examination. With the development of image technology, CT imaging technology will be greatly improved, and CT examination is relatively convenient, inexpensive, and non-invasive, which should become the mainstream choice for diagnosing ED in the future.

4 Physical therapy of ED

Currently, physical methods commonly used to treat ED include Vacuum erectile devices (VED), low-frequency electrical stimulation, low-intensity extracorporeal shock waves, Chinese acupuncture, and other treatment methods.

4.1 VED

Most VED consist of a shrink ring, a cylinder and a pump powered manually or by battery power (Beaudreau et al., 2021). It employs negative pressure to dilate the cavernous venous sinus, increase the perfusion of cavernous artery and venous blood, and ultimately achieve the goal of producing penile erection. An external constriction ring is placed at the base of the penis to prevent blood flow during intercourse in order to maintain an erection, but the ring should not be placed for more than half an hour (Yuan et al., 2010; Lin and Wang, 2013). It is used to promote the recovery of penis function and maintain penis length (Ma et al., 2021). Animal experiment by Ma et al. had demonstrated that the erectile response induced by the vacuum erection device may increase the smooth muscle/collagen ratio by decreasing hypoxia-inducible factor-1 and transforming growth factor-1, thereby improving penile blood flow (Yuan et al.,

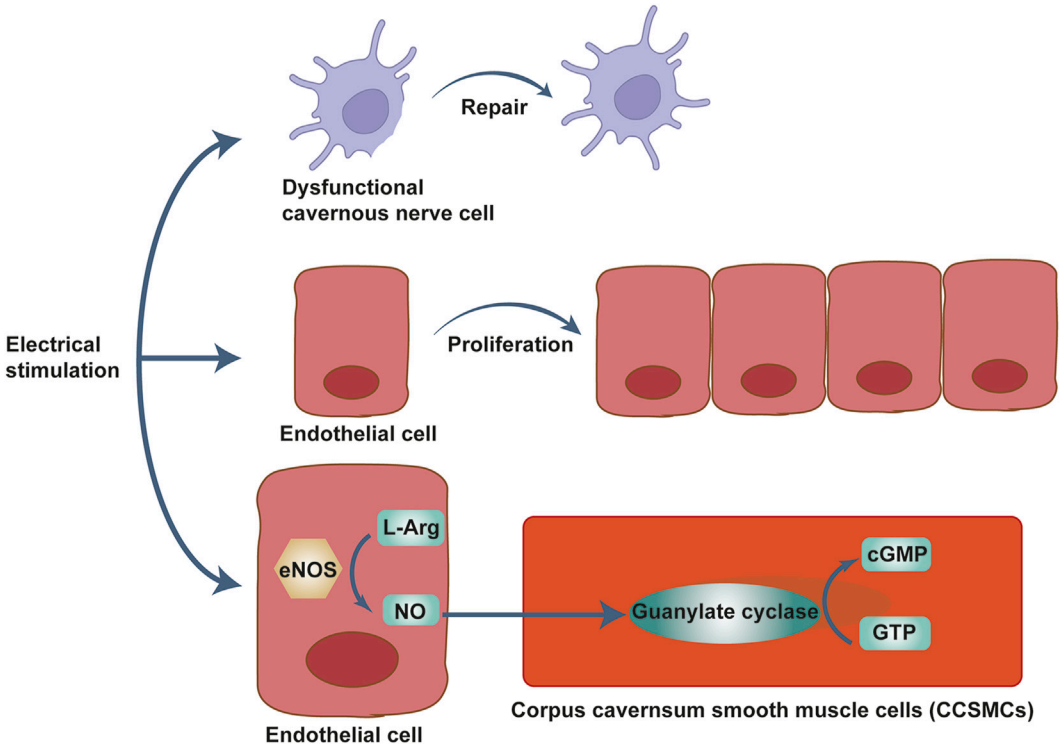

FIGURE 2
Mechanism of ES for the treatment of ED. Electrical stimulation treats erectile dysfunction by promoting cavernous smooth muscle proliferation, repairing cavernous nerves, and increasing endothelial cell NO.

2010; Brison et al., 2013). In addition, the device can only increase the oxygenation of the corpus cavernosum without the use of a shrink ring. This method can bypass the limitations of oral drugs and directly achieve an artificial erection, but it requires a normal and intact cavernous nerve to produce an erection (Lin and Wang, 2013; Beaudreau et al., 2021). Compared to other penile rehabilitation therapies, VED therapy has the advantages of being non-invasive and having fewer systemic side effects (Brison et al., 2013; Sultana et al., 2022) (Figure 2).

The study by Sherry A Beaudreau et al. found that the correct use of the device resulted in an erection to complete normal intercourse in 90.7% of patients (49 of 54 patients), all of whom indicated that they would recommend the device to other ED patients. Approximately 93.9% of patients reported that their quality of sex life was satisfactory or very satisfactory after treatment with the VED (Beaudreau et al., 2021). In a study by Khayyamfar et al., the erectile success rate of the subjects reached 100%, and it is verified that VED is not affected by the etiology of ED (diabetic, venous occlusion dysfunction, arterial origin, *etc.*) in achieving erection (Khayyamfar et al., 2014). Although most patients and their partners are satisfied with the device, studies had shown that the use of the device causes a lot of discomfort for some patients. For example, insufficient lubrication may lead to bruising of the penis, numbness and/or pain of the penis, cold sensation of the penis and inability to ejaculate (Brison et al., 2013; Lin and Wang, 2013; Beaudreau et al., 2021). About 25% of the patients in a study reported some physical discomfort during and after use of the device (Beaudreau et al., 2021). According to some studies, the negative pressure suction device also brings some psychological discomfort to patients, such as frustration and lack of autonomy (Brison et al., 2013; Lin and Wang, 2013; Beaudreau et al., 2021; Ma et al., 2021; Sultana et al., 2022).

VED has become a common method of postoperative penile rehabilitation (Lima et al., 2021). After radical prostatectomy, at least 85.8% of ED patients received penile rehabilitation, including VED (Tal et al., 2011; Lima et al., 2021; Zhang et al., 2022). Studies have demonstrated that ligation of the internal paraarteries of the genitals during radical prostatectomy may lead to nerve damage and decrease arterial inflow, which is a potential cause of ED after radical prostatectomy (Lin and Wang, 2013; Zhang et al., 2022). In terms of efficacy, Dalkin et al. conducted a study of 42 patients who had undergone nerve-preserving radical prostatectomy and discovered that only one of 36 patients who received VED had a penis length reduction more than 1 cm. Regular use of VED in the early postoperative period has been verified to be beneficial for the preservation of penile length (Karakus and Burnett, 2020). The Pajoovic et al. (Pajovic et al., 2017)study included 50 patients with type I and type II diabetes ED, treated with VED and found that 85% of patients had positive returns. Therefore, VED therapy can be used as an alternative to pharmacotherapyz (Dalkin and Christopher, 2007; Karakus and Burnett, 2020). Some studies have shown that VEDs can also be used in combination with laser illumination and are more effective than either alone (Moskvin and Ivanchenko, 2014).

VEDs are non-invasive and very effective in treating ED and improving sexual partner relationships, with a high success rate and very few side effects (Liu et al., 2017), particularly in patients with ED after radical prostatectomy. In ED patients after radical prostatectomy, early treatment with VED can significantly improve erectile function (Qin et al., 2018b). Common adverse effects of VEDs include penile

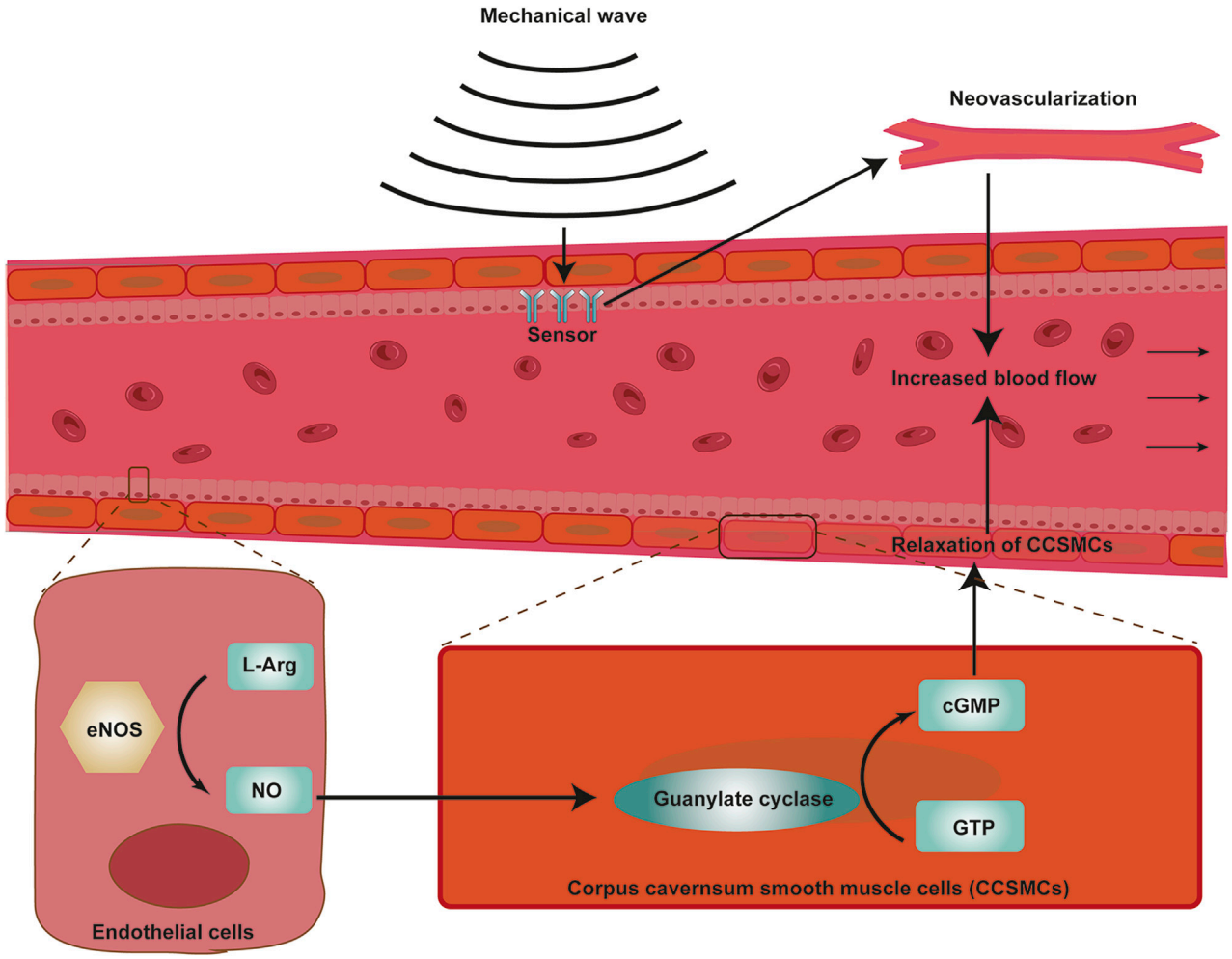

FIGURE 3
Mechanism of Li-ESWT for the treatment of ED. Mechanical waves stimulate the endothelial mechanosensors of the blood vessels, resulting in neovascularization. It also stimulates nNOS production in vascular endothelial cells, smooth muscle cells, and nerve cells, and cGMP production increases. Together, these promote increased blood flow to the penile corpus cavernosum and penile erection.

contusion due to improper use, especially in patients who are taking or have recently taken anticoagulants, penile numbness and/or pain, penile coldness, and ejaculation disorders (Fode et al., 2020). The device may also cause some psychological discomfort to the patient, such as frustration and feelings of lack of autonomy. Therefore, combining negative pressure suction device treatment with psychotherapy will be more effective. Moreover, the optimal duration of VED treatment for ED, the oxygen saturation in the cavernous body during treatment, and other factors need to be further studied.

4.2 Electrical stimulation (ES)

Electrical stimulation is one of the emerging technologies in clinical practice. It is a physical method that relies on the output of low-frequency pulsed current to treat diseases and is now widely used in urology, male surgery, gastrointestinal surgery, obstetrics, and gynecology, *etc.* (Qu et al., 2017; Wang et al., 2018b; Li et al., 2021). Especially in the field of treatment of ED, increasing numbers of studies have shown that the use of electrical stimulation techniques for ED can achieve positive outcomes (Figure 3).

According to some studies, the main principle of functional electrical stimulation for the treatment of ED include inducing penile endothelial proliferation and cavernous smooth muscle regeneration, promote NO release from cavernous endothelial cells (Gratzke et al., 2010), and producing cGMP, which can relax cavernous smooth muscle and raise cavernous body pressure (Hurt et al., 2002). According to the current state of clinical research, most studies are limited to the efficacy of functional electrical stimulation (FES) on ED. Some scholars believe that ES can stimulate peripheral nerve regeneration (Willand et al., 2016), which can dramatically improve the recovery of nerve function, and improve ED symptoms in turn. Regenerative electrical stimulation (RES) can treat ED patients with cavernous nerve damage through promoting nerve regeneration and restoring damaged nerve function (Balog et al., 2019). For example, in a study of a rat model of ED after nerve dissection, Shapira et al. found that short courses of electrical stimulation administered early in the course of nerve

injury promoted recovery of nerve function (Shapira et al., 2019). Mendez et al. found that RES after facial nerve injury in rats accelerated facial nerve function and improved regeneration of facial nerve-specific pathways, and that ES significantly increased brain-derived neurotrophic factor (BDNF) expression in the nucleus of the cell body of motor neurons after injury, promoting repair of damaged nerve cells (Mendez et al., 2018). In summary, the main mechanism of RES is the upregulation of BDNF and its receptor, tyrosine kinase B (trkB), in motor neurons (Al-Majed et al., 2000; Balog et al., 2019), BDNF and the binding of trkB can promote nerve regeneration and the recovery of damaged nerve function (English et al., 2014).

Carboni et al. (Carboni et al., 2018) initially investigated the effects of FES on ED. They found that after 4 weeks of FES treatment, the patients' IIEF-5 and Erection Hardness Score (EHS) significantly improved, indicating that FES had a positive therapeutic effect on ED. The study Shafik et al. also found that transcutaneous perineal ES can treat neurogenic ED (Shafik et al., 2008). Van et al. demonstrated that pelvic floor muscle function training combined with ES can produce positive results in the treatment of ED, with nearly half of the patients in the trial regaining normal erectile function (Van Kampen et al., 2003). Rislan et al. compared the therapeutic effects of ES and aerobic exercise on ED and found that ES was significantly more effective than aerobic exercise in the treatment of ED (Rislanu et al., 2020). Based on summarizing the existing relevant studies and experiments, due to the continuous progress of current electrical stimulation, ES for ED has increasingly obvious advantages, including: a. It is a simple and non-invasive physical therapy program (Lin and Chen, 2017); b. Clinically, ES treatment is much cheaper than other methods, and the patient compliance is relatively high (Azevedo Coste et al., 2017).

The advantages of low-frequency ES for ED include (Salonia et al., 2021): few side effects, non-invasive (Ismail and El-Sakka, 2016), easy operation; low cost, and a short single treatment time. The disadvantages include (Salonia et al., 2021): a lack of a systematic and standardized treatment plan, a lack of sufficient clinical case verification (Ismail and El-Sakka, 2016), the difficulty of applying individualized treatment for different causes. Therefore, the authors concluded that ES combined with akupunktur for ED could produce better results than ES treatment alone.

At present, akupunktur combined with electrophysiological technology is relatively mature. The update of equipment needs to closely match the development of electrophysiological technology, and ES treatment also requires more advanced techniques and equipment. It can be combined with drugs, multi-mechanism, and multi-target therapy. It can be used to treat erectile dysfunction by electrically stimulating the acupoints.

4.3 Low-intensity extracorporeal shock wave therapy (Li-ESWT)

Since its initial introduction in 2010, Li-ESWT has gained popularity as a treatment for ED (Vardi et al., 2010) (Figure 4). Li-ESWT can achieve the purpose of treating ED primarily by stimulating tissue repair and vascular regeneration (Stoykov et al., 2020). Li-ESWT was initially introduced as urinary system lithotripsy (Wang and Zhou, 2015), however, many studies reported the benefits of Li-ESWT in different medical fields such as musculoskeletal diseases, wound, and bone healing disorders, ischemic heart disease, and spastic tension (Dumfarth et al., 2008; Gadomski et al., 2018; Yue et al., 2021). At present, Li-ESWT has been widely used in the clinical treatment of ED.

The mechanism of Li-ESWT treatment for ED is still unknown. Its effects could be caused by the induction of mechanical stress, which could result in neovescularization, the recruitment of stem cells and growth factors, an improvement in blood flow, and nerve regeneration (Gruenwald et al., 2013; Mason et al., 2022). Some studies have shown that Li- ESWT can promote the expression of neuronal nitric oxide synthase (nNOS) in endothelial cells, smooth muscle cells, and nerve cells (Yao et al., 2022). However, according to some studies, Li-ESWT does not rely on nNOS and guanosine cyclic phosphate to improve erectile function (Assaly-Kaddoum et al., 2016). Up to now, no study has confirmed the specific mechanism of Li-ESWT in the treatment of ED.

Oginski n et al. Carried out a study in which 50 ED patients were treated with Li-ESWT once a week for 6 weeks. It was considered a successful treatment if the IIEF-5 score increased by ≥ 5 points or the erectile stiffness score increased by ≥ 3 points. Among them, 56% of patients were shown to be treated effectively; 50% of patients were improved in the first 3 months, and in which 16% continued for 6 months. Another 3 cases had improved erectile function 6 months after the treatment. In addition, the effect was significantly improved for patients with cardiovascular risk factors (p = .026) (Oginski et al., 2022). This study proves that Li-ESWT is an effective but short-term treatment method

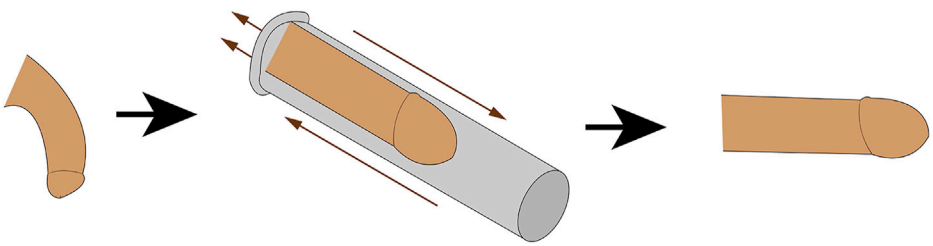

FIGURE 4
Mechanism of VED for the treatment of ED. Negative pressure dilates the cavernous sinuses, increasing their arterial and venous blood perfusion and ultimately achieving penile erection.

for ED patients, especially those with cardiovascular diseases. Liu et al. (Liu et al., 2022) conducted a meta-analysis to assess the efficacy of Li-ESWT for ED and found that Li-ESWT significantly improves erectile function in patients with mild and moderate ED. Moreover, its safety is very high, and so far there are few reports of adverse effects (Pai et al., 2021). There is increasing evidence that Li-ESWT causes minor damage to vital organs such as the heart while improving myocardium, bladder, joints, and penis function (Jiang et al., 2021).

A study by Zewin et al. investigated the role of Li-ESWT in penile rehabilitation after nerve-preserving radical prostatectomy in men. The Li-ESWT treatment group in the study showed a significant increase in total IIEF score, sexual satisfaction, overall satisfaction domain score, and EHS score throughout the follow-up period, demonstrating its clinical therapeutic properties (Zewin et al., 2018). Moreover, in a study of 350 ED patients by Leonid Spivak et al., Li-ESWT improved phosphodiesterase five inhibitor sensitivity in 55% of patients who did not respond to phosphodiesterase five inhibitors (Spivak et al., 2021). Li-ESWT is relatively safe for short-term treatment in multiple studies, but there is a lack of long-term studies confirming the safety of Li-ESWT. With the shortcoming of expensive, Li-ESWT has not been approved by the FDA for the treatment of ED.

4.4 Akupunktur treatment

ED is called impotence in Traditional Chinese medical (TCM). Some TCM methods are frequently used to treat ED, and the most commonly used method is akupunktur (Tan et al., 2021). Akupunktur is the umbrella term for acupuncture and moxibustion. Acupuncture is the procedure of inserting filiform needles into specific points on the patient's body, known as acupoints, and using acupuncture techniques such as twisting and lifting to treat disease (Zhou et al., 2021). Moxibustion is the practice of smoked burning the skin with burning Ai velvet according to certain acupoints and using the heat to stimulate the treatment of diseases. TCM believes that these acupoints can control and regulate the flow of qi, as well as its distribution and excretion in the viscera, in order to maintain the balance between the internal and external environments (Zhou et al., 2021). It has been reported that akupunktur can control the release of nitric oxide and some neuropeptides involved in the erectile process (Wang et al., 2022b). Acupuncture can also improve blood circulation and regulate the sensitivity of nerves to relieve the symptoms of ED patients (Wang et al., 2019b). However, the available evidence is still insufficient to demonstrate that akupunktur is an effective method for ED. Therefore, the therapeutic effect of akupunktur on ED requires further investigation (Li et al., 2017).

4.5 Discussion

Overall, the current treatment for ED includes medication, physical therapy, psychotherapy, and surgery. Medication, especially PDE-5 inhibitors, is still the first-line treatment (Yuan et al., 2021), among which sildenafil, vardenafil, tadalafil, and avanafil have better efficacy (Liu et al., 2020a). Although the results are encouraging, many patients do not respond to these medications, are unable to tolerate the side effects, or relapse after discontinuation. Therefore, it is critical to research physical therapy methods and mechanisms related to ED. It could also be used as a springboard for the development of more clinically relevant physical therapy approaches for the treatment of ED. However, each method has its advantages and limitations. At present, the cost of various physical therapies varies in clinical practice. ES, VED, and acupuncture are relatively inexpensive for most patients, while Li-ESWT treatment is relatively expensive. When faced with patients with ED in clinical settings, physicians should establish a treatment plan that considers the severity of the patient's ED and the patient's financial situation. It is possible to start with a relatively inexpensive treatment modality, alone or in combination, such as ES or a combination of ES and VED. When the program does not work, then switch to other modalities.

As the prevalence of ED increases, there is a greater incentive to find safer, more effective, and simpler treatments. Pharmacological and physical therapies paid more attention on symptoms control but not the function restore. More attention has recently focused on the latest technologies of gene therapy and stem cell transplantation. Bone marrow-derived and adipocyte-derived stem cells have been used in animal models with dramatic results. We are looking forward that these treatments could help a large number of ED patients to regain their strength in the coming years.

5 Conclusion

We have now reviewed literatures about the pathogenesis of ED or some of the factors that influence the development of the disease. In this review, the authors detailed the various etiologies of ED and the pathogenesis of each etiology, and presented the various common clinical diagnostic and physical therapy devices currently available for ED and the advantages and disadvantages of each device. This will enable clinicians to provide individualized treatment plans for each patient based on the different etiopathogenic factors, and to improve the condition of ED patients in the future.

Author contributions

Conceptualization, KP, DP and HX; Literature review, KP, DP, HX, YM, JW, HW, and GZ; writing -original draft preparation, DP, HX, and JW; drawing, JW, PX, and HW; writing -review and editing, KP and GZ; supervision, KP and GZ. 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.

Publisher's note

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Glossary

ED: erectile dysfunction
NO: nitric oxide
cGMP: cycloguanosine monophosphate
RhoA: ras homolog gene family
AGEs: advanced glycation end products
PKG-1: cGMP dependent protein kinase-1
NPT: nocturnal penile tumescence
PDEi-5: phosphodiesterase inhibitor-5
PPDU: pharmaco penile duplex ultrasonography
PGE1: prostaglandin 1
PSV: peak systolic velocity
EDV: end-diastolic velocity
IPA: internal pudendal arteriography
DSA: digital subtraction angiography
D-e CTA dual-energy CT arteriography
4D-CTA: 320-Detector row dynamic volume CT
VED: vacuum erectile device
ES: electrical stimulation
FES: functional electrical stimulation
RES: regenerative electrical stimulation
BDNF: brain-derived neurotrophic factor
trkB: tyrosine kinase B
EHS: erection hardness score
Li-ESWT: low-intensity extracorporeal shock wave therapy
nNOS: neuronal nitric oxide synthase
TCM: traditional chinese medical

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Cisplatin causes erectile dysfunction by decreasing endothelial and smooth muscle content and inducing cavernosal nerve senescence in rats

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Introduction: Cisplatin (cis-diamminedichloroplatinum II, CDDP), a drug widely used for cancer worldwide, may affect erectile function, but its side effects have not received enough attention. To investigate the effect of CDDP on erectile function and its possible mechanism.

Methods: Sprague–Dawley rats were intraperitoneally administered CDDP (CDDP group) or the same volume of normal saline (control group). Erectile function was evaluated after a one-week washout. Then, histologic changes in the corpus cavernosum and cavernous nerve (CN) were measured. Other Sprague-Dawley rats were used to isolate the major pelvic ganglion and cavernous nerve (MPG/CN). RSC96 cells were then treated with CDDP. SA-β-gal staining was used to identify senescent cells, and qPCR was used to detect the senescence-associated secretory phenotype (SASP). Finally, the supernatant of RSC96 cells was used to culture MPG/CN. Erectile function was measured after administration of CDDP. The cavernosum levels of α-SMA, CD31, eNOS, and γ-H2AX, the apoptosis rate and the expression of p16, p21 and p53 in CN were also assayed. The senescent phenotype of RSC96 cells treated with CDDP was identified, and neurite growth from the MPG/CN was photographed and measured.

Results: The CDDP group had a significantly lower ICP/MAP ratio than the control group. Compared to the control group, the CDDP group exhibited significantly lower α-SMA, CD31 and eNOS levels and significantly higher γ-H2AX and apoptosis rates in corpus cavernosum. In addition, CDDP increased some senescence markers p16, p21 and p53 in CN. *In vitro*, CDDP induced RSC96 senescence and SASP, and the supernatant of senescent cells slowed neurite outgrowth of MPG/CN.

Discussions: CDDP treatment could induce erectile dysfunction, by affecting the content of endothelial and smooth muscle and causing SASP in CN. The results indicate that CDDP treatment should be considered as a risk factor for ED. Clinicians should pay more attention to the erectile function of cancer patients who receive CDDP treatment.

KEYWORDS
cisplatin, erectile dysfunction, apoptosis, senescence, cancer survivor

Introduction

In 2020, there were 19.3 million new cases of cancer and 10.0 million cancer deaths worldwide. The global cancer burden is expected to rise 47% from 2020, with 28.4 million cases in 2040 (1). Given the increasing incidence and prevalence of cancer, chemotherapy is a well-established treatment strategy. The role of chemotherapy will continue to expand and play a more important role in improving both the survival and quality of life of patients. CDDP is a widely used drug to treat various solid cancers such as testicular (2), ovarian (3), head and neck (4), bladder (5), lung (6), cervical cancer (7), lymphomas (8) and several others (9). However, the adverse effects of CDDP limit its effectiveness and become an inherent challenge for its application.

Erectile dysfunction is not usually mentioned as a side effect. However, 42% of colorectal cancer patients who were treated with oxaliplatin had erectile problem (10). Tomoya Kataoka et al. reported that oxaliplatin causes erectile dysfunction in rats due to endothelial dysfunction (11), providing further confirmation that platinum-based drugs can indeed induce ED and that this problem is often overlooked by clinicians. CDDP, a main chemotherapy agent for testicular tumors, has been reported to be associated with erectile dysfunction (12, 13). Moreover, testicular tumors are the most common tumors in young men. Therefore, it is necessary to clarify the relationship between CDDP and erectile function.

In this study, we studied erectile function in rats after administration of CDDP. Illustrating the mechanism, underlying the effect of CDDP on erectile function, will help doctors take measures to protect erectile function in cancer survivors.

Materials and methods

Cells and drugs

RSC96, the Schwann cell line of the rat sciatic nerve, was purchased from Procell (Wuhan, China). Briefly, cells were maintained in DMEM (Gibco Life Technologies, CA, USA) supplemented with 10% fetal bovine serum (Gibco Life Technologies, CA, USA) and 1% penicillin-streptomycin (Gibco Life Technologies, CA, USA) at 37 ˚C in a humidified atmosphere of 5% CO_2. CDDP was acquired from APExBIO (Houston, USA) and dissolved in normal saline.

