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The mechanism of sudden unexpected death in epilepsy and the specific forensic diagnostic indicators in sudden death with a negative autopsy

Edited by
Beixu Li, Likun Wang and Bin Tu

Published in
Frontiers in Neurology

ISSN 1664-8714
ISBN 978-2-8325-3396-3
DOI 10.3389/978-2-8325-3396-3

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The mechanism of sudden unexpected death in epilepsy and the specific forensic diagnostic indicators in sudden death with a negative autopsy

Topic editors

Beixu Li — Shanghai University of Political Science and Law, China
Likun Wang — Affiliated Hospital of Guizhou Medical University, China
Bin Tu — Columbia University, United States

Citation

Li, B., Wang, L., Tu, B., eds. (2023). *The mechanism of sudden unexpected death in epilepsy and the specific forensic diagnostic indicators in sudden death with a negative autopsy*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-3396-3

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frontiers | Frontiers in Neurology

TYPE Editorial
PUBLISHED 18 August 2023
DOI 10.3389/fneur.2023.1265787

OPEN ACCESS

EDITED AND REVIEWED BY
Fernando Cendes,
State University of Campinas, Brazil

*CORRESPONDENCE
Beixu Li
libeixu@shupl.edu.cn
Likun Wang
769070308@qq.com
Bin Tu
bt2261@columbia.edu

RECEIVED 23 July 2023
ACCEPTED 11 August 2023
PUBLISHED 18 August 2023

CITATION
Li B, Wang L and Tu B (2023) Editorial: The mechanism of sudden unexpected death in epilepsy and the specific forensic diagnostic indicators in sudden death with a negative autopsy. *Front. Neurol.* 14:1265787. doi: 10.3389/fneur.2023.1265787

Editorial: The mechanism of sudden unexpected death in epilepsy and the specific forensic diagnostic indicators in sudden death with a negative autopsy

Beixu Li[1,2]*, Likun Wang[3]* and Bin Tu[4]*

[1]School of Policing Studies, Shanghai University of Political Science and Law, Shanghai, China, [2]Shanghai Fenglin Forensic Center, Shanghai, China, [3]Emergency Department of Internal Medicine-Neurology, Affiliated Hospital of Guizhou Medical University, Guiyang, Guizhou, China, [4]Comprehensive Epilepsy Center, Department of Neurology, College of Physicians and Surgeons, Columbia University, New York, NY, United States

KEYWORDS

sudden unexpected death, places of confinement, sudden unexpected death in epilepsy, molecular mechanism, forensic diagnostic indicators, artificial intelligence

Editorial on the Research Topic
The mechanism of sudden unexpected death in epilepsy and the specific forensic diagnostic indicators in sudden death with a negative autopsy

Research on sudden unexpected death (SUD) is essential to clinical and forensic medicine. The diagnosis of the cause of SUD is challenging and is a research hotspot in forensic medicine, especially in cases without specific diagnostic indicators and deaths from non-natural causes, including SUD in epilepsy (SUDEP). Further, the accurate diagnosis of the cause and manner of SUD is necessary for judicial purposes to ascertain facts, settle disputes, and resolve conflicts, particularly in confined places such as prisons, detention centers, and rehabilitation centers, and in cases of SUD of healthy middle-aged and young adults.

SUDEP was defined in 2012 as a category of death and is classified into seven subtypes: [1] definite SUDEP, characterized by a sudden, unexpected, witnessed or unwitnessed, non-traumatic, and non-drowning death, occurring in benign circumstances in individuals with epilepsy with or without evidence of seizures and excluding documented status epilepticus (seizure duration longer than 30 min or seizures without recovery), in which postmortem examination does not reveal a definite cause of death, [2] definite SUDEP plus, [3] probable SUDEP/probable SUDEP plus, [4] possible SUDEP, [5] near-SUDEP/near-SUDEP plus, [6] non-SUDEP, and [7] unclassified (1). SUDEP is diagnosed by excluding other causes that may have led to death. Diagnosis is controversial because of the lack of specific forensic markers and the tendency to occur more frequently among young people (2), as demonstrated in the present study. Mechanisms underlying SUDEP are incompletely understood but may be associated with seizure-related cardiac dysfunction, respiratory depression, autonomic nervous dysfunction, and brain dysfunction in the postictal phase (3).

This study focuses on the characteristics of SUDEP, underlying molecular mechanisms, and predictors of SUD with negative autopsy. The study evaluated seven original research articles and two reviews, including six studies from China, two from Canada, and one from Italy.

Retrospective and comparative studies provided autopsy data on SUDEP, elucidating its pathological characteristics. Patients who underwent SUDEP show no lethal pathological changes but rather exhibit mild neurological, respiratory, and cardiovascular abnormalities, in line with the definition of SUDEP and its association with young age and prone position. Zhang, Zhang et al. innovatively compared autopsy and toxicological findings of SUDEP and other causes of death in individuals with epilepsy. Yan et al. evaluated three cases of SUDEP from their forensic center from 2011 to 2020 and 385 reported cases of SUDEP. Moreover, these authors discussed the importance of performing comprehensive brain examinations for suspected cases of SUDEP and evaluating the safety and effectiveness of antiepileptic drugs. Argo et al. analyzed four cases of SUDEP providing valuable data for the diagnosis of SUDEP in terms of pathological characteristics, circumstance factors, and the relationship between antiepileptic drugs and SUDEP. These autopsy data elucidate the pathogenesis and causes of SUDEP.

Two review articles evaluated the mechanism and diagnostic practices of SUDEP. Sun et al. found that SUDEP was associated with inherited cardiac ion channel diseases and severe obstructive sleep apnea. Underlying mechanisms involved decreased heart rate variability (HRV) and prolonged QT interval, potentially leading to arrhythmias; laryngospasm; amygdala activation; adenosine neuromodulation; and the inhibition of 5-HT neuronal activity. Nonetheless, little is known about the molecular mechanisms of SUDEP and risk predictors. Sun and Wang discussed current forensic methods for SUDEP diagnosis, the reasons for the low rate of diagnosis of SUDEP, and the prospects of molecular autopsy in forensic pathology. The authors suggested standardizing a testing protocol for SUDEP to facilitate data sharing and research collaboration worldwide.

Zhang, Ma et al. identified miRNA-mRNA regulatory networks associated with the glutamatergic system in a rat model of post-traumatic epilepsy (PTE) by transcriptome sequencing and bioinformatics analysis. Some miRNA-mRNA interaction pairs were involved in the development of PTE and are thus potential predictors of the risk of SUDEP. Lamrani et al. measured EEG parameters and HRV to assess autonomic function and the risk of SUDEP. HRV patterns and unusual cardiorespiratory manifestations indicated autonomic abnormalities that could predict an increased risk of SUDEP. Based on clinical data prior to death in SUDEP and non-SUDEP patients, Gravitis et al. used single spectrum analysis, independent component analysis, and cross-frequency phase-phase coupling to develop a novel metric to assess non-linear interactions between two ECG rhythms and predict the risk of SUDEP. These clinical data can help predict, prevent, and diagnose SUDEP.

Artificial intelligence, deep learning, and big data technology can assist in SUDEP prediction, prevention, and diagnosis (4). Argo et al. analyzed the risk of SUDEP using information technology as SUDEP and related research are gaining increased attention. Tong et al. performed a bibliometric analysis of studies on SUDEP. *Frontiers in Neurology* ranks sixth in the number of SUDEP-related articles published in the past 20 years, and its impact factor ranks third among these six journals. The United States, Europe, and Asia are leading the research on this topic (5). In turn, African studies on this topic are limited. The study by Argo et al. proved that. The present study discusses various aspects of SUDEP research including predictors of the risk of SUDEP in forensic pathology and underlying mechanisms, the incorporation of new technologies, and the current state of global research on the subject. The study aims to advance our understanding of SUDEP mechanisms and foster the identification of specific forensic diagnostic indicators associated with this condition.

Author contributions

BL: Funding acquisition, Validation, Writing—original draft, Writing—review and editing. LW: Validation, Writing—review and editing. BT: Validation, Writing—review and editing.

Funding

This work was supported by the Summit-Climbing Plan of School of Policing Studies, Shanghai University of Political Science and Law.

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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frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 09 December 2022
DOI 10.3389/fneur.2022.1077624

OPEN ACCESS

EDITED BY
Beixu Li,
Shanghai University of Political Science and Law, China

REVIEWED BY
Xie Yuquan,
Shanghai Jiao Tong University, China
Haixiang Xu,
Kunshan Traditional Chinese Medicine Hospital, China

*CORRESPONDENCE
Jianhua Zhang
zhangjh@ssfjd.cn
Donghua Zou
zoudh@ssfjd.cn
Zhengdong Li
lzdadv@163.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Epilepsy, a section of the journal Frontiers in Neurology

RECEIVED 23 October 2022
ACCEPTED 14 November 2022
PUBLISHED 09 December 2022

CITATION
Zhang X, Zhang J, Wang J, Zou D and Li Z (2022) Analysis of forensic autopsy cases associated with epilepsy: Comparison between sudden unexpected death in epilepsy (SUDEP) and not-SUDEP groups. *Front. Neurol.* 13:1077624. doi: 10.3389/fneur.2022.1077624

Analysis of forensic autopsy cases associated with epilepsy: Comparison between sudden unexpected death in epilepsy (SUDEP) and not-SUDEP groups

Xian Zhang[1], Jianhua Zhang[2*†], Jinming Wang[2], Donghua Zou[2*†] and Zhengdong Li[2*†]

[1]Department of Cardiology, Kunshan Hospital of Integrated Traditional Chinese and Western Medicine, Jiangsu, China, [2]Shanghai Key Laboratory of Forensic Medicine, Academy of Forensic Science, Ministry of Justice, Shanghai, China

Background and aims: Epilepsy is a common and chronic neurological disorder characterized by seizures that increase the risk of mortality. SUDEP is the most common seizure-related category of death. The study aimed to evaluate the key characteristics between SUDEP and not-SUDEP death cases.

Methods: A retrospective study of forensic autopsy cases from 2002 to 2021, performed by the Academy of Forensic Science (Ministry of Justice, China), identified a total of 31 deaths associated with epilepsy. We compared the different characteristics between individuals who died of SUDEP (SUDEP group) and individuals with epilepsy died suddenly due to unrelated causes (not-SUDEP group).

Results and conclusions: 13 cases met the general accepted definition of SUDEP; and 18 cases were classified as not-SUDEP. The mean age of the not-SUDEP group was significantly higher than that of the SUDEP groups ($p < 0.05$) and there were more cases without a clear cause of epilepsy in the SUDEP group than in the not-SUDEP group ($p < 0.05$). Death position differed significantly between the two groups, with more cases dying in the prone position in the SUDEP group ($p < 0.05$). Complete autopsies were performed in 24 of the 31 cases. There were no significant differences in heart, lungs and brain weights, or in ventricular thickness ($p > 0.05$) between the SUDEP and not-SUDEP groups. In addition, compared to the not-SUDEP group, the SUDEP group featured a significantly more cases with coronary lesions (grades 1-3, $p < 0.05$). Neuropathological lesions were identified in 12 of the 13 SUDEP cases (92.3%), cardiac lesions were present in 10 cases (76.9%) and pulmonary edema and pulmonary congestion were present in all cases. The primary cause of death in 13 of the 31 cases was seizure disorder or epilepsy. The primary mechanism of death in SUDEP group was mainly asphyxia while that in the not-SUDEP group was cardiopulmonary failure ($p < 0.05$). Patients in the prone position had a significantly higher risk of asphyxia than those who were

not. Here, we investigated the key characteristics between SUDEP and not-SUDEP death cases, which may help to facilitate forensic diagnosis in presumed SUDEP cases.

KEYWORDS

sudden unexpected death in epilepsy (SUDEP), forensic pathology, autopsy, post-mortem findings, cause of death

Introduction

Epilepsy is a common neurological disease that represents a serious threat to human health, affecting ∼70 million people globally (1). The weighted median of the standardized death ratio (SMR) in patients with epilepsy is 2.3 in high-income countries and 2.6 in low-income countries, thus indicating a significantly higher risk of mortality than that in the general population (2). Leading causes of death in epilepsy include the sudden death of unknown causes, status of epilepsy, accidental injury, and suicide (3).

Sudden unexpected death in epilepsy (SUDEP) is defined as a sudden, unexpected death, witnessed or unwitnessed, of a person with epilepsy, for whom a complete postmortem examination does not reveal a specific cause of death (4). This nomenclature was initially defined in 1997 by Annegers (5) and (6). The definition of SUDEP was unified in 2012. According to this definition, SUDEP is defined as a category of death and can be classified into seven subtypes: (1) definite SUDEP, (2) definite SUDEP plus, (3) probable SUDEP/probable SUDEP plus, (4) possible SUDEP, (5) near-SUDEP/near-SUDEP plus, (6) not-SUDEP, and (7) unclassified (7). The new definition showed the extension and refinement of the understanding of the disease. Deaths caused by SUDEP remain a serious public health concern (8). SUDEP is the leading cause of epilepsy-related premature mortality and accounts for 8–17% of deaths among people with epilepsy (9). The calamity of SUDEP preferentially targets young people (10).

Because the diagnosis of SUDEP is made by exclusion of other causes of death, it requires a clinical history of epilepsy, witness statements, details of the scene and circumstances of death, and complete postmortem examinations including toxicology. Therefore, forensic medicine study has also played an important role in SUDEP research, and it provides many important clues to elucidate the mechanism of SUDEP as well (11). The neuropathology and cardiac pathology findings in SUDEP are the main concerns of the autopsy (12–16). However, there are no specific neuropathological and cardiac alterations that can categorically confirm SUDEP. The postmortem examination of SUDEP in the future will be an integration of clinical, pathological, and molecular genetic investigation conducted by both forensic experts and neuropathologists.

Obtaining insight into its pathophysiological mechanisms is a cardinal step toward the prevention and reduction of the incidence of SUDEP. The exact mechanism of SUDEP is unknown but postictal disturbed cardiac or respiratory physiology is implicated (17). Seizures that arise in the cortical region can spread to involve the subcortical regions of the central autonomic network. The ictal activity of the central autonomic network can disrupt the functional connectivity of this network by inhibiting or activating autonomic areas, causing diverse autonomic manifestations, including respiratory and cardiovascular dysfunction (18). Adverse effects of adenosine signaling may also potentiate a fatal outcome in the form of SUDEP by suppressing breathing and arousal in the postictal period (19).

Clinical studies on death, including SUDEP, are challenging. It can hardly be carried out in a trial, but only through patient history reviews, medical records, or surrogate measures such as cardiac and respiratory abnormalities (20). Furthermore, retrospective research is still complicated by the loss of data. Previous studies have either compared individuals who had died of SUDEP to individuals who were alive and had epilepsy (20), or compared individuals who died of SUDEP to individuals who died suddenly due to some unrelated causes without epilepsy. Therefore, in this study, we analyzed forensic autopsy cases associated with epilepsy and compared the characteristics of individuals who died of SUDEP to individuals with epilepsy who died of other causes.

Materials and methods

Case data

This study was approved by the Academic Committee of the Academy of Forensic Science (AFS), Ministry of Justice, China. We conducted a retrospective study of all cases investigated by the AFS between 2002 and 2021. The cases, in which the cause of death was listed as a seizure or a clear medical history of epilepsy was confirmed, were sifted out from the AFS autopsy case database. Data for forensic pathological identification, including detailed investigation records, surveillance videos, medical history, and autopsy findings, had been collected by AFS, and all case information was never published in any literature.

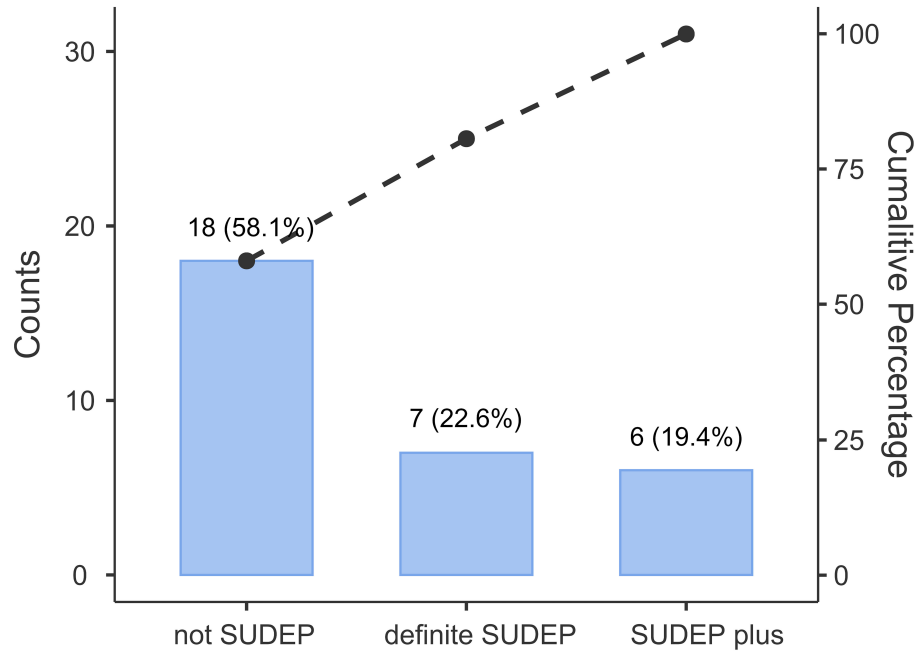

FIGURE 1
Classification of the collected 31 cases and the respective percentages.

Methods

Electronic searches revealed 31 cases for analysis. We reviewed investigation reports and autopsy findings for each of these 31 cases. Cases were then classified according to the latest definition of SUDEP (7). The definitions of each classification are described as below: (1) "definite SUDEP": a sudden, unexpected, witnessed or unwitnessed, non-traumatic and non-drowning death, occurring in benign circumstances, in an individual with epilepsy, with or without evidence for a seizure and excluding documented status epilepticus (seizure duration $\geq$30 min or seizures without recovery), in which postmortem examination does not reveal a definite cause of death; (2) "SUDEP plus": satisfying the definition of definite SUDEP, if a concomitant condition other than epilepsy is identified before or after death, if the death may have been due to the combined effect of both conditions, and if autopsy or direct observations/recordings of terminal event did not prove the concomitant condition to be the cause of death; (3) "probable SUDEP": same as definite SUDEP but without autopsy; (4) "possible SUDEP": a competing cause of death is present; (5) "near-SUDEP": a patient with epilepsy survives resuscitation for more than 1 h after a cardiorespiratory arrest that has no structural cause identified after investigation; (6) "not-SUDEP": a clear cause of death is known; (7) "unclassified": not possible to classify. The classification results for the 31 cases are shown in Figure 1. The deceased cases were assigned into two groups: (1) those that met the diagnostic criteria for SUDEP (either definite SUDEP or SUDEP plus) or (2) death unrelated to epilepsy (not-SUDEP). Analysis of etiology was based on the Classification of International League against Epilepsy (ILAE) (21).

Data were then analyzed from several different perspectives: (1) demographic data of the subject, such as age and gender; (2) personal information obtained from records of investigation, including medical history and history of drug or alcohol use; (3) information acquired from the evaluation of the scene and the circumstances of death such as the time of death, the location of death, and the position of the deceased when found; and (4) the cause of death and the mechanism of death.