Cell viability assay (MTT)

Cell viability was evaluated at different time points (48 hours, 72 hours) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. RSC96 cells were seeded in 96-well plates at a density of 3×10^3 cells/well and cultured for 24 hours. Then, the cells were cotreated with different concentrations of CDDP in the medium. At different time points, 10 μL of MTT was added to each well and incubated at 37°C for an additional 4 hours. Then, the supernatant was carefully removed, and dimethyl sulfoxide was added to each well to dissolve the crystals by gentle agitation for 10 minutes. For each well, the absorbance at 490 nm was estimated on a microplate reader (Bio-Tek, VT, USA).

SA-β-gal staining

Cells were seeded in 6-well plates at a density of approximately 20 000 cells/well for 24 hours, and then treated with different concentrations of CDDP for 2 days. Thereafter, the medium was removed and replaced with complete medium (without CDDP) for another 2 days. Then, the cells were fixed and stained using a Senescence β-Galactosidase Staining Kit (Solarbio Life Science, Beijing, China).

RNA extraction and quantitative reverse transcription-PCR

For qPCR, total RNA was extracted using the RNA Kit (Omega Bio-Tek, USA) according to the manufacturer's instructions. Reverse transcription was performed using a HiScript II 1st strand cDNA synthesis kit (Vazyme, China). qPCR was performed on a step-one plus real-time PCR system (Bio-Rad, CA, USA) using ChamQ universal SYBR qPCR master mix (Vazyme, China). All primers were synthesized by Ribobio Co., Ltd. (Guang Zhou, China). Primer sequences are listed in Table 1. β-actin was used as an internal control. The relative levels of RNAs were calculated using the 2-ΔΔCt method.

Major pelvic ganglion and cavernous nerve culture

Bilateral MPG/CN (entire MPG with a 2-mm length CN attached) complexes from each rat were isolated and excised intact. Each MPG/CN complex was placed in a 6-well plate and then covered with 50 μL of Matrigel (Corning, NY, USA) as previously described (14). After incubation at 37 °C for 5 minutes, 2.0 mL of complete culture medium and 2.0 ml supernatant from RSC96 treated with or without CDDP were added according to the grouping. RSC96 cells were treated with CDDP for 2 days, and the medium was removed and replaced with complete culture medium (without CDDP) for another 2 days. The supernatant was then used to culture the MPG/CN complex.

TABLE 1 Primers for qPCR.

Gene symbol	5′-3′
IL6-F	GTCAACTCCATCTGCCCTTCAG
IL6-R	GGCAGTGGCTGTCAACAACAT
TGFB1-F	GCGCCTGCAGAGATTCAAGTCAAC
TGFB1-R	GTATCAGTGGGGGTCAGCAGCC
β-actin -F	TCAGGTCATCACTATCGGCAAT
β-actin -R	AAAGAAAGGGTGTAAAACGCA
TGFB2 -F	TGCTGAGAACCTTTTTGCTCC
TGFB2-R	GTCGAGGGTGCTGCAGGTA
ICAM-1-F	GTCGGTGCTCAGGTATCCATC
ICAM-1 -R	TCGTCTTTCATCCAGTTAGTCTCC
IL1A -F	AAATACTCAGCTCTTTGTGAGTGC
IL1A -R	TGTGATGAGTTTTGGTGTTTCC
CCL2 -F	CTCTTCCTCCACCACTATGC
CCL2 -R	CTCTGTCATACTGGTCACTTC
CTGF -F	CAGGCTGGAGAAGCAGAGTCGT
CTGF -R	CTGGTGCAGCCAGAAAGCTCAA
PAI1 -F	CCATCTCCGTGCCCATGAT
PAI1 -R	GTCATGTTGCTCTTCCATTGTCT

Animal treatment

All animal experiments in this study were approved by the Institutional Animal Care and Use Committee of the Fifth Affiliated Hospital of Sun Yat-sen University.Twenty 12-week-old male Sprague–Dawley rats were used for this study. The rats were divided into 2 groups: The CDDP group rats (n = 10) were intraperitoneally administered 2 mg/kg body weight of CDDP on Days 1, 2, 8, 9, 15, 16, 22, and 23 (Figure 1A). And the others (n=10) injected with normal saline as the control group. The dose was based on some scholars' recommendation about the dose translation from animals to humans (15, 16). The erectile function was evaluated after a one-week washout.

Measurement of erectile function

Intracavernous pressure (ICP) was measured by electrical stimulation, as previously reported (17, 18). All rats were anesthetized by injection with pentobarbital (40 mg/kg). Then, the carotid artery was exposed and cannulated with a PE-50 tube which was connected to a pressure transducer to continuously monitor arterial pressure. A 25-gauge needle containing 100 IU/mL heparin solution was inserted into the right penile crura and the other end of the tube was also connected to a pressure transducer to monitor intracavernous pressure (ICP). The cavernous nerve was electrically stimulated at 1 V, 20 Hz, with a pulse width of 5 ms for 1 minute and a 5-minute interval before subsequent stimulation. The ratios of

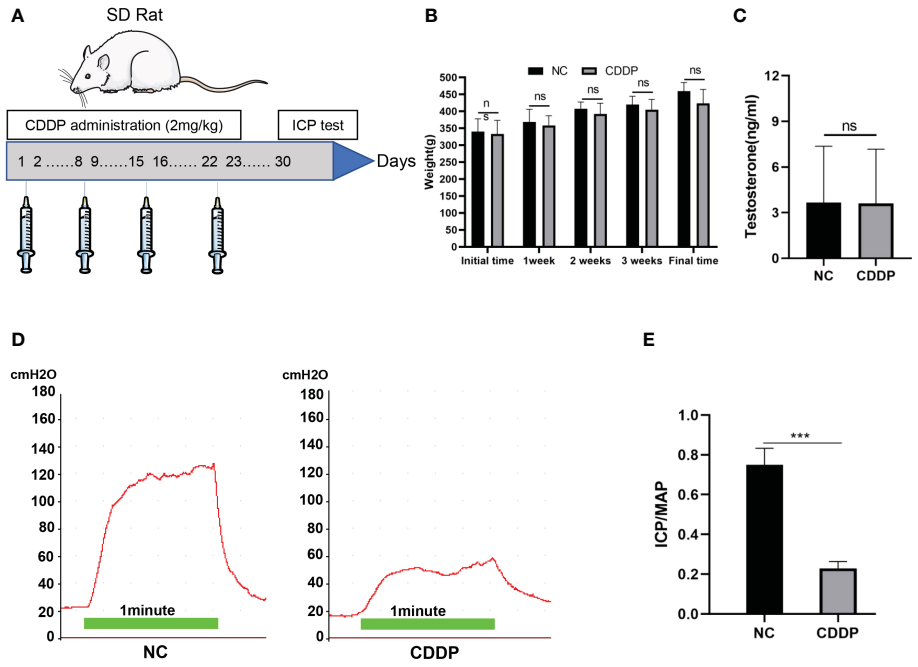

FIGURE 1
Characterization of the CIED rat model. **(A)** Experimental scheme for the development of the CIED rat model. CDDP was administered i.p. twice a week for 4 weeks. After a week washout, animals were evaluated to assess erectile function. **(B)** Changes in rat body weight over time. Initial weight levels in the 2 groups of rats after 7 days of adaptive feeding; final weight after 7 days of washout at 4 weeks. **(C)** Testosterone levels of each group were shown. **(D)** Representative images of ICP in response to electrical stimulation of the cavernosal nerve; **(E)** Results of the ratio of MaxICP to MAP in each group. ns: not significant, ***$P < 0.001$.

maximal ICP (MaxICP) to mean arterial pressure (MAP) were calculated to evaluate erectile function *in vivo*.

Measurement of serum concentration of testosterone

After the ICP test was completed, clinical needles and vacuum tubes were used to collect blood through the inferior vena cava.The serum was separated immediately and stored at 80°C. The levels of testosterone were assessed using ELISA kits from Solarbio (Beijing, China).

Histologic examinations

H&E staining

The staining procedures were reported in our previous study (18, 19). Slides containing tissue sections were deparaffinized using a dry oven at 60°C for 30 minutes and in 2 changes of xylene for 10 minutes, and then rehydrated in a series of decreasing concentrations of alcohol. Finally, the slides were washed in tap water. After that, Harris's hematoxylin reagent was used to stain for 8 minutes. The slides were then destained in 0.5% acid-alcohol for 3 seconds and washed with running water. The slides were counterstained with 0.5% eosin for another 1 minute. After dehydration with different concentrations of alcohol and xylene, the slides were observed with a microscope for histopathologic examination.

Masson trichrome staining

The staining procedures were reported in our previous study (18). Tissue sections of the middle part of the penis were stained according to Masson kit instructions (Solarbio, Beijing, China). The collagen tissues (stained blue) of the penile sponge and smooth muscle tissues (stained red) were observed under a light microscope. The cavernous smooth muscle/collagen ratio was analyzed using ImageJ software.

Immunofluorescence

Slides containing tissue sections were deparaffinized with xylene and rehydrated with ethanol. Then, antigen retrieval was performed by placing the slides in Tris/EDTA buffer (Solarbio Life Science, Beijing, China) before heating for 10 minutes. The slides were then treated with endogenous peroxidase blocker for 10 minutes and normal 10% goat serum (Solarbio Life Science, Beijing, China) was utilized for 30 minutes to block nonspecific binding sites. Different antibodies were applied to the slides and incubated overnight at 4 °C. After the hybridization of secondary antibodies, DAPI was used to stain the cell nucleus. The slides were observed using a fluorescence microscope (OLYMPUS, Tokyo, Japan). Antibodies against γ-H2AX, p16, p21 and p53 were obtained from Abclonal Biotechnology (Wuhan, China); CD31 antibody was purchased from Bioworld Technology (Nanjing, China); eNOS antibody was obtained from Abcam Biotechnology (Cambridge, UK). α-SMA antibody was purchased from Affinity (OH, USA).

TUNEL staining

TUNEL staining was carried out according to previous studies (18). Apoptosis of the corpus cavernosum of each group was detected using a TUNEL apoptosis detection kit (KeyGEN BioTECH, Nanjing, China) following the manufacturer's instructions. Each sample was randomly selected from 4 fields of view. Cells with green staining were counted as apoptotic, and the ratio of apoptotic cells to the total number of cells in the field of view yielded the rate of apoptosis.

Statistical analysis

Results were analyzed using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA) and expressed as the mean ± standard deviation (SD). Statistical analyses were performed using the two-tailed Student's t test. Differences among groups were considered significant at a P value less than 0.05.

Results

CDDP weakens the erectile function of rats

As shown in Figures 1B, C, the mean body weight and testosterone levels did not differ significantly between the two groups, but erectile function had obvious difference (Figures 1D, E). Erectile function was assessed by MaxICP and MaxICP/MAP. The results showed that both MaxICP and MaxICP/MAP revealed a significant decrease in the CDDP group compared to control rats.

Effects of CDDP on smooth muscle contents and endothelium function in corpus cavernosum

The morphological changes and smooth muscle (SM)-to-collagen ratios of the different groups were detected with H&E and Masson's trichrome staining, as shown in Figures 2A–C. CDDP significantly caused structural disorders and decreased smooth muscle mass in the corpus cavernosum. Moreover, Immunofluorescence staining with an anti-α-SMA antibody showed a significant reduction in smooth muscle content in CDDP-treated rats compared with normal control rats (Figure 2D). Cavernosal endothelial dysfunction is recognized as a hallmark of the disease pathology. Tomoya Kataoka found that oxaliplatin causes erectile dysfunction in rats due to endothelial dysfunction (11). Consistent with the findings, we found the endothelial cell content and eNOS was severely decreased in the CDDP group (Figure 2E).

CDDP induces DNA damage and apoptosis in corpus cavernosum

CDDP binds to DNA to cause a biological effect by forming CDDP-DNA adducts and induceing DNA damage response. Phosphorylated H2AX (γ-H2AX) is known to be a marker to investigate the effects on DNA damage (20). As shown in Figure 3A, CDDP treatment significantly increased γ-H2AX levels compared with the control group in the corpus cavernosum. The apoptotic pathway may be triggered in cells after CDDP treatment. We measured the apoptosis level in corpus cavernosum by TUNEL (Figures 3B, C). In the CDDP group, there were a dramatically larger

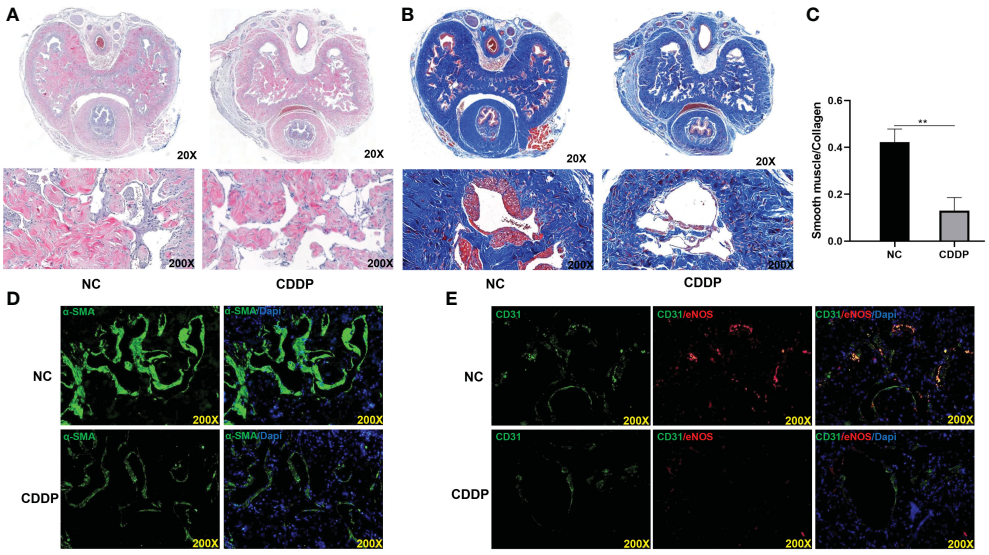

FIGURE 2
Effect of CDDP on smooth muscle and endothelium contents in the corpus cavernosum **(A)** H&E staining. A thinner smooth muscle layer was found in all CDDP-treated rats; **(B)** Masson trichrome staining. Smooth muscle manifested as red, and connective tissue was blue. **(C)** The ratio of smooth muscle to collagen. **(D)** Representative images of immunofluorescence staining of α-SMA in each group. **(E)** Representative images of immunofluorescence staining of CD31(green) and eNOS (red) in the corpus cavernosum from age-matched control and CDDP-treated rats. $^{**}P < 0.01$.

percentage of apoptosis cells than the control group. The aforementioned results indicates that CDDP can induces DNA damage and apoptosis in corpus cavernosum.

CDDP causes senescence in CN

ED is considered a complication of CDDP-induced peripheral neuropathy (12). Aina Calls found that CDDP-induced peripheral neuropathy was associated with neuronal senescence-like response (21). In senescent cells, the levels of cell cycle inhibitors, including p16, p21, and p53 were augmented. Following CDDP treatment, the expression levels of the p16, p21, and p53 were increased in CN (Figure 4).

CDDP induces Senescence and SASP in RSC96 cells

To determine RSC96 cells response to CDDP, an MTT assay was performed. Notably, the proliferation viability of cells decreased with increasing concentration (Figure 5A). The SA-β-gal staining assay confirmed our hypothesis that senescence occurred in RSC96 cells treated with CDDP (Figure 5B). Immunofluorescence illustrated that CDDP upregulated the expression of senescence-related genes: p16, p21 and p53 (Figure 5C). SASP is another typical feature of senescent cells. CDDP promoted the expression of SASP-related genes, IL-6, TGFβ1, ICAM1, TGFβ2, CCL2, IL-1α and CXCL1 (Figure 5D).

Senescent Schwann cells affects the function of CN

To investigate the effect of senescent Schwann cells on axonal growth of the CN, we used an in vitro model of MPG/CN culture (14). As shown in Figure 6A, the supernatant of RSC9 cells was used to treat the MPG/CN system. We dissected the MPG with 2 mm of CN attached and cultured the tissue in vitro. After 120 hours, new neurite outgrowths from the end of the CN were measured. CDDP significantly restrained the neurite outgrowth of CN (Figures 6B–D).

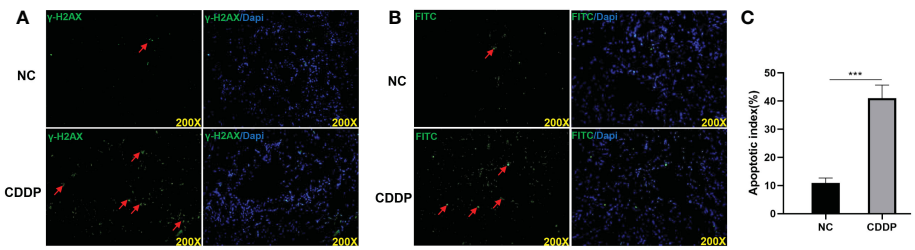

FIGURE 3
CDDP increased DNA damage and apoptosis in the corpus cavernosum. **(A)** Representative images of immunofluorescence staining of γ-H2AX, a DNA damage marker, in the corpus cavernosum from age-matched control and CDDP-treated rats. **(B)** Representative images of TUNEL staining in the corpus cavernosum from age matched control and CDDP-treated rats; **(C)** The apoptotic index, the percentage of apoptotic cells (stained green) of all cells, to quantify the cavernous apoptosis level. Red arrows showed the positive signals, $^{***}P < 0.001$.

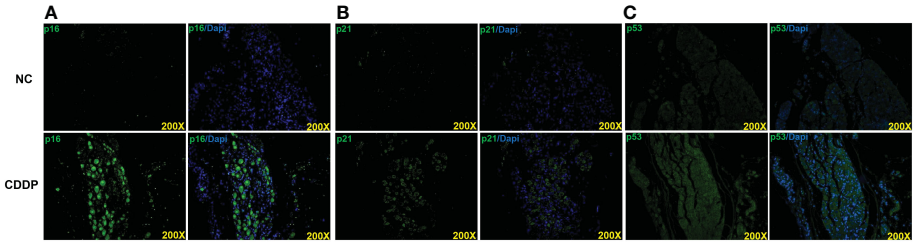

FIGURE 4
CDDP induced senescence in CN. Representative images of immunofluorescence staining of p16 **(A)**, p21 **(B)** and p53 **(C)** in each group.

Discussion

Many anticancer agents used in cancer chemotherapy possess either cytotoxic or cytocidal activity. However, they can injure both the cancer cells and the normal tissue and cells of patients because of their nonselectivity. The dysfunctions of normal cells and organs caused by these agents are called side effects. A variety of complications that cause suffering and lower quality of life make it harder for doctors. Many side effects have been reported and could be overcome. However, there are still problems to be resolved (22). Several studies have demonstrated that platinum-based chemotherapy can induce ED (10, 12, 13, 23, 24), but ED has not gained the attention of clinicians as a complication. Some scholars have found that oxaliplatin can induce ED in rats by inducing damage to the endothelium of the corpus cavernosum (11). CDDP is a conventional chemotherapeutic agent for numerous tumors. In this study, administration of CDDP to rats resulted in a decrease in the MaxICP/MAP ratio, endothelium and smooth muscle content and eNOS level. In addition, CDDP induces senescence in Schwann cells, which triggers the SASP phenotype, thereby affecting CN function. Therefore, CDDP is confirmed to be able to induce ED by affecting the corpus cavernosum and CN. CDDP exposure can cause testicular damage and a significant reduction in testosterone level (25, 26). In our study, the CDDP-treated rats showed no different serum testosterone levels than the control group, which might be caused by the use of a lower concentration of CDDP. eNOS has an indispensable role in the erectile response (27) and endothelial dysfunction is common in ED induced by diabetes mellitus (28), bilateral cavernous nerve injury (29) and oxaliplatin (11). Long-term cardiovascular events have often been reported to increase in patients with platinum-based chemotherapy due to vascular toxicity and endothelial dysfunction. In our results, CD31 staining and eNOS levels in the corpus cavernosum were decreased after CDDP treatment. CDDP can induce apoptosis in HUVECs (30). Furthermore, CDDP also upregulates NF-κB/ICAM-1 to affect the production of nitric oxide (NO) and cGMP, which leads to endothelial dysfunction (31). Moreover, CDDP also caused loss of smooth muscle. CDDP exerts anticancer activity *via* multiple mechanisms but the generation of DNA lesions is the most acceptable mechanism, followed by activation of the apoptosis

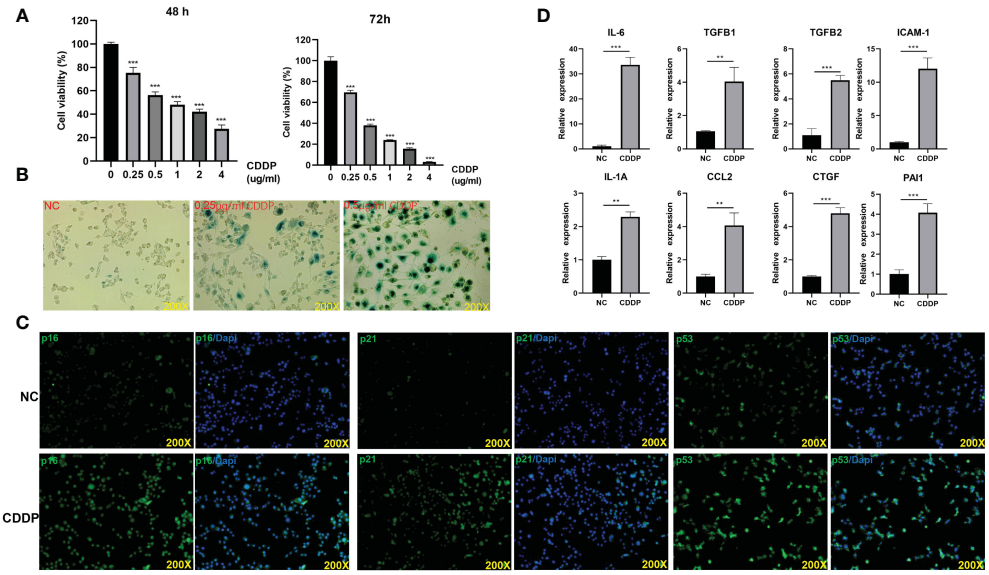

FIGURE 5
CDDP could induce senescence in RSC96 cells. **(A)** The effect of different concentrations of CDDP on the viability of RSC96 cells was detected by MTT at 48 and 72 hours. **(B)** RSC96 cells were treated with the indicated concentrations and then stained for SA-β-gal activity at 96 hours. **(C)** After RSC96 cells were treated with CDDP (0.5 μg/ml), immunofluorescence staining was performed to detect the expression of p53, p21 and p16 in RSC96 cells. **(D)** qPCR was used to detect the SASP-related genes after RSC96 cells were treated with CDDP (0.5 μg/ml). $^{**}P < 0.01$, $^{***}P < 0.001$.

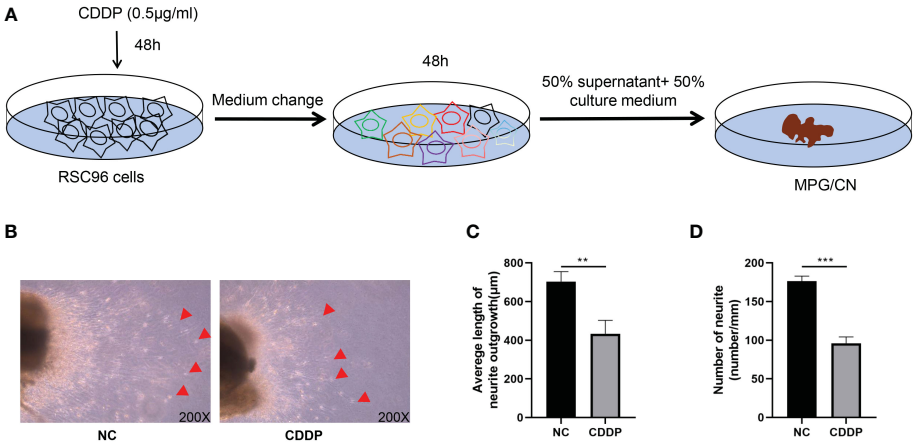

FIGURE 6
Senescent Schwann cells reduced neurite outgrowth from CN *in vitro*. **(A)** A schematic diagram of supernatant isolation and MPG treated with supernatant. **(B)** Neurite outgrowth from CN 120 hours after seeding. Original magnification x200. The red arrow indicates outgrowing fibers, **(C)**. The supernatant of senescent cells inhibited fiber outgrowth from CN. **(D)**. The supernatant of senescent cells decreased the number of fibers from CN. $^{**}P < 0.01$, $^{***}P < 0.001$.

pathway (9, 32). Correspondingly, our results showed that γ-H2AX, a DNA damage marker, and TUNEL staining were augmented in corpus cavernosum of CDDP group. Hence, we hypothesized that CDDP might damage DNA and cause endothelial and smooth muscle cell apoptosis, which impairs the erectile function of rats. Of course, the specific mechanism by which CDDP affects the endothelial and smooth muscle cells of the corpus cavernosum deserves further study.

Peripheral neurotoxicity is a common side effect of platinum-based chemotherapy (33). Some researchers have even considered ED as a neuropathic subscale-related symptom (10). A recent study found that CDDP-induced peripheral neuropathy is associated with neuronal senescence-like response (21). In our results, the expression of senescence-related markers was up-regulated in CN after CDDP treatment. Schwann cells help nerve cells to transmit information faster by wrapping their long extensions in myelin. Abnormal production of myelin can perturb signal transmission between nerve cells, which leads to neurological defects (34). Schwann cells in the peripheral nervous system play a pivotal role in nerve repair (35). In addition, dysfunction of Schwann cells plays an important role in the pathogenesis of diabetic peripheral neuropathy (36, 37). *In vitro*, we found that CDDP was indeed able to induce senescence in RSC96 cells and generate SASP. Inflammation is a key driver of pathological changes in many peripheral neuropathy (38). We used the supernatant of RSC96 cells to culture MPG/CN and indeed found that the growth of CN decelerated in the CDDP group, which indirectly indicated that the substances secreted by senescent Schwann cells could affect the function of CN.

In summary, our findings indicate that the CDDP-induced reduction in endothelial and smooth muscle content and SASP, which leads to CN dysfunction, are responsible for ED in rats.

In this study, we used normal rats administered CDDP, and the absence of cancer model experiments was one limitation of the present study. In addition, the effects of different dose and frequency of administration of CDDP on erectile function should be evaluated in the future. Another limitation of this study is that the direct effects of CDDP on nerves were not investigated. The underlying mechanism of effects of CDDP on CN needs to be further explored.

Conclusion

Our study revealed that CDDP treatment could induce erectile dysfunction by affecting the content of endothelial and smooth muscle and causing SASP in CN. Thus, erectile function in cancer survivors receiving CDDP chemotherapy should receive the attention of clinicians.

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 animal study was reviewed and approved by the Ethics Committee of the Fifth Affiliated Hospital, Sun Yat-sen University.

Author contributions

Conceptualization, YY, YD and YT; Data curation, YY, JZ, LZ and YD, Formal analysis, YY, JZ, LZ, BN and JP, Funding acquisition, LZ, YZ and YT, Methodology, YY, HH, MX, and JP, Project administration, YD and YT, Supervision, YD and YT, Validation, YY, BN and YZ; Writing – Original Draft, YY, YD and YT, Writing – Review & Editing, YY, YD and YT. 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.

Publisher's note

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Uncovering the genetic links of diabetic erectile dysfunction and chronic prostatitis/chronic pelvic pain syndrome

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Background: Clinical associations between erectile dysfunction and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) have been noticed, but the common pathogenic mechanisms between them remain elusive. The aim of the study was to mine shared genetic alterations between ED and chronic prostatitis/chronic pelvic pain syndrome.