Cases that met any of the following criteria were excluded to ensure comparability of measurements: (1) The case was an external examination only; (2) The decedent was under 14 years old; and (3) The decedent was decomposing to such a degree as to alter normal organ weights. Finally, the exclusion of 7 cases above left 24 autopsies. All of the 24 cases received a complete autopsy, including histopathological examinations and postmortem toxicological analysis (22). In each autopsy, the heart was dissected 1–2 cm above the aorta and pulmonary trunk. The heart mass was determined by weighing the fresh heart on a metric pan scale after blood and clots were removed from the heart. The epicardial fat was left intact for weighing.

The presence of anti-epileptic drugs (AEDs) was detected by gas chromatography/mass spectrometry (GC/MS). Several AEDs were routinely detected, including carbamazepine,

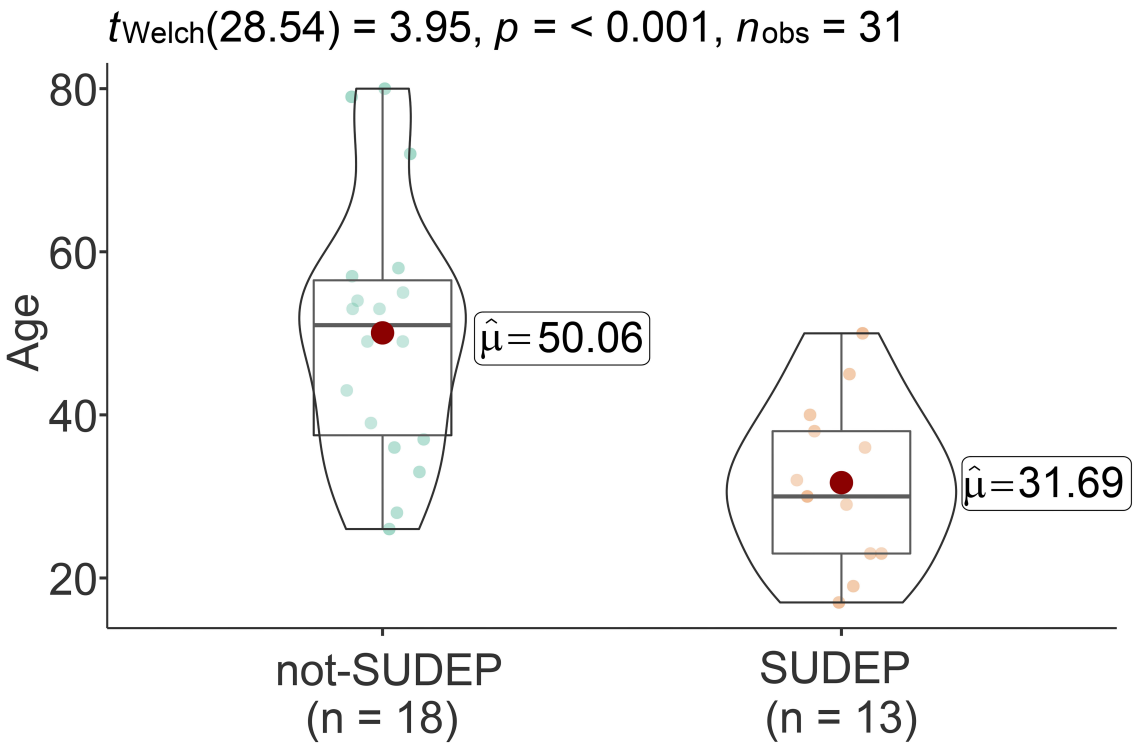

FIGURE 2
Age comparison between SUDEP and not-SUDEP groups (red dot represents mean value).

lamotrigine, phenytoin, phenobarbitone, valproic acid, levetiracetam, hydroxycarbazepine, and primidone. For the purpose of forensic identification and this study, therapeutic concentrations were considered as follows: carbamazepine, 4–8 mg/L; lamotrigine, 3–14 mg/L; phenytoin, 10–20 mg/L; phenobarbitone, 10–30 mg/L; valproic acid, 50–100 mg/L; levetiracetam, 10–37 mg/L; zonisamide, 20–30 mg/L; topiramate, 3.4–5.2 mg/L; and hydroxycarbazepine, 12–30 mg/L (23, 24).

Statistical analyses

Statistical analyses were performed using Jamovi 2 (jamovi.org) (25) and ggstatsplot (26). We checked normal distribution with the Shapiro-Wilk test and verified the homogeneity of variances for each set of data. Normally distributed data are presented as means ± standard error, categorical data are presented as numbers (percentages), and the continuity variables that are not normally distributed are represented by M (Q1, Q3). Statistical testing involved Welch's *t*-test and the Mann-Whitney *U*-test for continuous variables and the chi-square test and Fisher's exact test for categorical variables. To predict whether death position could have exerted an impact on the mechanism of death, we used binomial logistic regression models. Statistical significance difference was defined as $p < 0.05$.

Results

Demographic characteristics

A total of 31 cases were classified into three groups according to the latest SUDEP definition; 7 cases (22.6%) met the definition of "definite SUDEP," 6 cases (19.4%) were classified as "SUDEP plus" group, and the rest 18 cases (58.1%) were defined as "not-SUDEP." The classification results for the 31 cases are shown in Figure 1.

Of the 31 cases, 22 were men and 9 were women, and the mean age at death was 42.4 ± 16.3 years (range: 17–80 years). Gender ratios did not vary significantly when compared between the SUDEP and not-SUDEP groups ($p > 0.05$). However, the age of the cases in the not-SUDEP group (50.1 ± 15.9 years) was significantly higher than that of the cases in the SUDEP group (31.7 ± 9.9) ($p < 0.01$). The age comparison results are shown in Figure 2.

Clinical history and the circumstances of death

In total, 11 cases were associated with epileptic lesions, including intracranial occupancy in three cases and brain trauma in eight cases. The metabolic causes of epilepsy were brain hypoxia and poisoning in four cases; 16 cases had no significant cause of epilepsy. The distribution of cases with or without definite etiology was significantly different when compared between the two groups and more cases in the SUDEP group did not have a definite etiology ($p < 0.05$). Table 1 shows that there were more cases (10/13) with undefined etiology of epilepsy in the SUDEP group than that in the not-SUDEP group (6/18).

Investigations indicated that 14 cases (45.2%) died in the hospital or the clinic, while 11 cases (35.5%) died at their residence. Other death locations included a jail cell, a driveway, a massage room, a working place, and in a sewer. The distribution of death locations did not differ significantly when compared between the SUDEP and not-SUDEP groups ($p > 0.05$), as shown in Table 1. By reviewing the case records, a total of 29 cases clearly stated the time of death; two cases did not specify the time of death. The time of death was classified as day and night (daytime was defined as 8:00 a.m. to 8:00 p.m.). No significant difference was detected in terms of the time of death when compared between the SUDEP and not-SUDEP groups ($p > 0.05$), as shown in Table 1. Death positions were analyzed according to case investigations and livor mortis distribution, while 28 cases had a determined death position, and 3 cases of the not-SUDEP group could not determine the death position. Results arising from the SUDEP group differed significantly from the not-SUDEP group. More cases died in the prone position in the SUDEP group (7/13) than that in the not-SUDEP group (1/15) ($p < 0.05$). Details of the death position are shown in Table 1.

TABLE 1 Etiology, death circumstances, and AEDs of the SUDEP cases.

	Not-SUDEP ($N = 18$*)	SUDEP ($N = 13$)	χ^2	P
Etiology of epilepsy			5.74	**0.017**
Not definite [N (%)]	6 (33.3)	10 (76.9)		
Definite [N (%)]	12 (66.7)	3 (23.1)		
Death location			4.64	0.098
Home	5 (27.8)	6 (46.2)		
Hospital	11 (61.1)	3 (23.1)		
Others	2 (11.1)	4 (30.8)		
Death time	$N = 16$		1.09	0.296
Night	8 (50)	9 (69.2)		
Day	8 (50)	4 (30.8)		
Death position	$N = 15$		7.6	**0.006**
Prone	1 (6.7)	7 (53.8)		
Not prone	14 (93.3)	6 46.2		
Types of AEDs#			1.18	0.555
Without	9 (50)	9 (69.2)		
With 1 type	5 (27.8)	2 (15.4)		
With 2 types	4 (22.2)	2 (15.4)		

*Except "death time" and "death position".
#Anti-epileptic drugs (AEDs). The bold values indicate the values which are less than 0.05, suggesting significant difference.

TABLE 2 Comparison of organ weight and ventricular thickness of the SUDEP cases.

	Not-SUDEP ($N = 11$)	SUDEP ($N = 13$)	T	p
Brain weight (g, $\overline{x} \pm$ s)	1287.4 ± 222.2	1390.6 ± 142.9	−1.228	0.242
Heart weight (g, $\overline{x} \pm$ s)	317.4 ± 80.5	344.5 ± 78.9	−0.783	0.445
Lung weight (g, $\overline{x} \pm$ s)	1410 ± 652.8	1119.5 ± 254.2	1.27	0.234
Left ventricular thickness (cm, $\overline{x} \pm$ s)	1.2 ± 0.2	1.2 ± 0.2	−0.422	0.678
Right ventricular thickness (cm, $\overline{x} \pm$ s)	0.3 ± 0.05	0.3 ± 0.09	−0.513	0.614

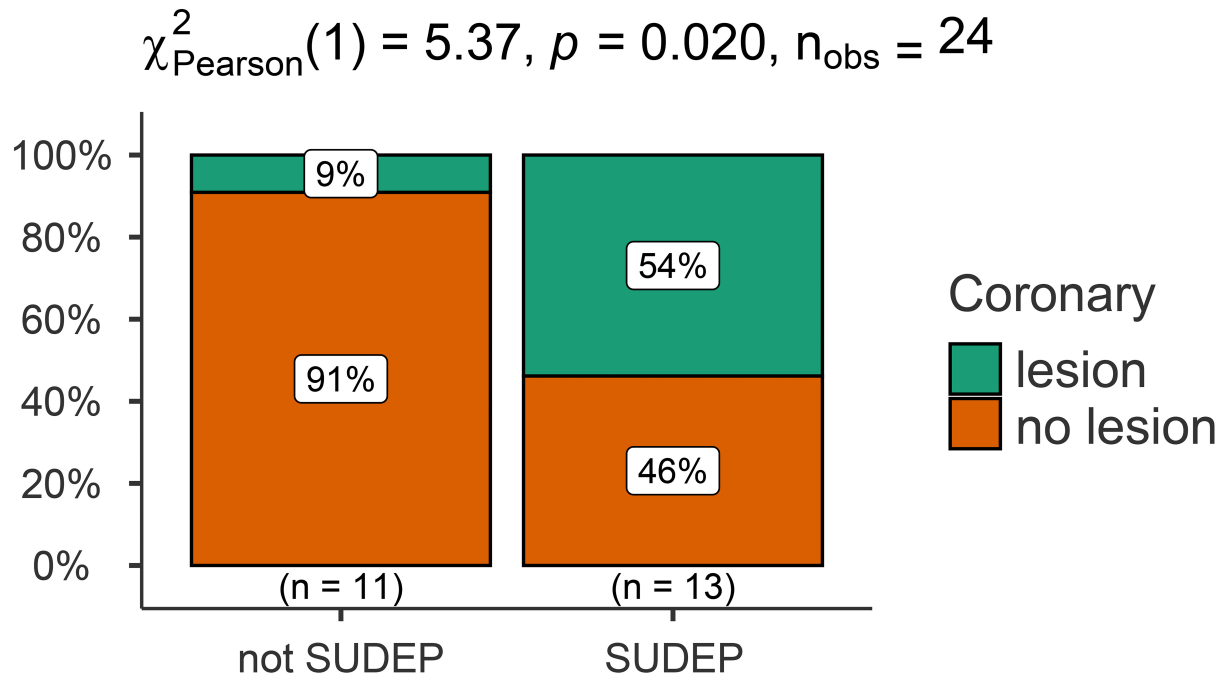

FIGURE 3
Comparison of the coronary artery lesions between SUDEP and not-SUDEP groups.

Anti-epileptic therapy, medications, and postmortem toxicological results

Postmortem toxicological analysis revealed that 13 subjects (41.9%) had detectable levels of antiepileptic drugs (AEDs), including valproate (6/13), carbamazepine (6/13), oxcarbazepine (4/13), topiramate (2/13), levetiracetam (1/13), and phenytoin (1/13). Only one case had a supra-therapeutic concentration of carbamazepine; the remaining 12 cases all showed therapeutic concentrations of AEDs. Of the cases taking epilepsy drugs, six took two drugs and seven took one drug. There was no significant difference between the two groups regarding the use of AEDs ($p > 0.05$), as shown in Table 1. One patient had undergone surgery for epilepsy and one other case received electrotherapy. Alcohol consumption was recorded for one case just before death, and traditional Chinese medicine injections were co-administered in another case.

Pathological findings

In total, 24 of the 31 subjects underwent a complete autopsy, including body surface examination, autopsy, and histopathological examinations.

Quantitative comparison of organ weight and ventricular thickness

There were no significant differences between the SUDEP and not-SUDEP groups in terms of the weights of the heart, lungs, and brains, or the ventricular thickness ($p > 0.05$). Further details are shown in Table 2.

Comparison of coronary artery lesions

Analysis showed that the SUDEP group featured a significantly higher number of cases with coronary lesions (grades 1–3) than that in the not-SUDEP group ($p < 0.05$); further details are shown in Figure 3.

Pathological findings in the SUDEP group

Next, we analyzed the main pathological findings of the 13 cases in the SUDEP group. Neuropathological findings were present in 12 cases (92.3%), and four typical epileptic lesions are presented in Figure 4; the remaining cases showed no gross or microscopic abnormalities. Ten cases showed cardiac pathological changes (76.9%), most of which involved subpericardial petechiae; the next most common condition was local myocardial fibrosis. Seven cases (53.8%) had coronary artery atherosclerosis with stenosis degrees of stages I to III. All subjects showed pulmonary congestion and edema.

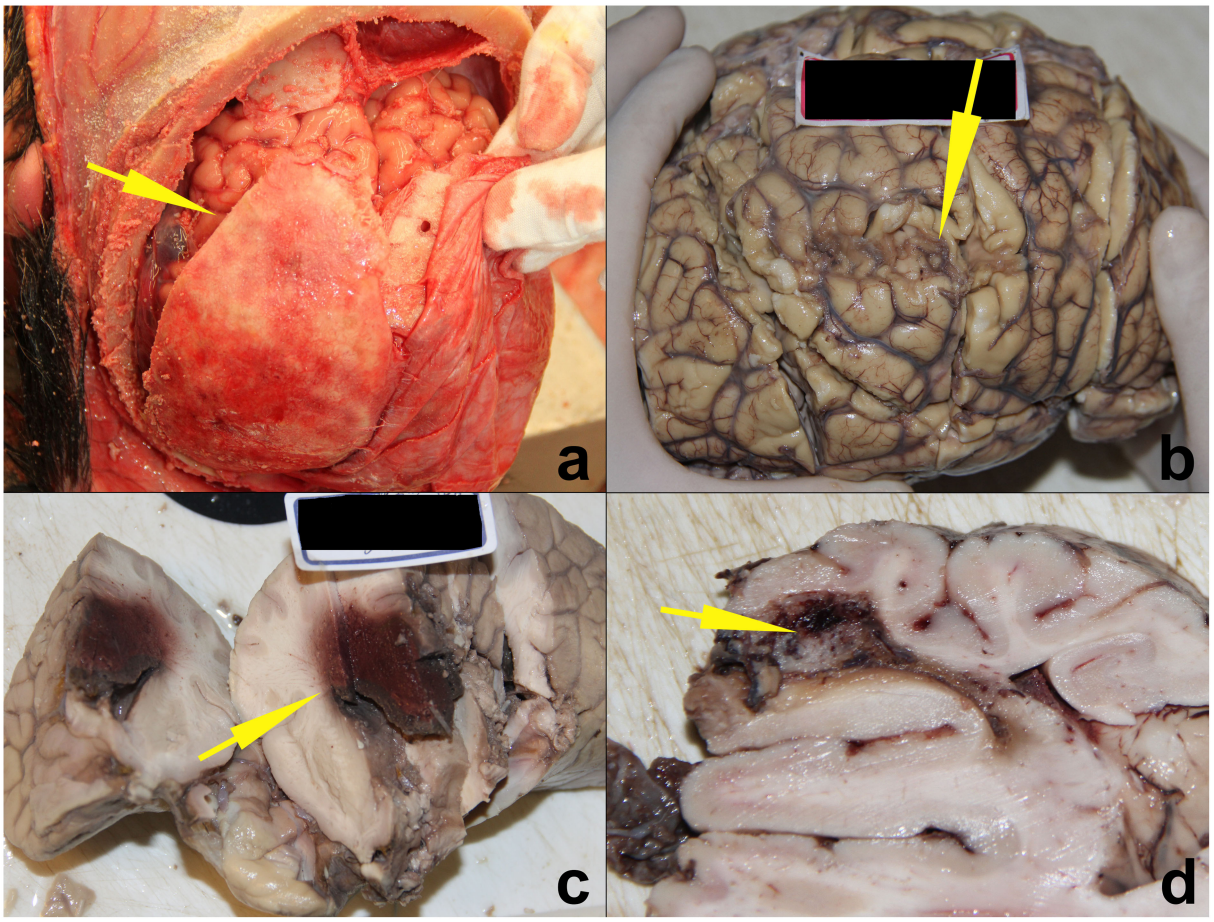

FIGURE 4
Four SUDEP cases with typical epileptic brain lesions. **(a)** Epilepsy secondary to chronic subdural hematoma ossification. **(b)** Epilepsy secondary to old cerebral contusions. **(c)** Epilepsy secondary to cerebral glioma. **(d)** Epilepsy secondary to vascular malformation. All lesions are indicated by yellow arrows.

Other pulmonary outcomes included hemorrhage and focal inflammation. Nail cyanosis and other pathological findings were also recorded and shown in Table 3.

Causes and mechanisms of death

Table 4 shows the causes and mechanisms of death, as identified by forensic pathologists. Of the 31 cases, SUDEP/epileptic state was listed as the primary cause of death in 14 cases. The mechanism of death was classified as cardiopulmonary failure in 15 cases, asphyxia in eight cases, central nervous system dysfunction in five cases, and sudden cardiac death in three cases.

Comparative analysis showed that there was a significant difference between the SUDEP and not-SUDEP groups in terms of the mechanisms of death ($p < 0.05$). The main mechanism of death in the SUDEP group was asphyxia; in the not-SUDEP group, the predominant mechanism was a cardiopulmonary failure. Comparative analyses of the mechanism of death are shown in Figure 5.

Regression analysis of death mechanisms and death positions

Next, we used logistic regression analyses to investigate the effect of death position on the mechanisms of death in the SUDEP group and found that death position had a significant influence on the mechanism of death by SUDEP. Patients in the prone position had a 57-fold higher risk of asphyxia than those in the non-prone position (95% confidence interval [CI]: 4.36–22.26, $p = 0.002$). There was a statistical difference ($p < 0.05$) in the classification of causes of death between the SUDEP and not-SUDEP groups, where the not-SUDEP group died mainly from cardiopulmonary failure (67%), while the SUDEP group

TABLE 3 Pathological findings of SUDEP cases.