Method: Transcriptome data of ED and chronic prostatitis/chronic pelvic pain syndrome-related genes (CPRGs) were retrieved from relevant databases and differentially expressed analysis was used to obtain significant CPRGs. Then function enrichment and interaction analyses were performed to show shared transcriptional signature, including gene ontology and pathway enrichment, the construction of protein-protein interaction (PPI) network, cluster analysis, and co-expression analysis. Hub CPRGs and key cross-link were selected by validating these genes in clinical samples, chronic prostatitis/chronic pelvic pain syndrome and ED-related datasets. Then the miRNA-OSRGs co-regulatory network was predicted and validated. Subpopulation distribution and disease association of hub CPRGs were further identified.

Result: Differentially expressed analysis revealed 363 significant CPRGs between ED and chronic prostatitis/chronic pelvic pain syndrome, functioning in inflammatory reaction, oxidative stress, apoptosis, smooth muscle cell proliferation, and extracellular matrix organization. A PPI network containing 245 nodes and 504 interactions was constructed. Module analysis depicted that multicellular organismal process and immune metabolic process were enriched. 17 genes were screened in PPI *via* topological algorithms, and reactive oxygen species as well as interleukin-1 metabolism were regarded as the bridging interactive mechanism. After screening and validation, a hub-CPRG signature consisting of COL1A1, MAPK6, LPL, NFE2L2 and NQO1 were identified and associated miRNA were verified. These miRNAs played an important role in immune and inflammatory response likewise. Finally, NQO1 was identified as a key genetic link between ED and chronic prostatitis/chronic pelvic pain syndrome. It was predominately enriched in corpus cavernosum endothelial cell, and correlated with other male urogenital and immune system diseases tightly.

Conclusion: We identified the genetic profiles as well as corresponding regulatory network underlying interaction between ED and chronic prostatitis/chronic pelvic pain syndrome *via* multi-omics analysis. These findings expanded a new

understanding for the molecular mechanism of ED with chronic prostatitis/chronic pelvic pain syndrome.

KEYWORDS

erectile dysfunction, chronic prostatitis, chronic pelvic pain syndrome, inflammation, biomarker

1 Introduction

Erectile dysfunction (ED) is a common sexual disorder in men, characterized by the insufficient ability to achieve acceptable sexual performance due to the absence of adequate obtainment or maintenance of penile erectile (Shamloul and Ghanem, 2013). Although it is less likely to threaten men's life, it troubles quality of life for couples to a great extent. The number of concerned people with ED increases with age, which will reach more than 300 million after 3 years (Ayta et al., 1999; Bacon et al., 2003). Since ED is a pathological process referring to vasculogenic, endocrinological, neurogenic, psychogenic, and other factors, phosphodiesterase type 5 inhibitors (PDE5Is), the first-line remedies tend to exhibit unsatisfactory effects in ED with combined or unknown etiological factors. It is a fact that clarifying the mechanism and distinguishing the specific etiology of ED are the key to the treatment of intricate ED.

Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) is the most common type of prostatitis based on the NIH category, which could cause great distress to males from all ages (Khan et al., 2017). It manifests a sense of repeated discomfort or pain in the pelvis accompanied with lower urinary tract symptoms (LUTS) in the absence of infection (Lee et al., 2008). What's more, its impact on sexual function is usually overlooked (Anderson et al., 2006). Cumulative studies have shown that CP/CPPS is tightly associated with ED (Tran and Shoskes, 2013; Li et al., 2021). Specifically, ED is present in 27%–40.5% of patients with CP/CPPS (Tran and Shoskes, 2013; Li and Kang, 2016). And men with ED had a possibility of previous CP/CPPS diagnosis three times more than control patients (Chung et al., 2012). Although epidemiological link between ED and CP/CPPS has been revealed, it isn't enough to explain their pathological relationship based on the current studies (Ma et al., 2020). Shoskes et al. (2011) found that men with CP/CPPS tend to have evidence of greater endothelial dysfunction and arterial stiffness. Another study noted that patients suffering from CP/CPPS had higher serum levels of androstenedione and testosterone (Dimitrakov et al., 2008). Moreover, some growth and inflammatory factors involved in ED also existed in CP/CPPS (Dahiya et al., 1999; Pontari and Ruggieri, 2008). Apart from organic factors, psychological factors propelled the pathological interaction in ED and CPPS (Tran and Shoskes, 2013). Also, as one of PDE5Is, tadalafil was proven to be effective in prostatitis (Hiramatsu et al., 2020). It seems that multidimensional pathomechanism coexists in these two diseases. Therefore, mining the overlapping genetic alternations at the molecular level will broaden our understanding of potential mechanisms in ED and CP/CPPS.

Based on these, in the present study, we explored the genetic interrelationships between ED and CP/CPPS based on cell, tissue and human researches for the 1 time. Besides, significant biomarkers and associated biological pathways were identified and analyzed. Our study would help to clarify the common mechanisms and critical regulators behind the interplay of these two diseases, and guide the treatment in the occurrence of ED and CP/CPPS for further research.

2 Materials and methods

2.1 Data acquisition

The datasets related to ED and CP/CPPS were searched in the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) with the key words "erectile dysfunction," "chronic prostatitis" and "chronic pelvic pain syndrome". After screening, five correlative datasets were obtained. Herein, GSE2457 contained expression profiling of corpus cavernosum in rats with ED and corresponding control group (Sullivan et al., 2005). GSE146078 deposited gene data of cavernous endothelial cells in high-glucose and normal-glucose conditions (Yin et al., 2021). Single-cell transcriptome of the corpus cavernosum and microRNA (miRNA) profiles in ED patients were exacted from GSE206528 (Zhao et al., 2022) and GSE182053 (Xu et al., 2021), respectively. In addition, genes related to CP/CPPS were retrieved in the GeneCards database (https://www.genecards.org/) and validated in GSE159438.

2.2 Differentially expressed analysis of CPRGs

Genes related to ED in GSE2457 were preprocessed and normalized first, and calibrated genes were processed by "limma" package (Ritchie et al., 2015) in R software (Version 4.2.1) to obtain differentially expressed genes (DEGs) between ED and control groups with the threshold of p-value <0.05. Then, the intersection of DEGs in ED and CP/CPPS in GeneCards was performed to obtain significant CP/CPPS-related genes (CPRGs). These results were presented with heatmap and Venn diagram by "pheatmap" package and EVenn (http://www.ehbio.com/test/venn/) (Chen et al., 2021a).

2.3 Functional enrichment analysis

After identification of significant CPRGs, Gene Ontology (GO) and pathway enrichment analyses were performed to reveal the possible biological roles of these genes with the threshold of adjusted p-value <0.05 and count >2 in the Database for Annotation, Visualization and Integrated Discovery (DAVID) online tool (http://david.ncifcrf.gov). GO was categorized into three subsections: biological process, cellular component, and molecular function, and pathways were conducted by Kyoto Encyclopedia of Genes and Genomes (KEGG) and Reactome. And the results were visualized by "ggpubr" and "ggplot2" packages, respectively.

2.4 PPI network and module analysis

Significant CPRGs were uploaded to the Search Tool for the Retrieval of Interacting Genes (STRING) database (http://string-db.org) to depict

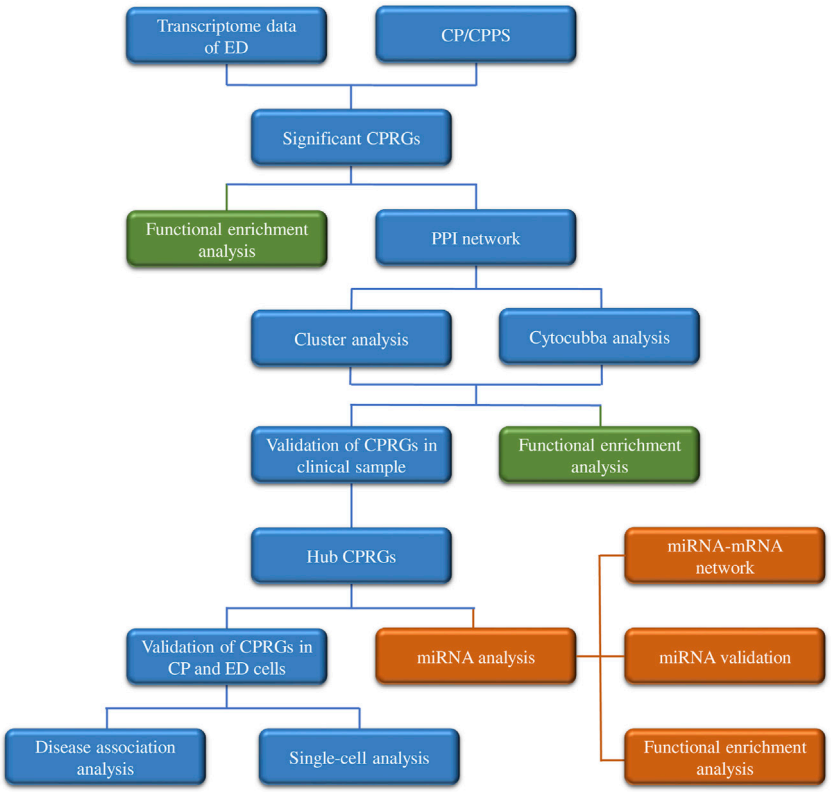

FIGURE 1
The analytic process of this research. ED, erectile dysfunction; CP, chronic prostatitis; CPPS, chronic pelvic pain syndrome; CPRGs, CP/CPPS-related genes; PPI, protein-protein interaction.

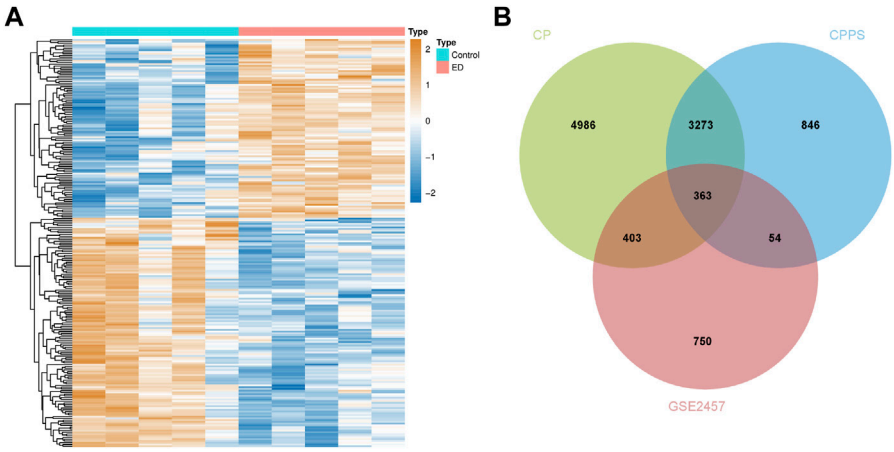

FIGURE 2
Differentially expressed CPRGs analysis. **(A)** The heatmap of significant CPRGs between the ED and control groups. **(B)** Intersection of DEGs of GSE2457 and CP/CPPS-related gene sets. ED, erectile dysfunction; CP, chronic prostatitis; CPPS, chronic pelvic pain syndrome; CPRGs, CP/CPPS-related genes; DEGs, differentially expressed genes.

the interactive functions. The screening criteria was set as an interaction score >0.7 and abandonment of disconnected nodes. Then a protein-protein interaction (PPI) network was constructed in Cytoscape (https://cytoscape.org/, version 3.7.1). In the present network, the Molecular Complex Detection (MCODE) plugin was utilized to conduct module analysis for representing specific molecular complexes with default values (Bader and Hogue, 2003). Additionally, the functional annotation and enrichment analysis of genes in specific modules were performed in

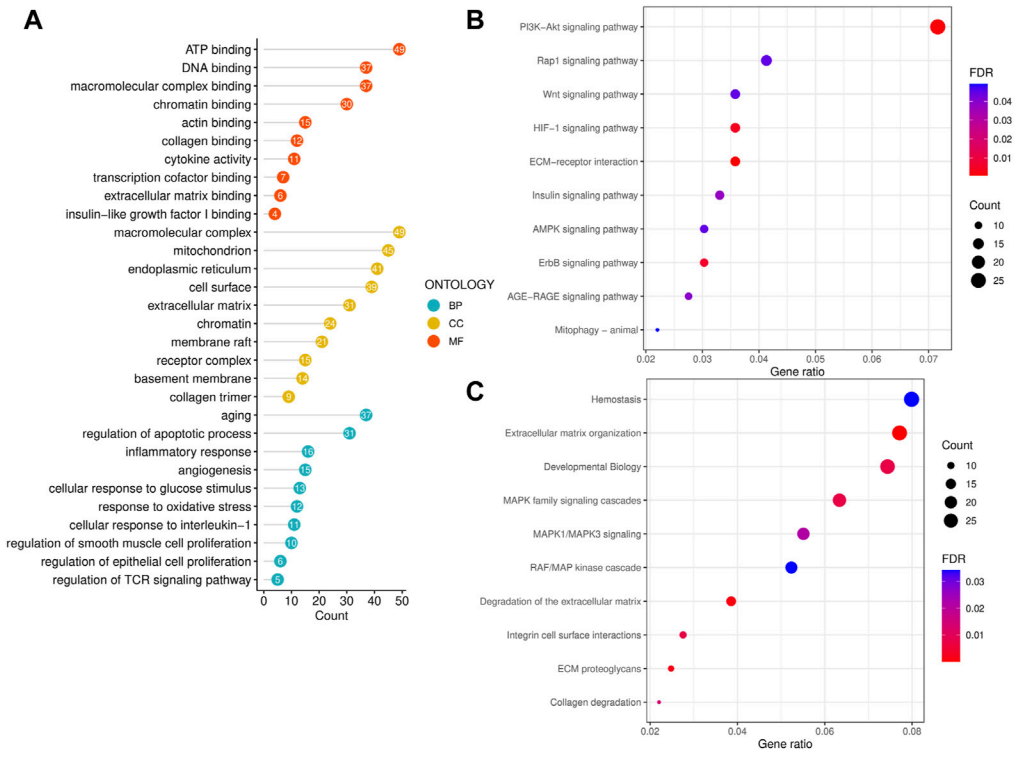

FIGURE 3
Functional enrichment analysis of significant CPRGs. **(A)** GO enrichment analysis of significant CPRGs. **(B)** KEGG enrichment analysis of significant CPRGs. **(C)** Reactome enrichment analysis of significant CPRGs. CP, chronic prostatitis; CPPS, chronic pelvic pain syndrome; CPRGs, CP/CPPS-related genes; GO, Gene Ontology; BP, biological process; CC, cellular component; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes; FDR, false discovery rate.

Metascape (http://metascape.org) to capture the relationship of enriched terms. It included GO biological processes, KEGG pathway, Reactome gene sets, CORUM and WikiPathways. Then these terms were collected and grouped into clusters based on their membership similarities. Terms with a p-value <0.01, a minimum count of 3, and an enrichment factor >1.5 were regarded as significant.

2.5 Significant CPRGs detection and functional interaction

The cytoHubba plugin in Cytoscape was applied to screen hub genes by different topological ranking algorithms including BottleNeck, Degree, DMNC, MCC, MNC, and Stress in the considered network (Chin et al., 2014). The overlapped genes in these six methods were further analyzed in the GeneMANIA tool (https://genemania.org/), which provided co-expression and functional analysis based on co-expression, co-localization and predicted interactions (Warde-Farley et al., 2010).

2.6 Identification of hub CPRGs and miRNA analysis

The expressed difference of overlapped genes in cytoHubba between ED and control groups was presented in a boxplot with the Wilcox test. Subsequently, genes with statistical significance were validated in GSE206528 from the Male Health Atlas database (http://www.malehealthatlas.cn/) (Zhao et al., 2022), which contained single cell sequencing profiling of corpus cavernosum from eight ED patients and relevant men with normal erection. Hub CPRGs were identified ultimately after screening by similar expressed trend.

Hub CPRGs-associated miRNAs were predicted in miRWalk database (http://mirwalk.umm.uni-heidelberg.de/) based on the reference databases of miRTarBase, TargetScan and miRDB. The expression profiling of these miRNAs was validated in clinical specimens of 20 ED patients and control groups in GSE182053. Then a miRNA–hub CPRGs regulatory network was constructed in Cytoscape. The biological functions of miRNAs were conducted in the miRNA Enrichment Analysis and Annotation Tool (miEAA 2.0) database with the threshold of $p < 0.05$ (https://ccb-compute2.cs.uni-saarland.de/mieaa2/).

2.7 External validation of hub CPRGs

To enhance authenticity, the expression profile of hub CPRGs was validated in CP/CPPS-related gene set of GSE159438 and ED-related cellular gene set of GSE146078 with the threshold of adjusted p-value <0.05 by "edegR" package, respectively. The final key genes were regarded as genetic links of ED and CP/CPPS. Then the key genes were analyzed in MHA data to show the distribution of cell clusters. Finally, to identify the association between key genes and diseases, the Comparative Toxicogenomics Database (CTD, http://ctdbase.org/)

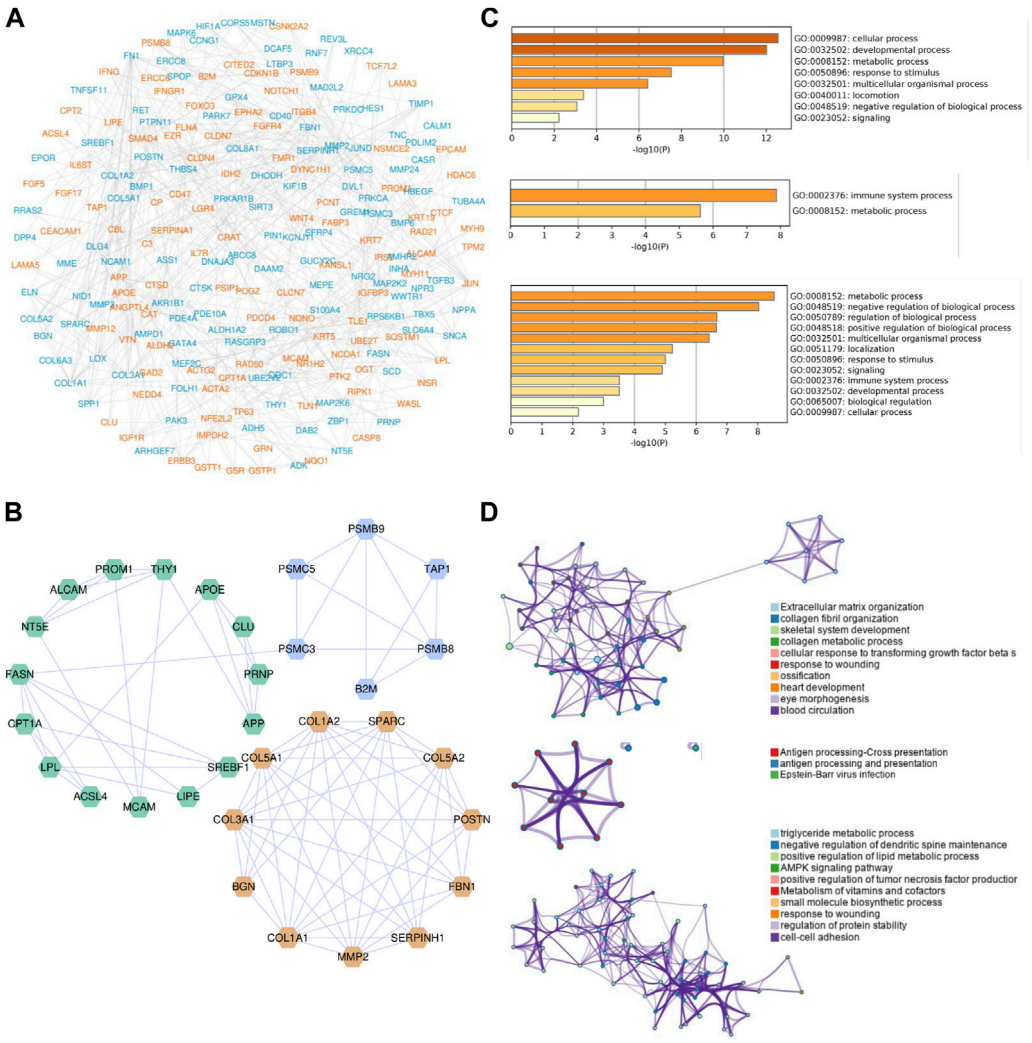

FIGURE 4
PPI network construction and module analysis of significant CPRGs. **(A)** PPI network of significant CPRGs. **(B)** The top three gene clusters based on module analysis. **(C)** GO enrichment analysis of the top three gene clusters. **(D)** Pathway and process enrichment analysis of the top three gene clusters. For **(A)**, yellow labels represent upregulated CPRGs and blue labels for downregulated CPRGs. For **(B)**, yellow, blue, and green labels represent gene model 1, 2, and 3, respectively. For **(D)**, each node represents an enriched term and is colored by cluster ID. PPI = protein-protein interaction. CP = chronic prostatitis; CPPS, chronic pelvic pain syndrome; CPRGs, CP/CPPS-related genes; GO, Gene Ontology.

(Davis et al., 2017) was employed to generate their relevance with the inference score >20.

3 Results

3.1 Identification of significant CPRGs

The analytic process of this research was depicted in Figure 1. After differentially expressed analysis in GSE2457, a total of 1,570 DEGs were confirmed between ED and control groups. The expression profile of DEGs was presented as a heatmap (Figure 2A). Meanwhile, 9,025 genes related to CP and 4,536 genes related to CPPS were obtained in GeneCards. Then a comparative analysis to determine the overlapped genes was conducted. And 363 significant CPRGs were found consisting of 173 upregulated and 190 downregulated genes for further analysis (Figure 2B).

3.2 Functional enrichment analysis of significant CPRGs

To reveal the biological functions and associated pathways of significant CPRGs, the function enrichment analysis was performed based on GO, KEGG and Reactome terms (Figure 3A). In GO analysis, biological process revealed significant enrichment of regulation of apoptotic process, smooth muscle cell proliferation, epithelial cell proliferation and TCR signaling pathway, response to interleukin-1, oxidative stress and inflammatory reaction. Cellular component contained extracellular matrix, chromatin, receptor complex, and so on. Molecular function was highly associated with chromatin binding, insulin-like growth factor binding and cytokine activity.

In KEGG analysis, shared pathways contained PI3K-Akt signaling pathway, HIF signaling pathway, and ECM-receptor interaction (Figure 3B). And extracellular matrix organization, integrin cell

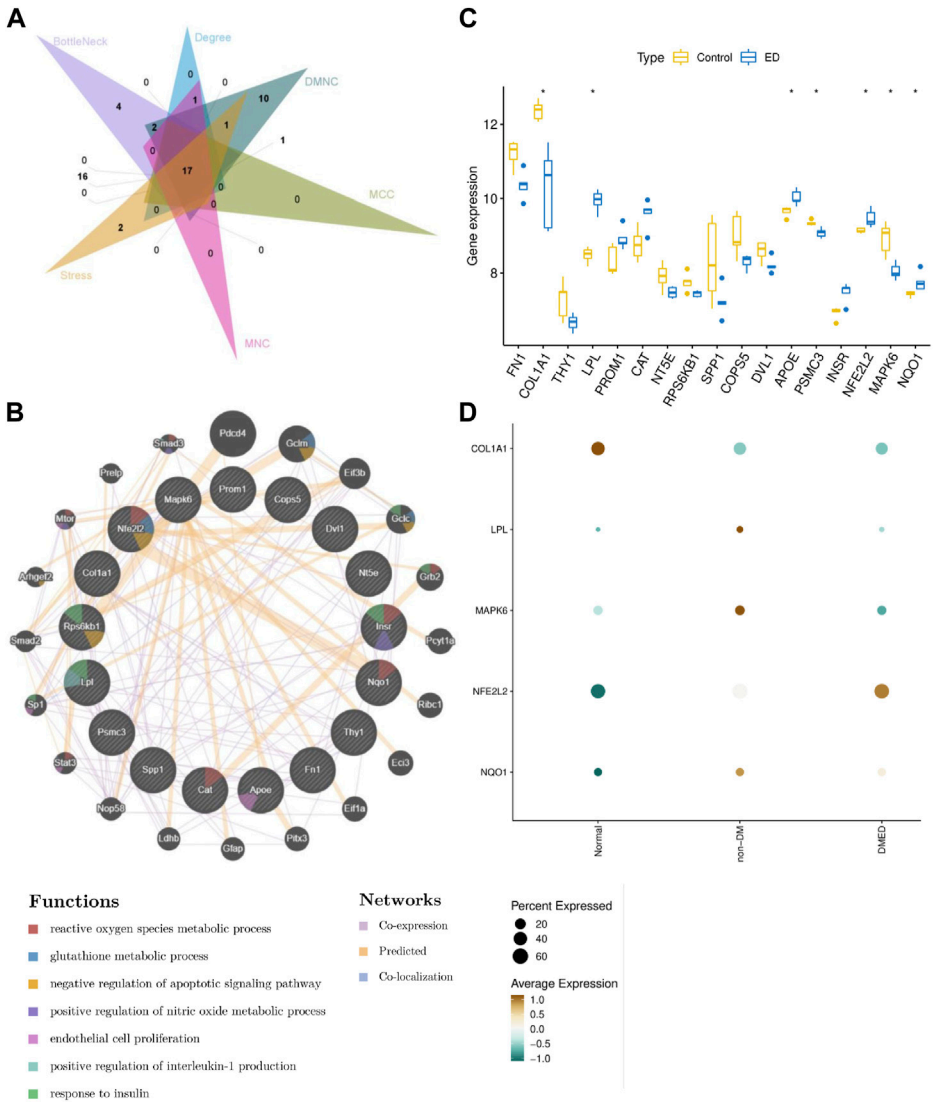

FIGURE 5
Analysis of comprehensive signature of significant CPRGs **(A)** The intersection of genes *via* six topological ranking algorithms in Cytohubba. **(B)** The interaction information for CPRGs in GeneMANIA. **(C)** The expressed pattern of CPRGs between ED and control groups. **(D)** The expressed pattern of CPRGs in clinical patients with impaired erectile function. For **(B)**, the 20 most frequently changed neighboring genes are shown. The associated neighboring genes are in the outer circle and CPRGs in the inner circle. CP, chronic prostatitis; CPPS, chronic pelvic pain syndrome; CPRGs, CP/CPPS-related genes; ED, erectile dysfunction.

surface interactions, collagen degradation and MAPK family signaling cascades were observed in Reactome analysis (Figure 3C).

3.3 Construction of PPI network and module analysis of significant CPRGs

To analyze the interplays among these significant CPRGs, a PPI network was constructed. It consisted of 245 nodes and 504 interactions (Figure 4A). Then gene modules in this network were excavated further to reveal differential biological signatures. MCODE analysis presented nine modules, of which the top three modules had a higher density score (Figure 4B). Biological process enrichment analysis showed that genes on module one participated in multicellular organismal process, and collagen metabolic as well as extracellular matrix organization were noted. Module two focused on immune metabolic process, including antigen processing and presentation. Similarly, multidimensional metabolism and immune process were enriched significantly in module three (Figures 4C, D).

3.4 Analysis of comprehensive signature of significant CPRGs for further analysis

PPI network provided an interactive landscape in these CPRGs. To find the core role in this network, six topological ranking algorithms were utilized to identify the most corresponding components. After comparative analysis, 17 genes exhibited great connectivity (Figure 5A). Furthermore, these genes and 20 their predicted co-expressed genes were displayed in a co-expression network. They were involved in multiple metabolic processes including

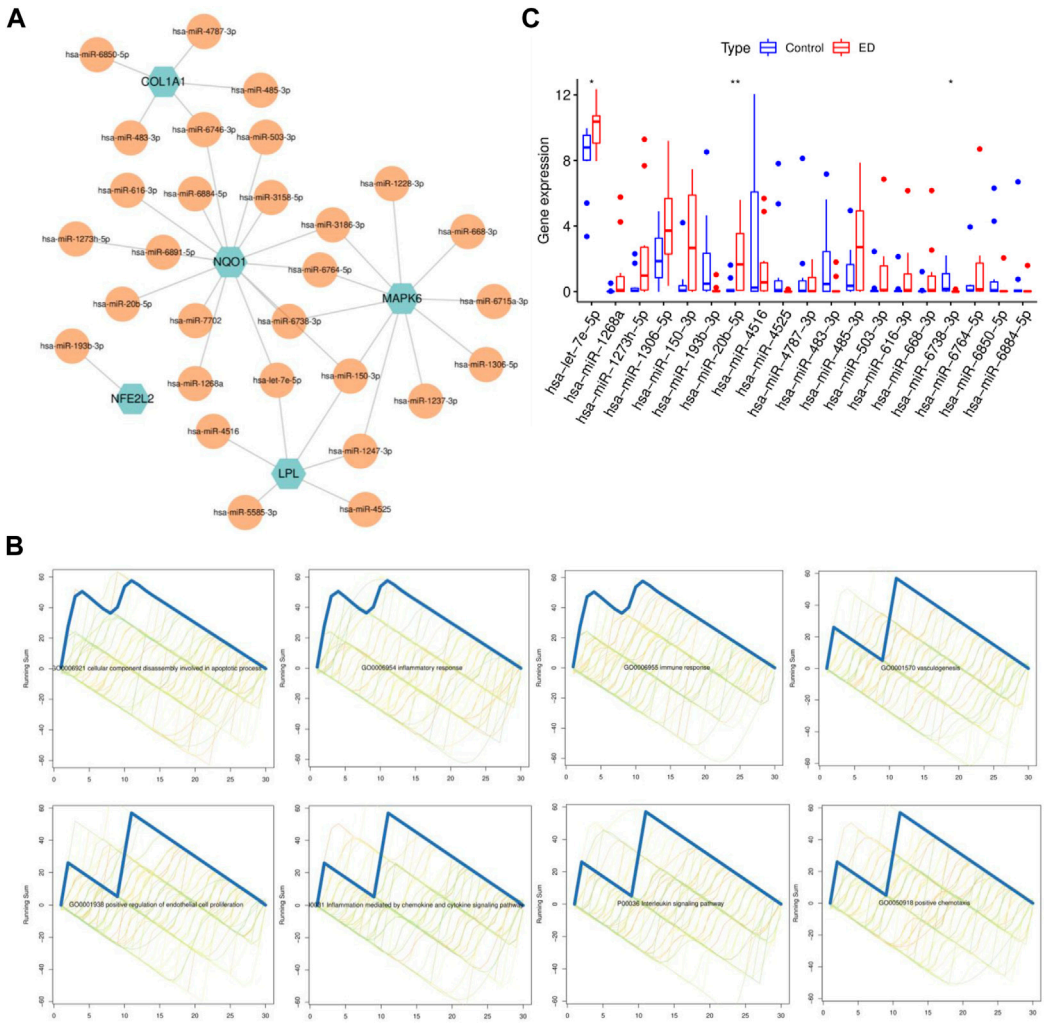

FIGURE 6
MiRNA-CPRGs network construction and functional enrichment analysis. **(A)** MiRNA-CPRGs co-regulatory network. **(B)** The functional enrichment analysis of miRNAs. **(C)** The expressed pattern of miRNAs between ED and control groups. For **(A)**, green labels represent CPRGs and yellow labels for miRNAs. CP, chronic prostatitis; CPPS, chronic pelvic pain syndrome; CPRGs, CP/CPPS-related genes; ED, erectile dysfunction.

reactive oxygen species, nitric oxide, interleukin-1, glutathione, and regulation of apoptosis as well as endothelial cell proliferation (Figure 5B).

The expressed pattern of 17 genes in GSE2457 was exhibited in a boxplot. The result showed that seven of 17 genes remained significant differences between ED and control groups (Figure 5C). In the meanwhile, their expressed signatures were also validated in clinical patients with impaired erectile function. Finally, five genes with consistent trends of expression were identified as hub CPRGs (Figure 5D). That's, COL1A1 and MAPK6 had a lower level of expression in ED group compared with control group. LPL, NFE2L2 and NQO1 showed contrary manifestations.

3.5 MiRNA-CPRGs network construction and functional enrichment analysis

Considering miRNAs could contribute to the process in the interaction of ED and CP/CPPS, miRNAs related to hub CPRGs were predicted first. After validation in clinical specimens, 29 of 862 miRNAs were obtained. Then a co-regulatory network with hub CPRGs as the core components miRNAs as edges was generated (Figure 6A). Consistent with mRNAs, functional enrichment showed that these miRNAs had a tight association with inflammatory response including positive chemotaxis and interleukin signaling pathway, immune response, vasculogenesis (Figure 6B), which suggested a synergetic relationship of CPRGs and miRNAs during the diseases process. Finally, hsa-let-7e-5p, hsa-miR-20b-5p and hsa-miR-6738-3p remained different expression between ED and control groups (Figure 6C).

3.6 External validation of hub CPRGs

Since CPRGs were screened from ED-related expression matrix and CP/CPPS-related gene sets progressively, to enhance authority and preciseness, hub CPRPs were validated further in external CP/CPPS and ED-related cellular gene sets simultaneously (Figures 7A, B). After performing differentially expressed analysis, overlapped

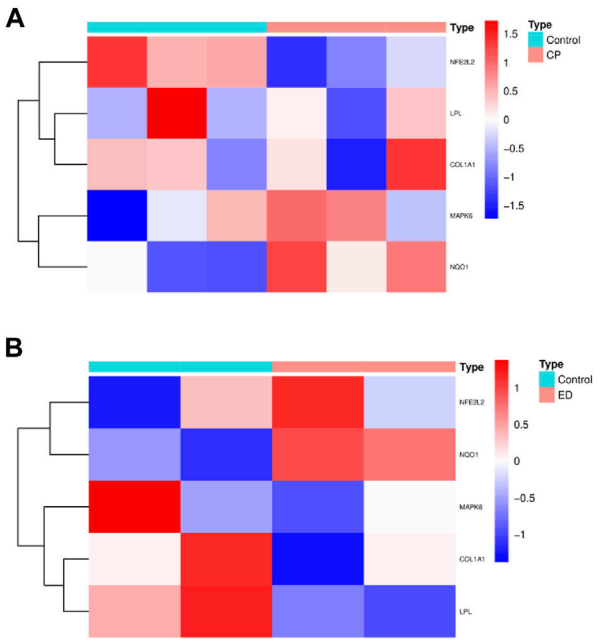

FIGURE 7
The profiling of hub CPRGs in CP/CPPS **(A)** and ED-related cellular **(B)** gene sets. CP, chronic prostatitis; CPPS, chronic pelvic pain syndrome; CPRGs, CP/CPPS-related genes; ED, erectile dysfunction.

DEGs in the two gene sets were compared with hub CPRGs. Finally, NQO1 was identified. It may serve as a key in the genetic links of ED and CP/CPPS for clinically and pharmacologically oriented research.

3.7 Subpopulation distribution and disease association of hub CPRGs

The subpopulation distribution of NQO1 in corpus cavernosum was explored in MHA. The result showed that seven types of cells with 11 subtypes existed in human corpus cavernosum for clustering analysis. They consisted of corpus cavernosum endothelial cell, vessel endothelial cell, PI16-positive fibroblast, APOC1-positive fibroblast, COMP-positive fibroblast, pericyte, corpus cavernosum smooth muscle cell, vessel smooth muscle cell, Schwann cell, macrophage and T cell (Figure 8A). NQO1 was predominately enriched in endothelial cell (Figure 8B).

Considering immune response was enriched in the biological functions of CPRGs and related miRNAs, disease association of NQO1 was utilized in CTD. In male urogenital disease, apart from ED and prostatitis, NOQ1 was associated with testicular and male infertility (Figure 8C). In immune system disease, NOQ1 may be involved in the process of autoimmune diseases and immunological deficiency syndrome (Figure 8D).

4 Discussion

High morbidity and prevalence of ED and CP/CPPS in men have been noticed in recent years (Ma et al., 2020). The coexistence of these conditions is attractive and elusive. ED was thought to account for a large proportion among men with prostatitis-like symptoms (Zhang et al., 2015). Also, the history of prostatitis was regarded as an independent risk factor for ED (Ma et al., 2020). There are no studies to investigate the genetic relationship between ED and CP/CPPS. Therefore, this study focused on exploring shared transcriptional alternations, interaction pathways and regulatory network through a systems biology approach. After a multi-omics analysis, hub biomarkers and associated molecular roles were well-mined and validated.

The initial analysis identified 173 upregulated and 190 downregulated shared genes in ED and CP/CPPS. Since both were caused by various etiological factors, to distinguish their heavy interconnection, functional enrichment analysis was conducted first. Obviously, apoptosis, oxidative stress, inflammatory response related to interleukin, and smooth muscle cell proliferation contributed to the predominant functions in these conditions. In a rat model of prostatitis, except for impaired erectile function, CP/CPPS could enhance oxidative stress and calcium imbalance and promote transformation from contractile to synthetic state in corpus cavernosum smooth muscle cells (Wang et al., 2020). Inflammation plays an important role in CP/CPPS and ED. Impaired endothelium could accelerate inflammation in penile vasculature, accompanied by increased inflammatory cytokines like TNF-α and IL-6 (Vlachopoulos et al., 2015; Huang et al., 2019). Hu et al. (Hu et al., 2016) reported that CP/CPPS rendered a systemic inflammatory response in the corpus cavernosum, characterized by increased levels of TNF-α, IL-6 and IL-1β. As we know, oxidative stress and associated apoptosis are crucial pathological phenotypes during the damage of corpus cavernosum endothelial and smooth muscle cells. Elevated production of reactive oxygen species and apoptotic process were noted in the corpus cavernosum of CP/CPPS (Hu et al., 2016). In pathway analysis, PI3K-Akt signaling pathway is a classic process in regulating the activity of endothelial nitric oxide synthase in ED (Li et al., 2017). Similarly, inflammation and oxidative stress could be mediated by PI3K/AKT/FOXO1 pathway in autoimmune prostatitis (Feng et al., 2021). HIF signaling pathway is predominant in hypoxia (Liang et al., 2021). These shared pathways should be considered in the shared pathology of ED and CPPS.

A total of 363 significant CPRGs were heterogeneous gene sets. Cluster analysis in PPI network showed different roles of these genes. It was notable that module two and three focused on immune metabolic process, including 21 genes and 40 interactions. Actually, CP/CPPS was more likely to be considered as an autoimmune disease. Many autoantigens have been identified in the pathogenesis of CP/CPPS (Hou et al., 2009; Liu et al., 2021). Chen et al. (Chen et al., 2021b) showed that the T helper type 1 (Th1) and Th17 cells promoted the progression of CP/CPPS. In detail, Th1 and Th17 immune responses specific to prostate antigens were associated with chronic inflammation of the male genital tract including prostate in patients with CP/CPPS (Motrich et al., 2020). However, apart from increased inflammatory biomarkers, immune process was seldom mentioned in ED. Immune cell infiltration was not observed obviously in diabetic ED (Wang et al., 2022). We speculated that immune response was a particular process in prostatitis complicated with ED.

Ultimately, a 5-gene signature comprising COL1A1, MAPK6, LPL, NFE2L2 and NQO1 were regarded as the hub CPRGs, in which NQO1 may serve as the genetic link in ED and CP/CPPS. COL1A1 encodes the pro-alpha1 chains of type I collagen as a major

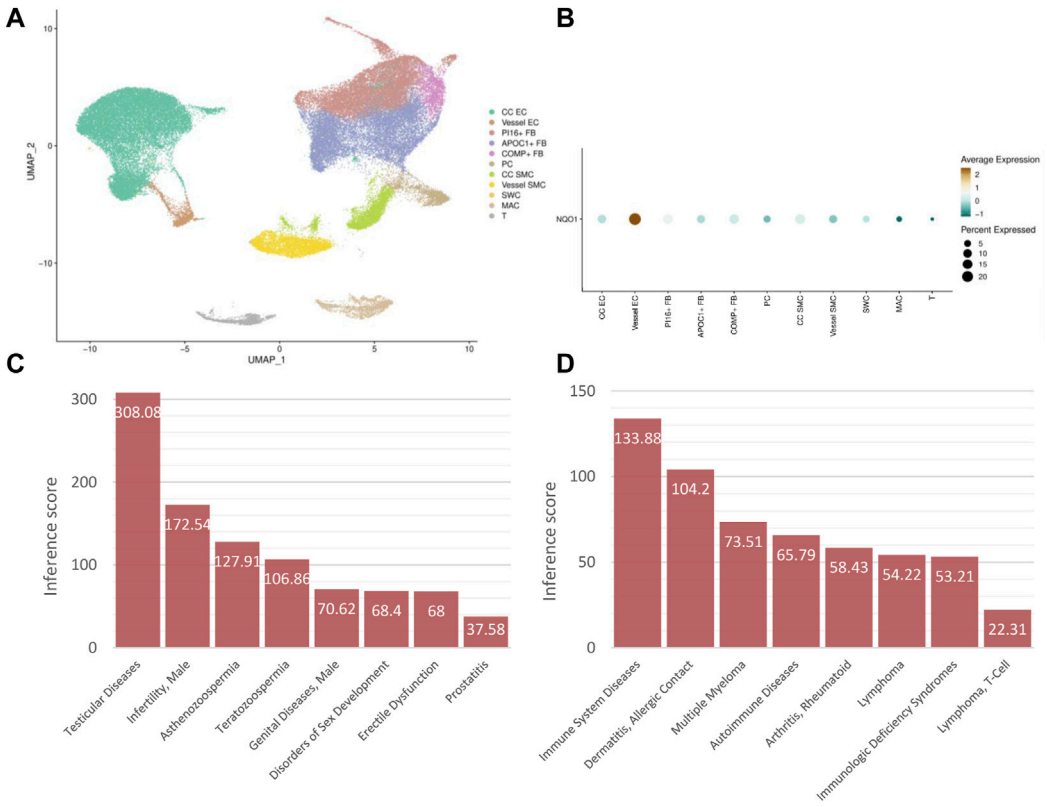

FIGURE 8
Subpopulation distribution and disease association of hub CPRGs **(A)** UMAP visualization of subpopulations clustering in human corpus cavernosum. **(B)** Expression distribution of NQO1 in ED patients grouped by cell types. **(C)** The association between NQO1 and male urogenital diseases. **(D)** The association between NQO1 and immune system diseases. CP, chronic prostatitis; CPPS, chronic pelvic pain syndrome; CPRGs, CP/CPPS-related genes; ED, erectile dysfunction.

component of extracellular matrix. It could affect apoptosis by regulating oxidative stress and autophagy in bovine cumulus cells (Fu et al., 2019), and serve as a reliable biomarker in tumors (Ma et al., 2019; Geng et al., 2021). The vital role of COL1A1 accounted for the extracellular matrix-related relationship in ED and CP/CPPS during functional enrichment analysis, and further research should focus on the associated changes. The protein encoded by MAPK6 is a member of the Ser/Thr protein kinase family, which plays an important role in immune and inflammation (Arthur and Ley, 2013). Studies *in vitro* and *in vivo* demonstrated that defects in chemotaxis of monocytes and neutrophils were noticed in MAPK6-deficient cells. MAPK6 was essential to produce several cytokines like IL-8 and activating protein 1 (Bogucka et al., 2020). Therefore, MAPK6 could be thought to construct the inflammatory relationship between ED and CP/CPPS, and worth exploring in depth.

LPL, NFE2L2 and NQO1 showed higher levels of expression in ED group compared with control group. LPL encodes lipoprotein lipase and modifies the regulation of lipid balance in energy homeostasis (Wang et al., 2011). LPL could increase uptake of modified LDL and impair both vascular and endothelial cells, inducing the decrease in eNOS expression. It had a vital impact on blood vessel relaxation (Sullivan et al., 2005). Huo et al. (Huo et al., 2019) found that lncRNA-MIAT downregulation protected erectile function by targeting LPL *via* activating miR-328a-5p in diabetic ED. Although it is not concerned in the available research of prostatitis, it reflected the shared energy metabolism in ED and CP/CPPS. NFE2L2 regulates genes containing antioxidant response elements in their promoters. Pharmaceutical research in ED found that NFE2L2 was a promising target by regulating oxidative stress and inflammatory response in treating ED (Draganski et al., 2018; Pierre et al., 2022). Similarly, in a model of chronic nonbacterial prostatitis, NFE2L2 was involved in the process that tadalafil alleviated inflammation and oxidative stress in RWPE-1 cells (Song et al., 2021). These results propelled the interplay between ED and CP/CPPS.

After comprehensive analysis and validation, a co-regulatory network between hub CPRGs and miRNAs was generated. And hsa-let-7e-5p, hsa-miR-20b-5p as well as hsa-miR-6738-3p remained different expression between ED and control groups. In ED research, Xu et al. (Xu et al., 2021) has revealed that hsa-let-7e-5p was one of the characteristic miRNAs as signature by machine learning method in diabetic ED. Besides, circulating hsa-let-7e-5 could serve as one of the peripheral biomarkers for major depression and bipolar disorders mood disorders (Gecys et al., 2022). And the latter propelled the pathological interaction in ED and CPPS. As for hsa-miR-20b-5p, it was regarded as one of the oncogenic miRNAs in T-cell acute lymphoblastic leukemia by repressing the expression of PTEN and BIM (Drobna et al., 2020). Besides, miR-20b-5p contributed to the dysfunction of aortic smooth muscle cells by targeting MAGI3 in hypertension (Xu and Yu, 2022). The role of hsa-miR-20b-5p in interacting with NOQ1 during the

interplay between ED and CP/CPPS should be considered in the further research. Finally, hsa-miR-6738-3p was seldom reported except for primary great saphenous vein varicosities and gastric cancer (Zhang et al., 2018; Fattahi et al., 2021), and its potential functions in ED and CP/CPPS would be analyzed in future experiments.

Our results identified NQO1 as the hub genetic link in ED and CP/CPPS after validation. NQO1 belongs to the NAD(P)H dehydrogenase family and encodes a cytoplasmic 2-electron reductase. Similar to LPL, NQO1 manifests a protective process against inflammatory stimuli and oxidative injury in multiple systems including endothelial and vascular smooth cells (Lee et al., 2021). The finding was consistent with the subpopulation distribution of NQO1 in corpus cavernosum of our results. Zhou et al. (Zhou et al., 2022) pointed out that ED was found in a rat model of hypospadias, accompanied by increased NRF2/Keap-1 ratio and NQO1 expression. Besides, it was regarded as a biomarker against oxidative damage in hydrocortisone-induced ED (Yu et al., 2020). Disease association analysis uncovered the tight correlation of NQO1 and male urogenital disease as well as immune system disease, which showed potential functions in these diseases. It is also significant to explore the specific mechanism of NQO1 in the cross-link of ED and CP/CPPS.

This study could provide new views into the genetic patterns of the shared transcriptional signature in ED and CP/CPPS. However, some limitations were exposed in the present analysis. This study performed relevant molecular excavation based on cell, animal, and human samples with validation, but approach bias *via* the bioinformatics was unavoidable. In addition, due to limited eligible datasets related to ED and CP/CPPS, recapitulating the shared genetic alternations wasn't persuasive enough. And different types of diabetes were used in the analysis of associated miRNAs. Considering the predominant roles of hub CPRGs in ED ad CP/CPPS, more experimental verification and prospective studies will be conducted in our further research.

5 Conclusion

We identified the genetic profiles underlying interaction between ED and CP/CPPS *via* multi-omics analysis. A hub gene signature consisting of COL1A1, MAPK6, LPL, NFE2L2, and NQO1 as well as corresponding regulatory network were well screened and validated. NQO1 was regarded as a key in the genetic links of the two conditions. These findings laid the groundwork for the molecular mechanism of ED with CP/CPPS, and paved the way to fueling the clinically oriented research.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Author contributions

Conceptualization: PY and YC; formal analysis: PY, TS, ZH, and QM; writing-original draft: PY; funding acquisition: PY and YC; data collection and analysis: PY, TS, and ZH; review and editing: PY, TS, ZH, and YC, QM; 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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Hypothalamic-pituitary-adrenal axis activity and its relationship to the autonomic nervous system in patients with psychogenic erectile dysfunction

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Background: Psychological stress and its two stress response systems, the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS), are closely related to psychogenic erectile dysfunction (pED). However, the analyses of perceived stress and stress systems in pED patients need to be more in-depth, especially the interactions between them.

Methods: Our study included 75 patients with pEDs and 75 healthy men. The International Index of Erectile Function-5 (IIEF-5) and the 10-item Perceived Stress Scale (PSS-10) were used for assessing the severity of ED and perceived stress. All participants collected saliva samples on three consecutive days at eight specific times with strict reference to the time of morning awakening for measuring cortisol parameters and wore electrocardiography for 24 h to derive heart rate variability (HRV).

Results: The PSS-10 scores of pED patients were significantly higher than the control group ($p<0.001$). Although PSS-10 and IIEF-5 scores were negatively correlated in pED patients, there was no statistical significance between them ($r=-0.049$, $p=0.677$). Compared with the control group, the HRV parameters of pED patients were significantly increased in LF/HF ratio ($p=0.014$) but significantly decreased in LF, HF, and pNN50 ($p<0.001$). However, the two groups had no statistically significant differences in cortisol variables (all $p>0.05$). The interaction between sympathovagal modulation (HF, rMSSD) and cortisol awakening response (CAR AUCi) explained significantly greater variance in perceived stress than either stress system alone. Higher parasympathetic activity combined with a higher cortisol awakening response was associated with greater perceived stress.

Conclusion: Our results suggested that the interrelation between ANS and HPA axis activity might enhance our comprehension of how stress affected the physical and mental health of pED patients.

KEYWORDS

psychogenic erectile dysfunction, autonomic nervous system, heart rate variability, HPA axis, cortisol, perceived stress

Introduction

Erectile dysfunction (ED) is the most common male sexual disorder, which refers to the persistent inability to attain or/and maintain an adequate penile erection to complete satisfactory sexual intercourse (1). Psychogenic ED (pED) is a primary subtype of ED caused by mental and psychosocial factors, which is diagnosed in the exclusion of organic factors and has severe impacts on the overall psychological health and quality of life (QOL) of patients and their partners (2). It even brings huge socio-economic burdens (3). ED is highly prevalent in modern societies, affecting approximately 30% of young men (4). pED patients account for 73.6% of ED patients under 40 years old in China (5). In recent years, pED has been the focus of researchers due to its extreme importance and high incidence (3).

Stress represents a necessary response that maintains *in vivo* homeostasis upon exposure to the threat of the environment and events (6). Chronic psychological stress has detrimental effects on both physical and mental health (7). It is known to cause physiological distress, leading to body balance perturbations associated with various metabolic and immune dysfunctions (8). Recent experimental evidence suggests that chronic stress is also closely related to ED (9–12). For instance, chronic stress might lead to the development of ED by reducing nitric oxide synthase expression in the penile constitution (9). The corpus cavernosum tissues of male rats under long-term stress had morphological changes (10). Chronic psychological stress impaired the neurogenic and endothelium-dependent relaxation function of the rabbit corpus cavernosum, resulting in ED (11). Additionally, men engaged in stress management had statistically significant reductions in perceived stress scores compared with men treated with tadalafil alone (12). Meanwhile, even if mental stress is not the cause of ED, ED can lead to psychological stress and further aggravate ED symptoms (13).

Psychological stress triggers a cascade of pathophysiological events mediated by the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis (14, 15). These two neural stress systems coordinate the responses of many other physiological systems to stressors, including the immune and cardiovascular systems, allowing the body to return to homeostasis (14). Dysregulation of the HPA axis or ANS can significantly disrupt homeostasis, causing cacostasis or allostasis, with various clinical manifestations. This could be a potential mechanism contributing to the pathogenesis of pEDs. However, so far, only a few studies have reported the changes in the ANS system in patients with ED. A previous study has shown that ED patients who were not classified by etiology exhibited relatively lower parasympathetic activity (16). Patients with pED further demonstrated significant cardiac sympathetic hyperactivity and severity-dependent vagal impairment (17). No reports have been found for the relationship between ED or pED and HPA axis change. Therefore, we intended to study the changes in these two stress response systems in pED patients simultaneously.

It is also worth noting that the ANS and HPA axis are highly coordinated and physically interconnected (18). The ANS and HPA axis activation in response to stress follows a coordinated, transient sequence. The ANS rapidly promotes physiological changes through the synaptic transmission of its two branches, the sympathetic and parasympathetic nervous systems. The parasympathetic nervous system promotes the sympathetic response to stress by eliminating its inhibitory effect, which enables physiological changes, including releasing norepinephrine by the locus coeruleus and stimulating sympathetic preganglionic neurons to increase heart rate (18). Whereas the HPA axis, as a hormonal system, exerts corresponding regulatory effects on the body a few minutes after activation. The HPA axis is initiated by releasing the corticotrophin-releasing hormone from the paraventricular nucleus of the hypothalamus, which leads to a cascade of endocrine events that ultimately lead to the release of cortisol from the adrenal cortex (18). Cortisol affects immune and metabolic systems and enhances the ANS activity, such as increasing the sympathetically mediated cardiovascular response to stress, manifested by an increased heart rate (19). The ANS works with the HPA axis to form biological and behavioral homeostasis. Thus, exploring the interrelation between ANS and HPA axis can provide more insight into the association between psychological stress and pED (18).

To deepen our understanding of the complexity of psycho-neuro-endocrine interactions in pED patients, in the present study, we aimed to determine whether and how both ANS and HPA axis changed in patients with pED and tested the hypothesis that inter-relation models, which included ANS and HPA axis interactions, would explain perceived stress better than models with ANS or HPA axis singularly.

Materials and methods

Participants

The study was carried out at the Urology Male Clinic of our hospital and ran from March 2016 to August 2022. Patients were interviewed to complete the Chinese version of the International Index of Erectile Function 5 (IIEF-5) (20). Inclusion criteria were partnered sexual activity, history of psychogenic ED for at least six months, a score of 5–21 in the IIEF-5 system, no conscious penile erection, poor hardness or non-lasting erection, and inability to complete normal sexual life; >18 or <50 years of age; genital examination showing no obvious developmental deformity; normal development of secondary sexual characteristics. Patients with vasculogenic and neurogenic ED (neurologic disease, pelvic surgery) were excluded. Participants using medications known to interfere with cardiovascular or endocrine function, such as tricyclic antidepressants and corticosteroids, were excluded. In addition, exclusion criteria also included other sexual dysfunction or sex hormone abnormalities or diseases that may influence ANS and HPA axis; psychosis; peripheral vascular disease; diabetes; spinal cord injury; coronary heart disease; hypertension; a history of alcohol or drug abuse, etc. All patients did not use drugs affecting sexual function in the past six months and were not administered drugs or other methods for ED treatment within three months. Healthy men were recruited as controls from advertisements in the communities surrounding the hospital.

Perceived stress

Perceived stress was assessed using the Perceived Stress Scale, the most widely used psychometric instrument to measure the perception of stress. Participants were administered the 10-item Perceived Stress Scale (PSS-10) (21), an abbreviated version of the 14-item Perceived Stress Scale with high validity and reliability, assessed using a 5-point Likert scale ranging from 0 (never) to 4 (very often). The PSS-10 evaluated how unpredictable, uncontrollable, and overwhelming an individual considers their life to be over the past month. It was translated into Chinese and tested through internal consistency, the construct validity presenting good psychometric qualities.

Salivary cortisol

Saliva samples were collected eight times per day for three consecutive weekdays, with strict reference to the time of morning awakening: 0, 15, 30, and 60 min after awakening, followed by four more samples at 3-h intervals throughout the day. Participants were given labeled 1.5-ml sterile Eppendorf tubes and adjustable alarm clocks to collect saliva samples. They were told not to eat, drink, chew gum, smoke, or brush their teeth 30 min before sampling and were instructed to place the cotton swabs under their tongues for at least 30 seconds and, when the swabs were saturated, to put them back into the Eppendorf tubes. Samples were stored in the participant's home refrigerator until all saliva samples were collected, which were then shipped to our laboratory and stored at -80°C until assayed. Participants recorded the date, the precise wake-up time, and the time each sample was taken in a daily log. To ensure compliance with saliva collection procedures, we adjust the alarm clock to beep at designated times for saliva collection. In addition, notification information was sent to participants *via* mobile phone text messages the night before each sample collection. This non-invasive technique was used for home or work collection to minimize disruption to everyday daily life.