Pathological findings	Cases (%) $N = 13$
Neuropathological findings	
Encephaledema	6 (46.2)
Traumatic lesions	2 (15.4)
Developmental abnormalities	2 (15.4)
Focal encephalomalacia	2 (15.4)
Subarachnoid hemorrhage	1 (7.6)
Vascular malformation	1 (7.6)
Cortical atrophy	1 (7.6)
Tumor	1 (7.6)
Post brain surgery	1 (7.6)
Hippocampi atrophy	1 (7.6)
No pathological findings	1 (7.6)
Cardiac findings	
Subplane-epicardial bleeding	6 (46.2)
Local myocardial fibrosis	4 (30.7)
No pathological findings	3 (23.1)
Coronary arteriosclerosis	
Grade 1	1 (7.6)
Grade 2	3 (23.1)
Grade 3	3 (23.1)
Grade 4	0 (0)
No lesions	6 (46.2)
Pulmonary findings	
Pulmonary congestion	13 (100)
Pulmonary edema	13 (100)
Subpulmonary hemorrhage	4 (30.7)
Pulmonary hemorrhage	2 (15.4)
Focal inflammation	2 (15.4)
Other findings	
Nail cyanosis	12 (92.3)
Palpebral conjunctiva congestion	5 (38.5)
Bronchial foam	5 (38.5)
Tongue between dentitions	4 (30.7)
Intraoral and nasal bleeding	4 (30.7)
Laryngeal edema	1 (7.6)
Facial cyanosis	1 (7.6)
Pale area around the mouth and nose	1 (7.6)

died mostly from asphyxia (46%). Further details are shown in Figure 6.

Discussion

Epilepsy is a common and chronic neurological disorder characterized by seizures (27). Epilepsy can cause death or contribute to the circumstances of death in numerous different ways: status epilepticus, complications following seizure such as aspiration pneumonia, injury or drowning, complications of treatment, or suicide (28).

TABLE 4 The cause and mechanism of death of the SUDEP cases.

Cause of death	Cases (%) $N = 31$
SUDEP/epileptic state	13 (41.9)
Trauma/external force	9 (29)
Other medical diseases	4 (12.9)
Toxicosis	3 (9.7)
Accident	2 (6.5)
The death mechanism	
Cardiopulmonary failure	15 (48.4)
Asphyxia	8 (25.8)
Central nervous system dysfunction	5 (16.1)
Sudden cardiac death	3 (9.7)

Sudden unexpected death in epilepsy (SUDEP) is defined as a sudden, unexpected death, witnessed or unwitnessed, of a person with epilepsy, where a complete postmortem examination does not reveal a specific cause of death (47). The definition of SUDEP has been revised over recent years; it is now recognized that SUDEP is actually a general term for a range of diseases. Thus, SUDEP was divided into seven groups by Nashef et al.: (1) definite SUDEP, (2) definite SUDEP plus, (3) probable SUDEP/probable SUDEP plus, (4) possible SUDEP, (5) near-SUDEP/near-SUDEP plus, (6) not-SUDEP, and (7) unclassified (7). The refined classification shows the complex presentation of SUDEP cases. The cases described in this study involved only three groups: definite SUDEP, SUDEP plus, and not-SUDEP groups; our analysis did not involve the other five classifications of SUDEP. It is clear that by definition the "probable SUDEP" and "near SUDEP" groups could not be in the forensic autopsy file records. This result may also be related to the relatively small number of cases in this study, while the small sample size of this study is due to the low autopsy rate in China. Since there is no clear conclusion that shows a different mechanism between the definite "SUDEP" and the "SUDEP plus" groups, we considered the two groups together in the experiment, which is called the SUDEP group, while those deaths with a clear cause of death were included in the not-SUDEP group. We performed a comparative analysis of the demographic profiles, death scenes, medical histories, histopathology results, and mechanisms of death between the SUDEP group and the not-SUDEP group, both of which had epilepsy. Such a grouping is significantly different from the previous studies. Previous studies have either compared individuals who had died of SUDEP to individuals who were alive and had epilepsy (20), or compared individuals who died of SUDEP to individuals without epilepsy who died suddenly due to some unrelated causes, and very few cases have

directly compared individuals who died of SUDEP to individuals with epilepsy who died due to other certain causes (29). We considered this comparison to be practically valuable, which revealed another perspective for studying the SUDEP. Because all the death cases are combined with epilepsy, it can eliminate the interference of disease background to the fullest extent, and the comparative results are more convincing in terms of death mechanism.

The risk factors of SUDEP include generalized tonic-clonic seizures, the levels of anti-epileptic drugs (AEDs), frequent seizures, sleep, the prone position, reduced heart rate variability (HRV), and concomitant channelopathies (30–33). However, our analyses identified some new aspects to consider.

Previous studies suggested that nocturnal seizures and the prone position can be related to SUDEP (34, 35). There is a strong association between SUDEP and sleep, with ∼70% of SUDEP cases identified during sleep (36). Our analysis of death scenes and medical history found that the SUDEP group differed from the not-SUDEP group in terms of the death position, as there were more deaths in the prone position in the SUDEP group. However, there was no significant difference between the two groups in terms of the time of death and the place of death. It should be noted that the time of death does not fully relate to whether a patient is in a state of sleep; this is because in many cases, the process of death is not witnessed. This may contribute to a divergence in the results of trials. Other case-control investigations of SUDEP postmortems also have shown no evidence for diurnal patterns with respect to SUDEP (37).

The effect of taking anti-epileptic medications on the occurrence of SUDEP may be influenced by the presence or absence of generalized tonic-clonic seizures (GTCS) episodes or sub-therapeutic levels of anti-seizure medications l (35, 38). In the present study, we found no significant difference between the SUDEP and not-SUDEP groups regarding the use of medications when considering postmortem toxicological analysis. Furthermore, we did not identify any cases with sub-therapeutic levels of anti-seizure medications; this may also suggest that the effect of AED use on SUDEP is limited without other qualifying conditions (39).

We also found some interesting phenomena when conducting demographic and etiological analyses. First, in terms of the age at death, we found that cases in the not-SUDEP group were significantly older than those in the SUDEP group ($p < 0.05$). Second, based on medical records, we identified more cases with undefined etiology of epilepsy in the SUDEP group than that in the not-SUDEP group. These findings might suggest that SUDEP may differ from other types of epilepsy in terms of etiology and pathogenesis (40).

A key research focus is whether patients with SUDEP have potentially fatal cardiovascular and cerebral diseases (41). Our statistical analyses found no significant difference in the weights

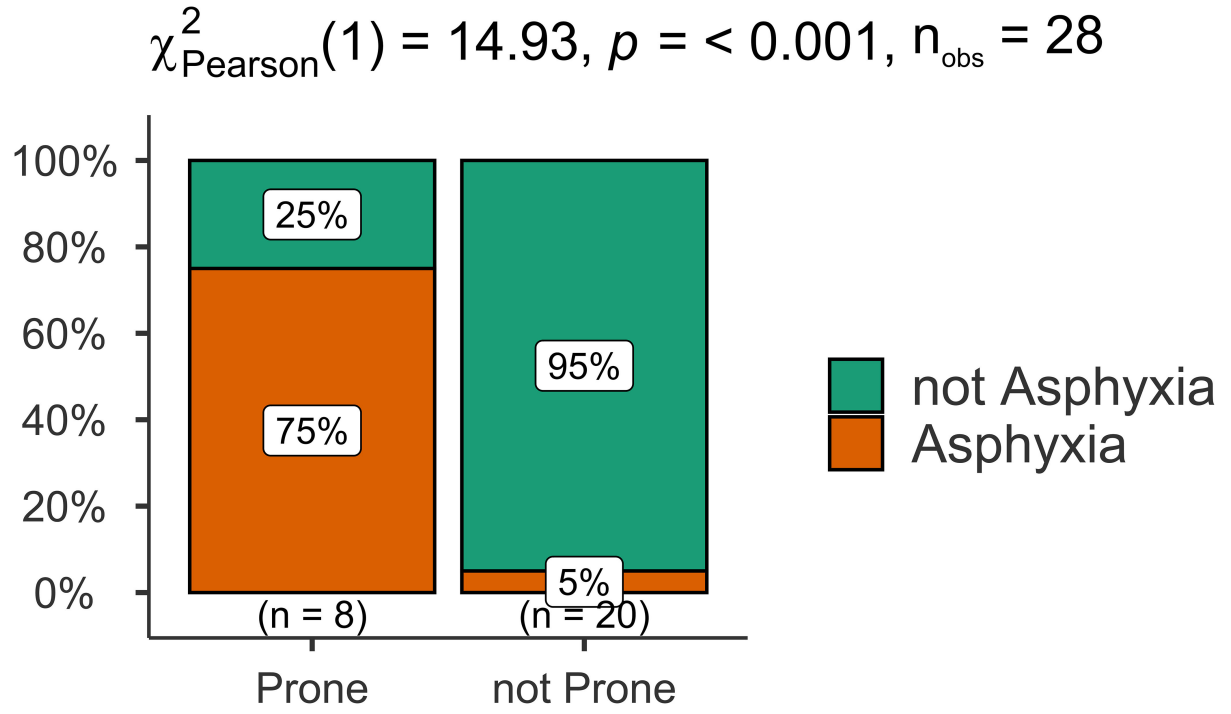

FIGURE 5
Comparison of the signs of asphyxia between prone and not-prone groups.

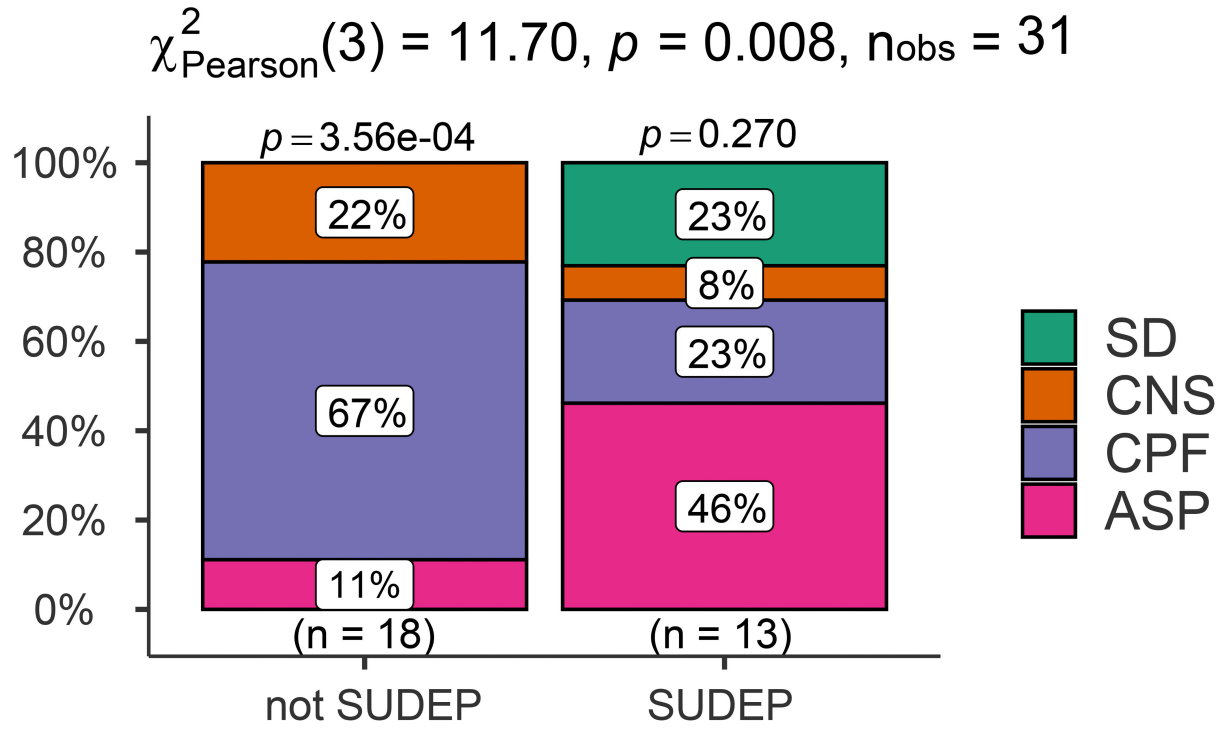

FIGURE 6
Comparison of the cause of death classification results in the SUDEP and not-SUDEP groups.

of the heart, lungs, and brains, or the thickness of the ventricles when compared between the SUDEP and not-SUDEP groups ($p > 0.05$). However, the proportion of cases with coronary lesions in the SUDEP group was significantly higher than that in the not-SUDEP group ($p < 0.05$). However, no significant pathological changes of myocardial infarction were detected in these cases with coronary lesions, thus indicating that coronary artery disease is not the main cause of death in SUDEP but may be involved in the nosogenesis of SUDEP. Myocardial ischemia due to coronary heart disease may induce abnormal ECG activity rather than myocardial infarction which could then participate in the development of SUDEP. This is potentially supported by the findings that T-wave alternans is considered a potential biomarker for SUDEP (42–44).

Analysis of the mechanism of death is an important aspect of forensic pathology practice and is also the focus of SUDEP (43). The mechanism of SUDEP involves neuropeptidergic, serotonergic, and adenosine systems, as well as alterations of the ventrolateral medulla, amygdala, hippocampus, and central autonomic regions, orchestrating autonomic dysfunction (45). In the present study, pooled analysis of 31 cases of SUDEP identified the mechanisms of death in epileptic patients as asphyxia, cardiopulmonary dysfunction, central nervous system dysfunction, and sudden cardiac death. Our results showed that the mechanisms of death differed between the definite SUDEP and not-SUDEP groups. Most of the cases in the definite SUDEP group showed signs of asphyxia, such as nail cyanosis, hemorrhage in the bulbar conjunctiva, pulmonary pleura, and the sub-epicardium. To explain this phenomenon, we performed regression analysis on the death position most likely to cause asphyxia and found a strong correlation between death position and asphyxia, thus suggesting that the prone position was the main factor associated with asphyxia in patients with SUDEP. Ictal and postical effects on autonomic function and accidental asphyxia are commonly considered potential factors of SUDEP (18). As such, SUDEP may share mechanisms similar to sudden infant death syndrome (31).

However, due to the small number of cases, the conclusions of this study may need to be verified by further studies with larger sample sizes. In addition, due to the lack of background data, and the lack of awareness of SUDEP in many cases during the autopsy, there may be a lack of intensive and detailed pathological examinations, such as immunohistochemistry studies for mild malformations of cortical development (MCD) (46). Thus, more detailed results concerning the mechanisms of death could not be obtained.

Conclusion

Based on the forensic death cases, we conducted a statistical analysis of deaths in patients with epilepsy, focusing specifically

on the comparative analysis of individuals who died of SUDEP (SUDEP group) to individuals with epilepsy who died due to other certain causes (not-SUDEP group). Significant differences were founded between the two groups: compared to the not-SUDEP group, the SUDEP group was younger in age; the SUDEP group has more cases with uncertain etiology of epilepsy, prone death position, and coronary lesions. The primary mechanism of death in the SUDEP group was mainly asphyxia while that in the not-SUDEP group was a cardiopulmonary failure. Additionally, asphyxia in the SUDEP group correlates significantly with the prone position. This will provide new ideas and directions for further research and forensic identification on SUDEP.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors.

Ethics statement

The studies involving human participants were reviewed and approved by Academic Committee of the Academy of Forensic Science (AFS), Ministry of Justice, PR China. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

XZ and ZL conceptualized the study. ZL responsible for the methodology. XZ and JZ responsible for the formal analysis. XZ wrote the original draft. DZ supervised the research. JW provided administration services. DZ and ZL acquired the funding. All authors read and agreed to the published version of the manuscript.

Funding

This study was financially supported by grants from the National Natural Science Foundation of China (Reference: 82171872), the Natural Science Foundation of Shanghai (Reference: 21ZR1464600), the Shanghai Key Laboratory of Forensic Medicine (Reference: 17DZ2273200), the Shanghai Forensic Service Platform (Reference: 19DZ2290900), and the Central Research Institute Public Project (References: GY2020G-4 and GY2021G-5).

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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TYPE Original Research
PUBLISHED 23 December 2022
DOI 10.3389/fneur.2022.1102672

OPEN ACCESS

EDITED BY
Beixu Li,
Shanghai University of Political Science and Law, China

REVIEWED BY
Zhang Gui,
Capital Medical University, China
Yiwu Zhou,
Huazhong University of Science and Technology, China

*CORRESPONDENCE
Jun Ma
✉ 13159543160@163.com
Tiantong Yang
✉ ytt_cupl@outlook.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Epilepsy, a section of the journal Frontiers in Neurology

RECEIVED 19 November 2022
ACCEPTED 05 December 2022
PUBLISHED 23 December 2022

CITATION
Zhang X, Ma Y, Zhou F, Zhang M, Zhao D, Wang X, Yang T and Ma J (2022) Identification of miRNA–mRNA regulatory network associated with the glutamatergic system in post-traumatic epilepsy rats. *Front. Neurol.* 13:1102672. doi: 10.3389/fneur.2022.1102672

Identification of miRNA–mRNA regulatory network associated with the glutamatergic system in post-traumatic epilepsy rats

Xiaoyuan Zhang[1,2†], Yixun Ma[3,4†], Fengjuan Zhou[1,2], Mengzhou Zhang[1,2], Dong Zhao[1,2], Xu Wang[1,2], Tiantong Yang[1,2*] and Jun Ma[1,5*]

[1]Key Laboratory of Evidence Science, Institute of Evidence Law and Forensic Science, China University of Political Science and Law, Ministry of Education, Beijing, China, [2]Collaborative Innovation Center of Judicial Civilization, Beijing, China, [3]College of Biological Science, China Agricultural University, Beijing, China, [4]Chinese Institute for Brain Research, Beijing, China, [5]Department of Radiology, Chui Yang Liu Hospital Affiliated to Tsinghua University, Beijing, China

Background: Glutamate is one of the most important excitatory neurotransmitters in the mammalian brain and is involved in a variety of neurological disorders. Increasing evidence also shows that microRNA (miRNA) and mRNA pairs are engaged in a variety of pathophysiological processes. However, the miRNA and mRNA pairs that affect the glutamatergic system in post-traumatic epilepsy (PTE) remain unknown.

Methods: PTE rats were induced by injecting 0.1 mol/L, 1 μL/min $FeCl_2$ solution. Behavioral scores and EEG monitoring were used to evaluate whether PTE was successfully induced. RNA-seq was used to obtain mRNA and miRNA expression profiles. Bioinformatics analysis was performed to screen differentially expressed mRNAs and miRNAs associated with the glutamatergic system and then predict miRNA–mRNA interaction pairs. Real-time quantitative reverse transcription PCR was used to further validate the expression of the differential miRNAs and mRNAs. The microRNA–mRNA was subject to the Pearson correlation analysis.

Results: Eight of the 91 differentially expressed mRNAs were associated with the glutamatergic system, of which six were upregulated and two were downregulated. Forty miRNAs were significantly differentially expressed, with 14 upregulated and 26 downregulated genes. The predicted miRNA–mRNA interaction network shows that five of the eight differentially expressed mRNAs associated with the glutamatergic system were targeted by multiple miRNAs, including *Slc17a6*, *Mef2c*, *Fyn*, *Slc25a22*, and *Shank2*, while the remaining three mRNAs were not targeted by any miRNAs. Of the 40 differentially expressed miRNAs, seven miRNAs were found to have multiple target mRNAs associated with the glutamatergic system. Real-time quantitative reverse transcription PCR validation and Pearson correlation analysis were performed on these seven targeted miRNAs—*Slc17a6*, *Mef2c*, *Fyn*, *Slc25a22*, and *Shank2*—and six additional miRNAs selected from the literature.

Real-time quantitative reverse transcription PCR showed that the expression levels of the mRNAs and miRNAs agreed with the predictions in the study. Among them, the miR-98-5p–Slc17a6, miR-335-5p–Slc17a6, miR-30e-5p–Slc17a6, miR-1224–Slc25a22, and miR-211-5p–Slc25a22 pairs were verified to have negative correlations.

Conclusions: Our results indicate that miRNA–mRNA interaction pairs associated with the glutamatergic system are involved in the development of PTE and have potential as diagnostic biomarkers and therapeutic targets for PTE.

KEYWORDS

post-traumatic epilepsy, glutamatergic system, RNA sequencing, miRNA, mRNA

1. Introduction

Post-traumatic epilepsy (PTE) is the most devastating sequela of traumatic brain injury (TBI), and seriously affects the quality of life of the injured (1, 2). PTE patients not only suffer from recurrent spontaneous seizures, but also from memory and cognitive loss, depression, and numerous other adverse effects (3). These signs and symptoms are caused by neuronal damage. An imbalance in the regulatory mechanisms of neuronal excitation and inhibition leads to excessive synchronous firing of neurons. In this process, glutamate and other neurotransmitters are widely involved in the regulation process between neuronal excitation and inhibition by activating corresponding receptors (4). The occurrence of PTE is related to excessive release of glutamate. After TBI, glutamate release is increased, and the regulatory mechanisms of neuronal excitation and inhibition are unbalanced, which further induces neuronal cell injury, death, and dysfunction, and finally induces PTE (5–7). Although more than 20 antiseizure medications are used in the clinical treatment of PTE, ∼30% of patients with PTE still experience drug-resistant seizures (8). Understanding the molecular mechanisms of PTE will help to identify new therapeutic strategies for PTE. The imbalance of the neuronal regulatory mechanism triggered by glutamate is an essential reason for the development of PTE. Therefore, it is necessary to study the molecular mechanism of glutamate related to PTE to identify new therapeutic strategies.

Abnormal expression of glutamatergic mRNA has been observed after TBI. γ-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the brain, which is formed by the decarboxylation of glutamic acid by glutamic acid decarboxylase (GAD). In the fluid-percussion injury model, $GABA_A$ receptor subunit mRNAs show abnormal expression in different regions of the rat brain. This may result in diminished $GABA_A$ receptor-mediated inhibition (9). However, the development of PTE will be affected after the normal level of glutamatergic mRNA expression is restored. For example, SLC1A2 expression was downregulated after TBI, and PTE symptoms were alleviated after normalizing SLC1A2 expression with ceftriaxone (10). Because TBI directly induces PTE, the abnormal expression of glutamatergic mRNA after TBI may be one of the molecular mechanisms of PTE. In addition to mRNA, microRNAs (miRNA) are also an important component of the molecular mechanism of TBI. As potential diagnostic and predictive biomarkers, miRNAs do not encode proteins, but can regulate the expression of target mRNAs at the post-transcriptional level and subsequently affect the expression level of proteins (11). During TBI and epilepsy, miRNAs are involved in neuronal cell apoptosis, the inflammatory response, synaptic remodeling, abnormal conduction pathway formation, and other neuronal cell damage and repair. For example, several studies have confirmed the abnormal expression of miR-21 and miR-92a after TBI (12–14), and miR-139-5p and others play a key role in various pathophysiological mechanisms of epilepsy (15–17). However, the aforementioned differences in mRNA and miRNA expression have not been verified in the PTE model, so the glutamate-related molecular mechanisms of PTE are still unknown.

The mRNA–miRNA regulatory network plays an important role in the study of several disease mechanisms. For example, a study on mesial temporal lobe epilepsy (mTLE) identified key mRNAs and miRNAs involved in mTLE by establishing and analyzing the mRNA–miRNA regulatory network. miR-27a-3p was identified as a potential diagnostic biomarker for mTLE (18). Some studies have established a circular RNA (circRNA)–miRNA–mRNA regulatory network to analyze the interactions between circRNA, miRNA, and mRNA in the control cortical shock (CCI) model, to understand the molecular mechanism of TBI and screen potential therapeutic targets (19). Considering the important role of the mRNA–miRNA regulatory network in the study of disease mechanisms, it is necessary to uncover the regulatory relationship of miRNA–glutamatergic mRNA to understand the pathogenic mechanisms of PTE and glutamatergic association.

To understand the differential expression of glutamatergic mRNA and miRNA during the development of PTE and the possible targeting relationship between them, and to

reveal the glutamatergic-related molecular mechanism of the development of PTE, we used transcriptome sequencing technology to identify differentially expressed miRNA and glutamatergic mRNA in the rat model of PTE. Bioinformatics methods were used to draw the miRNA–mRNA targeting network, and real-time quantitative reverse transcription PCR was used to verify the negative targeting relationship between miRNA and mRNA. Our results suggest that miRNA–mRNA interaction pairs in the glutamate system are involved in the development of PTE, and have potential as diagnostic biomarkers and therapeutic targets for PTE.

2. Materials and methods

2.1. Ethics statement

The Animal Research Ethics Committee of the Institute of Evidence Science, China University of Political Science and Law, approved the animal study (#2019012). All the animal experiments and procedures were performed following the guidelines of the Weatherall Committee and the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs; London, UK).

2.2. Animals and sample preparation

A total of 15 male Sprague-Dawley rats (7–8-months old, weighing 210–230 g) were purchased from Beijing Laboratory Animal Research Center (<city>Beijing</city>, China) and housed with a 12-h light/dark cycle with free access to water and food. After a 72-h acclimatization period, the rats were randomly divided into sham and PTE groups (n = 6/group). A $FeCl_2$-induced PTE model was established as previously described (20). Briefly, the rats were anesthetized with 2% pentobarbital sodium (40 mg/kg) *via* intraperitoneal injection and placed in a stereotactic device (Nanjing Medease Science and Technology, Jiangsu, China). The electrodes were fixed to the rat frontal and occipital lobes and connected with a biosignal acquisition system (Shanghai creaform3d information, Shanghai, China) for EEG collection. The rats in the PTE group were injected with 10 μl $FeCl_2$ (0.4 mol/L, 1 μl/min) at the right frontal cortex (2.0 mm anterior to bregma, 3.0 mm from midline, and 2.0 mm depth) using a microinjector. The sham group underwent the same procedure except for the injection. The rats were killed on the 13th day after the operation. The brain tissue samples surrounding the injection sites were collected and stored at −80°C for further experiments.

2.3. Behavioral assessment and electroencephalography monitoring

The severity of seizures was assessed at 1 h before the operation, 1 h after the operation, and once daily thereafter for 30 consecutive days in accordance with the Racine's scale (21): 0, no abnormality; 1, staring; 2, head nodding or wet-dog shaking with or without facial tics; 3, unilateral forelimb clonus; 4, bilateral forelimb clonus and continuous head nodding; 5, exacerbated bilateral forelimb clonus, loss of balance and falling, or generalized tonic–clonic seizures.

A 1-h EEG, consisting of the first 15-min and 10-min blocks at 5-min intervals, was recorded at 1 h before surgery and at 1, 7, and 30 days after surgery.

The criteria for a successful PTE model include: (1) Racine's score > 4, (2) sharp waves or spike waves on EEG, and (3) paroxysmal or continuous abnormal discharges on EEG.

2.4. Total RNA isolation, library construction, and sequencing

Total RNA was isolated from frontal lobe brain tissues adjacent to the $FeCl_2$ injection region of rats using TRIzol™ reagent (#15596026, Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's protocol. The RNA quality was monitored on 1% agarose gels. The purity and quantity of RNA were determined using a NanoPhotometer® device (Implen, Camarillo, CA, USA) and a Qubit® 2.0 kit, respectively. The RNA integrity was examined using an RNA Nano 6,000 kit on a Bioanalyzer 2100 system (Life Technologies, Carlsbad, CA, USA).

For each sample, a total of 3 μg of RNA was loaded to generate the sequencing library using a NEBNext® Ultra™ RNA library prep kit for Illumina® (NEB, USA) following the manufacturer's instructions. The RNA was purified using an AMPure XP system, and the RNA quality was evaluated on an Agilent Bioanalyzer 2100 system. Gene clustering was performed using the TruSeq PE Cluster Kit v3-cBot-HS (Illumina, San Diego, CA, USA). Paired-end reads of 150 bp were generated for mRNA sequencing, and 50-bp single-end reads were generated for miRNA sequencing, using an Illumina HiSeq 2500 sequencer.

2.5. RNA sequencing data analysis

Clean reads were obtained from raw reads (FASTQ format) by removing adapter- or poly-N-containing reads and low-quality reads. The Q20, Q30, and GC contents were calculated. Clean reads were aligned with the reference genome (rat release-91) downloaded from the Ensembl database using

Bowtie v2.2.3 (22) and TopHat v2.0.12 (23). The fragments per kilobase of transcript per million mapped reads of each transcript was calculated using Cuffdiff v2.1 (24). miRNA expression was compared with the expression of miRNA precursors and corresponding mature miRNAs in miRbase v22 (25) using miRDeep2 (26). Cuffdiff v2.1 was used to analyze the differentially expressed mRNA. The miRNA differential expression was analyzed using the DESeq R package. Genes with an adjusted P-value < 0.05 were identified as differentially expressed genes. The miRNA–mRNA interactions were predicted using RNAhybrid, PITA, and miRanda (27). Target genes of the differentially expressed miRNAs were subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis using GOseq (28) and KEGG Orthology Based Annotation System (29).

2.6. Quantitative real-time PCR

Quantitative real-time (qRT)-PCR was conducted to measure the expression of differentially expressed miRNAs and mRNAs. For mRNA expression determination, complementary DNA was synthesized using SuperScript™ III SuperMix (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Primers for *Slc17a6*, *Mef2c*, *Fyn*, and *Slc25a22* were designed using the National Center for Biotechnology Information primer-blast (http://www.ncbi.nlm.nih.gov/tools/primer-blast/), and the sequences are summarized in Table 1. Glyceraldehyde 3-phosphate dehydrogenase was used as an internal control. PCR was performed using SYBR Power Plus Master Mix (Thermo Fisher Scientific). The conditions for real-time PCR were 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, and 60°C for 60 s. Melting curve analysis was performed from 65 to 95°C in increments of 0.5°C. For miRNA expression determination, complementary DNA was synthesized using a TaqMan® miRNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's protocol. PCR was performed using TaqMan® miRNA Assays and TaqMan® Universal PCR master mix (Applied Biosystems) on an Applied 7,500 device. Conditions for real-time PCR were 95°C for 10 min, followed by 50 cycles of 95°C for 15 s and 60°C for 60 s. Melting curve analysis was performed from 65 to 95°C in increments of 0.5°C. The miRNA primers are summarized in Table 2. U6 small nuclear RNA was used as an internal control. The mRNA or miRNA expression was quantified using the $2^{-\Delta\Delta Ct}$ method.

2.7. Statistical analysis

Data are expressed as mean ± standard deviation. Statistical analysis was performed using SPSS 25.0 (IBM, Armonk, NY USA). Graphs were generated using GraphPad Prism 9 (San Diego, CA, USA). The correlation between mRNA and miRNA was assessed using Pearson's correlation coefficient. Different groups were compared using a one-way ANOVA followed by a t-test of least significant difference. A P-value < 0.05 was considered statistically significant.

TABLE 1 Primers for quantitative real-time PCR.

Primer name	Primer sequence
GAPDHqPCRF	5′-CACCAGCATCACCCCATT-3′
GAPDHqPCRR	5′-CCATCAAGGACCCCTTCATT-3′
Slc17a6qPCRF	5′-TACGGTACCACCAAATCCTACGG-3′
Slc17a6qPCRR	5′-CTCGGTCCTTATAGGCGTACG-3′
Mef2cqPCRF	5′-AGCAGCAGCACCTACATAACAT-3′
Mef2cqPCRR	5′-TAGGAACTGCTACAGCTGCTCA-3′
FynqPCRF	5′-ATGGGCTGTGTGCAATGTAAG-3′
FynqPCRR	5′-GAAGCTGGGGTAGTGCTGAG-3′
Slc25a22qPCRF	5′-GCCAGCCAAGCTCATCAATG-3′
Slc25a22qPCRR	5′-GAGGCAGTCGGACATGCTC-3′

TABLE 2 MicroRNA primers for quantitative real-time PCR.

MicroRNA	Primer sequence
miR-1224	5′-CTCCACCTCCCCAGTCCTCAC-3′
miR-137-3p	5′-CTACGCGTATTCTTAAGCAATAA-3′
miR-19b-3p	5′-TCAGTTTTGCATGGATTTGCACA-3′
miR-190a-5p	5′-ACCTAATATATCAAACATATCA-3′
miR-20a-5p	5′-CTACCTGCACTATAAGCACTTTA-3′
miR-211-5p	5′-AGGCAAAGGATGACAAAGGGAA-3′
miR-298-3p	5′-AGCAGAGAGAAGGCTAGTTCCT-3′
miR-30e-5p	5′-CTTCCAGTCAAGGATGTTTACA-3′
miR-335	5′-ACATTTTTCGTTATTGCTCTTGA-3′
miR-449a-5p	5′-ACCAGCTAACAATACACTGCCA-3′
miR-466c-5p	5′-CATGTACATACACACATCACA-3′
miR-494-3p	5′-AGAGGTTTCCCGTGTATGTTTCA-3′
miR-98-5p	5′-AACAATACAACTTACTACCTCA-3′
RNU6	5′-TTGCGTGTCATCCTTGCGCAGG-3′

3. Results

3.1. Successful establishment of a post-traumatic epilepsy rat model

To assess whether the PTE rat model was successfully established, we examined behavioral seizures and EEGs in rats before and after $FeCl_2$ injection. We found that, compared

with sham rats, rats in the PTE group developed behavioral seizures after $FeCl_2$ injection, including paroxysmal binocular immobility, staring, head nodding or wet-dog shaking, facial twitching, and generalized tonic–clonic seizures. Behavioral seizures occurred frequently within 3 days of the injection and declined thereafter until a regular behavioral seizure developed around 15 days after the injection. The total Racine's score of the PTE group was 810, with a mean value > 4 for each rat. In addition, compared with the EEG of the sham rats (frequency 5–10 Hz, amplitude < 200 μV; Figure 1A), the EEG of the PTE rats exhibited multiple epileptiform discharges, such as sharp-wave polyspikes (Figure 1B), waves (Figure 1C), spikes (Figures 1E, F), and continuous abnormal discharges (Figure 1D) with a maximum amplitude of 1,000 μV. Together, these results suggest that $FeCl_2$ injection successfully induces PTE in rats.

3.2. Differentially expressed messenger RNAs and miRNAs in frontal lobes of post-traumatic epilepsy rats

In this study, transcriptome sequencing was used to identify differentially expressed mRNAs and miRNAs in the frontal brain tissue of PTE rats. After comparing the mRNA and miRNA expression levels in the frontal brain tissue of rats in the PTE group and the normal control group, we found that 91 mRNAs and 40 miRNAs were abnormally expressed in the brain tissue of rats in the PTE group. According to Figures 2A, B, among the 91 mRNAs with abnormal expression, 59 mRNAs were upregulated (65%) and 32 mRNAs were downregulated (35%). Of these, eight differentially expressed mRNAs were associated with glutamatergic energy, six of which were upregulated and two of which were downregulated. According to Figures 2C, D, of the 40 miRNAs with abnormal expression, 14 were upregulated (35%) and 26 were downregulated (65%). The transcriptome sequencing results showed that multiple mRNAs and miRNAs were abnormally expressed in the brain tissue of rats with PTE, suggesting that mRNAs and miRNAs are involved in the occurrence and development of PTE and may play an important regulatory role.

3.3. Messenger RNAs by gene ontology annotation and kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis

To understand the function of the differentially expressed mRNAs in PTE, GO and KEGG enrichment analyses were performed on mRNAs with abnormal expression. Visualization of both the GO and KEGG enrichment analyses were performed with bubble diagrams (Figure 3A). The abscissa of the bubble plot is the ratio, the left ordinate is the GO term/metabolic pathway, the right ordinate is the *P*-value, and the size of the bubble indicates the number of genes. GO analyses showed that the functions of the 91 differential mRNAs dysregulated in PTE were mainly enriched in neuron death, regulation of neuron death, neuron process, hormone development, hormone development transport, negative regulation of neuron death, regulation of neuron process, and regulation of dendritic spine GO terms such as development. Thus, dysregulated mRNAs in PTE mainly participate in the processes of neuronal death and apoptosis. KEGG enrichment analyses (Figure 3B) showed that the 91 differential mRNAs dysregulated in PTE were mainly involved in metabolic pathways including GnRH development, cholinergic synapse, transcriptional misregulation in cancer, insulin secretion, the GnRH signaling pathway, and glutamatergic synapse. Figure 3C shows a network diagram of differential genes in PTE, showing the relationship between enrichment function and gene inclusion. According to Figure 3C, differentially expressed genes such as *Mef2c*, *Fyn*, *En1*, *En2*, *Aspa*, *Camk2b*, *Barh11*, and *Cacna1d* may participate in PTE in various ways.

3.4. Construction of miRNA–messenger RNA regulatory network

Table 3 shows the differential mRNAs and functional annotations related to the glutamatergic system in PTE, with a total of eight dysregulated mRNAs related to the glutamatergic system. Among the eight mRNAs, six mRNAs were upregulated, namely *Slc17a6*, *Mef2c*, *Fyn*, *Tas1r2*, *Aspa*, and *Cacna1d*, and two mRNAs were downregulated, namely *Slc25a22* and *Shank2*. The targeting relationship prediction results showed that the dysregulation of eight mRNAs and 31 miRNAs related to the glutamatergic system was related to PTE. After statistical analysis, 33 pairs of miRNA–mRNA negative regulatory relationships related to the glutamatergic system in PTE were found, and the results are shown in Figures 4A, B. Because we did not find miRNAs targeting *Aspa*, *Cacna1d*, and *Tas1r2* differentially represented in PTE, the predictions for these mRNAs are not shown in the targeting relationship diagram. Further statistical analysis of the negative regulatory relationships between miRNA–mRNA from the miRNA perspective revealed that three downregulated miRNAs, miR-137-3p, miR-190a-5p, and miR-335, have negative regulatory relationships with *Slc17a6* and *Fyn*. Both miR-30e-5p and miR-98-5p have negative regulatory relationships with *Slc17a6*, *Mef2c*, and *Fyn*, and both miR-1224 and miR-211-5p have negative regulatory relationships with *Slc25a22* and *Shank2*.