The free cortisol concentration in saliva was assayed in duplicate using a ^{125}I spectral radioimmunoassay kit (Beijing North Institute of Biotechnology, Beijing, China), according to the manufacturer's specifications. Assay sensitivity was estimated at 0.1 nmol/l. The intra- and inter-assay coefficients of variation were less than 6 and 10%, respectively. Unconverted cortisol values were used to calculate four cortisol measurements per day: the areas under the awakening response relative to dynamic increase (AUCi) and ground (AUCg) of cortisol awakening response (CAR), the diurnal cortisol slope (DCS) and diurnal cortisol AUCg. CAR AUCi indicated changes (positive or negative) in cortisol concentration, thus signifying HPA axis reactivity and response to arousal stress. However, the CAR AUCg value reflected the total cortisol secretion within 1h after awakening. DCS represented the change in cortisol secretion across the day, estimated by fitting a line that best matched all cortisol values. Finally, the diurnal AUCg showed the total salivary cortisol secretion and total HPA axis activity during the day. The three-day average of each cortisol measure was used in the analyses.

Heart rate variability

By monitoring HRV, we could quantify the activity of the autonomic nervous system (ANS) and its sympathetic and parasympathetic modulation due to workload. The HRV was recorded using wireless Holter (BMS Century 3,000; Biomedical Systems, St. Louis, MO, United States) monitoring to obtain a 24-h electrocardiogram (ECG). During the recording, the participants followed their daily life and completed a time-activity diary. To avoid the influence of the circadian cycle, we scheduled all measurements to start at 7–8 a.m. and end at 24 h later. An ECG analysis software (CardioScan 12 Satellite; DM Software Inc., Beijing, China) was used to determine the frequency and time domain parameters of HRV. Arrhythmias and noise were automatically identified and filtered by the same software prior to the analysis of HRV parameters and then validated or modified, if necessary, by a trained cardiologist. Frequency-domain measures included: power in very low frequency (VLF; 0.01–0.04 Hz), in low frequency (LF; 0.04–0.15 Hz), in high frequency (HF; 0.15–0.40 Hz), and the LF/HF ratio. Time-domain parameters included: the standard deviation of normal-to-normal R-R intervals (SDNN), root mean square of the successive differences of R-R intervals (rMSSD), the proportion of the number of pairs of successive R-R intervals that differ by more than 50 ms divided by the total number of R-R intervals (pNN50) and standard deviation of the average R-R intervals calculated over 5 min (SDANN).

Statistical analysis

SPSS 17.0 (SPSS Inc., Chicago, IL, United States) was used for all statistical analyses. Kolmogorov-Smirnov test, normal curve histogram, and P-P plots were used to test the normality of all continuous variables. Continuous variables were expressed as median and range or mean ± standard deviation (SD), depending on the (non-) normal distribution of the measured variables. Discontinuous variables were described as numbers (percentages). The independent-sample t-test was used to compare normal distribution continuous variables, and the Kruskal-Wallis test was used to compare non-normal variables. The IIEF-5 scores were adjusted for age because the ED is known to be age dependent. The chi-square test or fisher's exact test was used for categorical variables.

Next, hierarchical linear regression analyses were conducted to examine whether the cortisol and HRV data were associated with perceived stress. A collinearity analysis was performed to check the prerequisites for regression analysis and showed no signs of collinearity (tolerance factor >0.1 and variance inflation factor<10). Due to concerns regarding multicollinearity, singular models tested the association between each stress system measure (HRV: VLF, HF, LF, LF/HR ratio, SDNN, SDANN, rMSSD, pNN50; Cortisol: CAR AUCi, CAR AUCg, diurnal AUCg, DCS) and perceived stress. Inter-relation models included the main effects and two-way interaction of HRV and cortisol measures. Simple slope analyses were used to interpret significant interactions. Tests were two-tailed, and $p<0.05$ was considered statistically significant.

Results

Demographic, symptomatic, and psychological characteristics

We recruited 92 patients with pEDs and 88 healthy men. Due to the withdrawal of consent, incomplete information, and other reasons, some participants discontinued the study, and finally, 75 pED patients and 75 healthy men completed our study. The ages of all subjects ranged from 20 to 49 (31.9 ± 7.1) years, with a median age of 30.5 years. The two groups were age-matched ($p=0.418$) and had a similar body mass index (BMI) ($p=0.380$). pEDs were all newly developed, and the disease course lasted from three to nine months, with a median of about five months. The distribution of occupational status ($p<0.001$) and marital status ($p=0.007$) were significantly different between the two groups. Compared with the control group, there were very few student patients and no single patients. Nevertheless, the distribution of educational attainment was roughly the same in both groups ($p=0.129$). Last but most important, the IIEF-5 and PSS-10 scores of pED patients were significantly different from those of healthy controls ($p<0.001$ for both). Although PSS-10 and IIEF-5 scores were negatively correlated in pED patients, there was no statistical significance between them ($r=-0.049$, $p=0.677$). The detailed characteristics of each group are presented in Table 1.

Measurement of cortisol and HRV parameters

Table 1 also showed the differences in cortisol and HRV parameters between groups. In the frequency domain analysis, the LF and HF power of the pED group were significantly lower than those of the control group (both $p<0.001$), but the pED patients had a significantly higher LF/HF ratio than the controls ($p=0.014$). In the time domain analysis, only pNN50 was significantly higher in the healthy control group than in the pED group ($p<0.001$). However, no statistically significant differences in changes between groups were seen in any cortisol variables during the study (all $p>0.05$).

Singular versus inter-relation models

A two-way interaction term tested whether each HRV parameter moderated the relation between cortisol measures and perceived stress. In pED patients, CAR AUCi, CAR AUCg, and diurnal cortisol AUCg demonstrated significant associations with perceived stress in the models. Inter-relation models with the interactions of HF*CAR AUCi and rMSSD*CAR AUCi accounted for significantly greater variance in perceived stress than the singular HRV or cortisol models. Other parameters had no significant interactions. The inter-relation of HF*CAR AUCi and rMSSD*CAR AUCi accounted for an additional 6.1% and 5.9% of the variance in perceived stress. Results are given in Table 2. However, in the control group, only pNN50 was associated with perceived stress in the singular HRV model. No significant interactions were found in all HRV and cortisol parameters (Table 3).

Simple slope test

A simple slope analysis was performed to identify patterns of the inter-relations between ANS and HPA axis activity and to show how HF and rMSSD modulated the association between CAR AUCi and perceived stress in pED patients. Specifically, higher HF combined with higher CAR AUCi was associated with higher perceived stress ($k=3.333$, $p=0.009$; Figure 1A). Similarly, higher rMSSD combined with greater CAR AUCi was associated with higher perceived stress ($k=3.065$, $p=0.009$; Figure 1B).

Discussion

Psychophysiological measures are reliable indicators of stress. PSS-10 is a widely used psychological tool to assess non-specific perceived chronic stress and to measure the extent to which a person's life situation is evaluated as stressful (21). As expected, pED patients in this study had significantly higher PSS-10 scores than age-matched asymptomatic men, suggesting that pED patients did experience more significant perceived stress; however, their

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TABLE 1 Descriptive statistics: demographics, symptomatic and psychological scores, cortisol and HRV measures.

Characteristics and parameters	pED	Control	*p* value*
Demographics			
Sample size, n	75	75	N/A
Age (years), mean ± SD	31.44 ± 7.12	32.39 ± 7.15	0.418
BMI (kg/m^2), mean ± SD	23.53 ± 1.80	23.83 ± 2.25	0.380
Employment, n(%)			<0.001
Student	3(4.0)	28(37.3)	
Employed	34(45.3)	21(28.0)	
Unemployed or retired	38(50.7)	26(34.7)	
Marital status, n(%)			0.007
Never married/single	0(0)	7(9.3)	
Married/cohabiting	73(97.3)	62(82.7)	
Divorced or separated	2(2.7)	6(8.0)	
Educational level, n(%)			0.129
Less than high school	13(17.3)	14(18.7)	
High school graduate/vocational school	14(18.7)	24(32.0)	
College graduate or higher education	48(64.0)	37(49.3)	
Symptomatic and psychological scores			
IIEF-5, mean ± SD	8.40 ± 2.34	23.12 ± 0.97	<0.001
PSS-10, mean ± SD	17.44 ± 4.75	13.97 ± 6.08	<0.001
Cortisol measures			
CAR AUCi (μg·min/dl), median (range)	85.20 (-123.98-486.83)	134.33(-550.80-1107.68)	0.744
CAR AUCg (μg·min/dl), median (range)	351.15 (93.00-577.20)	368.25 (93.00-1109.03)	0.972
Diurnal AUCg (μg·min/dl), median (range)	2211.45 (805.58-4834.13)	2111.63 (553.80-10345.88)	0.970
DCS (μg/dl/h), median (range)	-0.15(-0.48-0.11)	-0.05 (-1.05-0.18)	0.784
Frequency-domain HRV			
VLF (ms^2), median (range)	2842.00 (146.00-6623.00)	2376.00 (240.00-5428.00)	0.219
LF (ms^2), median (range)	670.00 (121.00-6289.00)	1893.00 (150.00-4876.00)	<0.001
HF (ms^2), median (range)	622.00 (127.00-9624.00)	3845.00 (1179.00-7496.00)	<0.001
LF/HF, median (range)	0.66 (0.07-14.05)	0.60 (0.03-3.47)	0.014
Time-domain HRV			
SDNN (ms), median (range)	146.47 (29.36-327.23)	136.40 (30.62-274.38)	0.660
SDANN (ms), median (range)	118.96 (20.22-349.58)	113.52 (20.55-272.82)	0.866
rMSSD (ms), median (range)	37.25 (20.79-120.39)	58.68 (16.35-108.04)	0.065
pNN50 (ms), median (range)	16.50 (5.74-39.22)	18.50 (6.98-39.22)	<0.001

SD, standard deviation; BMI, body mass index; IIEF-5, International Index of Erectile Function-5; PSS-10, Perceived Stress Scale-10; CAR, cortisol awakening response; AUCi, area under the curve with respect to increase; AUCg, area under the curve with respect to ground; DCS, diurnal cortisol slope; HRV, heart rate variability; VLF, very low frequency; LF, low frequency; HF, high frequency; SDNN, standard deviation of all R-R intervals; SDANN, standard deviation of the average R-R intervals calculated over 5 min; rMSSD, root mean square of successive differences; pNN50, percent of R-R intervals differing more than 50 ms from each other; pED, psychogenic erectile dysfunction.
*p value from an independent-sample t-test for age, BMI, symptom, and psychological scores, Kruskal-Wallis test for salivary cortisol and HRV values, and the chi-square test or fisher's exact test for discontinuous variables. N/A, not applicable.

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TABLE 2 Singular versus inter-relation model comparisons in pED patients.

HRV	Cortisol	Singular models HRV			Singular models cortisol			Inter-relation models					Model comparison	
		β_{hrv}	F	R^2	β_{cort}	F	R^2	β_{hrv}	β_{cort}	β_{int}	F	R^2	ΔF	ΔR^2
VLF		-0.080	0.465	-0.007	–	–	–	–	–	–	–	–	–	–
	CAR AUCi	–	–	–	0.183	2.538	0.020	-0.033	0.189	0.079	1.081	0.003	0.334	0.005
	CAR AUCg	–	–	–	**0.263***	5.412	0.056	-0.093	**0.275***	-0.054	1.920	0.036	0.142	0.002
	Diurnal AUCg	–	–	–	0.217	3.617	0.034	-0.003	0.217	0.048	1.238	0.010	0.122	0.002
	DCS	–	–	–	-0.075	0.413	-0.008	-0.094	-0.095	0.024	0.348	-0.027	0.038	0.001
LF		0.153	1.740	0.010	–	–	–	–	–	–	–	–	–	–
	CAR AUCi	–	–	–	–	–	–	0.046	0.203	0.127	1.403	0.016	0.877	0.012
	CAR AUCg	–	–	–	–	–	–	0.067	0.241	0.005	1.872	0.034	0.001	0.000
	Diurnal AUCg	–	–	–	–	–	–	0.196	0.291	0.108	2.005	0.039	0.430	0.006
	DCS	–	–	–	–	–	–	0.142	-0.058	-0.008	0.643	-0.015	0.004	0.000
HF		0.069	0.353	-0.009	–	–	–	–	–	–	–	–	–	–
	CAR AUCi	–	–	–	–	–	–	-0.108	**0.384***	**0.348***	2.545	0.059	4.798	0.061
	CAR AUCg	–	–	–	–	–	–	-0.062	**0.312***	0.124	1.947	0.037	0.489	0.006
	Diurnal AUCg	–	–	–	–	–	–	0.154	**0.399***	0.217	1.904	0.035	1.363	0.018
	DCS	–	–	–	–	–	–	0.065	-0.054	0.025	0.216	-0.033	0.033	0.000
LF/HF		-0.052	0.199	-0.011	–	–	–	–	–	–	–	–	–	–
	CAR AUCi	–	–	–	–	–	–	-0.165	0.252	-0.198	1.464	0.018	1.651	0.022
	CAR AUCg	–	–	–	–	–	–	-0.018	**0.261***	-0.002	1.762	0.030	0.000	0.000
	Diurnal AUCg	–	–	–	–	–	–	-0.002	0.213	0.015	1.179	0.007	0.005	0.000
	DCS	–	–	–	–	–	–	0.115	-0.064	-0.234	0.934	-0.003	2.282	0.031
SDNN		0.081	0.481	-0.007	–	–	–	–	–	–	–	–	–	–
	CAR AUCi	–	–	–	–	–	–	0.031	0.171	-0.028	0.878	-0.005	0.053	0.001
	CAR AUCg	–	–	–	–	–	–	-0.015	**0.268***	-0.043	1.814	0.032	0.143	0.002
	Diurnal AUCg	–	–	–	–	–	–	0.087	0.231	0.055	1.390	0.016	0.195	0.003
	DCS	–	–	–	–	–	–	0.041	-0.059	-0.077	0.365	-0.026	0.369	0.005
SDANN		0.074	0.406	-0.008	–	–	–	–	–	–	–	–	–	–
	CAR AUCi	–	–	–	–	–	–	0.016	0.167	-0.099	1.087	0.004	0.688	0.009
	CAR AUCg	–	–	–	–	–	–	-0.009	**0.263***	-0.061	1.866	0.034	0.263	0.003
	Diurnal AUCg	–	–	–	–	–	–	0.069	0.223	0.037	1.302	0.012	0.090	0.001
	DCS	–	–	–	–	–	–	0.033	-0.063	-0.069	0.321	-0.028	0.284	0.004
rMSSD		0.009	0.006	-0.014	–	–	–	–	–	–	–	–	–	–
	CAR AUCi	–	–	–	–	–	–	-0.059	**0.274***	**0.266***	2.424	0.055	4.640	0.059
	CAR AUCg	–	–	–	–	–	–	-0.007	**0.254***	0.041	1.794	0.031	0.109	0.001
	Diurnal AUCg	–	–	–	–	–	–	0.137	**0.301***	0.188	1.987	0.038	2.063	0.027
	DCS	–	–	–	–	–	–	-0.005	-0.076	-0.009	0.136	-0.036	0.006	0.000
pNN50		0.086	0.548	-0.006	–	–	–	–	–	–	–	–	–	–
	CAR AUCi	–	–	–	–	–	–	0.051	0.202	0.099	1.116	0.005	0.664	0.009

(Continued)

TABLE 2 Continued

HRV	Cortisol	Singular models HRV			Singular models cortisol			Inter-relation models					Model comparison	
		β_{hrv}	F	R^2	β_{cort}	F	R^2	β_{hrv}	β_{cort}	β_{int}	F	R^2	ΔF	ΔR^2
	CAR AUCg	–	–	–	–	–	–	0.065	**0.239***	-0.063	1.936	0.037	0.277	0.004
	Diurnal AUCg	–	–	–	–	–	–	0.157	**0.280***	0.096	1.769	0.030	0.526	0.007
	DCS	–	–	–	–	–	–	0.073	-0.090	-0.037	0.339	-0.028	0.071	0.001

HRV, heart rate variability; VLF, very low frequency; LF, low frequency; HF, high frequency; SDNN, standard deviation of all R-R intervals; SDANN, standard deviation of the average R-R intervals calculated over 5 min; rMSSD, root mean square of successive differences; pNN50, percent of R-R intervals differing more than 50 ms from each other; CAR, cortisol awakening response; AUCi, area under the curve with respect to increase; AUCg, area under the curve with respect to ground; DCS, diurnal cortisol slope; β_{hrv}, standardized beta coefficient for HRV measure; β_{cort}, standardized beta coefficient for cortisol measure; β_{int}, standardized beta coefficient for the interaction term. Bold values indicate significance: $^*p < 0.05$.

TABLE 3 Singular versus inter-relation model comparisons in healthy men.

HRV	Cortisol	Singular models HRV			Singular models cortisol			Inter-relation models					Model comparison	
		β_{hrv}	F	R^2	β_{cort}	F	R^2	β_{hrv}	β_{cort}	β_{int}	F	R^2	ΔF	ΔR^2
VLF		0.129	1.231	0.003	–	–	–	–	–	–	–	–	–	–
	CAR AUCi	–	–	–	0.034	0.082	-0.013	0.128	0.024	0.014	0.418	-0.024	0.014	0.000
	CAR AUCg	–	–	–	0.091	0.610	-0.005	0.129	0.093	0.059	0.719	-0.012	0.254	0.003
	Diurnal AUCg	–	–	–	0.131	1.279	0.004	0.131	0.148	0.066	1.029	0.001	0.315	0.004
	DCS	–	–	–	-0.095	0.658	-0.005	0.130	-0.103	-0.076	0.825	-0.007	0.415	0.006
LF		-0.136	1.372	0.005	–	–	–	–	–	–	–	–	–	–
	CAR AUCi	–	–	–	–	–	–	-0.141	0.054	-0.010	0.509	-0.020	0.006	0.000
	CAR AUCg	–	–	–	–	–	–	-0.132	0.077	0.035	0.647	-0.015	0.088	0.001
	Diurnal AUCg	–	–	–	–	–	–	-0.115	0.117	-0.026	0.750	-0.010	0.048	0.001
	DCS	–	–	–	–	–	–	-0.119	-0.052	-0.038	0.584	-0.017	0.085	0.001
HF		0.042	0.131	-0.012	–	–	–	–	–	–	–	–	–	–
	CAR AUCi	–	–	–	–	–	–	0.037	0.057	-0.041	0.110	-0.037	0.088	0.001
	CAR AUCg	–	–	–	–	–	–	0.044	0.072	0.082	0.386	-0.026	0.459	0.006
	Diurnal AUCg	–	–	–	–	–	–	0.009	0.065	0.237	1.773	0.030	3.827	0.050
	DCS	–	–	–	–	–	–	0.009	-0.074	-0.038	0.246	-0.032	0.078	0.001
LF/HF		-0.108	0.855	-0.002	–	–	–	–	–	–	–	–	–	–
	CAR AUCi	–	–	–	–	–	–	-0.108	0.050	0.022	0.315	-0.029	0.022	0.000
	CAR AUCg	–	–	–	–	–	–	-0.098	0.030	-0.078	0.518	-0.020	0.272	0.004
	Diurnal AUCg	–	–	–	–	–	–	-0.069	0.053	-0.147	1.047	0.002	1.066	0.014
	DCS	–	–	–	–	–	–	-0.101	-0.012	0.114	0.636	-0.015	0.654	0.009
SDNN		0.111	0.910	-0.001	–	–	–	–	–	–	–	–	–	–
	CAR AUCi	–	–	–	–	–	–	0.093	0.036	-0.069	0.407	-0.025	0.310	0.004
	CAR AUCg	–	–	–	–	–	–	0.124	0.068	0.091	0.698	-0.012	0.555	0.008
	Diurnal AUCg	–	–	–	–	–	–	0.141	0.154	0.131	1.356	0.014	1.283	0.017
	DCS	–	–	–	–	–	–	0.092	-0.072	-0.136	1.007	0.000	1.201	0.016
SDANN		0.097	0.695	-0.004	–	–	–	–	–	–	–	–	–	–

(Continued)

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TABLE 3 Continued

HRV	Cortisol	Singular models HRV			Singular models cortisol			Inter-relation models					Model comparison	
		β_{hrv}	F	R^2	β_{cort}	F	R^2	β_{hrv}	β_{cort}	β_{int}	F	R^2	ΔF	ΔR^2
	CAR AUCi	–	–	–	–	–	–	0.077	0.043	-0.092	0.421	-0.024	0.555	0.008
	CAR AUCg	–	–	–	–	–	–	0.109	0.079	0.068	0.547	-0.019	0.309	0.004
	Diurnal AUCg	–	–	–	–	–	–	0.138	0.155	0.114	1.139	0.006	0.951	0.013
	DCS	–	–	–	–	–	–	0.078	-0.068	-0.158	1.090	0.004	1.652	0.022
rMSSD		0.156	1.813	0.011	–	–	–	–	–	–	–	–	–	–
	CAR AUCi	–	–	–	–	–	–	0.160	0.013	0.037	0.631	-0.015	0.089	0.001
	CAR AUCg	–	–	–	–	–	–	0.148	0.061	0.045	0.796	-0.008	0.118	0.002
	Diurnal AUCg	–	–	–	–	–	–	0.164	0.144	0.069	1.290	0.012	0.341	0.005
	DCS	–	–	–	–	–	–	0.175	-0.119	0.114	1.066	0.003	0.845	0.011
pNN50		**0.244***	4.626	0.047	–	–	–	–	–	–	–	–	–	–
	CAR AUCi	–	–	–	–	–	–	**0.247***	0.016	0.016	1.518	0.021	0.014	0.000
	CAR AUCg	–	–	–	–	–	–	**0.249***	0.000	0.147	2.085	0.042	1.234	0.016
	Diurnal AUCg	–	–	–	–	–	–	**0.235***	0.070	0.154	2.565	0.060	1.469	0.019
	DCS	–	–	–	–	–	–	**0.238***	-0.079	-0.006	1.681	0.027	0.002	0.000

HRV, heart rate variability; VLF, very low frequency; LF, low frequency; HF, high frequency; SDNN, standard deviation of all R-R intervals; SDANN, standard deviation of the average R-R intervals calculated over 5 min; rMSSD, root mean square of successive differences; pNN50, percent of R-R intervals differing more than 50 ms from each other; CAR, cortisol awakening response; AUCi, area under the curve with respect to increase; AUCg, area under the curve with respect to ground; DCS, diurnal cortisol slope; β_{hrv}, standardized beta coefficient for HRV measure; β_{cort}, standardized beta coefficient for cortisol measure; β_{int}, standardized beta coefficient for the interaction term. Bold values indicate significance: $^*p < 0.05$.

perceived stress levels were not correlated to pED severity. pED patients with the most severe symptoms did not necessarily show the highest perceived stress levels, and other psychosocial factors may also be responsible for pED.

Recent studies have confirmed a close correspondence between perceived stress levels and physiological or hormonal stress (HRV and cortisol) parameters (22, 23). Our results showed that compared with the control group, in addition to the significant LF/HF ratio increase, the LF, HF, and pNN50 were significantly decreased in pED patients. However, no changes in cortisol parameters were observed. Mathematically, the significant increase in LF/HF ratio may be due to the greater power decline of HF than LF. Chen et al. (17) reported strikingly congruent results to our findings. They found that patients with non-organic ED had significantly lower HF and significantly higher LF/HF than the healthy group. However, in patients with erectile problems, only a statistically significant increase in LF/HF ratio was found (16). LF reflects parasympathetic and sympathetic nervous system activity, and HF mainly reflects vagal activity. LF/HF estimates the relative balance between sympathetic and parasympathetic response; rMSSD and pNN50 mainly reflect the tension of the parasympathetic nerve (24). In the present study, the decrease of LH, HF, and pNN50 and the increase of LF/HF ratio reconfirmed the theory that there might be an ANS imbalance in pED patients, which was mainly manifested as impaired parasympathetic tone, and ultimately, the imbalance was still sympathetic dominance (16, 17, 24).

Salivary cortisol, an indicator of the hormonal stress system, is usually considered a reliable and non-invasive marker for assessing HPA axis activity (25). Most studies have described increased activation of the HPA axis in response to various stressors (26–28). However, our results showed no significant differences in the CAR and diurnal cortisol profiles between pED patients and healthy individuals. Three possible reasons may explain the lack of change in pED patients' HPA axis activity. First, we speculated that acute or physical stress was more likely to stimulate the hyperactivity of the HPA axis. However, chronic mental stress associated with pED could keep the basal activity of the HPA axis intact (25). Second, since the pED patients we recruited were new cases with a relatively short course of the disease, the accumulation of stressors was insufficient to activate the HPA axis. Third, a smaller sample size may not capture subtle changes in HPA axis activity during statistical analysis.

The above results emphatically analyze the changes in psychophysiological parameters related to stress in pED patients compared with healthy men. However, the relationship between perceived stress and the stress response system is still unclear. In the current study, the results of regression analyses partially supported the hypothesis that the inter-relation between measures of ANS (HF, rMSSD) and the HPA axis (CAR AUCi) in pED patients was better associated with perceived stress than the singular effect of either ANS or HPA axis activity. Meanwhile, significant interactions between rMSSD or HF and CAR AUCi accounted for a relatively high 5.9% or 6.1% variance in perceived stress. In some child and adolescent psychology studies, it is also suggested that there is an interrelation between autonomic and HPA axis activity. Rotenberg et al. (18) found that the interaction of cardio-autonomic control

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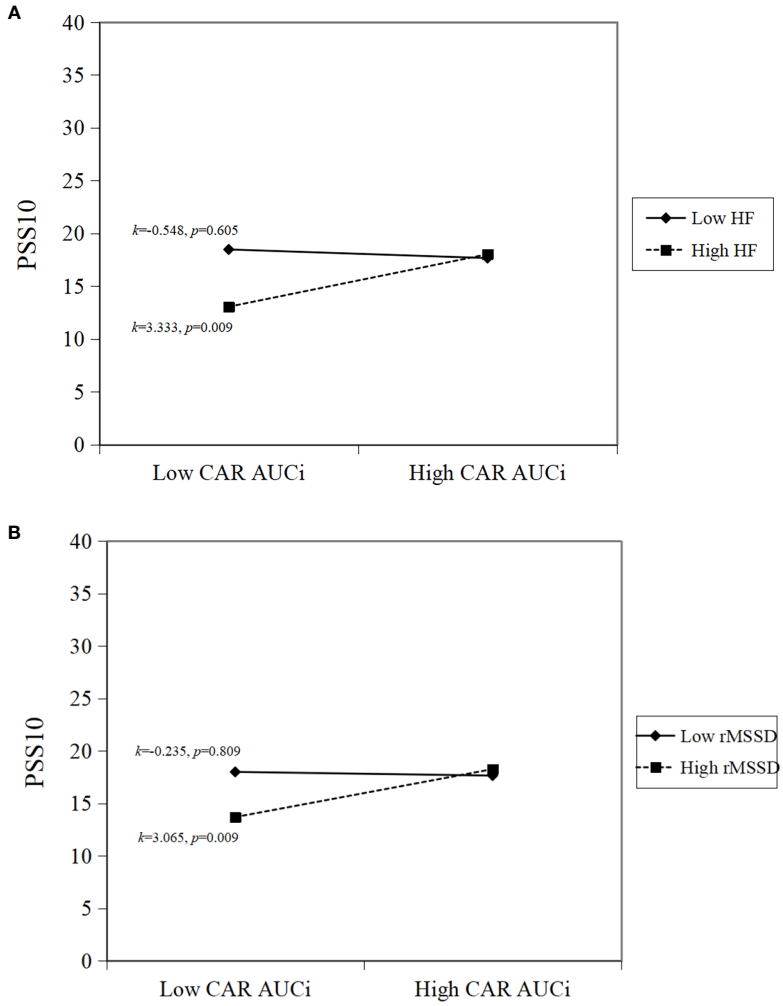

FIGURE 1
Simple slope interpretations of significant inter-relations. Higher sympathovagal modulation and higher cortisol awakening response are associated with greater perceived stress. In the panels, **(A)** higher HF and higher CAR AUCi; **(B)** higher rMSSD and higher CAR AUCi, are associated with higher perceived stress.

and the HPA axis accounted for 2–4% of the variance in perceived stress. El-Sheikh et al. (29) reported that the interaction of HF and cortisol accounted for 2-14% of the variance in anxiety and depressive symptoms. These results, including ours, support emerging theories in stress physiology, which emphasize the importance of considering the interrelation between the physiological components of the stress response system (18, 30). Interestingly, no significant interaction between HRV and cortisol measures related to perceived stress was found in healthy individuals. It is inferred indirectly that the distribution of PSS-10 scores and physiological or hormonal stress parameters in healthy men is more concentrated and tight, and the distribution range is narrower.

Because HF and rMSSD reflect parasympathetic or vagus nerve activity and CAR AUCi represents HPA axis response and arousal stress response, simple slope analyses of significant interactions in the present study indicated that pED patients with higher parasympathetic activity and cortisol awakening response had greater perceived stress. On the contrary, it could be understood that, with the decrease in parasympathetic activity, the effect of HPA axis activity on perceived stress in pED patients was gradually weakened. This finding is another explanation for changes in HRV and cortisol parameters in pED patients compared to normal men. Namely, the impaired parasympathetic tone in pED patients resulted in the attenuated influence of the HPA axis on perceived stress. Therefore, sympathovagal modulation played a major role in regulating perceived stress. Park et al. (31) reported that forest environments could lower cortisol concentrations, lower pulse rate, lower blood pressure, increase parasympathetic nerve activity, and lower sympathetic nerve activity compared with city settings, and finally aid in effectively relaxing the human body. Vieira et al. (32) found that auriculotherapy therapy could relieve university students' anxiety before exams by activating the parasympathetic nervous system and reducing salivary cortisol levels. Moreover, yoga stretching enhanced parasympathetic activity and decreased salivary cortisol levels, which could compensate for the lack of exercise and increase life expectancy in the general population (33). These researches suggest that if we increase parasympathetic

activity in pED patients while maintaining normal levels of stress hormones, it may be possible to reduce perceived stress and even promote penile erection.

Our study has the following limitations. First, we could not verify and compare our findings due to the lack of other studies on the relationship between HPA axis activity and perceived stress in patients with ED (34). Comparisons with other stress-related studies in specific populations are difficult because we used hierarchical multiple linear regression analysis, interaction effect analysis, and simple slope analysis, which may have increased our chances of finding meaningful associations between perceived stress and stress response systems. Second, more measures are needed to evaluate the stress and the activity of the ANS and HPA axis. In addition to perceived stress, chronic stress and stressful life events are also associated with adverse outcomes (35). Future research should consider measuring stress from a multidimensional perspective (e.g., longer duration, early life adversity) (18). Furthermore, we used only HRV and salivary cortisol to assess the ANS and HPA axis activity. Future studies should consider other autonomic measures (e.g., salivary alpha-amylase, pre-ejection period) and other stress hormones (e.g., cortisone, dehydroepiandrosterone) to more comprehensively characterize the ANS and HPA axis (15, 18). In summary, future research would benefit from extending the current findings by examining how the inter-relation between the ANS and the HPA axis is associated with other stress components, a larger sample size over a more extended period, and using additional measures of autonomic and HPA activity. Third, because the analyses were cross-sectional, our results cannot determine any causal direction for the associations found. Future longitudinal studies for pED patients are necessary to investigate further the interrelation between autonomic and HPA axis functioning and its relationship with stress (25).

In conclusion, we first studied the changes in perceived stress and two neural stress systems in pED patients. Then we provided evidence for the importance of considering the inter-relation between the ANS and the HPA axis when investigating the relationship between stress and the stress response system. Our descriptive statistics found that the PSS-10 score of pED patients was significantly higher than that of healthy controls, and there was no correlation with the IIEF-5 score. In terms of the stress response system, our results demonstrated that compared with healthy individuals, the HRV parameters of pED patients decreased. However, the cortisol parameters did not change, suggesting that the stress response of pED patients was characterized by ANS imbalance rather than HPA dysregulation. The ANS imbalance was mainly caused by significantly impaired parasympathetic vagal tone (decreased HF and pNN50 index), which eventually led to the dominance of sympathetic tone (increased LF/HF ratio). Moreover, regression analyses showed that the interrelation between ANS and the HPA axis was more related to perceived stress than the singular correlation of either the ANS or HPA axis. pED patients with higher parasympathetic activity and a higher cortisol awakening response had greater perceived stress. These results suggest that the interaction between the ANS and HPA axis is uniquely related to perceived stress. Identifying different patterns of stress responses and linking these patterns to adverse health outcomes is vital to advance the current understanding of how stress affects the physical and mental health of pED patients. Further large-scale, longitudinal, multi-center investigations and animal experiments might confirm and generalize our findings.

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.

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Review Board of Tongji Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

SW, JL, LJ, and JB contributed to the conception and design of the study. ML, BH, SY, YC, and XD collected clinical data and specimens. KR, ML, JY, and XL analyzed the data. JX wrote the first draft of the manuscript. TW, YC, KR, and LG wrote sections 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.

Publisher's note

Supplementary material

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Erectile dysfunction and exosome therapy

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Erectile dysfunction (ED), as a common male disease, can seriously reduce the life quality of men and their partners. With the improvement of human living standards, ED is considered to be an important health issue that plagues men. However, it is difficult for existing therapeutic approaches to meet the needs of all patients, so it is necessary to develop novel treatment strategies. Exosomes, as a class of vesicles secreted by cells with bilayer membrane structure, are involved in various physiological and pathological processes in human body and considered to have great therapeutic potentials. This review summarizes the recent advances on exosome therapy with animal models of ED, and proposes the prospect of future research in order to provide a basis for clinical trials and clinical translation.

KEYWORDS

erectile dysfunction, exosome, extracellular vesicle, diabetes, cavernous nerve injury

Background

More than 5,000 years ago, erectile dysfunction (ED) was mentioned in ancient Egyptian scriptures (1). It was defined until 1993 by the National Institutes of Health (NIH) as the persistent or recurrent failure to attain or maintain a sufficient penile erection for successful penetration (2, 3). ED is a widespread medical issue that seriously affects male health, with 150 million men suffering from it in varying degrees worldwide (4, 5). With males over the age of 40 having a higher prevalence of this issue, it is anticipated that there will be more than 322 million cases of ED worldwide by 2025 (1). In the past, it was frequently believed that psychiatric disorders contribute significantly to the development of ED, but many researches have revealed that organic etiology accounts for more than 80% of ED cases. Cardiovascular diseases, diabetes, dyslipidemia, hypogonadism and nerve damage are independent risk factors for ED (6). More importantly, ED is no longer just related to sexual dysfunction, it may also signal potential vascular endothelial dysfunction, acting as an early indicator of cardiovascular disease (7). Selective phosphodiesterase type 5 inhibitors (PDE5i), like sildenafil citrate, have been widely utilized as the first-line treatment for ED since they enhanced erectile function in 63% of patients and were exploited based on the role of nitric oxide (NO) in cavernous smooth muscle relaxation (8). However, because of the complexity of the pathway that regulates penile erection, up to

35% of patients do not respond to the pharmacological therapy (1). As current treatments do not provide maximum benefits to patients, it is crucial to investigate novel strategies for ED treatments (9).

Extracellular vesicles (EVs) are cell-derived membrane structure with a diameter of 40 nm to 1000 nm. Exosomes and microvesicles are two types of EVs that are released from the endosomal system or shed from plasma membrane, respectively (10). Exosomes are nanosized particles with a diameter of roughly 40-160 nm secreted by various cells under physiological or pathological conditions (11). Exosomes have a bilayer structure that is made by the plasma membrane through encapsulating extracellular components and membrane proteins as well as intersecting with other vesicles and organelles (10, 12). Therefore, exosomes contain many constituents such as metabolites, proteins, lipids and nucleic acids (13, 14). Exosomes are particularly heterogeneous population due to their origin from different cells, as well as the notable differences in their size, composition and effect on the function of recipient cells (15, 16). Exosomes are involved in a variety of physiological and pathological processes in the human body (17), and can be exploited as molecular and signal carriers in intercellular communication (18). Exosome biogenesis enables cells to rapidly and selectively remove proteins from the plasma membrane, facilitating procedures like sperm-egg binding (19). Exosomes are linked to the process of viral infection and are involved in the induction of innate and adaptive immune responses (20). Studies on exosomes and diseases have revealed that exosomes may be involved in cardiovascular and metabolic disorders, take a role in the pathogenesis of neurological disorders, and dynamically affect the growth of cancers (11, 21).

Exosomes are frequently investigated in clinical studies. Exosomal non-coding RNAs (ncRNAs) have been revealed to be expressed differently in most human disorders, which paves the way for their potential application as biomarkers in early stage of diseases (22). Exosomes also serve as drug delivery systems (DDS) for the treatment of cancer (23). For instance, exosomes containing cisplatin inhibited the progression of hepatocellular carcinoma (24). In animal experiments, transplanted bone marrow promoted the regeneration of injured β cell by releasing exosomes enriched with miR-106b-5p and miR-222-3p (25). Exosomal miRNAs produced by mesenchymal stem cells have been found to exhibit anti-atherosclerotic properties (26). Therefore, it is valuable to further explore the application of exosomes in ED patients, which might be caused by diabetes or cardiovascular disease.

In this review, we introduced the biosynthesis and contents of exosomes, and concentrated on the application of exosomes as well as other types of EVs in the treatment of ED.

The biogenesis of exosomes

The exact mechanisms of exosome formation are still unclear, but existing studies suggest that the process of exosome biogenesis may be similar in different cell types. Generally, the biogenesis of exosomes is a continuous dynamic process and consists mainly of two invaginations of plasma membrane and the formation of multivesicular bodies (MVBs) containing intraluminal vesicles (ILVs) (11).

First of all, exosomes originate from the inward budding of plasma membrane (that is the first invagination of plasma membrane) to form early sorting endosomes (ESEs) with cell surface proteins and extracellular constituents (27, 28). In some cases, the generated ESEs may be directly merged with the preformed ESEs (11). Afterwards, ESEs either fuse with the plasma membrane for the recycling of sequestered cargoes or convert into late sorting endosomes (LSEs), which can give rise to MVBs with the involvement of endoplasmic reticulum and Golgi complex (29, 30). During the maturation of MVBs, cytoplasmic cargoes including nucleic acids, proteins, lipids, amino acids and metabolites can enter into LSEs by inward budding of endosomal membrane (that is the second invagination of plasma membrane), which leads to the generation of ILVs (27, 29–31). Finally, some MVBs are degraded by the autophagosome or lysosome to maintain cellular homeostasis, while others fuse with the plasma membrane *via* the exocytotic pattern to secrete ILVs, which are also considered as exosomes (32, 33). Notably, exosomes derived from other cells can be taken up by the cells and further fused with ESEs (11).

However, in addition to this canonical model, several studies also revealed the other mechanisms involving in the biogenesis of exosomes. Exosomes can be formed immediately by outward budding through the plasma membrane or delayed released by budding through the deep invagination of plasma membrane (17). Electron microscopy experiments in mesenchymal stem cells and human immune cells have confirmed the existence of the above phenomenon (17, 34–36).

Many factors are associated with the process of exosome biogenesis. Endosomal-sorting complex required for transport (ESCRT) machinery is a key regulator for the conversion of ESEs to LSEs/MVBs, while sometimes this process is independent of ESCRT complex (10, 37). ESCRT machinery is composed of four complexes (ESCRT-0, ESCRT-I, ESCRT-II and ESCRT-III) and the associated proteins (VPS4, VTA1, ALIX and TSG101) (29, 31, 38). ESCRT-0 binds to the ubiquitinated cargoes on the membrane of MVBs followed by the recruitment of ESCRT-I and ESCRT-II thus initiating the invagination of endosomal membrane (10, 39, 40). ESCRT-III is responsible for the scission of ILVs into MVBs (10, 39, 40). Moreover, it is reported that Munc13-4, NEH6, heat shock protein αB-crystallin (HSPB5) and Rab GTPases are required for the endosome maturation and exosome release (41–43).

After exosomes are released by donor cells, they either fuse directly with the plasma membrane of recipient cells to transfer cargoes, or interact with the receptors of recipient cells to activate the corresponding signaling pathways, or undergo endocytosis by recipient cells to release cargoes or fuse with the endosomes to take part in the biogenesis of exosomes in recipient cells (11, 27, 31).

The contents of exosomes

Exosomes contain not only extracellular substances and plasma membrane proteins, but also a variety of cytoplasmic substances such as DNAs, RNAs, proteins, lipids and metabolites, some of

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which can be used as biomarkers of exosome (27, 30, 31). The content of exosomes is not constant and varies widely in different cell microenvironments.

Exosomal proteins include: (i) proteins located on the membrane of exosome such as tetraspanins (CD9, CD63, CD81), integrins, MHC class I, II, glycoproteins and other signaling receptors (tumor necrosis factor (TNF) receptor, transferrin receptor), as well as (ii) proteins located in the lumen of exosome such as HSPs (HSP60, HSP70, HSP90), ESCRT machinery (ALIX, TSG101), cytoskeletal proteins (actin, tubulin), enzymes, growth factors and cytokines (27, 44, 45). For instance, transforming growth factor-beta 1 (TGF-β1) and bone morphogenetic protein 2 (BMP2) were detected in plasma exosomes from gastric cancer patients and gastric cancer cell-derived exosomes, respectively (45). These exosomal cytokines were demonstrated to activate the SMAD or PI3K/AKT signaling pathway and give rise to the differentiation of other cells to fibroblasts in gastric cancer (45). Prostate specific membrane antigens (PSMA; PSMA6 and PSMA7) were also identified in blood exosomes from atherosclerosis patients (46). Under the stimulation of lipopolysaccharide macrophage-derived exosomes contain TNF-α and interleukin-1β (IL-1β), which both regulate the inflammatory responses (47).

Exosomal nucleic acids include DNA, mRNA and non-coding RNA (miRNA, lncRNA and circRNA), among which miRNA is one of the most abundant nucleic acids in exosomes. More and more miRNAs contained in exosomes are identified in scientific researches, and they regulate the function of recipient cells or the expression of target mRNA in recipient cells (22, 32). The overexpression of miR-934 in colorectal cancer cell-derived exosomes induced M2 macrophage polarization, activated PI3K/AKT signaling pathway and decreased the PTEN expression (48). High levels of miR-205 were found in circulating exosomes from ovarian cancer patients promoting the angiogenesis and tumor growth (49). Moreover, it was reported that exosomal miRNA from M2 macrophages such as miR-328 promoted the proliferation of pulmonary interstitial fibroblasts and induced the development of lung fibrosis (50). In addition, lipids including cholesterol, ceramides, sphingomyelin and phosphatidylinositol are also contained in exosomes (27).

The therapeutic potential of exosomes in ED

Most studies on exosomes have focused on the field of oncology, while the correlation between exosomes and ED is less studied. The investigation of effects of exosome on the amelioration of ED was analyzed for the first time in 2017 (51). Details of exosome therapy studies in ED models are displayed in Figure 1 and Table 1.

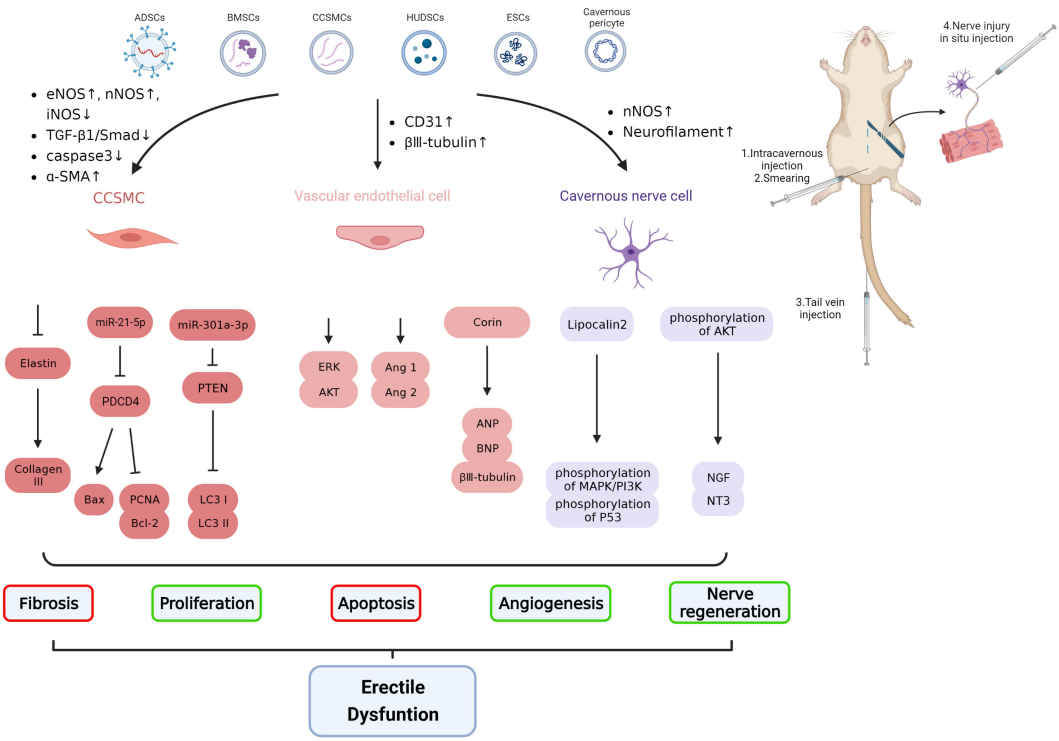

FIGURE 1
Schematic diagram of the molecular mechanism of exosome therapy for ED. CCSMC, corpora cavernosum smooth muscle cell; eNOS: endothelial nitric oxide synthase; nNOS: neuronal nitric oxide synthase; iNOS: inducible nitric oxide synthase; Ang 1, angiotensin 1; Ang 2, angiotensin 2; ANP: atrial natriuretic peptide; BNP: brain natriuretic peptide; TGF-β1, transforming growth factor-β 1; NT3: neurotrophin 3; NGF, nerve growth factor.

TABLE 1 Therapeutic strategies of exosomes in rat models of ED.

Origin	Model	Modification	Contents of exosome	Validation of exosome	Management of exosome	Indicators of treatment	Outcomes of treatment	Study
ADSCs	STZ-induced type II diabetic rats	/	/	TEM; protein markers (CD63, CD81 and calnexin)	Intracavernous injection: 100 μg	4 weeks later ICP/MAP ratio; the expression of Bcl-2, cleaved-caspase 3, CD31 and α-SMA	Prohibiting the apoptosis in cavernous endothelial cells and smooth muscle cells; enhancing erectile function	(51)
ADSCs	STZ-induced type I diabetic rats	/	miR-126, miR-130a, miR-132, miR-let7b, miR-let7c	TEM; protein markers (CD9 and CD63)	Intracavernous injection: 0, 10 or 100 μg	4 weeks later The ratio of ICP to MAP, tube formation; the protein expression of endothelial marker vWF and the relative area of smooth muscle to collagen	Increasing endothelial content and angiogenic activity, reversing fibrosis and improving the erectile function in a dose-dependent manner	(52)
BMSCs	STZ-induced diabetic rats	miR-21-5p-agomir	miR-21-5p	TEM; protein markers (CD9, CD63 and TSG101)	Tail vein injection: 100 μg	4 weeks later ICP/MAP ratio; apoptosis markers (Bcl-2, Bax, cleaved-caspase 3) and CCSMCs content	Inhibiting expression of PDCD4 and CCSMCs apoptosis; attenuating the erectile dysfunction.	(53)
ADSCs	STZ-induced diabetic rats	Corin siRNA	Corin	TEM; protein markers (CD9, CD31, CD63 and CD81)	Intravenous injection: 200 μg	2 weeks later ICP/MAP ratio; the expression of nNOS, ANP, BNP, CD31 and beta-III tubulin; the levels of inflammatory factors (TNF-α, IL-1β, IL-6)	Promoting angiogenesis and nerve content; ameliorating inflammation; improvement of erectile function	(54)
CCSMCs, ADSCs, BMSCs	STZ-induced type I diabetic rats	/	/	TEM; protein markers (CD9, CD63, Calnexin and TSG101)	Intracavernous injection: 100 μg	4 weeks later The ratio of ICP/MAP; the expression of nNOS, eNOS and fibrotic markers; the level of NO/cGMP	CCSMC-EXOs have higher peak concentration and longer retention time, restore erectile dysfunction through inhibiting corporal fibrosis and modulating the NO/cGMP pathway	(55)
ADSCs	BCNI rats	polydopamine thermosensitive hydrogel	/	TEM; protein markers (CD9 and CD63)	Intracavernous injection: 300 μg	3 weeks later ICP/MAP ratio; the expression of eNOS, nNOS and α-SMA	Healing endothelial cells and neurons; improving the erectile function	(56)
ADSCs	BCNI rats	thermo-sensitive hydrogel	/	TEM; protein markers (CD9, CD63 and TSG101)	Nerve injury *in situ* injection: 100 μg	4 weeks later ICP/MAP ratio; the neurite outgrowth; the expression of eNOS and nNOS,	Promoting the repair of nerve injury; improving the erectile function	(57)
ADSCs, BMSCs	BCNI rats	/	/	TEM; protein markers (CD63, CD81 and HSP70)	Intracavernous injection: 100 μg	3 weeks later ICP/MAP ratio; the expression of nNOS in DNP and MPG; the level of vWF and the relative area of smooth muscle to collagen	Both alleviating the distortion of normal neural anatomy, smooth muscle atrophy, collagen deposition and erectile dysfunction	(58)
BMSCs	BCNI rats	/	/	TEM; protein markers (CD63, Flotillin-1 and TSG101)	Intracavernous injection: 100 μg	4 weeks later ICP/MAP ratio; the expression of nNOS, Caspase-3 and α-SMA; the apoptosis of CCSMCs	Inhibiting apoptosis in CCSMCs; improving the erectile function	(59)

(Continued)

TABLE 1 Continued

Origin	Model	Modification	Contents of exosome	Validation of exosome	Management of exosome	Indicators of treatment	Outcomes of treatment	Study
BMSCs	Internal iliac artery injury rats	/	/	TEM; protein markers (CD9 and TSG101)	Intracavernous injection: 50 or 100 μg	4 weeks later The ratio of ICP to MAP; the expression of CD31, VEGFA and OCT4; the protein level of eNOS, nNOS, iNOS and SOD; the ratio of smooth muscle to collagen	Promoting cavernous endogenous stem cells to differentiate into cavernous sinus endothelial cells; reducing the oxidative stress damage of corpus cavernosum; improving the erectile function	(60)
Human urine-derived stem cells	TGF-β1 induced Peyronie's disease rats	/	/	TEM; protein markers (CD9, CD63, Alix and TSG101)	Intratunical injection: 100 μg	4 weeks later ICP/MAP ratio; the ratio of smooth muscle to collagen; the expression of α-SMA, TGF-β1 and p-Smad2/3; the gene level of TIMPs and MMPs; the activity of MMPs	Ameliorating the tunica albuginea fibrosis; improving the erectile function	(61)
ADSCs	Chronic intermitent hypoxia rats	miR-301a-3p mimics	miR-301a-3p	TEM; protein markers (CD9, CD63 and TSG101)	Intracavernous injection: 400 μg	8 weeks later ICP/MAP ratio; the protein level of eNOS, nNOS and iNOS; the level of apoptosis and autophagy; the ratio of smooth muscle to collagen; the expression of PTEN and TLR4 in DNP	Affecting the apoptosis and autophagy of CCSMCs; improving the erectile function	(62)

ADSC, adipose-derived stem cell; ANP, atrial natriuretic peptide; BCNI, bilateral cavernous nerve injury; BMSC, bone marrow-derived stem cell; BNP, brain natriuretic peptide; CCSMCs, corpus cavernosum smooth muscle cells; cGMP, cyclic guanosine monophosphate; DNP, dorsal nerve of the penis; eNOS, endothelial nitric oxide synthase; ICP, intracavernous pressure; IL, interleukin; iNOS, inducible nitric oxide synthase; MAP, mean arterial pressure; MMP, matrix metalloproteinase; MPG, major pelvic ganglion; MSC, mesenchymal stem cell; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; Smad, contraction of Sma and Mad (Mothers against decapentaplegic); SOD, superoxide dismutase; STZ, streptozotocin; TEM, transmission electron microscopy; TGF, transforming growth factor; TIMP, tissue inhibitor of matrix metalloproteinase; TNF, tumor necrosis factor; TSG101, tumor susceptibility gene 101.

Diabetic ED

ED is considered as one of the long-term complications of diabetes, which increases the risk of developing ED by 2.5-fold, and more than 50% of people with diabetes are affected by ED (68). Diabetic patients are mainly manifested with hyperglycemia and insulin resistance, usually accompanied by the metabolic syndrome (obesity, hypertension, dyslipidemia), hypogonadism, cardiovascular diseases and neuropathy (69). These manifestations and comorbidities affect the levels of androgen, inflow of arteries, outflow of veins as well as nerve signaling, and ultimately influence the production of reactive oxygen species, NO, cyclic guanosine monophosphate (cGMP) and nitric oxide synthase (NOS), and the function of endothelial cells and corpus cavernosum smooth muscle cells (CCSMCs) during the erection of penis (69, 70).

Chen et al. established a rat model of type II diabetes and isolated exosomes from adipose-derived stem cells (ADSCs) by ultracentrifugation (51). They found that ADSC-derived exosomes promoted the recovery of erectile function by increasing the ratio of maximal intracavernous pressure (ICP) to mean arterial pressure (MAP), elevating the endothelium and smooth muscle contents and decreasing the apoptosis in cavernous endothelial cells and smooth muscle cells (51). In addition to type 2 diabetes, ED was also induced following type 1 diabetes. Zhu et al. injected type 1 diabetic ED rats with three different doses of ADSC-derived exosomes (0, 10 or 100 μg, respectively) (52). It was demonstrated that exosomes improved the erectile function by reversing fibrosis, increasing endothelial content and angiogenic activity in a dose-dependent manner. Interestingly, they also performed miRNA sequencing on the extracted exosomes and found that some functional miRNAs were contained in these exosomes, including proangiogenic miRNAs (miR-126, miR-130a, miRNA-132) and antifibrotic miRNAs (miR-let7b and miR-let7c). Although the role of these miRNAs in exosomes in the treatment of ED has not been validated *in vitro* or *vivo*, these findings revealed the potential mechanisms of exosome therapy in ED, which opened up a new perspective for the future investigation of exosomes in the treatment of ED.

Notably, donor cells can be modified by transgenic methods so that exosomes released by these cells can contain a large number of specific cargoes. For example, bone marrow-derived stem cells (BMSCs) treated with miR-21-5p-agomir resulted in the increase of miR-21-5p in BMSC-derived exosomes, which decreased the expression of target gene programmed cell death 4 (PDCD4) in CCSMCs, attenuating the erectile dysfunction through leading to the proliferation and apoptosis inhibition of these cells (53). Meanwhile, exosomes derived from ADSCs transfected with corin siRNA promoted the neurovascular function and suppressed the levels of inflammatory factors including TNF-α, IL-1β and IL-6 (54). Our group also investigated the

therapeutic effects of exosomes in ED. Distinct from the above study, we isolated exosomes from CCSMCs and found that this type of exosomes was more easily retained in the corpus cavernosum and better ameliorated the diabetes-induced erectile dysfunction compared with exosomes from ADSCs and BMSCs (55).

Bilateral cavernous nerve injury-induced ED

Prostate cancer surgery and other pelvic surgeries often result in damages to the cavernous nerve, which originates from the major pelvic ganglion (MPG) and controls the relaxation and contraction of CCSMCs, thus regulating the erection of the penis (71). It was reported that up to 80% of prostate cancer patients suffer from ED after radical prostatectomy (72). Many molecules and signaling pathways contribute to the development of ED during the injury of cavernous nerve, for instance TGF-β, hydrogen sulfide, NO pathway, RhoA/ROCK pathway or oxidative stress-related pathway (73, 74). The dysregulation of these factors after BCNI leads to the tissue fibrosis, as well as phenotypic transformation and apoptosis of CCSMCs (73).

Exosomes isolated from ADSCs and BMSCs have been applied not only to diabetic ED but also to BCNI-induced ED. Ouyang et al. found that four weeks after injection with exosomes into the corpus cavernosum of BCNI-induced ED rats, the erectile function was obviously improved *via* inhibiting apoptosis in CCSMCs (59). Similarly, ADSC-derived and BMSC-derived exosomes have been shown to be effective in recovering erectile dysfunction in BCNI rat model (58). Both of them can alleviate the distortion of normal neural anatomy, smooth muscle atrophy and collagen deposition, which impaired the erection of penis.