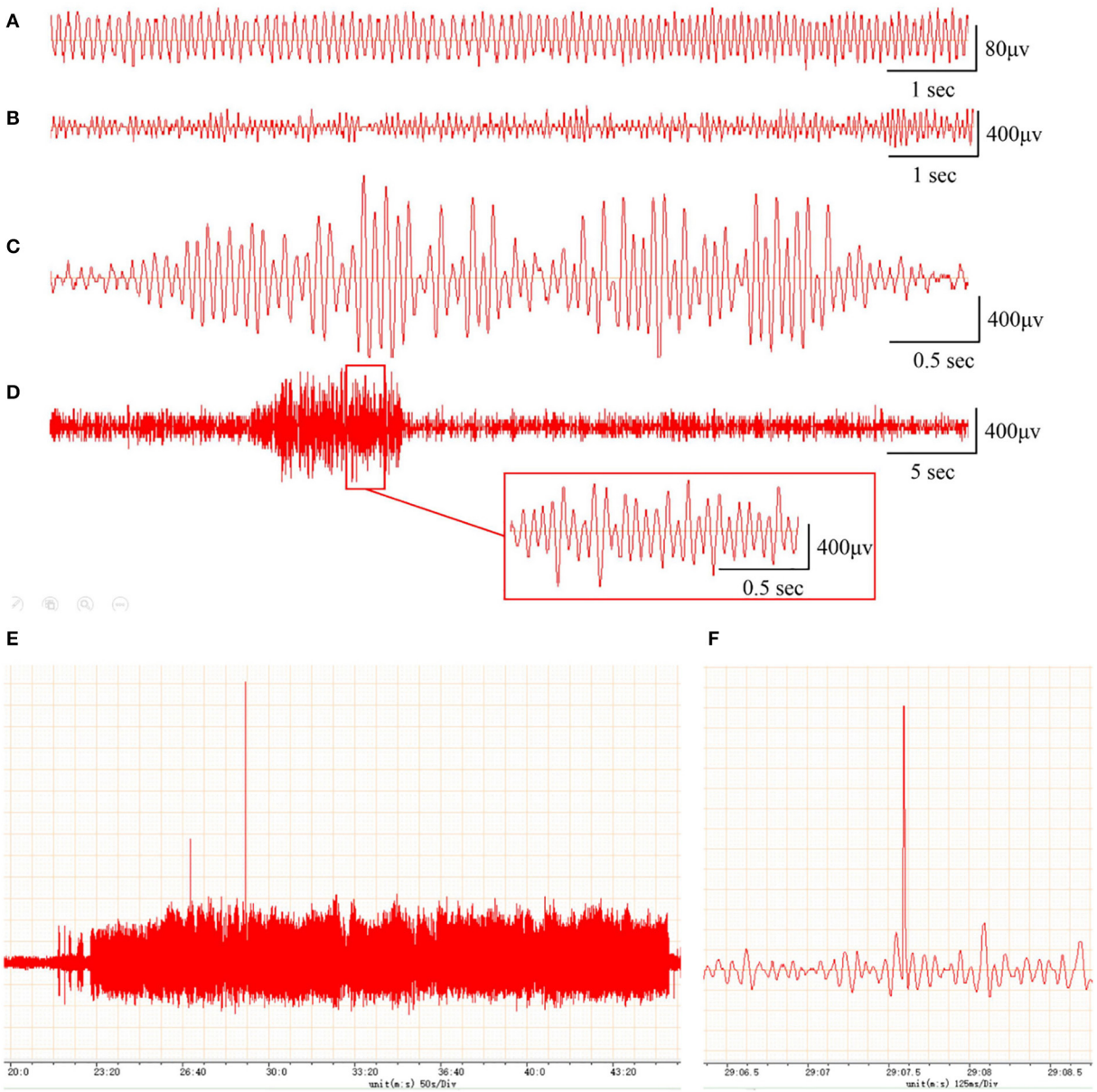

FIGURE 1
Rats with $FeCl_2$ injection-induced post-traumatic epilepsy (PTE) showed epileptiform discharges on electroencephalogram (EEG). A total of 12 rats were randomly divided into sham and PTE groups ($n = 6$/group). Rats in PTE group were injected with 10-μl $FeCl_2$ (0.4 mol/L, 1 μl/min) at right frontal cortex. Sham group underwent same procedures except for injection. A 1-h EEG, including first 15 min and blocks of 10 min at 5-min intervals, was recorded at 1 h before injection and at 1 h and 1, 7, and 30 days after injection. Representative EEGs are shown. **(A)** Normal EEG at 60 min before injection. Multiple spikes **(B)**, two- and three-phase sharp waves **(C)**, 10-s continuous abnormal discharges **(D)**, 20-min explosive abnormal discharges with occasional high-amplitude spikes **(E)**, and a sudden high-amplitude spike **(F)** were observed on day 30 after injection.

3.5. qRT-PCR validation and correlation evaluation of miRNA and glutamatergic messenger RNAs

To verify the accuracy of the above transcriptome sequencing results, in combination with literature mining of miRNA function and differential multiples, we applied qRT-PCR to verify the expression differences of the above five mRNAs and 13 mRNAs with targeting relationships. qRT-PCR verification results of the mRNA (Figure 5A, Table 4) showed that, compared with the normal control group, the expression levels of *Slc17a6*, *Mef2c*, and *Fyn* in the PTE experimental

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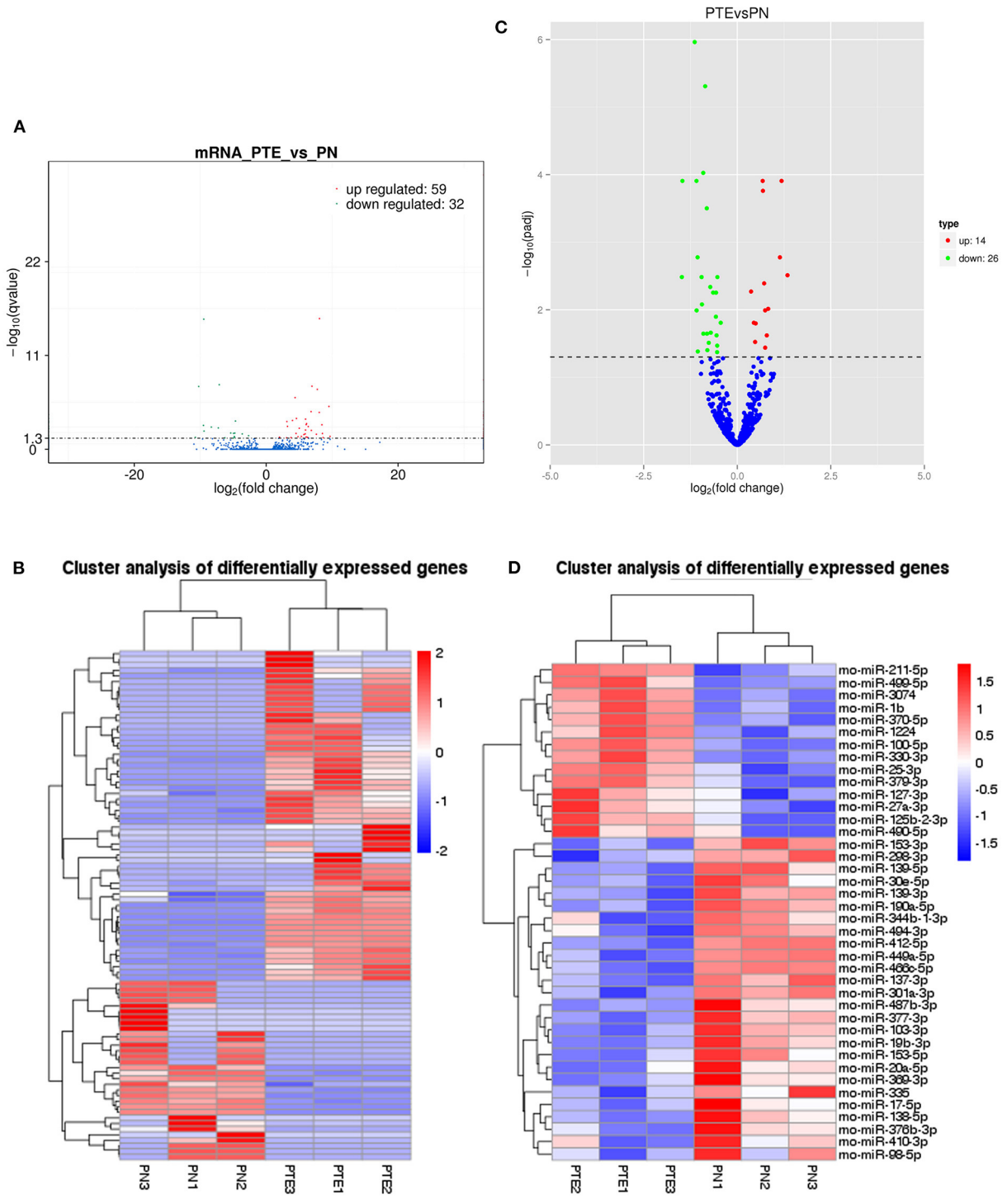

FIGURE 2
mRNA and miRNA transcriptome analysis in rat frontal lobe by RNA sequencing (RNA-Seq). **(A)** A volcano plot of mRNA sequence transcriptome data transcriptome data displays gene expression values of PTE rats compared with those of sham rats. Significantly differentially expressed genes (adjusted *P*-value <0.05) are highlighted in red (upregulated) or blue (downregulated). Non-differentially expressed genes are highlighted in green. $n = 6$. **(B)** Heat map of 59 significantly upregulated and 32 significantly downregulated mRNAs in PTE rats compared with sham rats. **(C)** A volcano plot of miRNA sequence transcriptome data displays gene expression values of PTE rats compared with those of sham rats. Significantly differentially expressed genes (adjusted *P*-value <0.05) are highlighted in red (upregulated) or blue (downregulated). Non-differentially expressed genes are highlighted in green. $n = 6$. **(D)** Heat map of 14 significantly upregulated and 26 significantly downregulated miRNAs in PTE rats compared with sham rats.

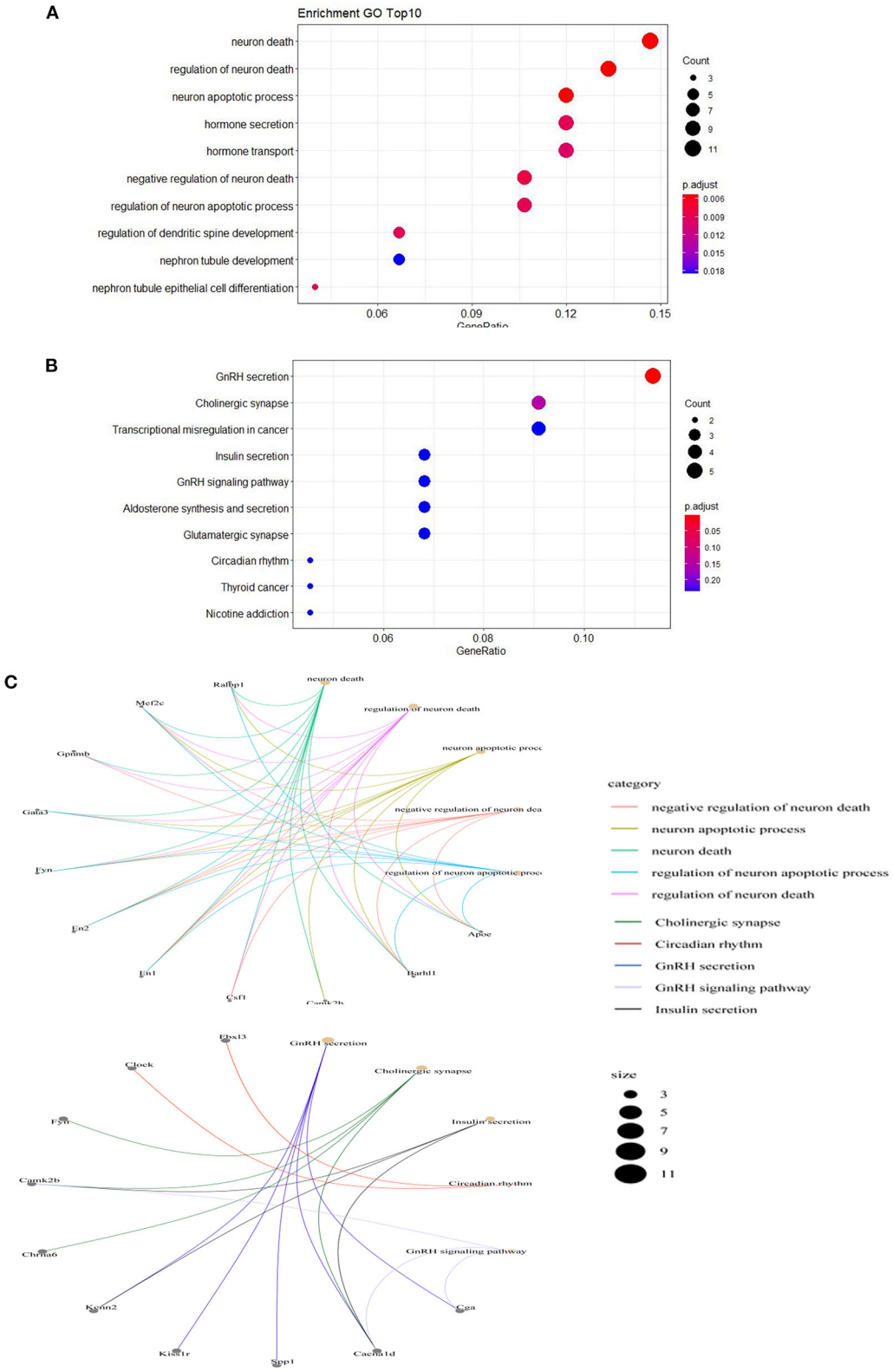

FIGURE 3
Gene Ontology annotation and Kyoto Encyclopedia of Genes and Genomes pathway analysis of differentially expressed mRNAs. **(A)** Top 10 Gene Ontology Annotation $P < 0.05$. **(B)** Top 10 Kyoto Encyclopedia of Genes and Genomes pathways $P < 0.05$. **(C)** The network diagram of relationship between enrichment function and gene inclusion.

TABLE 3 Differential mRNAs and functional annotations related to the glutamatergic system in PTE.

Gene_ID	Gene_name	Gene_description
ENSRNOG00000016147	Slc17a6	Glutamatergic synapse
ENSRNOG00000033134	Mef2c	Regulation of synaptic transmission, glutamatergic, synaptic transmission, glutamate receptor signaling pathway, regulation of glutamate receptor signaling pathway, regulation of N-methyl-D-aspartate selective glutamate receptor activity, regulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate selective glutamate receptor activity
ENSRNOG00000000596	Fyn	Glutamate receptor binding, type 5 metabotropic glutamate receptor binding, G-protein coupled glutamate receptor binding, glutamate receptor signaling pathway, ionotropic glutamate receptor signaling pathway
ENSRNOG00000061876	Tas1r2	Glutamate metabolic process, proline catabolic process to glutamate, aspartate and glutamate metabolism
ENSRNOG00000019659	Aspa	Succinyl glutamate desuccinylase, aspartate and glutamate metabolism
ENSRNOG00000013147	Cacna1d	Glutamatergic synapse
ENSRNOG00000018450	Slc25a22	High-affinity glutamate transmembrane transporter activity, L-glutamate transmembrane transporter activity, L-glutamate transport
ENSRNOG00000050206	Shank2	Glutamatergic synapse

group were significantly upregulated, and the expression level of *Slc25a22* was significantly downregulated, and the differences were statistically significant ($P < 0.05$). This was consistent with the transcriptome sequencing results. However, no significant difference in *Shank2* expression was detected. The qRT-PCR verification results for the miRNAs (Figure 5B, Table 4) showed that, compared with the normal control group, in the PTE group, the expression levels of miR-137-3p, miR-19b-3p, miR-190a-5p, miR-20a-5p, miR-298-3p, miR-30e-5p, miR-335, miR-449a-5p, miR-466c-5p, miR-494-3p, and miR-98-5p were significantly upregulated, while the expression levels of miR-1224 and miR-211-5p were significantly downregulated, and the differences were statistically significant ($P < 0.05$). This was consistent with the results of the transcriptome sequencing.

Pearson correlation analysis showed that expressions of miR-98-5p ($r = -0.90$, $P < 0.05$), miR-335 ($r = -0.95$, $P < 0.05$), and miR-30e-5p ($r = -0.92$, $P < 0.05$) were significantly negatively correlated with *Slc17a6* expression (Figures 6A–C); and miR-1224 ($r = -0.97$, $P < 0.05$) and miR-211-5p ($r = -0.94$, $P < 0.01$) were negatively correlated with *Slc25a22* expression (Figures 6D, E). However, we did not observe significant negative correlation between miR-137-3p ($r = -0.78$, $P > 0.05$) /miR-190a-5p ($r = -0.77$, $P > 0.05$) and *Slc17a6*/*Fyn* expression; and miR-30e-5p ($r = -0.55$, $P > 0.05$) and miR-98-5p ($r = -0.55$, $P > 0.05$) were significantly negatively correlated with *Mef2c* and *Fyn* (Figures 6F–M).

4. Discussion

PTE is an acquired form of epilepsy caused by trauma. Studies have confirmed that increased glutamate responses modulate neuronal microcircuits after TBI, which correlates with increased seizure-like activity near the injury site (30). Considering the important role of glutamatergic dysregulation in the development of PTE, we used transcriptome sequencing technology, miRNA–mRNA regulatory network construction, GO and KEGG enrichment analyses, and other bioinformatics methods to analyze the mRNAs and miRNAs with abnormal expression in PTE to understand the molecular mechanism of the glutamatergic system in PTE. Based on the predicted targeting relationships, qRT-PCR was performed to validate the five mRNAs with anomalous expression and associated glutamatergic energy and the 13 mRNAs targeted by them. The results showed that there were indeed abnormal expression and regulation of miRNA–mRNA related to the glutamatergic system in the PTE model, which is very likely to play an important regulatory role in the occurrence of PTE. These abnormally expressed RNA molecules are likely to be involved in the pathogenesis of PTE, which may not only become a new diagnostic biomarker of PTE in the future but also a potential therapeutic target for clinical treatment of PTE. Considering that the changes to miRNAs and mRNAs after TBI may affect the development of PTE, combined with the neuropathological changes after TBI, we will discuss the mechanism of abnormal miRNA and mRNA expression in the PTE model involved in the development of PTE.

After TBI, Ca^{2+} channels are activated, and a large amount of Ca^{2+} influx induces neuronal apoptosis (31). However, massive influx of Ca^{2+} simultaneously causes rapid depolarization, and abnormal excitation of nerve cells directly leads to seizures (32). Therefore, the neuronal apoptosis induced by massive Ca^{2+} influx after TBI leads to the decrease of inhibitory neurotransmission, and the increase of excitatory neurotransmission caused by Ca^{2+} influx can directly cause the

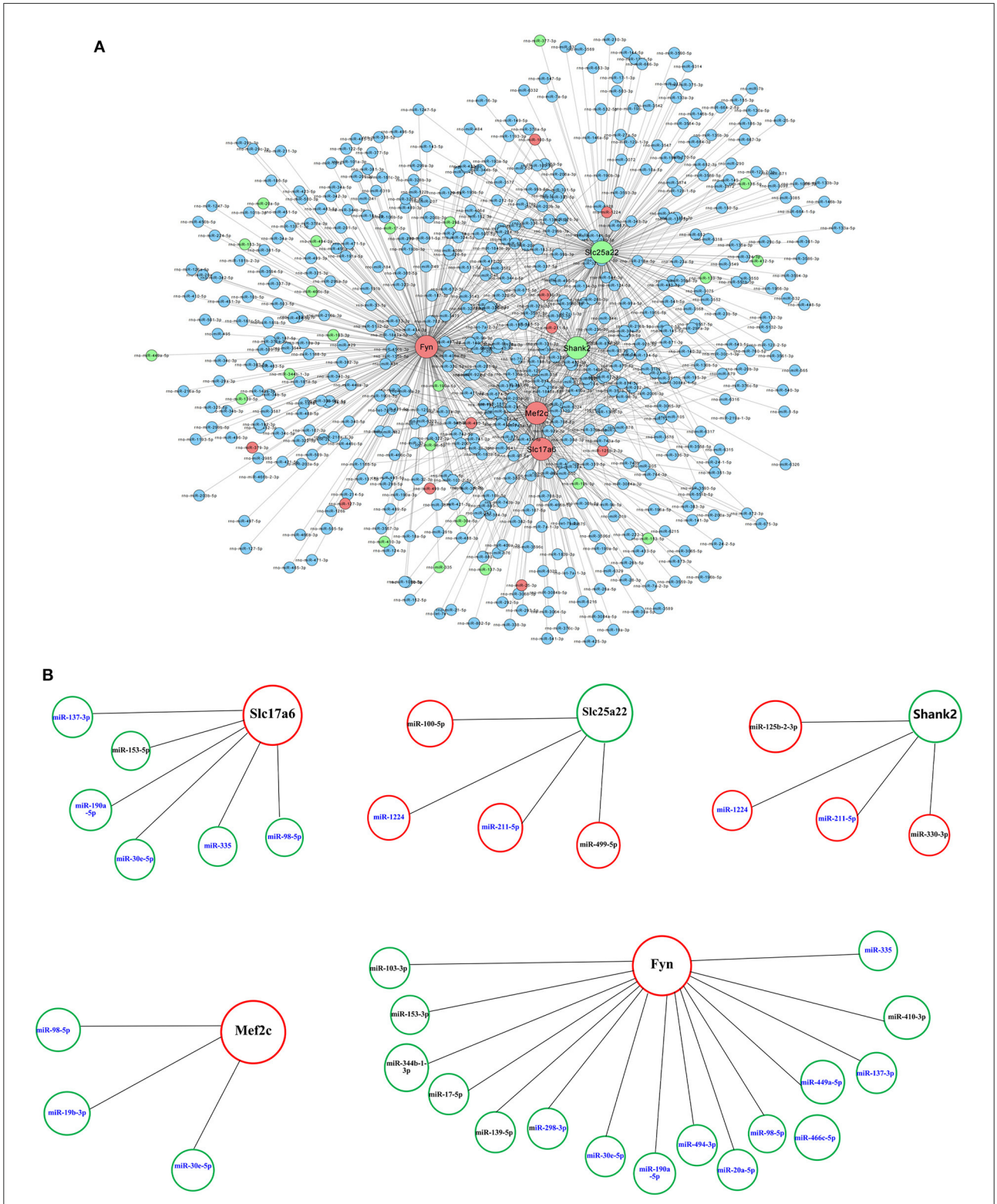

FIGURE 4
Prediction of microRNA–mRNA interaction. **(A)** Interaction network of microRNAs with 8 glutamatergic-related mRNAs. **(B)** Interaction network of 31 microRNAs with five glutamatergic-related mRNAs. Larger dots indicate mRNAs. Smaller dots indicated microRNAs. Red indicates upregulated mRNAs or microRNAs. Green indicated downregulated mRNAs or microRNAs. Blue indicates unchanged mRNAs or microRNAs.