In order to enhance the uptake of exosomes and the efficacy of treatment, some research teams have developed specific hydrogels and mixed them with exosomes for the treatment of ED. Liang et al. fabricated polydopamine thermosensitive hydrogels, which exhibited sol-gel transition at body temperature and allowed exosomes to be released slowly within two weeks (56). *In vivo* experiments confirmed that ADSC-derived exosomes loaded within polydopamine thermosensitive hydrogel improved the erectile function by healing the endothelial cells and neurons in penis. On the other side, ADSC-derived exosomes encapsulated into the thermosensitive hydrogel significantly repaired the cavernous nerves injury in rats, thus restoring erectile function (57).

In addition, a study showed that Schwann cell-derived exosomes promoted nerve regeneration of MPG and cavernous nerve with MPG, suggesting that they may provide potential therapeutic options for ED treatment (75). Since this study only involved ex vivo experiments, appropriate *in vivo* experiments are needed to further confirm the findings.

Other types of ED

So far, exosomes have been introduced to treat some other types of ED in addition to diabetic ED and BCNI-induced ED. Vascular ED accounts for a large proportion in elderly patients with ED, which can be caused by atherosclerosis, trauma and surgery. It has been shown that in internal iliac artery injury-induced ED rats, BMSC-derived exosomes promoted cavernous endogenous stem cells to differentiate into cavernous sinus endothelial cells while effectively reduced the oxidative stress damage of corpus cavernosum (60). These findings provided a novel insight and strategy for the clinical treatment of severe arterial injury ED.

Since erectile dysfunction is often observed in patients with obstructive sleep apnea, the researchers developed a rat model of chronic obstructive hypoxia-induced ED. The miR-301a-3p-enriched ADSC-derived exosomes affected the apoptosis and autophagy of CCSMCs by targeting PTEN and TLR4, and ultimately improved erectile function (62). Furthermore, Yang et al. demonstrated that human urine-derived stem cell (HUSC)-derived exosomes ameliorated the fibrosis in tunica albuginea and restored erectile function in Peyronie's disease rats (61).

The therapeutic potential of other subsets of EVs in ED

Due to the low production of EVs, Kwon et al. developed embryonic stem cell (ESC)-derived EV-mimetic nanovesicles (ESC-NVs), and diabetic mice were received intravenous injection of 0.1, 0.5, 1, 2 or 5 μg ESC-NVs for 2 times, respectively. The results revealed that those ESC-NVs enhanced penile neurovascular regeneration by boosting the expression of angiogenic and neurotrophic factors (63). A team of researchers from South Korea extracted EV-NVs from cavernous pericytes, and they found that these NVs can significantly promote neurovascular regeneration and ameliorate erectile dysfunction in both diabetic and BCNI-induced ED rats (64, 65). The studies also compared the efficacy of different doses of NVs and found that the higher the dose, the better the recovery of pathological changes and erectile dysfunction. Moreover, EVs derived from HUSC (HUSC-EVs) have been proven to ameliorate erectile dysfunction effectively (66, 67). In contrast to the conventional intracavernous or intravenous injection, Zhuang et al. mixed HUSC-EVs with hyaluronic acid and smeared them on the glans of the rats several times (67). The results showed that this administration can also improve apoptosis, angiogenesis, and smooth muscle regeneration as the conventional injection Table 2.

Future prospects

Basic and preclinical researches have shown that exosomes have the potential to treat ED. Since the research in this field is still at a preliminary stage, there are no ongoing or completed clinical trials. Although exosomes exhibit good therapeutic effects in rat and mouse, rodent models are insufficient to predict human clinical outcomes. Exosome therapy in ED needs to be validated in higher-order animals that more closely mimic the physiological and clinical characteristics of the human body. In fact, a large number of clinical

TABLE 2 Therapeutic strategies of other subsets of EVs in rat models of ED.

Origin	Model	Modification	Contents of EVs	Validation of EVs	Management of EVs	Indicators of treatment	Outcomes of treatment	Study
ESCs	STZ-induced diabetic mice	/	/	TEM; protein markers (CD63, CD81, GM130 and TSG101)	Intracavernous injection: 0.1, 0.5, 1, 2 or 5 μg for 2 times	2 weeks later The ratio of total ICP or maximum ICP to MSBP; the content of cavernous pericyte, smooth muscle cell and endothelial cell; the level of tube formation and aortic ring micro-vessel outgrowth; the expression of angiogenenic and neurotrophic factors	Enhancing penile neurovascular regeneration; ameliorating erectile dysfunction	(63)
Cavernous pericyte	CNI rats	/	/	TEM; protein markers (CD81, GM130, Alix and TSG101)	Intracavernous injection: 0.2, 1 or 5 μg	2 weeks later ICP/MSBP ratio; the content of cavernous pericyte and endothelial cell; the content of nNOS and neurofilament in DNB; the level of tube formation; the expression of neural and neurovascular regeneration markers	Promoting neurovascular regeneration; improving erectile dysfunction	(64)
Cavernous pericyte	STZ-induced diabetic mice	/	/	TEM; protein markers (CD81, GM130, Alix100 and TSG101)	Intracavernous injection: 0.5, 1 or 5 μg	2 weeks later The ratio of total ICP or maximum ICP to MSBP; the expression of PECAM-1 and α-SMA; the level of tube formation and aortic ring micro-vessel outgrowth; the expression of neural and neurovascular regeneration markers	Promoting penile angiogenesis and neural regeneration; improving erectile dysfunction	(65)
Human urine-derived stem cells	STZ-induced diabetic rats	/	miR-21-5p, let-7 family, miR-10 family, miR-30 family, miR-148a-3p	TEM; protein markers (CD63 and Calnexin)	Intracavernous injection: 100 μg	4 weeks later ICP/MAP ratio; the expression of nNOS and eNOS; the relative area of smooth muscle to collagen	Alleviating the fibrosis; attenuating the erectile dysfunction.	(66)
Human urine-derived stem cells	STZ-induced type II diabetic rats	hyaluronic acid	/	TEM; protein markers (CD9, CD63, TSG101 and Calnexin)	Smearing on the glans: 2×10^9 particles for 5 or 10 times	4 weeks later ICP/MAP ratio; the formation of HUVECs capillary-like Structure; the gene level of Bcl-2, Bax and SOD2; the expression of nNOS, eNOS and iNOS; the relative area of smooth muscle to collagen	Improving apoptosis, angiogenesis, and smooth muscle regeneration; enhancing erectile function	(67)

CNI, cavernous nerves injury; DNB, dorsal nerve bundle; eNOS, endothelial nitric oxide synthase; ESC, embryonic stem cell; HUVEC, human umbilical vein endothelial cell; ICP, intracavernous pressure; iNOS, inducible nitric oxide synthase; MAP, mean arterial pressure; MSBP, mean systolic blood pressure; nNOS, neuronal nitric oxide synthase; SOD2, superoxide dismutase 2; STZ, streptozotocin; TEM, transmission electron microscopy; TSG101, tumor susceptibility gene 101.

trials based on exosomes have been completed in many other diseases (76–83), so we need to speed up the pace to more comprehensively confirm the therapeutic effect of exosomes on ED and promote the translation from animal studies to clinical trials. In addition, it is worth noting that most of the existing studies only focus on the therapeutic effects of exosomes on ED and related mechanisms, but there are relatively few explorations of exosome contents. In the future, we should take actions to further understand the role of key molecules identified in exosomes in the treatments of ED.

There are still several key issues to be resolved, including long-term safety, optimal source of exosomes, optimal therapeutic method, dose and course, appropriate delivery system and elucidation of the specific mechanism. Most of the studies used exosomes derived from allogeneic cells to treat ED through intracavernous injection. Tail vein injection, intratunical injection and nerve injury *in situ* injection are also working with exosomes during the treatment of ED, although there are only a few reports in the literature. Considering the low yield of human exosomes, how to obtain sufficient exosomes for future clinical use is worth thinking. The sources of exosomes and therapeutic doses need recognized standards, and the comparison of the effects of exosomes from different sources requires more rigorous evidence to support it. Meantime, the doses of exosomes in previous studies vary greatly, which makes the appropriate dose necessary. Most researchers have injected exosomes at a dose of 100 μg and achieved good results, but

this only applies to rodent models. Despite the fact that exosomes do not express MHC proteins and will not cause immune tolerance and cell malignancies (84), it is still unknown whether there will be other adverse advents in the further research owing to the fact that the contents of exosomes have not been fully clarified. While exosomes may not be able to completely reverse the pathological changes in the corpus cavernosum, they can delay the progression of the disease, which is enough to improve sexual function and improve the quality of life. Due to the complexity of ED pathophysiology, combination therapy may be more effective, such as oral medications and physical therapy.

Moreover, exosomes have some applicative advantages. First, exosomes can be stored for a long time at low temperature, which is convenient for storage and transportation (84). Secondly, the protein, nucleic acid and other contents in exosomes are encapsulated by lipids, which is the structural basis for good stability (85). It is important to note that exosomes may circumvent many issues related to ethics. The exosome may be the future of ED treatment, nevertheless research is still at the preliminary stage. Basic researches and clinical trials of exosomes in the treatment of ED lay a solid foundation for the clinical translation of exosome therapy.

Author contributions

HF: Conception, methodology, data investigation & manuscript draft. WP: Conception, data investigation & manuscript draft. ZD: Conception, data investigation & manuscript draft. JL: Manuscript reviewing & project supervision. TW: Manuscript reviewing & project supervision. 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.

Publisher's note

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Erectile dysfunction and associated factors among patients with diabetes attending follow-up at a public hospital, Harar, Eastern Ethiopia. A cross-sectional study design

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Background: The global prevalence of erectile dysfunction among patients with diabetes is high. It is the most underestimated problem but has a great physical, psychological, and social impact on the individual with the disease, family, and society in general. Thus, this study aimed to assess the magnitude of erectile dysfunction and associated factors among patients with diabetes attending follow-up at a public hospital, Harar, Eastern Ethiopia.

Methods: Facility-based cross-sectional study was conducted on selected 210 adult male patients with diabetes attending follow-up at a public hospital, Harar, Eastern Ethiopia, from 1 February to 30 March 2020. Simple random sampling was used to select study participants. A pre-tested interviewer-administered structured questionnaire was used to collect the data. The data were entered to EpiData version 3.1 and exported to SPSS version 20 for analysis. Bivariate and multivariable binary logistic regression were carried out, and a P-value of <0.05 was taken as statistically significant.

Result: A total of 210 adult male patients with diabetes participated in the study. The overall magnitude of erectile dysfunction was 83.8%, with 26.7% suffering from mild, 37.5% mild to moderate, 29% moderate, and 6.8% severe erectile dysfunctions. Age 46–59 years [adjusted odds ratio (AOR): 2.560; 95% confidence interval (CI) (1.73, 6.53)], age ≥ 60 years [AOR: 2.9; 95% CI (1.48, 5.67)], and poor glycemic control [AOR: 2.140; 95% CI (1.9, 7.44)] were significantly associated with erectile dysfunction among patients with diabetes.

Conclusion: The present study revealed a high magnitude of erectile dysfunction among population with diabetes. The age categories of 46–59 and ≥60 and having poor glycemic control were the only variables significantly associated with erectile dysfunction. Thus, routine screening and management for erectile dysfunction in patients with diabetes should be part of routine medical care particularly for adult male patients and those with poor glycemic control.

KEYWORDS

magnitude, erectile dysfunction diabetes mellitus, Eastern Ethiopia, complication of diabetes, patient

Introduction

Diabetes mellitus (DM) is one of the most common and serious non-communicable disease affecting the lives and wellbeing of individuals with the disease, families, and societies at large. In 2019, approximately 463 million (9.3%) people were living with diabetes, and it was estimated that, by 2045, the prevalence will rise to 10.9% (700 million) (1). Its complications have major and long-lasting impacts at different levels (2). One of the most common and underestimated complications among DM is erectile dysfunction (ED) (3). According to the National Institutes of Health Consensus Development Conference, ED is defined as the persistent inability to achieve or maintain an erection sufficient for satisfactory sexual performance (4). ED is two to three times more common among individuals with DM than those without DM (5, 6).

The global prevalence of ED was 3%–76.5% (7). There is a great variation in the prevalence of ED among male patients with DM. On the basis of different epidemiological data, its prevalence was 52% (3). Different studies were carried out in different parts of the world, where the prevalence of ED among patients with DM ranges from 6.8% in Ethiopia to 95% in South Africa (8–21). In Africa, its general prevalence among patients with DM was 71.45% (22). Different studies on the prevalence of ED among patients with DM in some parts Ethiopia were carried out, and the prevalence was from 6.8% to 85.5% (8, 11, 19, 21, 23–25).

Although the number of people living with diabetes is rising in Ethiopia, there are only few studies on the complications of DM particularly ED. ED has a significant impact on the individual both physically and psychologically. It can also impair the quality of life of the patients, as well as their partners and families in general (5, 26). Factors including depression, older age, low educational status, poor quality of life, lack of regular physical activity, longer duration of DM, history of cardiovascular disease, cigarette smoking, hypercholesterolemia, poor glycemic control, obesity, taking beta blockers, and comorbidity were stated as the determinant factors for ED in patients with DM in different studies (8, 9, 11, 13, 16–19, 21, 23–25, 27).

ED is a treatable condition. If effective care and management including lifestyle modification, psychosexual therapy, and pharmacotherapy is applied, then it can be cured for up to 95% of cases (28). However, talking about sexual practice and experience is a very sensitive issue in Ethiopia where it is considered as a shameful act. This makes the diagnosis and treatment of ED difficult. The poor culture of openly discussing the sexual health problem among the community may mask the real magnitude of the problem in the population with diabetes. No study on ED in the eastern part of the country was carried out, and knowing its current magnitude and determinant factors may be important for early detection, managing the problem, and improving the quality of life of the patients. Thus this study aimed to assess the magnitude of ED and associated factors among patients with diabetes attending follow-up at a public hospital, Harar, Eastern Ethiopia.

Materials and methods

Study setting and period

A facility-based cross-sectional study was conducted at HiwotFana Comprehensive Specialized Hospital (HFCSH) from 1 February to 30 March 2020. The hospital is found in Harar city, Harari region, which is found at a distance of 526 km southeast of the capital city, Addis Ababa. HFCSH is a teaching hospital with a catchment of 5.2 million populations. It has a total of 185 inpatient beds distributed among four major departments.

Study population

All adult male patients with diabetes on follow-up at HFCSH during the study period were the source population, and male patients with diabetes who were on follow-up clinic during the

Abbreviations: AOR, adjusted odds ratio; BMI, body mass index; CI, confidence interval; COR, crude odds ratio; DM, diabetic mellitus; ED, erectile dysfunction; ETB, Ethiopian birr; FBS, fasting blood sugar; IIEF-5, International Index of Erectile Function-5; IDF, International Diabetic Federation; HFCSH, Hiwot Fana Comprehensive Specialized Hospital; HTN, hypertension; SD, standard deviation; WHO, World Health Organization.

study period, who are ≥ 18 years of age, and who are willing to participate in the study were included in the study. However, patients who were critically ill, have serious mental illness, had paraplegia from any cause, had past lower urinary tract and prostate surgery, and were not sexually active not because of ED for the previous 6 months prior to the study were excluded from the study.

Sample size determination and sampling procedure

The sample size was determined using the single population estimation formula by considering margin of error of 5%, confidence level of 95%, and the proportion of ED among patients with DM of 85.5% (24). The calculated sample size was 191, and, by adding 10% non-response rate, the final sample size became 210. To select the study participants, the number of male patients with diabetes expected to have follow-up during the study period in the follow-up clinic was taken from follow-up registration book; then, a systematic random sampling method was used to select study participants.

Data collection tools and method

The data were collected by using an interviewer-administered structured questionnaire. The questionnaire was first prepared in English and then translated to Afan Oromo and Amharic languages and then translated back to English by language experts to check for consistency. The questionnaire was taken from previous similar studies in Ethiopia and from the abridged five-item version of the International Index of Erectile Function (IIEF-5) (29). The instrument produced high sensitivity and specificity, 92.2 and 92.1%, respectively. It has a total of five questions scored out of 25. Accordingly, individuals who scored 1–21 were reported as having ED. While those who scored 22–25 were reported as not having ED. Those who scored 1–7, 8–11, 12–16, and 17–21 out of 25 points were classified as having severe ED, moderate ED, mild to moderate ED, and mild ED, respectively (29). Four medical intern students collected the data.

Data quality control

The quality of data was assured by proper designing of the questionnaire and pre-testing on 10% of the total sample size before 1 week of the actual data collection, and amendments were made based on the information obtained. One-day training was given to the data collectors and supervisors by the principal investigator. During data collection, each questionnaire was reviewed for completeness and consistency by supervisor, and all the necessary feedback was given to the data collectors immediately.

Data processing and analysis

The collected data were first checked for its completeness, cleaned and entered into EpiData version 3.1, and then exported to SPSS version 20 for analysis. Descriptive statistics such as frequency, percentage, mean, and standard deviation (SD) were computed. Bivariate binary logistic regression analysis was carried out to determine the association between each independent variable and dependent variable. Variables with $p < 0.2$ in bivariate analysis were entered for multiple logistic regressions. Finally, binary logistic regression was carried out, and a P-value of <0.05 was used to declare the statistical significance. To measure the strength of association, adjusted odds ratio (AOR) with their corresponding 95% confidence interval (CI) was used. The fitness of the model was checked using Hosmer and Lemeshow test, and multicollinearity was checked by using variance inflation factor of <10 and tolerance of >0.2.

Result

Sociodemographic characteristics of the study participants

A total of 210 adult male patients with diabetes participated in the study with a 100% response rate. The mean age of the study participants was 54.53 (± 13.73 SD) with a majority (79; 37.6%) of the study participants in the age group between 46 and 59 years. From the total participants, a majority (197; 93.8%) of them were married. Regarding their educational status, 86 (41%) had elementary education and 80 (40.5%) were government employees. Almost two-thirds (142; 67.6%) of the respondents, had an average monthly income of 1,501–3,000 Ethiopian birr (Table 1).

Behavioral characteristics of the study participants

From the total participants, almost all of them did not have unsafe alcohol consumption. Majority of them (121; 57.6%) chew khat, and 32 (15.2%) were smokers. Regarding physical exercise, only about one-fifth (44; 21%) of the participants had regular physical exercise (Figure 1).

Medical characteristics of the study participants

Inadequate glycemic control was present at the time of the investigation, as evidenced by the mean fasting blood sugar (FBS) of 162 mg/dl (+19.9 SD). The mean body mass index (BMI) of the participants was 24.6 kg/m^2 (± 3.3 SD), and almost half of them (103; 49%) had a BMI of 18.5 to 24.96 kg/m^2. Majority (54.3%) of the respondents lived with diabetes for < 5 years with mean duration of 5.8 (± 4.21 SD) years. Majority of the participants (117; 55.7%) were within normal range of blood pressure (<140/90). Almost half (104; 49.5%) of the respondents had chronic diseases other than diabetes and were taking medications other than for diabetes (Table 2).

TABLE 1 Sociodemographic characteristics of adult male patients with diabetes attending follow-up at Hiwot Fana Comprehensive Specialized University Hospital, Harar, Eastern Ethiopia, 2020 (n = 210).

Variable	Frequency	Percentage (%)
Age (years)		
18–30	8	3.8
31–45	50	23.8
46–59	79	37.6
≥60	73	34.8
Marital status		
Married	197	93.8
Others*	13	6.2
Educational status		
Illiterate	33	15.7
Elementary school (1–8)	86	41.0
High school (9–12)	55	26.2
College and above	36	17.1
Occupational status		
Government employed	85	40.5
Farmer	53	25.2
Merchant	53	25.2
Others**	19	9.0
Income		
<1,500 ETB	21	10.0
1,501–3,000 ETB	142	67.6
>3,000 ETB	47	22.4

ETB, Ethiopian birr; Other*: single, widowed, and divorced; Others**: private employee, no occupation, and retired.

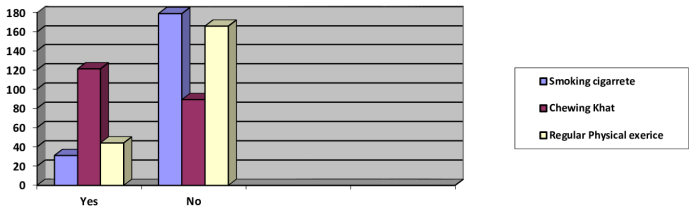

FIGURE 1
Behavioral characteristics of male patients with diabetes attending diabetic clinic at Hiwot Fana Comprehensive Specialized University Hospital (N = 210), Harar, Eastern Ethiopia, 2020 (n = 210).

Erectile dysfunction among study participants

The magnitude of ED in this study was found to be 83.8% (95% CI: 78.1, 88) of which 37.5% had mild to moderate ED and only 14 (7.95%) had ever sought treatment for the problem (Table 3).

Factors associated with erectile dysfunction among patients with diabetes

Factors associated with ED among patients with diabetes attending follow-up were assessed in the current study. Independent variables in the bivariable logistic regression analysis

TABLE 2 Medical conditions of adult male patients with diabetes attending follow-up at Hiwot Fana Comprehensive Specialized University Hospital, Harar, Eastern Ethiopia, 2020 (n = 210).

Variables	Mean(SD)	
Mean SBP		128.8 (± 15.8 SD)
Mean DPB		78.6 (± 10.3 SD)
Mean weight in kilograms		72 (± 9.20 SD)
Mean height in meters		1.72 (± **0**.042 SD)
Variable	**Frequency**	**Percentage (%)**
History of chronic diseases		
Yes	104	49.5
No	106	50.5
BMI		
<18.5	6	2.9
18.5–24.9	103	49.0
25–29.9	93	44.3
>30	8	3.8
Blood pressure		
Normal	117	55.7
Abnormal	93	44.3
Glycemic control		
Good	142	67.6
Poor	68	32.4
Duration of DM since diagnosed		
<5	114	54.3
5–10	61	29.0
>10	35	16.7
Takes medication other than DM		
Yes	104	49.5
No	106	50.5

that had a p value of less than 0.2 were passed for inclusion in the multivariable logistic regression analysis. Age and glycemic control were identified as the independent predictors significantly associated with ED. Adult male patients with diabetes who were more than 60 years old were 2.9 times more likely to experience ED compared with those in the age category of 18–30 years [AOR: 2.9; 95% CI (1.48, 5.67)]. Participants who were in the age category of 46–59 years were 2.56 times more likely to have ED than those who were in the age category of 18–30 years [AOR: 2.56; 95%CI: (1.73, 6.53)]. Likewise, adult male patients with diabetes who had poor glycemic control were 2.14 times more likely to have ED than those who had good glycemic control [AOR: 2.140; 95% CI (1.9, 7.44)] (Table 4).

Discussion

The current study assessed the magnitude and factors associated with ED among male patients with diabetes attending follow-up at HFCSH. The study found that an overall magnitude of ED was 83.8%. The finding is comparable with studies conducted in China (14), King Saudi University-Medical City in Saudi Arabia (14), and Bahirdar in Ethiopia (24), which showed ED prevalence of 79.1%, 80.5%, and 85.5%, respectively. However, The finding is comparable with studies conducted in Iran, 59.5% (20); Srilanka, 68% (16); Jamaica, 64% (17); Tanzania, 55.1% (15); Jimma, Ethiopia, 6.8% (21); and Central and Northwestern of Tigray, Ethiopia, 69.9% (19).

TABLE 3 Magnitude of erectile dysfunction among adult male patients with diabetes attending follow-up at Hiwot Fana Comprehensive Specialized University Hospital, Harar, Eastern Ethiopia, 2020 (n = 210).

Variable	Frequency	Percentage (100%)
Erectile dysfunction		
Yes	176	83.8
No	34	16.2
Category of erectile dysfunction (N = 176)		
Severe	12	6.8
Moderate	51	29.0
Mild to moderate	66	37.5
Mild	47	26.7
Ever seek medical care for ED (N = 176)		
Yes	14	7.95
No	162	92.05

TABLE 4 Multivariable logistic regression analysis for factors associated with erectile dysfunction among adult male patients with diabetes attending follow-up at Hiwot Fana Comprehensive Specialized University Hospital, Harar, Eastern Ethiopia, 2020 (n = 210).

Explanatory variables	Erectile dysfunction		COR (95% CI)	AOR (95% CI)	P-value
	Yes	No			
Educational status					
Illiterate	29	4	4.09 (1.177–14.262)	2.58 (0.501–10.97)	0.089
Elementary (1–8)	77	9	4.835 (1.83–12.74)	6.061 (0.28–12.846)	0.247
High school (9–12)	47	8	3.32 (1.20–9.13)	3.539 (0.521–8.2)	0.146
College and above	23	13	1	1	
Age (years)					
18–30	6	2	1	1	
31–45	21	29	0.24 (0.005–0.087)	0.32 (0.4–2.3)	0.06
46–59	71	8	2.953 (0.125–7.56)	2.560 (1.73–6.53)*	0.004
≥60	65	8	2.7 (0.125–4.756)	2.9 (1.48–4.67)*	0.001
Income					
Less than 1,500 EBR	12	9	0.316 (0.102–0.977)	0.072 (0.002–2.960)	0.166
1,501–3,000 EBR	126	16	1.865 (0.763–4.558)	1.651 (0.108–3.928)	0.640
More than 3,000 EBR	38	9	1	1	
Glycemic control					
Good	112	30	1		
Poor	60	8	2.008 (0.079–6.92)	2.140 (1.9–7.44)*	0.023
Regular physical exercise					
Yes	30	14	0.294 (0.133–0.645)	0.491 (0.513–0.932)	0.175
No	146	20	1		

(Continued)

TABLE 4 Continued

Explanatory variables	Erectile dysfunction		COR (95% CI)	AOR (95% CI)	P-value
	Yes	No			
Chewing khat					
Yes	107	14	2.215 (1.050–4.676)	2.368 (0.056–2.426)	0.299
No	69	20	1		
Duration of DM since diagnosis					
<5	86	28	0.396 (0.129–1.221)	0.159 (0.2–2.438)	0.267
5–10	59	2	3.806 (0.660–21.952)	3.581 (0.03–11.295)	0.720
>10	31	4	1	1	

*statistically significant at $p < 0.05$; COR, crude odds ratio; CI, confidence interval: AOR, adjusted odds ratio.

This inconsistency might be due to sociocultural difference among the study population that talking about sexual issue is not such embarrassing in Iran, Srilanka, and Tanzania as it is in our country, so the population in those studies might get diagnosed and treated accordingly before the study was conducted; different data collection methods and tools to determine the magnitude of ED might be the other reason for the discrepancy that the study that was conducted in the Jimma, Ethiopia, used card review, but this study used interview.

The result of the present study presented that age was significantly associated with ED. Adult male patients with diabetes who were more than 60 years old were 2.9 times more likely to experience ED than those in the age category of 18–30 years, and those participants who were in the age of 46–59 years were 2.56 times more likely to have ED that those who those in the age category of 18–30 years. This current finding is consistent with studies conducted in Turkey (9) and Dessie in Ethiopia (8), where male patients with diabetes whose age were greater than 60 years were more likely to experience ED than those who were less than 60 years old. Old age was also associated with ED among male patients with diabetes in Mizan-Tepi University Teaching Hospital and Tepi General Hospital, Ethiopia (23), and Jimma Medical Center, Southwest Ethiopia (11), where the prevalence was higher among patients greater than 40 years of age than those who were less than 40 years old. Older age was also associated with ED in a study conducted in Bahir-dar Ethiopia where both age group 45–59 and >60 years were associated with ED (24).

Glycemic control is another independent predictor in the present study that exhibited significant association with ED. Adult male patients with diabetes who had poor glycemic control were 2.14 times more likely to experience ED compared to those who had good glycemic control. This is consistent with studies carried out in Italy (10) and Saudi Arabia (9), which revealed a significant association of ED with poor glycemic control. However, in the studies conducted in Turkey (13), Northern Srilanka (16), southwest Ethiopia (23), and Jimma Medical Center Ethiopia (11), there was no association found between ED and glycemic control among adult male patients with diabetes. This variation might be due to differences among the study population, the methodology used, time of study, and diverse population culture.