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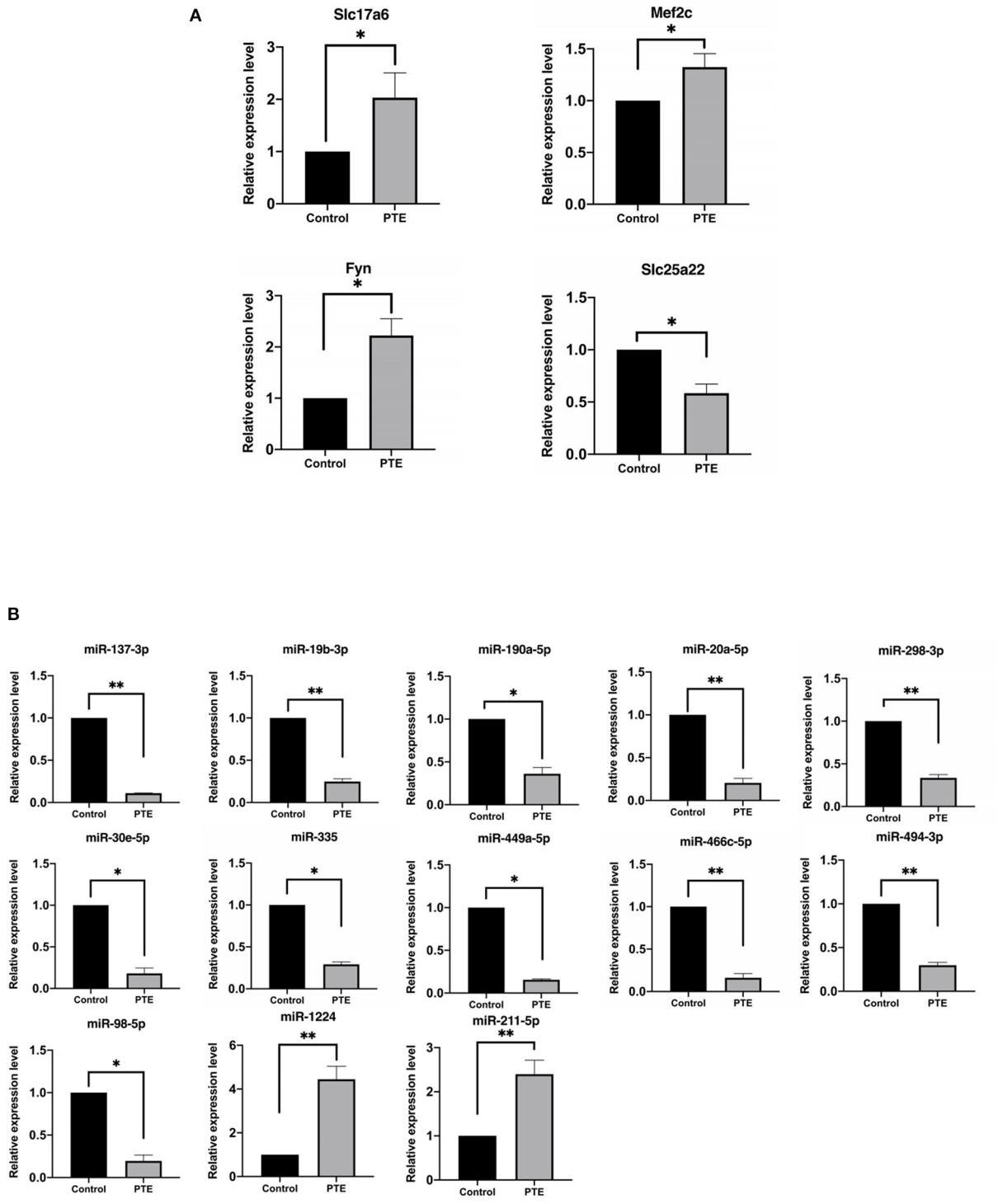

FIGURE 5
Quantitative real-time PCR verification of differentially expressed glutamatergic-related mRNAs and microRNAs. *$P < 0.05$, **$P < 0.01$ vs. sham group. **(A)** The expression of verified mRNAs (Slc17a6, Mef2c, and Fyn) in PTE group was significantly increased compared with the control group, and the expression of verified mRNAs (Slc25a22) in PTE group was significantly decreased compared with the control group. **(B)** The expression of verified microRNAs (miR-137-3p, miR-19b-3p, miR-190a-5p, miR-20a-5p, miR-298-3p, miR-30e-5p, miR-335, miR-449a-5p, miR-466c-5p, miR-494-3p, miR-98-5p) in the PTE group was significantly decreased compared with the control group, and the expression of verified mRNAs (miR-1224 and miR-211-5p) in PTE group was significantly increased compared with the control group.

TABLE 4 Relative expression level of mRNA and miRNA detected in PTE experimental group.

Name of mRNA and miRNA		Relative expression level (mean ± SD)	*P*-value	Regulation trend
mRNA	Slc17a6	2.03 ± 0.48	<0.05	↑
	Mef2c	1.32 ± 0.13	<0.05	↑
	Fyn	2.22 ± 0.33	<0.05	↑
	Slc25a22	0.58 ± 0.09	<0.05	↓
miRNA	miR-137-3p	0.11 ± 0.00	<0.01	↓
	miR-19b-3p	0.25 ± 0.03	<0.01	↓
	miR-190a-5p	0.36 ± 0.07	<0.05	↓
	miR-20a-5p	0.21 ± 0.05	<0.01	↓
	miR-298-3p	0.34 ± 0.04	<0.01	↓
	miR-30e-5p	0.21 ± 0.06	<0.05	↓
	miR-335	0.29 ± 0.03	<0.05	↓
	miR-449a-5p	0.20 ± 0.07	<0.05	↓
	miR-466c-5p	0.16 ± 0.05	<0.01	↓
	miR-494-3p	0.30 ± 0.03	<0.01	↓
	miR-98-5p	0.20 ± 0.07	<0.05	↓
	miR-1224	4.45 ± 0.6	<0.01	↑
	miR-211-5p	2.40 ± 0.31	<0.01	↑

neuronal excitatory/inhibitory (E/I) imbalance. This imbalance is likely to be the mechanism of PTE.

The results of this study suggest that expression of the *Mef2c* gene is upregulated in the frontal brain tissue of PTE rats. Expression of the MEF2C transcription factor triggers upregulation of NMDA receptor subunit 1 (NR1) (33). However, NMDA receptors are unique dual-gated channels, activation of which can lead to massive influx of Ca^{2+} and rapid depolarization of neuronal cells, which can directly lead to seizures (32). Combined with the phenomenon of massive Ca^{2+} influx after TBI, we hypothesized that after TBI, the expression of the *Mef2c* gene is upregulated and the NMDA receptor is activated, leading to massive Ca^{2+} influx, which is then involved in the occurrence of PTE. However, TBI and microglia-mediated inflammation inhibit the expression of more than 200 cortical neuron genes, including MEF2C, and MEF2C-HET mice exhibit excitatory/inhibitory (E/I) imbalance resulting from decreased inhibitory neurotransmission and increased excitatory neurotransmission (34, 35). These findings suggest that *Mef2c* may bidirectionally regulate neuronal death and promote apoptosis after TBI. Therefore, although our experimental results confirmed that the upregulation of *Mef2c* gene expression in the PTE model could explain the mechanism of PTE through neuronal excitatory/inhibitory (E/I) imbalance, microglia may inhibit the expression of *Mef2c* because microglia activation is a common feature of TBI and PTE. Therefore, further studies are needed to clarify the mechanism of *Mef2c* in the development of PTE (36).

Caspase-3 colocalizes with tau accumulation in CCI rats, indicating a possible correlation between apoptosis and tau in the chronic phase of TBI (37). A decrease in tau levels is accompanied by a decrease in NMDA receptor-dependent Ca^{2+} influx, which leads to diminished neuroexcitoxic effects, and thus abnormal tau accumulation induced by TBI may also be associated with the development of PTE (38). Our experimental results show that the expression of *Fyn* is upregulated in the PTE model. The FYN protein is one of the nine members of the Src family of non-receptor tyrosine kinases (SFK), which can phosphorylate metabolic and ionic glutamate receptors and regulate their subcellular distribution and function, thus regulating glutamatergic synaptic signaling (39–41). However, the phosphorylation of tau at tyrosine 18 (pY18) mediated by FYN protein determines the Ca^{2+} influx and the degree of nerve excitation associated with tau protein. Therefore, upregulation of *Fyn* may lead to changes in FYN protein expression and the consequent abnormal accumulation of tau protein, which is involved in the development of PTE (38). However, some scholars believe that tau plays an independent role from FYN in regulating the Ca^{2+} response (42). Therefore, although our experiments confirm the upregulation of *Fyn* gene expression in

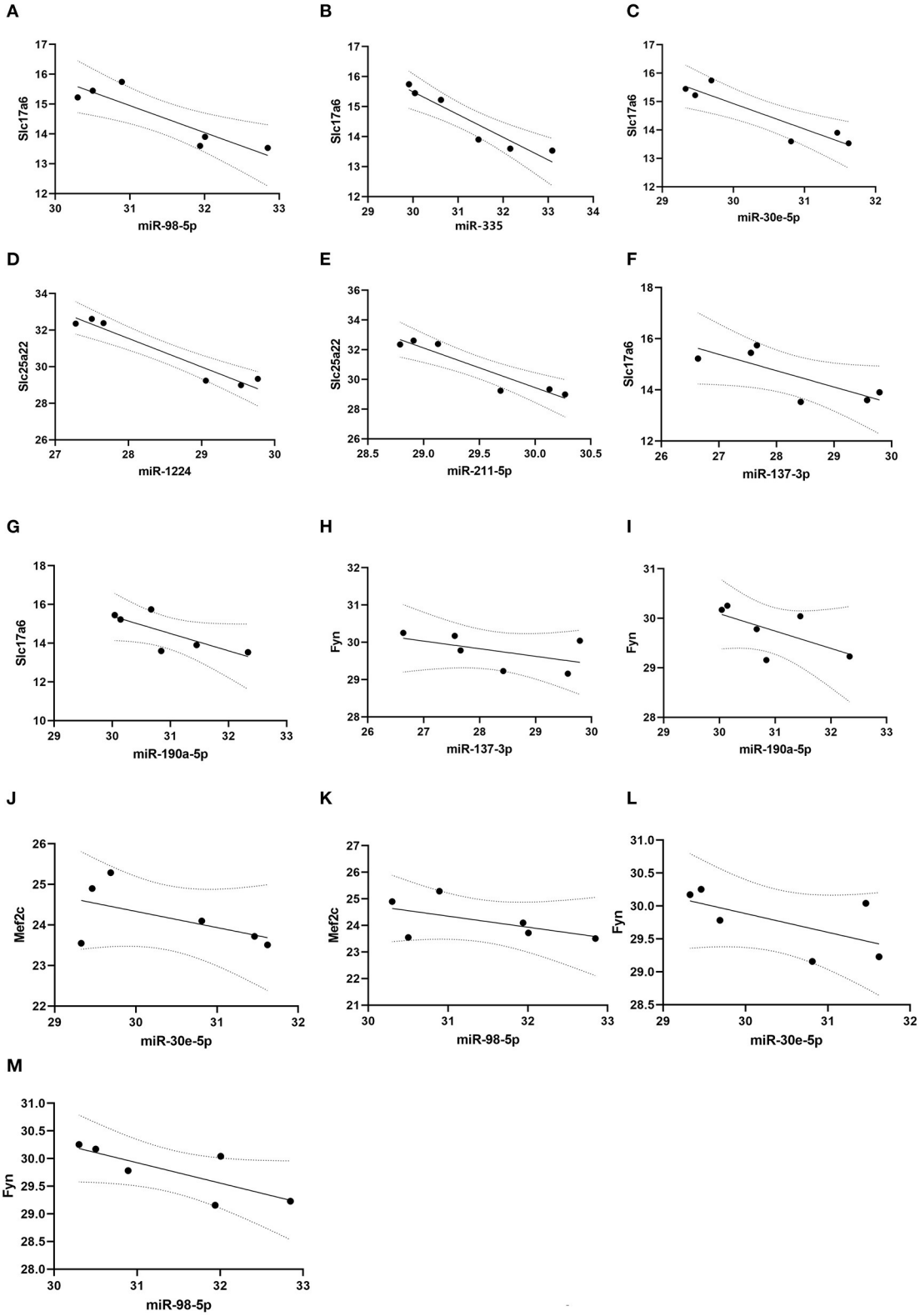

FIGURE 6
Correlation analysis. Pearson correlation analysis was conducted to evaluate the correlation between microRNAs and glutamatergic-related mRNAs. **(A–C)** miR-98-5p, miR-335 and miR-30e-5p expression were significantly and negatively correlated with Slc17a6 expression; **(D, E)** miR-1224 and miR-211-5p expression were negatively correlated with Slc25a22 expression. **(F–I)** miR-137-3p and miR-190a-5p expression were no significantly and negatively correlated with Slc17a6 and Fyn expression. **(J–M)** miR-30e-5p and miR-98-5p expression were no significantly and negatively correlated with Mef2c and Fyn expression.

the PTE model, whether *Fyn* is involved in the occurrence of PTE by mediating the tau protein remains to be confirmed by further studies.

Furthermore, astrocytes play an important role in post-injury brain repair, and astrocyte-mediated mechanisms involved in synaptic development may play an important role in neuronal injury-induced synaptic remodeling. However, intermittent reduction or loss of neuronal excitatory input after TBI can induce production of TNFα by reactive astrocytes, and overexpression of TNFα is associated with the development of epilepsy (43). The expression levels of FYN protein and phosphorylated extracellular signal-regulated kinase ERK1/2 (p-ERK1/2) were significantly increased in epilepsy models, and the expression of p-ERK1/2 was significantly decreased when FYN expression was downregulated, suggesting that the activation of the ERK1/2 signaling pathway may be regulated by *Fyn* expression (44). ERK1/2 signaling is involved in the activation and proliferation of astrocytes after TBI, which can affect the occurrence and recurrence of epilepsy (45). Therefore, in addition to the induction of PTE through tau, *Fyn* may also trigger astrocyte activation and proliferation through activation of the ERK1/2 signaling pathway, thereby enhancing susceptibility to PTE after TBI.

With the exception of *Mef2c* and *Fyn*, our experimental results show that *Slc17a6* expression is upregulated while *Slc25a22* expression is downregulated. *Slc17a6* encodes the VGLUT2 protein, a glutamate transporter that plays a key role in regulating the release of glutamate neurotransmitters. Changes in VGLUT2 protein levels can affect glutamate signaling (46). Therefore, upregulation of VGLUT2 protein leads to abnormal neuronal discharge, which may be an important mechanism for the occurrence and development of PTE. *Slc25a22 (*solute carrier family protein 25 member 22) is a mitochondrial glutamate transporter that is mainly expressed in astrocytes. *Slc25a22* mRNA and protein expression are significantly upregulated in tumor tissues and cell lines of gallbladder cancer, and downregulation of *Slc25a22* suppresses tumor cell migration and proliferation and promotes apoptosis. This apoptosis-promoting mechanism of *Slc25a22* may be achieved by downregulating the MAPK/ERK pathway (47). At the same time, downregulation of *Slc25a22* reduces glutamate catabolism in astrocytes and leads to accumulation of glutamate in cells. Therefore, the accumulation of glutamate triggered by *Slc25a22* downregulation may also be the initiating factor of abnormal neuronal excitation (48).

miRNAs may target multiple mRNAs individually, leading to multiple biological mechanisms. Combined with the existing results, we predict that the abnormal expression of some miRNAs may be involved in the occurrence of PTE by promoting apoptosis. The expression of miR-137-3p and miR-335 are downregulated in the PTE model used in this study. Studies have confirmed that miR-137-3p is significantly downregulated in both prostate cancer and colorectal cancer and can target specific proteins to inhibit tumor growth and metastasis and promote apoptosis (49). In addition, miR-137-3p targets GRIN2A, a target of NMDA receptors. Therefore, miR-137-3p may also be involved in PTE development by affecting NMDA receptors in addition to apoptosis (50). miR-335 is a classical tumor suppressor, and its expression level is significantly upregulated in serum of TBI patients. miR-335 is abnormally expressed in both TBI and PTE, suggesting that it is involved in the development of epilepsy after TBI (51). These findings suggest that miR-137-3p and miR-335 may be involved in the development of PTE by promoting apoptosis. Aside from miR-137-3p and miR-335, our results suggest that miR-1224 is also upregulated. miR-1224 has been reported to be abnormally expressed in a variety of tumors, and its overexpression can significantly inhibit the proliferation and metastasis of cancer cells and increase their apoptosis, so it is likely that miR-1224 is also involved in the development of PTE in the same way (52, 53).

In addition to the mechanisms that promote apoptosis, some abnormally expressed miRNAs may participate in the development of PTE by promoting neuroinflammation and inhibiting apoptosis. Our study shows that miR-30e-5p is downregulated and miR-211-5p is upregulated in the PTE model. In the cardiomyocyte hypoxia model, miR-30e-5p expression was significantly downregulated, and miR-30e-5p overexpression was found to inhibit inflammation by inhibiting the expression of target genes, thereby alleviating myocardial injury (54). miR-30e-5p is overexpressed in acute kidney injury models and directly targets the *Beclin1* gene, a key regulator of autophagy, to inhibit autophagy and induce apoptosis (55). Thus, the downregulation of miR-30e-5p may be involved in the occurrence of PTE through the above two mechanisms. It should be noted that unlike cardiac or brain tissue, miR-30e-5p expression is upregulated in saliva and cerebrospinal fluid after TBI, but the exact mechanism is unknown. TBI is the basis of PTE damage, so the mechanism of the involvement of miR-30e-5p in the development of PTE needs to be further studied (56). In the rat middle cerebral artery blockade and reperfusion model, miR-211-5p expression was decreased in the cerebral cortex of rats, while miR-211-5p overexpression significantly reduced cell apoptosis and lactate dehydrogenase (LDH) release rate, thereby improving cell viability (57). It has also been demonstrated that miR-211-5p expression is upregulated in the cerebral cortex of rats with a chronic inflammatory endophenotype induced by TBI (58). In combination with the upregulation of miR-211-5p expression in the PTE model, we hypothesize that miR-211-5p may be involved in the occurrence of PTE by inhibiting apoptosis or enhancing LDH release.

The involvement of miR-98-5p in PTE may be more complex than other miRNAs. miR-98-5p is downregulated in the mouse middle cerebral artery occlusion reperfusion model (MCAO/R) and the serum of stroke patients. Upregulation of miR-98-5p can alleviate the symptoms of cerebral ischemia in

MCAO/R mice and reduce oxidative stress injury by inhibiting the production of reactive oxygen species (ROS). Apoptosis was also inhibited by reducing protein kinase 1 (DAPK1), B-cell lymphoma/leukemia-2 (Bcl-2)-associated X protein (BAX), and cleaved caspase-3 levels (59). In addition, miR-98-5p can negatively regulate the expression of IL-6 and is related to the inflammatory response (60). Moreover, tumor-related studies have also confirmed that miR-98-5p regulates cell proliferation and apoptosis (61). This all suggests that miR-98-5p may be involved in PTE through mechanisms such as inhibition of oxidative stress, altered cell proliferation, apoptosis, and inhibition of the inflammatory response.

In addition to screening PTE differential genes, this study also constructed a miRNA–mRNA regulatory network related to the PTE glutamatergic system based on transcriptome detection results, and performed qRT-PCR experiments to validate some miRNA–mRNA negative regulatory pairs in the regulatory network. It is possible that miR-137a-3p, miR-190a-5p, and miR-335 may be involved in the occurrence of PTE by modulating the expression of *Slc17a6* and *Fyn*. Based on the prediction and validation results of the targeting relationship, it is possible that miR-137a-3p, miR-190a-5p, and miR-335 may be involved in the occurrence of PTE. Similarly, miR-30e-5p and miR-98-5p may be involved in the development of PTE by modulating the expression of *Slc17a6*, *Mef2c*, and *Fyn*, and miR-19b-3p may be involved in the development of PTE by upregulating *Mef2c*. miR-20a-5p, miR-298-3p, miR-449a-5p, miR-466c-5p, and miR-494-3p may be involved in the occurrence of PTE by upregulating *Fyn*. The expression of miR-1224 and miR-211-5p in the PTE model is significantly upregulated, and they may be involved in the occurrence of PTE by downregulating the expression of *Slc25a22*. Need to add that, miR-19b-3p, miR-20a-5p, miR-449a-5p and miR-494-3p are related to apoptosis (62–65). And it is noteworthy that miR-298-3p and miR-466c-5p are rarely reported (66, 67). Although their functions are poorly understood at present, they are promising diagnostic markers of PTE as newly discovered regulatory factors.

Statistical analysis showed that miR-98-5p ($r = -0.90$, $P < 0.05$), miR-335 ($r = -0.95$, $P < 0.05$), and miR-30e-5p ($r = -0.92$, $P < 0.05$) were negatively correlated with *Slc17a6* expression, and miR-1224 ($r = -0.97$, $P < 0.05$) and miR-211-5p ($r = -0.94$, $P < 0.01$) were negatively correlated with *Slc25a22* expression. Of the above predicted regulatory pairs, only the direct targeting relationship between miR-190a-5p and *Slc17a6* has been confirmed by previous studies. Whether other regulatory relationships have direct targeted regulatory relationships and how specific regulatory mechanisms work are still at the prediction stage and need to be validated by repeated *in vivo* experiments in cells and animals.

Our study has some limitations, including lacks of verification regarding the physical bindings between miRNAs and mRNAs, the miRNA function and pathway analysis results associated with mRNA, the record of EEG for 24 h continuously for 30 days, and small sample size. In addition, about the experimental model, of the existing chemicals used to induce PTE, $FeCl_2$ has been extensively practiced and accepted as the most successful chemical stimulator for PTE (68, 69). This is mainly because during the formation of PTE, hemosiderin plays an important role. Previous studies found that extravasation and dissolution of red blood cell and deposition of hemosiderin in neural network (CNN) occur following TBI, which are typical symptoms of TBI and closely related to epilepsy (70). As a result of trauma, subarachnoid hemorrhage and cerebral parenchymal hemorrhage often because blood accumulated in the cortical tissue, which leads to high risk of seizures. Animal experiments indicated that the epileptic effect of Fe ions is related to its redox reactions (71). In addition, because the process of producing epileptic susceptibility of FPI model is long (that is, several months after injury), and the generation of controlled cortical impact (CCI) model requires complex technical equipment, both methods have shortcomings (2). Therefore, we used $FeCl_2$ model due to multiple factors. However, it is not clear whether the differences in the models used so far affect the results. These limitations will be addressed in future studies.

To sum up, in this study, we found glutamatergic-related mRNAs of *Slc17a6*, *Mef2c*, *Fyn*, and *Slc25a22*, as well as miR-137-3p, miR-190a-5p, miR-335, miR-30e-5p, miR-98-5p, miR-19b-3p, miR-20a-5p, miR-298-3p, miR-449a-5p, miR-466c-5p, miR-494-3p, miR-1224, and miR-211-5p. miR-98-5p ($r = -0.90$, $P < 0.05$), miR-335 ($r = -0.95$, $P < 0.05$), and miR-30e-5p ($r = -0.92$, $P < 0.05$) expression were negatively correlated with *Slc17a6* expression, while miR-1224 ($r = -0.97$, $P < 0.05$) and miR-211-5p ($r = -0.94$, $P < 0.01$) were negatively correlated with *Slc25a22* expression. We analyzed the association between abnormally expressed genes and GO and KEGG enrichment analyses to provide insights into the molecular mechanisms of glutamatergic enrichment in PTE. Our findings suggest that these miRNAs and miRNA-regulated alterations in glutamatergic mRNA expression are involved in the development of PTE, providing potential diagnostic biomarkers and therapeutic targets for the treatment of PTE.

Data availability statement

The data presented in the study are deposited in the NCBI website repository, accessible with the following link, the Bioproject ID is PRJNA667324 (http://www.ncbi.nlm.nih.gov/bioproject/667324).

Ethics statement

The animal study was reviewed and approved by the Animal Research Ethics Committee of the Institute of Evidence Science, China University of Political Science and Law.

Author contributions

TY and JM conceived and designed the study. XZ and YM analyzed the data and wrote the initial draft of the manuscript. FZ, MZ, and DZ conducted the experiments and collected the data. XW contributed to refining the ideas. All authors were involved in revising the manuscript.

Funding

This study was supported by the Opening Project of Key Laboratory of Evidence Science (China University of Political Science and Law), the Ministry of Education (2021KFKT08), and the Capital Health Research and Development of Special (2022-2-7031).

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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frontiers | Frontiers in Neurology

TYPE Mini Review
PUBLISHED 06 February 2023
DOI 10.3389/fneur.2023.1137182

OPEN ACCESS

EDITED BY
Beixu Li,
Shanghai University of Political Science and Law, China

REVIEWED BY
Hui Li,
Shanghai Criminal Science and Technology Institution, China
Heng Zhang,
Anhui Medical University, China

*CORRESPONDENCE
Yehui Lv
✉ lvyh_15@sumhs.edu.cn

SPECIALTY SECTION
This article was submitted to Epilepsy, a section of the journal Frontiers in Neurology

RECEIVED 04 January 2023
ACCEPTED 20 January 2023
PUBLISHED 06 February 2023

CITATION
Sun X, Lv Y and Lin J (2023) The mechanism of sudden unexpected death in epilepsy: A mini review. *Front. Neurol.* 14:1137182. doi: 10.3389/fneur.2023.1137182

The mechanism of sudden unexpected death in epilepsy: A mini review

Xinyi Sun[1], Yehui Lv[1,2]* and Jian Lin[2,3]

[1]School of Basic Medical Sciences, Shanghai University of Medicine and Health Sciences, Shanghai, China, [2]Institute of Wound Prevention and Treatment, Shanghai University of Medicine and Health Sciences, Shanghai, China, [3]Chongming Hospital Affiliated to Shanghai University of Medicine and Health Sciences, Shanghai, China

Sudden unexpected death in epilepsy (SUDEP) is defined as a sudden, unexpected, non-traumatic, non-drowning death in a person with epilepsy. SUDEP is generally considered to result from seizure-related cardiac dysfunction, respiratory depression, autonomic nervous dysfunction, or brain dysfunction. Frequency of generalized tonic clonic seizures (GTCS), prone posture, and refractory epilepsy are considered risk factors. SUDEP has also been associated with inherited cardiac ion channel disease and severe obstructive sleep apnea. Most previous studies of SUDEP mechanisms have focused on cardiac and respiratory dysfunction and imbalance of the neural regulatory system. Cardiac-related mechanisms include reduction in heart rate variability and prolongation of QT interval, which can lead to arrhythmias. Laryngospasm and amygdala activation may cause obstructive and central apnea, respectively. Neural mechanisms include impairment of 5-HT and adenosine neuromodulation. The research to date regarding molecular mechanisms of SUDEP is relatively limited. Most studies have focused on p-glycoprotein, catecholamines, potassium channels, and the renin-angiotensin system, all of which affect cardiac and respiratory function.

KEYWORDS

epilepsy, mortality, sudden unexpected death in epilepsy, mechanism, P-glycoprotein, catecholamines, K^+ channel, renin-angiotensin system

Introduction

Sudden unexpected death in epilepsy (SUDEP) is defined as a sudden, unexpected, non-traumatic, non-drowning death in a person with epilepsy, witnessed or unwitnessed, in which an autopsy does not reveal an anatomical or toxicological cause of death (1). In a large Chinese community cohort of 1,562 epileptic patients, 15 experienced suspected SUDEP during the 5-year follow-up period; SUDEP incidence was 2.34 per 1,000 person-years (2). Sudden epileptic death is believed to be related to cardiac dysfunction, respiratory depression, autonomic nervous dysfunction, and brain dysfunction during seizure; however, the exact mechanism is unclear (3–5). The purpose of this review is to summarize the current knowledge regarding mechanisms of SUDEP.

SUDEP risk factors

The frequency of generalized tonic-clonic seizures (GTCS) is considered the most important clinical risk factor for SUDEP (3, 6–8): the higher the GTCS frequency, the higher the risk of SUDEP. Data from a pooled analysis of SUDEP risk factors indicate that patients who have one to two GTCS per year are nearly three times as likely to experience SUDEP than patients who do not have GTCS; patients who have more than 50 GTCS per year are more than 14 times as likely

to experience SUDEP (8). Prone positioning during seizures is an important risk factor for accidental death. Most instances of SUDEP occur after a generalized seizure; patients are usually found in the prone position (9, 10). In one study, 73.3% of SUDEP patients died in the prone position and prone position was significantly associated with SUDEP (11). Refractory epilepsy is also a risk factor for SUDEP (12). SUDEP accounts for 5–30% of deaths in all epileptic patients and up to 50% of deaths in patients with refractory epilepsy (13). Furthermore, risk of SUDEP is also higher in males, patients who have had epilepsy for many years, patients with ion channel or arrhythmia-related gene mutations, patients with neurological comorbidities, and patients taking multiple antiepileptic agents (8, 14, 15, 17). The occurrence of SUDEP is also associated with genes related to cardiac arrythmia and ion channels, especially the mutation related to long QT syndrome (LQTS), which may increase the risk of sudden death when combined with epilepsy (16–18). Severe obstructive sleep apnea has also been associated with increased risk of SUDEP (19). Seizure incidence is significantly lower in obstructive sleep apnea patients who receive positive airway pressure therapy than patients who are untreated (20). Structural brain damage may also be a SUDEP risk factor. Changes in brain structures and networks involved in central autonomic nerve and respiratory control have been observed in SUDEP patients and those at high-risk for SUDEP (21). These changes are mainly changes in gray matter volume in the hippocampus, amygdala, and thalamus (13, 22–24).

SUDEP mechanisms

Most studies which have examined the mechanisms underlying SUDEP have focused on cardiac and respiratory dysfunction and imbalance within the neural regulation system.

Cardiac dysfunction

Epilepsy may induce various transient cardiac effects, including heart rate changes, heart rate variability (HRV), arrhythmia, cardiac arrest and other electrocardiographic abnormalities (25). Acute and adaptive changes in heart rhythm in epileptic patients is one potential pathogenic SUDEP mechanism (18). To some extent, HRV reflects the balance of the sympathetic and parasympathetic autonomic nervous system divisions. An increase in HRV indicates increased parasympathetic activity while a decrease in HRV indicates a relative increase in sympathetic activity (26). In addition, HRV in epileptic patients decreases in the interictal period, especially in patients with temporal lobe epilepsy and drug resistant epilepsy (27). Moreover, reduction in HRV is associated with higher risk of SUDEP (28). Prolongation of the QT interval may be an important cause of ventricular arrhythmias in epileptic patients (29). Chahal et al. (17) reported that a prolonged QT interval in epileptic patients was associated with increased mortality. In particular, long QT syndrome, an inherited cardiac ion channel disease, is characterized by prolonged ventricular repolarization and ventricular arrhythmia, which may cause syncope or sudden cardiac death (30). In a dog model of long QT syndrome, anticonvulsant drugs can trigger torsade de pointes (31), which can progress to ventricular fibrillation and sudden death (32). Repeated seizures may also cause structural changes in the heart, which is another potential SUDEP mechanism. Pansani et al. (33) reported that repeated seizures in rats with epilepsy may damage the function and structure of the heart through regulation of microRNA that leads to myocardial cell hypertrophy and myocardial fibrosis. Similar pathological changes have also been reported in autopsy studies of SUDEP patients (34).

Respiratory dysfunction

Central and obstructive apnea and respiratory arrest have also been suspected as mechanisms underlying SUDEP. Hypoxemia caused by obstructive laryngospasm and subsequent respiratory arrest may be a mechanism of accidental sudden death in epileptic patients (35, 36). Tavee et al. (37) reported severe laryngospasm, continuous inspiratory wheezing, and cyanosis during a GTCS in a patient with refractory epilepsy. In animal studies, laryngospasm has been associated with seizure-associated reflux of gastric acid into the throat (35, 38) as well as seizure-associated increased recurrent laryngeal nerve discharge (36). Reflux is probably the cause of epilepsy-associated laryngeal spasm. In a rat epilepsy model, ST segment elevation on electrocardiography, intermittent apnea, and electroencephalography narrowing due to hypoxia were observed after gastric reflux entered the throat (38). Another rat study reported that blocking reflux into the esophagus could eliminate sudden epileptic death (35).

Amygdala activation may cause central apnea and sudden death in epileptic patients. Spread of seizure activity to the amygdala induces central apnea and decreased oxygen saturation (39–41). An area in the human amygdala that inhibits respiration and elicits apnea has been identified in children with epilepsy (42). Many patients with epilepsy are completely unaware of their apnea and do not report dyspnea (41). In addition, in a mouse model of SUDEP, electrolytic damage of the amygdala significantly reduced the incidence of seizure-induced respiratory arrest (S-IRA) and death (43). However, other studies have reported that seizures involving the amygdala are not accompanied by apnea/hypoventilation or that apnea/hypoventilation precedes the seizure. These findings indicate that amygdala involvement may not be important for induction of apnea/hypoventilation in all seizures (44).

Pulmonary edema/congestion is the most common pathological lung finding in SUDEP patients (34). In a forensic analysis of nine SUDEP cases, all exhibited pulmonary edema, pulmonary congestion, alveolar hemorrhage, and pulmonary small bronchiole wall contraction (45). GTCS are associated with neurogenic pulmonary edema (NPE). In post-ictal pulmonary edema, GTCS are the most frequently reported type (46). In one post-ictal neurogenic pulmonary edema study, five of 47 patients had symptoms of pulmonary edema and all five had GTCS (47). Moreover, the presence of an abnormality on chest radiography is significantly associated with the duration of the preceding GTCS (48). In animal models of epilepsy, pulmonary vascular pressure increases in proportion to the duration of seizure. This induced hypertension discharges fluid from the vascular compartment into the pulmonary parenchyma, causing pulmonary edema (49). This may be the mechanism of neurogenic pulmonary edema caused by epilepsy.

Neurotransmitter dysfunction

5-hydroxytryptamine (5-HT) and adenosine may participate in the pathophysiological mechanism of SUDEP (50). 5-HT plays a neuroregulatory role in respiratory control. It provides tension and excitability drive for multiple components of the respiratory network, detects changes in tissue pH/CO_2, and regulates ventilation by affecting neurotransmitter release (51). In DBA/1 mice, S-IRA is related to a defect in 5-HT neurotransmission in the dorsal raphe nucleus (52). Light stimulation of 5-HT neurons in the dorsal raphe nucleus and use of selective serotonin reuptake inhibitors and the antiepileptic drug fenfluramine can enhance the effect of 5-HT and reduce S-IRA incidence (52–54). 5-HT_3 and 5-HT_4 receptors may be involved in the above mechanism (53, 54). In a rat epilepsy model, seizures induced by pilocarpine can cause depletion of 5-HT in the hippocampus and significantly damage serotonergic neurons in the raphe nucleus (55). Adenosine signaling has a variety of beneficial and harmful effects in the context of epilepsy. Inhibition of adenosine, which leads to respiratory dysfunction during seizure, may be an important SUDEP mechanism (56). In DBA/2 epileptic mice, blockade of adenosine metabolism was significantly associated with increased incidence of S-IRA, while adenosine A_2 receptor antagonists were significantly associated with lower incidence (57). A_1 receptor activation with specific agonists can inhibit drug-resistant epileptic events in human temporal cortex slices from drug-resistant patients (58). In patients with temporal lobe epilepsy and hippocampal sclerosis, density of cortical A_{2A} receptors was significantly lower in those with a higher risk of SUDEP, which suggests impaired neuroglial dysfunction and adenosine regulation in these patients. In addition, amygdala A_1 receptor density was increased in the high-risk patients, which may contribute to peri-ictal amygdala dysfunction in SUDEP (59). Adenosine is closely associated with SUDEP and adenosine receptors may play an important role.

Molecular mechanism

P-glycoprotein

P-glycoprotein (P-gp) may be involved in SUDEP, which is the main cause of death in patients with refractory epilepsy (60). Multidrug resistance in patients with refractory epilepsy is primarily related to overexpression of ABC transporters such as P-gp (61, 62). Regardless of metabolic biotransformation, the biological distribution of antiseizure medications and their metabolites depends on functional expression of ABC transporters in the blood–brain barrier, intestine, liver, and kidney (61). However, high-frequency uncontrolled seizures can induce expression of ABC transporters such as P-gp in excretory organs and cells which normally do not express them such as neurons and cardiomyocytes; this increases the risk of refractory epilepsy (61). The expression of P-gp in neurons and myocardial cells can significantly reduce the resting membrane potential (−60 to −10 mV) and affect function in a manner that predisposes to epilepsy, malignant arrhythmia, and sudden accidental death (61, 62). Auzmendi et al. (60) reported that repeated induction of seizures in Wistar rats causes P-gp expression, electrocardiography changes, and increased mortality; these findings may be related to depolarization caused by myocardial cell P-gp expression.