Strength and limitation

This study has established some important points that will help us generate a hypothesis. It showed the magnitude of ED among patients with diabetes, which increases from time to time and needs attention. It was also used to see the relationship between the factors and ED among patients with diabetes. Because this study used a cross-sectional study design, cause-and-effect relationship cannot be reported.

Conclusion

The overall finding of the current study revealed a high magnitude of ED among male patients with diabetes. Majority of the participants experienced mild to moderate ED. Health institutions and healthcare providers should include assessment and management of ED as part of routine medical care in diabetic follow-up clinics. Patients who are of old age and who had poor glycemic control require special attention in screening for ED.

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.

Ethics statement

Ethical clearance was obtained from Institutional Health Research Ethical Review Committee (IHRERC) of the College of Health and Medical Science of Haramaya University. The ethical and supportive letters were submitted to Hiwot Fana Comprehensive Specialized Hospital, and consent was obtained from hospital administrator before data collection. A brief introductory orientation was given to the study participants prior to data collection, and written informed voluntary consent was obtained. Moreover, to maintain privacy, the names of the patients

were not written on the questionnaire, and patients were interviewed alone in a separate room.

Author contributions

All the authors had made significant contribution in idea generation, study design, analysis, and interpretation. They participated in drafting and reviewing 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.

Publisher's note

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Mendelian randomization study reveals the effect of idiopathic pulmonary fibrosis on the risk of erectile dysfunction

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Background: Several studies have found that erectile dysfunction (ED) is associated with interstitial lung disease. However, the causal relationship between idiopathic pulmonary fibrosis (IPF) and ED risk remains unclear. The present two-sample Mendelian randomization (MR) study aimed to reveal the causal effect of IPF on ED risk.

Methods: This study included two GWAS summary statistics of IPF (1,028 cases and 196,986 controls) and ED (6,175 cases and 217,630 controls) of European ancestry. The inverse-variance weighted (IVW) was applied as the primary method, and MR-Egger, weighted median, weighted mode, and simple mode were applied as complementary methods to estimate the causal impact of IPF on ED risk. The MR-PRESSO global test and MR-Egger regression were applied to evaluate the pleiotropy. The Cochran's *Q* test was applied to examine heterogeneity. The leave-one-out analysis ensured the robustness and reliability of the results.

Results: Twenty-one genetic variants were obtained as IPF instrumental variables without pleiotropy and heterogeneity. MR analysis using the IVW showed a potential causal relationship between IPF and increased ED risk (OR_{IVW}=1.046, 95% CI: 1.020–1.073, p=0.001), and consistent results were obtained with MR-Egger, weighted median, and weighted mode. The leave-one-out analysis showed that no instrumental variables unduly influenced the results.

Conclusion: This study suggested that IPF may increase the ED risk of the European population.

KEYWORDS

erectile dysfunction, idiopathic pulmonary fibrosis, Mendelian randomization, GWAS, instrumental variable

1. Introduction

Sexual health is a marker of overall health and good quality of life, of which erectile dysfunction (ED) has gained widespread attention worldwide (1). ED is defined as failing to achieve or sustain a penile erection sufficient for sexual intercourse (2). Current research on the prevalence of ED has found that it is a pervasive problem and increases with age. One survey found that the percentage of men aged under 40 consulting ED increased from 5% in 2010 to more than 15% in 2015 (3). Moreover, 52% of men aged 40–70 had some degree of ED (4). ED imposes a heavy burden on men and has become a health problem that cannot be ignored (5). As a result, it's essential to discover ED risk factors and evaluate individuals who may need early intervention or prevention.

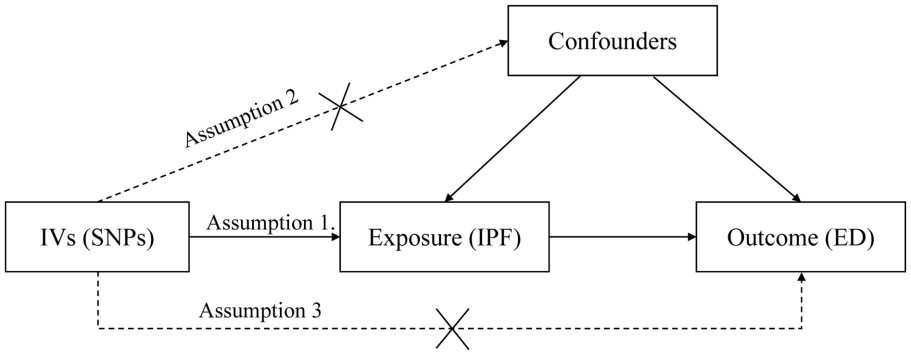

FIGURE 1
Three assumptions for IVs in MR analysis. ED, erectile dysfunction; IPF, idiopathic pulmonary fibrosis; MR, Mendelian randomization; IV, instrumental variable; SNP, single-nucleotide polymorphism.

At the onset of ED, various associated factors have been identified, such as depression, hypertension, diabetes, obesity, and smoking (6–9). Several observational studies have found that ED is a common problem in patients with interstitial lung disease (ILD), especially in idiopathic pulmonary fibrosis (IPF) patients (10, 11). IPF is a chronic fibrotic interstitial pneumonia marked by dyspnea and a gradual decline in lung function (12). Some studies have found that IPF is often combined with chronic hypoxia, which seems to have a shared pathogenic basis with ED (11). Considering that the causal link between IPF and ED risk remains unclear and that observational studies may be influenced by confounding factors or inverse causality to draw unreliable results, further clarification of the causal relationship between IPF and ED risk is necessary.

Two-sample Mendelian randomization (MR) examines the causal link between exposure and outcome by extracting genetic variants as instrumental variables (IVs) (13). Because genetic variants precede disease onset, MR analysis avoids reverse causality and the effects of unmeasured confounders (14), which overcomes the shortcomings present in observational studies. The present two-sample MR study aimed to estimate the causal effect of IPF on ED risk.

2. Materials and methods

2.1. Ethics statement

Our analyses used summary data from published studies or available genome-wide association studies (GWAS) and did not require ethics committee approval. The institutional ethics review board corresponding to GWAS approved each research, and all subjects signed informed consent.

2.2. Study design

Three critical assumptions of the MR study are illustrated in Figure 1. Assumption 1 is that IVs are reliably correlated with IPF. Assumption 2 is that IVs must be independent of any confounders. Assumption 3 is that IVs affect ED risk only through IPF but not through other pathways (14).

2.3. Data source

To identify genetic variants associated with IPF, we used the IPF GWAS study in the FinnGen Biobank (1,028 cases and 196,986 controls) (15). To avoid overlap in exposure and outcome populations, we used the summary data from a publicly available GWAS study of ED with populations primarily from UK Biobank, the Partners HealthCare Biobank cohort, and the Estonian Genome Center of the University of Tartu cohorts, including 6,175 ED patients and 217,630 controls (16). Both GWAS subjects are of European ancestry. The IEU open GWAS database (17)[1] provides the two GWAS summary data (IPF ID: finn-b-IPF; ED ID: ebi-a-GCST006956).

2.4. Selection of IVs

We performed strict quality control to obtain qualified instrumental single-nucleotide polymorphisms (SNPs) from the IPF GWAS study. First, based on assumption 1, we extracted SNPs significantly related to IPF ($p < 5 \times 10^{-6}$) (18). Second, to keep all IVs of the IPF from being in a linkage disequilibrium (LD) state, the clumping parameter is set as $R^2 < 0.001$ and window size = 10,000 kb (19). Third, we excluded SNPs with minor allele frequencies of less than 0.01. Fourth, we extracted the chosen SNPs from the ED GWAS. If an instrumental SNP does not exist in ED GWAS, we look for a SNP that is in LD status with it instead. Fifth, the harmonization process removed the palindromic SNPs from the IVs. Sixth, to ensure that IVs affect ED risk only through IPF, we examined and removed SNPs in IVs associated with possible confounders such as diabetes, obesity, hypertension, smoking, and metabolic syndrome through the PhenoScanner V2 database (20, 21). In addition, to avoid bias from weak IVs, we calculated the F-statistic ($F = \text{Beta}^2/\text{SE}^2$) (22). If the F-statistic of IVs is much larger than 10, the possibility of bias from weak IVs is slight (23).

1 https://gwas.mrcieu.ac.uk/datasets/

2.5. MR analysis

In the study, we estimated the causal relationship between IPF and ED risk using different methods, including the inverse-variance weighted (IVW) (24), MR-Egger (25), weighted median (26), weighted mode (27), and simple mode (28). These methods have different priorities under different conditions. The IVW method combines SNPs' Wald estimators to assess the impact of IPF on ED risk. When no pleiotropy exists for IVs or balanced pleiotropy exists, we acquire reliable causal estimates primarily through the IVW approach. If there was significant heterogeneity in the IVs ($p < 0.05$), we utilized a random effect model. Otherwise, a fixed effect model was utilized (19). The MR-Egger regression approach yields reliable estimates when pleiotropy exists for IVs (29). The weighted median approach still yields causal estimates when less than 50% of the IVs contravene the critical MR assumptions (26). The weighted model approach can perform MR causal inference when most IVs are valid (27). The simple mode is less potent than IVW (30). MR analysis was carried out in RStudio 4.2.1 using the R package TwoSampleMR (version 0.5.6) (31). $p < 0.05$ was considered significant.

2.6. Pleiotropy, heterogeneity, and sensitivity analysis

The MR-PRESSO global test (32) and MR-Egger regression (25) were utilized to evaluate the pleiotropy of IVs, and IVs were considered to have pleiotropy when $p < 0.05$. Heterogeneity was quantified by the Cochran's Q statistic and deemed significant when $p < 0.05$ (33). Additionally, to determine the presence of SNPs with bias effects, we conducted the leave-one-out analysis (34), which ensured the stability of our results.

3. Results

3.1. Selection of IVs

According to the screening criteria of the instrumental SNPs, we obtained 21 LD-independent SNPs from the GWAS of IPF (Supplementary Table S1). None of these 21 SNPs were associated with possible confounders of ED, such as diabetes, obesity, hypertension, smoking, and metabolic syndrome. Moreover, all of these 21 SNPs could be extracted in the GWAS of ED (Supplementary Table S2). Ultimately, we obtained these 21 SNPs as IVs of the IPF with $F > 10$ for each instrumental-exposure association, indicating a low likelihood of weak IV bias (Table 1). In addition, the genes corresponding to each instrumental SNP were shown in Supplementary Table S3.

3.2. Pleiotropy test

We used the MR-Egger regression and MR-PRESSO global test to detect the pleiotropy of IVs. The MR-Egger regression

TABLE 1 IVs for IPF.

SNP	Chr	Pos	Beta	SE	Effect_allele	Other_allele	EAF	F	p
rs7583252	2	228,838,054	0.216	0.046	A	G	0.423	21.9	2.92E-06
rs12638862	3	169,477,506	0.262	0.051	G	A	0.283	25.94	3.47E-07
rs9848175	3	8,743,076	0.808	0.176	G	A	0.021	21.12	4.35E-06
rs6847916	4	63,661,051	0.766	0.158	G	A	0.023	23.64	1.16E-06
rs558341636	5	1,584,923	2.210	0.365	C	T	0.007	36.71	1.37E-09
rs113548226	5	146,708,779	1.770	0.380	A	T	0.005	21.64	3.29E-06
rs145315307	5	168,904,005	1.019	0.129	G	T	0.037	62.84	2.20E-15
rs80236851	5	52,800,060	0.971	0.187	G	A	0.018	26.85	2.20E-07
rs10069690	5	1,279,790	−0.250	0.050	T	C	0.295	24.76	6.42E-07
rs79479138	5	1,238,988	1.037	0.186	T	C	0.018	30.99	2.59E-08
rs2076295	6	7,563,232	0.215	0.047	G	T	0.383	20.82	4.96E-06
rs116515165	6	2,392,366	0.958	0.208	T	C	0.014	21.23	4.06E-06
rs35000338	11	1,805,022	0.259	0.054	C	G	0.245	23.26	1.39E-06
rs11222805	11	99,979,997	−0.370	0.078	C	A	0.101	22.61	1.97E-06
rs11246335	11	866,133	0.784	0.123	A	G	0.041	40.54	1.91E-10
rs35705950	11	1,241,221	1.605	0.085	T	G	0.103	359.1	3.88E-80
rs72843931	11	1,875,886	0.899	0.132	T	C	0.036	46.47	9.29E-12
rs12605893	18	10,439,563	−0.216	0.046	A	C	0.464	22.32	2.32E-06
rs6423444	20	62,272,411	0.247	0.051	A	G	0.285	23.44	1.27E-06
rs9979837	21	15,359,048	0.227	0.048	T	C	0.363	22.4	2.17E-06
rs192474413	22	44,625,792	0.634	0.136	C	T	0.031	21.69	3.19E-06

IVs, instrumental variables; IPF, idiopathic pulmonary fibrosis; Chr, chromosome; SE, standard errors; EAF, effect allele frequency; Pos, position; SNP, single-nucleotide polymorphism.

suggested that IVs have no pleiotropy ($p = 0.064$, Table 2), which was confirmed by the MR-PRESSO global test ($p = 0.600$, Table 2). Therefore, our IVs are unlikely to be associated with confounders, and we validated this result in the PhenoScanner V2 database. Therefore, all these 21 IPF genetic variants can be used as valid IVs.

3.3. Heterogeneity test

The Cochran's Q test was used to detect heterogeneity in IVs. The Cochran's Q statistic suggested that IVs have no heterogeneity ($P_{IVW} = 0.549$, $P_{MR\text{-}Egger} = 0.741$, Table 3). Therefore, we primarily use the fixed effect model to perform MR causal estimates.

TABLE 2 Pleiotropy test of MR.

Method	Effect size	p
MR-Egger regression	−0.020 (egger_intercept)	0.064
MR-PRESSO global test	19.707 (RSSobs)	0.600

MR, Mendelian randomization; RSS, residual sum of squares.

TABLE 3 Heterogeneity test of MR.

Method	Q	Q_df	p
MR-Egger	14.713	19	0.741
Inverse-variance weighted	18.588	20	0.549

MR, Mendelian randomization.

3.4. MR estimates

After testing for pleiotropy and heterogeneity, we obtained 21 SNPs as IVs to assess the genetic association of IPF and ED risk, and the forest plot displays each SNP's causal impact on ED (Figure 2). Table 4 displayed the MR effect sizes for various approaches to evaluating the causal impact of IPF on ED risk. The IVW result revealed a causality of IPF on ED risk (OR = 1.046, 95% CI: 1.020–1.073, $p = 0.001$). Also, MR-Egger (OR = 1.074, 95% CI: 1.035–1.114, $p = 0.001$), weighted median (OR = 1.052, 95% CI: 1.017–1.088, $p = 0.003$) and weighted mode (OR = 1.047, 95% CI: 1.010–1.086, $p = 0.022$) approaches also obtained consistent results. In addition, the MR estimates of SNPs on both IPF and ED are shown in the scatter plot (Figure 3).

3.5. Leave-one-out analysis

The leave-one-out analysis was performed by excluding each SNP one by one and then observing whether the results changed. The leave-one-out analysis ensures that a particular SNP does not unduly influence the results. The result indicated no SNPs that significantly influenced ED risk and thus biased the causal estimates of MR. Therefore, our results were robust and not significantly biased (Figure 4).

4. Discussion

Epidemiological studies have found that ED has a high prevalence in men and increases with age. ED has become an

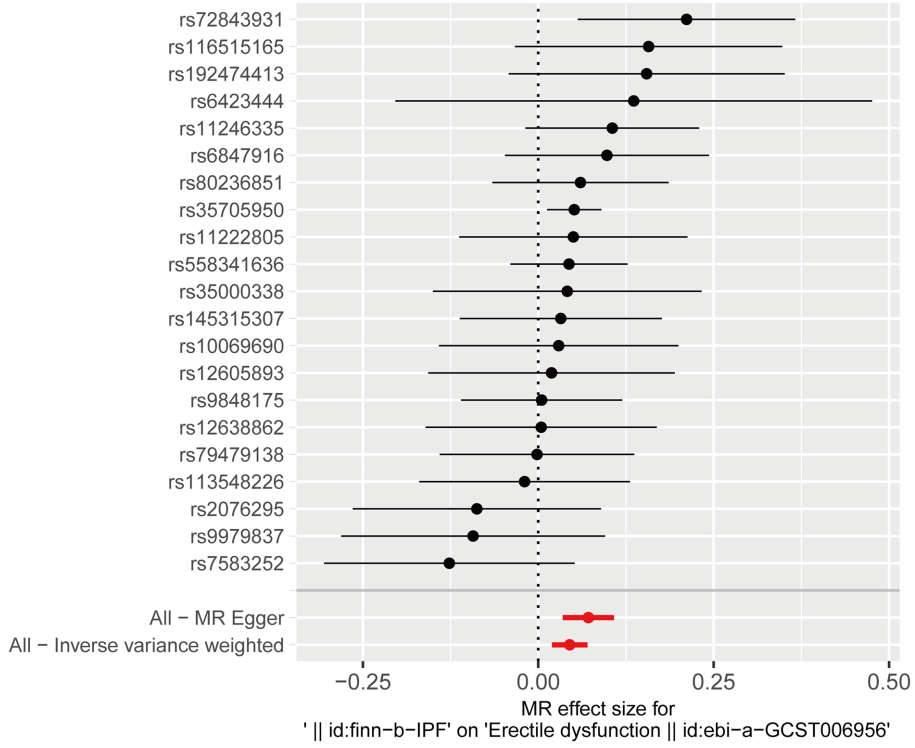

FIGURE 2
Forest plot for the causal effect of each SNP on ED risk. ED, erectile dysfunction; SNP, single-nucleotide polymorphism.

important health issue due to its severe impact on men's quality of life and psychosocial health (6). Therefore, the study of risk factors for ED is of great value for prevention and early intervention in ED.

Our study used the two-sample MR analysis to determine the causal link between IPF and ED risk. The results suggested that IPF causally increases the risk of ED, which is robust and reliable. A study investigating the prevalence of ED in ILD patients found that ED prevalence was much higher in ILD patients than in the background population and that ED prevalence increased with reduced pulmonary diffusion capacity (11). In another study of eight men with IPF-related hypoxia, nearly all of these men suffered from sex hormone suppression and ED (10). Although all of these studies suggest an association between IPF patients and increased ED risk, none can clarify the causal effects of IPF on ED risk. Moreover, observational studies are vulnerable to confounders or inverse causality. In contrast, our study provides causal evidence of IPF increasing ED risk within the MR design framework.

Although this study established a causal relationship between IPF and ED risk, the mechanisms by which IPF increases ED risk are unclear and may include common disease mechanisms of IPF and ED. Some instrumental SNPs have been shown to be closely associated with IPF, for example, the specific mutation rs35705950 (MUC5B) associated with fibrosis. However, the effect of these IVs on ED risk is via IPF rather than directly associated with ED, and IVs cannot be associated with ED susceptibility factors (confounders), including diabetes, obesity, hypertension, smoking, and metabolic syndrome. So, through what pathways does IPF increase ED risk? A recent study of COPD patients suffering from ED found that hypoxemia may be the mechanism by which COPD patients develop ED (35). In contrast, long-term oxygen therapy reversed ED in patients with hypoxemia (36). And patients with IPF-related hypoxia also

TABLE 4 Causal effect of IPF on ED risk.

Method	*N* (SNP)	Beta	SE	*p*	OR	OR_lci95	OR_uci95
MR-Egger	21	0.071	0.019	0.001	1.074	1.035	1.114
Weighted median	21	0.051	0.017	0.003	1.052	1.017	1.088
Inverse-variance weighted	21	0.045	0.013	0.001	1.046	1.020	1.073
Simple mode	21	0.032	0.031	0.324	1.032	0.971	1.097
Weighted mode	21	0.046	0.019	0.022	1.047	1.010	1.086

ED, erectile dysfunction; IPF, idiopathic pulmonary fibrosis; SNP, single-nucleotide polymorphism; SE, standard error; OR, odds ratio; OR_lci95, lower limit of 95% confidence interval for OR. OR_uci95, upper limit of 95% confidence interval for OR.

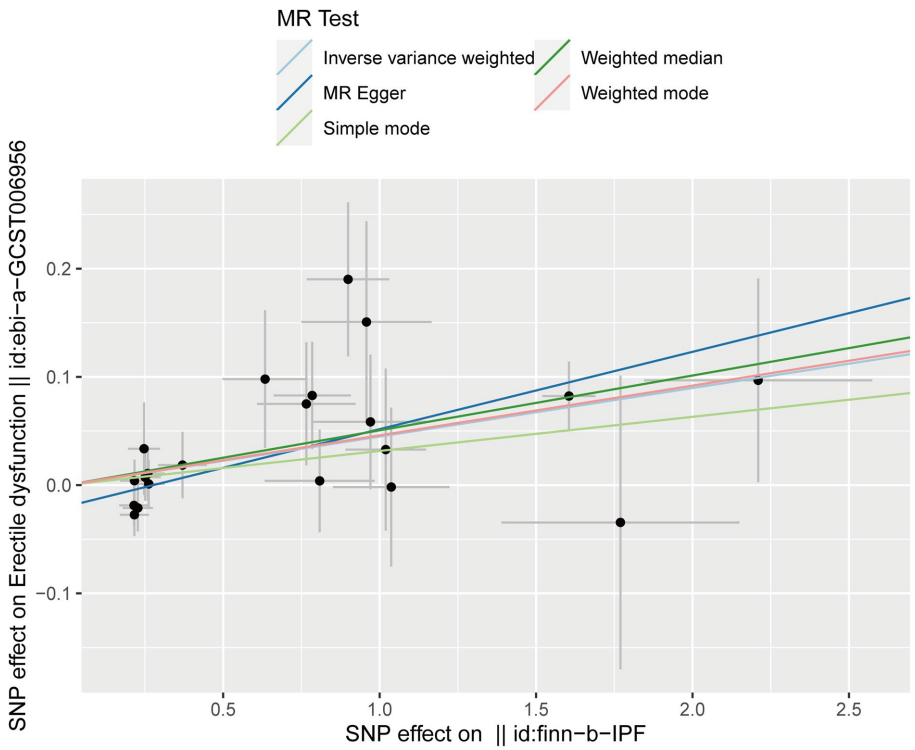

FIGURE 3
Scatter plot for the causal effect of IPF on ED risk. ED, erectile dysfunction; IPF, idiopathic pulmonary fibrosis.

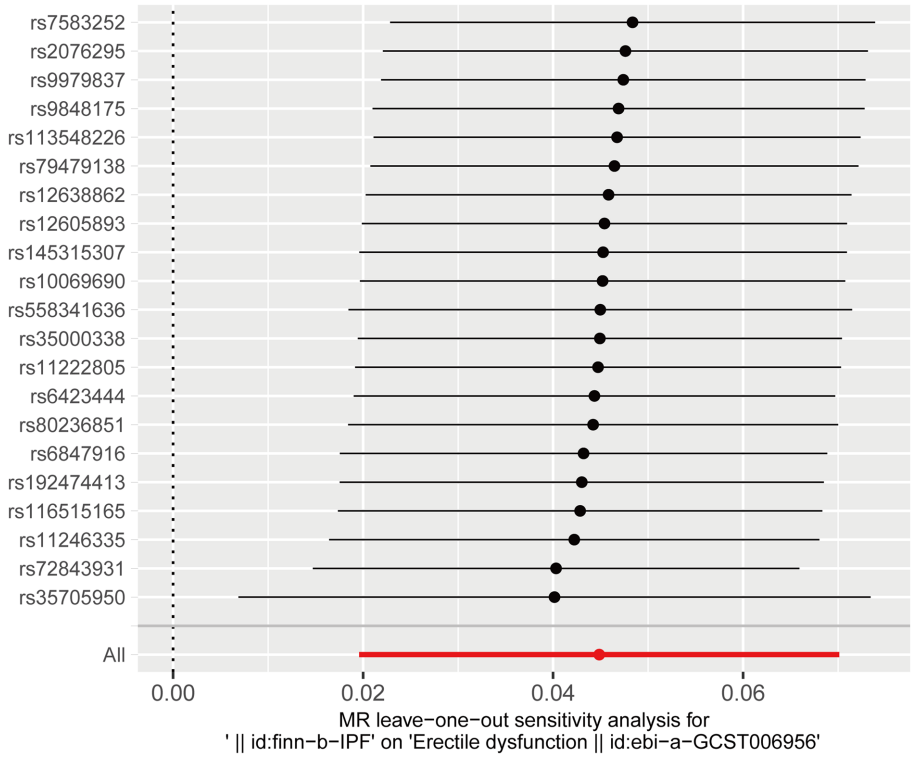

FIGURE 4
Leave-one-out analysis of the effect of IPF on ED risk. ED, erectile dysfunction; IPF, idiopathic pulmonary fibrosis.

developed sex hormone suppression and ED (10). In addition, the progression of IPF was associated with vascular endothelial dysfunction (37), which is characteristic of ED (38). Therefore, vascular endothelial dysfunction and hypoxemia may mediate IPF to increase ED risk.

Notably, our analysis is the first MR study of IPF on ED risk. We selected strongly correlated and independently inherited LD-independent SNPs as IVs to estimate the causal effect of IPF on ED risk. The strength of the link between IVs and IPF was assessed using an F-statistic that was much larger than 10, suggesting a low likelihood of weak IV bias (23). Several robust analysis methods provided robust inferences for our MR analysis. In addition, the current MR analysis used GWAS data from two large samples of European populations, providing sufficient power to estimate causality while avoiding demographic bias. The populations of these two GWAS studies were from two databases, minimizing the possibility of subject overlap, and we minimized the bias of sample overlap by using strong instruments ($F > 10$) (39). Finally, MR analysis revealed the effect of genetically predicted IPF on ED risk, overcoming the shortcomings of observational studies that are susceptible to confounders.

The study also has some limitations. First, the summary data of the GWAS involved only European ancestry, and our conclusions may only apply to European populations. Therefore, we ought to use our findings with caution in racially and ethnically diverse populations. Second, since the exact functions of some SNPs in IVs are not known, there may be residual bias. Furthermore, given the binary assessment of IPF and the lack of individual statistics, it was impossible to explore nonlinear associations between IPF and ED risk, perform stratified analyses, and adjust for other covariates.

5. Conclusion

In this study, we showed an apparent causal effect of genetically predicted IPF on ED risk in the European population using a two-sample MR analysis. However, further mechanistic studies are necessary to explain the deeper link between IPF and increased ED risk. Most importantly, our study reminds clinicians that measures to prevent and intervene early in ED should be considered when diagnosing male patients with IPF.

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

KZ and MC conceived the study and wrote the manuscript. KZ, JZ, and AL collected the data and conducted the analysis. All authors contributed to the article and approved the submitted version.

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.2023.1162153/full#supplementary-material

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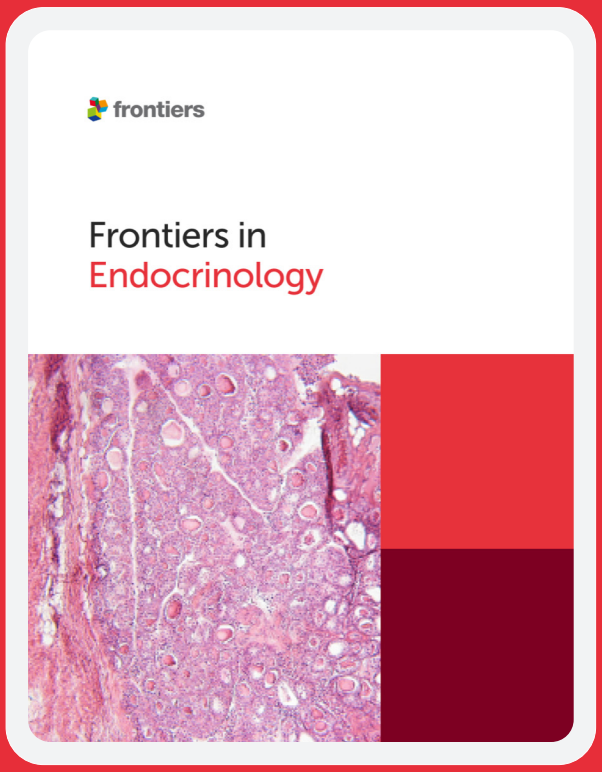

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