Catecholamines

Status epilepticus causes release of a large amount of catecholamines (63), which can cause myocardial ischemia, calcium ion overload, oxidative stress, and mitochondrial dysfunction that lead to cardiac damage (64). In animal studies, repeated induction of S-IRA in DBA/1 mice can cause ventricular calcification necrosis; the incidence and lesion size depend on the total number of S-IRA episodes (65). Verrier et al. (66) introduced the concept of "epileptic heart," which is "a heart and coronary vasculature damaged by chronic epilepsy as a result of repeated surges in catecholamines and hypoxemia leading to electrical and mechanical dysfunction."

K^+ channel

Kv1.1 belongs to the Shaker subfamily of voltage-gated potassium channels and is widely expressed throughout the nervous system, serving as a key regulator of neuronal excitability (67, 68). In wild-type mice, Kv1.1 can be detected in brain nuclei associated with heart and lung function including the basolateral amygdala nucleus, dorsal respiratory group nuclei, dorsal motor nucleus of the vagus nerve, nucleus ambiguus, ventral respiratory column nuclei, and the pontine respiratory group nuclei. It is also found in the posterior trapezoidal nucleus and central area nucleus, which are crucial for chemical sensing (69). Neurons in the posterior trapezium nucleus directly regulate respiration in response to CO_2/hydrogen ion changes in tissues and control respiration by integrating information from several respiratory centers, including the raphe medulla (70). Kv1.1 subunits control spontaneous excitatory synaptic activity of pyramidal neurons in the basolateral amygdala (71). Kcna knockout mice lack the Kv1.1 subunit and are used as a genetic model of SUDEP (72). In these mice, the inhibitory control of interneurons in the central lateral amygdala nucleus is reduced and abnormal parasympathetic transmission leads to impaired neural control of cardiac rhythm (69, 71, 73). With Kv1.1 deficiency, seizures may cause proliferation of glial cells in the nuclei of the heart and lung centers, which may cause abnormal breathing (69). Kv1.1 deficiency also reduces the inhibitory control of interneurons in the central lateral amygdala and the overexcitation related to seizure inhibition (71). In Kv1.1-deficient mice, epileptic seizures cause abnormal parasympathetic nerve transmission, which leads to impaired neural control of heart rhythm and malignant arrhythmias (73).

In animals with chronic epilepsy, levels of the Kv4.2 myocardial voltage-gated potassium channel are decreased (74). The Kv4.2 subunit contributes to the pore-forming region of channels that express a transient A-type potassium ion current in hippocampal CA1 pyramidal cell dendrites. It is the main medium of hyperpolarized A-type current in the brain, plays an important role in signal processing and synaptic integration, and is a key regulator of neuronal excitability (75, 76). Compared with wild-type mice, the latency to seizure and status epilepticus onset is lower in Kv4.2 knockout mice (76). Silencing of Kv4.2 is mediated by miR-324-5p (75, 77). In epileptic mice, increased Kv4.2 mRNA silencing causes decreased Kv4.2 protein level and production of type A current; its role in regulating neuronal excitability is also limited (77). Inhibition of miR-324-5p can reduce the frequency

of spontaneous seizures and epileptic spikes between seizures and produce neuroprotective and antiepileptic effects (75, 77).

Renin-angiotensin system

The renin-angiotensin system has also been implicated in SUDEP (78). Angiotensin II is the main peptide of the system. Various signal pathways in the central nervous system are stimulated by angiotensin II receptor-1 (ATR1) and angiotensin II receptor-2 (ATR2) (79). Activation of ATR1 is pro-inflammatory and pro-epileptogenic (80). Angiotensin converting enzyme and ATR1 are upregulated in the brain of rats with repetitive seizures (81). We speculate that repeated seizures will lead to upregulation of ATR1 in the brain, causing pro-inflammatory and pro-epileptogenic effects that may lead to SUDEP. Other renin-angiotensin system pathways in the nervous system include angiotensin-(1–7) binding to the receptor Mas (82, 83). Angiotensin-(1–7) participates in the learning and memory process that takes place in the central marginal region of the brain. In chronically stimulated epileptic rats, levels of thimet oligopeptidase [the main enzyme involved in generation of angiotensin-(1–7)], angiotensin-(1–7), and receptor Mas transcripts are elevated (82). However, the effect of this on risk of SUDEP is unknown.

Other mechanisms

Oxygen-conserving reflexes (OCR), amygdala rapid kindling (ARK), and central nervous system damage owing to repeated GTCS may also be potential mechanisms of SUDEP. Biggs et al. (84) reported that in epileptic rats, induction of OCR causes fluctuations in heart rate and respiratory rate similar to human SUDEP. The ability of the carotid body to stimulate respiratory restart appears to be impaired during seizures (85). Totola et al. (86) reported that in epileptic rats, the number of Fos-immunoreactive neurons in the posterior trapezoidal nucleus, raphe magnus nucleus, and nucleus tractus solitarius decreased after ARK; in addition, the ventilatory volume decreased significantly. ARK damages respiratory neurons in the brain stem, resulting in respiratory dysfunction. After a single GTCS, the blood–brain barrier exhibits signs of inflammation, neuronal damage, and transitory destruction (87). Therefore, repeated GCS attacks may cause central nervous system damage and SUDEP.

To sum up, catecholamine, P-gp and ATR1 may participate in SUDEP as important molecular biomarkers. The gene mutation related to potassium channel may also involve in its occurrence (Figure 1).

Discussion

Among the various SUDEP risk factors, frequency of GTCS appears to be the most important. Prone position, male sex, chronic epilepsy, ion channel or arrhythmia-related gene mutations, neurological comorbidities, polytherapy, long QT syndrome, obstructive sleep apnea, and structural brain damage are other potential factors.

Previous studies of SUDEP mechanisms have focused on malignant arrhythmias, myocardial cell hypertrophy, myocardial fibrosis, central and obstructive apnea, pulmonary edema, and abnormal regulation of the neurotransmitters 5-HT and adenosine. Research of underlying molecular mechanisms has been limited; therefore, SUDEP is often difficult to distinguish from other causes of sudden death (88).

There are many possible molecular mechanisms of SUDEP. First, epileptic seizures induce the expression of P-gp in neurons and myocardial cells, which reduces resting membrane potential and

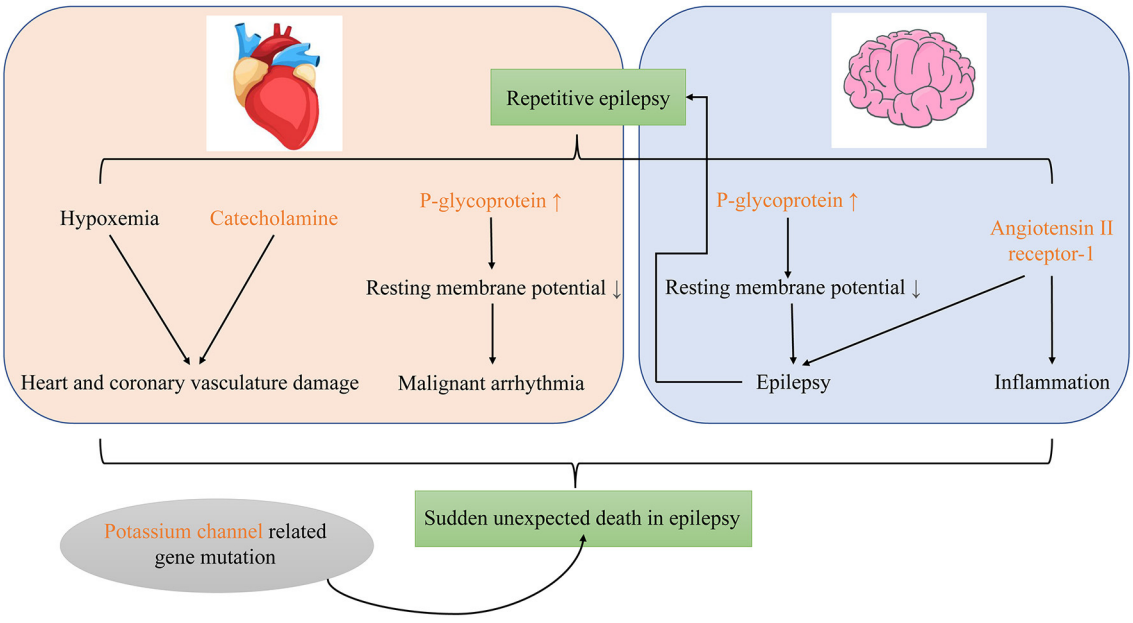

FIGURE 1
Possible molecular mechanisms of sudden unexpected death in epilepsy.

predisposes to development of epilepsy, malignant arrhythmias, and sudden death. Second, after status epilepticus, a large amount of catecholamines are released, which can cause myocardial ischemia, calcium overload, oxidative stress, and mitochondrial dysfunction. In turn, these may cause myocardial damage. Third, abnormal potassium channels may increase the risk of cardiopulmonary dysfunction during seizures. Fourth, repeated seizures lead to upregulation of ATR1 in the brain, causing pro-inflammatory and pro-epileptogenic effects. Finally, OCR, ARK, and central nervous system damage caused by repeated GTCS may be involved as well.

The mechanism of SUDEP is complex and most previous studies have focused on cardiac and respiratory dysfunction and imbalance of the neural regulatory system. Through a systematic literature review, we speculate on the mechanism of certain SUDEP cases as follows: (1) Repeated seizures cause chronic structural damage to the brain, especially the respiratory and cardiac centers. This cumulative damage causes cumulative increased risk of sudden death. (2) During seizures, especially GTCS, sudden neurological disorder and cardiac respiratory dysfunction may cause sudden death. (3) Deletions in ion channel genes deletion increase the risk of cardiopulmonary dysfunction during seizures. Overall, this review summarizes the existing mechanisms and molecular mechanisms of SUDEP, hoping to update the research progress and provide useful reference for forensic scholars in routine cases.

Author contributions

XS and YL designed the framework of the review and drafted the manuscript. YL and JL provided supervision and contributed to manuscript writing and editing. All authors have read and approved the latest version of the manuscript.

Funding

This work was funded by the Shanghai Sailing Plan (21YF1418800), the National Innovative Foundation Project for Students (202210262058) and the Shanghai Key Laboratory of Forensic Medicine, Academy of Forensic Science (KF1902).

Acknowledgments

We thank Edanz for editing the language of a draft of this manuscript.

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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frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 16 February 2023
DOI 10.3389/fneur.2023.1126652

OPEN ACCESS

EDITED BY
Beixu Li,
Shanghai University of Political Science and Law, China

REVIEWED BY
Meng He,
Fudan University, China
Dou Yin,
Shanghai Jiao Tong University, China

*CORRESPONDENCE
Fengping Yan
✉ tomjiangxi@163.com
Yuanyuan Chen
✉ chen_yuanyuan2008@163.com

SPECIALTY SECTION
This article was submitted to Epilepsy, a section of the journal Frontiers in Neurology

RECEIVED 18 December 2022
ACCEPTED 23 January 2023
PUBLISHED 16 February 2023

CITATION
Yan F, Zhang F, Yan Y, Zhang L and Chen Y (2023) Sudden unexpected death in epilepsy: Investigation of autopsy-based studies. *Front. Neurol.* 14:1126652. doi: 10.3389/fneur.2023.1126652

Sudden unexpected death in epilepsy: Investigation of autopsy-based studies

Fengping Yan[1,2]*, Fu Zhang[3], Yanan Yan[1,2], Le Zhang[4] and Yuanyuan Chen[1,2]*

[1]Department of Forensic Medicine, School of Basic Medical Sciences, Gannan Medical University, Ganzhou, Jiangxi, China, [2]Key Laboratory of Prevention and Treatment of Cardiovascular and Cerebrovascular Disease of Ministry of Education, Gannan Medical University, Ganzhou, Jiangxi, China, [3]Key Laboratory of Forensic Pathology, Ministry of Public Security and Criminal Technology Center of Guangdong Province Public Security Bureau, Guangzhou, Guangdong, China, [4]Forensic Center of Gannan Medical University, Ganzhou, Jiangxi, China

Epilepsy is a common neurological disorder that is associated with increased morbidity and mortality. Sudden unexpected death in epilepsy (SUDEP) is one of the most common causes for epilepsy-related deaths and its characteristics remain largely unknown, particularly from a forensic autopsy perspective. The present study aimed to investigate the neurological, cardiac, and pulmonary findings for a total of 388 SUDEP decedents, encompassing three cases from our forensic center during 2011–2020 and 385 literature-reported autopsy cases. In the cases mentioned in this study, two of them presented with only mild cardiac abnormalities, such as focal myocarditis and mild coronary atherosclerosis of the left anterior coronary artery. The third one was negative of any pathological findings. After pooling together these SUDEP cases, we found that neurological changes (n = 218 cases, 56.2%) were the most common postmortem findings associated with SUDEP, with cerebral edema/congestion (n = 60 cases, 15.5%) and old traumatic brain injury (n = 58 cases, 14.9%) being the major findings. Interstitial fibrosis, myocyte disarray/hypertrophy, and mild coronary artery atherosclerosis were the most common findings related to primary cardiac pathology, documented in 49 (12.6%), 18 (4.6%), and 15 (3.9%) cases, respectively. Non-specific pulmonary edema was the major finding in the lungs. This is an autopsy-based study that reports the scenario of postmortem findings for SUDEP cases. Our study paves the way for understanding the pathogenesis of SUDEP and the interpretation of death.

KEYWORDS

epilepsy, SUDEP, autopsy, cause of death, forensic pathology

1. Introduction

Sudden unexpected death in epilepsy (SUDEP) was defined as "sudden, unexpected, witnessed or un-witnessed, non-traumatic and non-drowning death in patients with epilepsy, with or without evidence for a seizure and excluding documented status epilepticus, in which postmortem examination does not reveal a toxicological or anatomic cause for death" (1). It is considered the main cause of death in patients with epilepsy and is the second most common neurological cause for potential years of life lost among all neurological diseases, second only to stroke (2, 3). The incidence rate of SUDEP was reported as 1.16 cases per 1,000 patients with epilepsy. SUDEP affected all age groups, but primarily young people with its incidence in the 20–45-year age group being 27 times higher than in control groups (4). Several risk factors have been identified, with generalized tonic–clonic seizures as the most important one. Other key risk factors include high seizure burden, lack of antiepileptic drug treatment, poly-therapy, intellectual disability, and prone position at the time of death (5).

In suspected SUDEP cases, a complete postmortem examination including both external and full internal examination, as well as toxicological analysis of antiepileptic drug (AED) levels should be made mandatory. Unfortunately, at postmortem investigation, a proportion of SUDEP cases were often unwitnessed and there was no information available on the victim's last moment of life and on the possible clues of seizures before death. Moreover, the majority of SUDEP cases were absent or at subtherapeutic levels of all AEDs (6). Owing to the limited evidence, it is often difficult to clarify whether epilepsy was the cause of death or not. Published case series did not suggest any definite pathological features or biomarkers for diagnosis of SUDEP (7). The most common mechanisms being studied are neuro-cardio-respiratory connections since ictal activity that arises in or spreads to the central autonomic network can disrupt functional connectivity of its network by inhibiting or activating autonomic areas, causing diverse autonomic manifestations, including cardiovascular and respiratory dysfunction, and brainstem damage (8, 9). There is also considerable evidence indicating that genetic factors may play a role. Cardiac genes associated with long QT syndrome, bradycardia, and sudden cardiac death can cause both epilepsy and arrhythmias or increase the risk of seizure-induced arrhythmias and have been linked to SUDEP (10, 11). Moreover, some AEDs may worsen patients' conditions, leading to other health complications including cardiovascular dysfunctions such as myocardial infarction, arrhythmias, and even cardiovascular death or SUDEP (12). All these studies provide a potential understanding of the mechanisms behind the SUDEP. However, the etiology and definite pathogenic mechanisms leading to SUDEP are still unknown.

The limited understanding of SUDEP pathogenesis is, at least in part, due to the scarce knowledge of postmortem findings for these cases. We present three cases of SUDEP individuals and summarized their epidemical and forensic characteristics. We also searched the literature on SUDEP that provided postmortem examination data, thereby summarizing common neurological, cardiac, or pulmonary pathologies with all these SUDEP cases, for the purpose of providing further insight into SUDEP from a forensic autopsy perspective.

2. Materials and methods

2.1. Study design

This is a retrospective and descriptive study with research interest in SUDEP cases undergoing a full autopsy examination. The study method was similar to those mentioned in previous studies (13, 14). We initially collected authentic SUDEP cases from our single center and then searched published literature to gather all relevant cases. We then described the neurological, cardiac, and pulmonary findings based on all the available SUDEP cases.

2.2. Case collection

The cases were collected between January 2011 and December 2020 in the Forensic Center of the Gannan Medical University. Data for this study were taken from the completed postmortem reports, including details of sex, age at death, scene at death, circumstances surrounding the event, autopsy findings, and postmortem toxicological results. Cases were collected based on the following criteria: (1) decedents had a clear record of epilepsy or seizure disorder; (2) decedents experienced a sudden, unexpected, witnessed or unwitnessed, non-traumatic, and non-drowning death; and (3) absence of definite anatomic or toxicological cause of death after complete examination.

Death certificate for these patients was made without controversy by three independent pathologists. In case of multiple pathological changes, the severity of each pathological change and its contribution to the death were seriously evaluated and independently decided by three pathologists. In case of suspected SUDEP, a neuropathologist was routinely consulted. In case of inconsistent conclusion, the case was consulted with another external pathologist to reach the final decision.

Each case was anonymized to protect the patient's privacy. This study only extracted patients' information from archived records without using patients' specimens. The review of patients' medical and forensic records was approved by the Ethical Review Board at the School of Basic Medical Science, Gannan Medical University (Approval No.: 2022-178).

2.3. Literature search strategy and selection criteria

To collect the most matched literature, we used a two-step screen strategy, to systematically obtain publications reporting on cases of patients with epilepsy who died suddenly and unexpectedly and underwent autopsy. Initially, we used terms such as "Epilepsy" and "autopsy" or "SUDEP" and "autopsy" to detect all publications that studied the SUDEP cases from a forensic autopsy perspective. The search was limited to articles published in the English language. The restriction in publication date was set from January 1980 to September 2022.

After initial screening, candidate articles were further evaluated by title and abstract, and then by full-text reading. Studies that did not report postmortem findings were excluded. Reference lists of the retrieved studies were also checked for potential additional articles. Types of studies include retrospective study, case-control study, prospective study, survey, and case report, only if they provided macroscopic and microscopic results of the brain, the heart, and the lungs, as well as toxicological results including serum AED concentrations.

3. Result

3.1. Basic characteristics and autopsy findings of the three SUDEP cases

A review of the files from our forensic center yielded eight cases that had a clear medical history of epilepsy. Among these, three (three out of eight) cases were identified to have died from SUDEP, the other five cases died from explainable causes, such as accidental drowning ($n = 1$), suicide ($n = 1$), status epilepticus ($n = 2$), and rupture of the aortic dissection ($n = 1$). Of the three SUDEP cases aged 30.5 ± 7.3 years, two were men and one was a woman. The etiology of epilepsy is unclear for all three cases. Two individuals died at their residence, unwitnessed, found in bed or

on the bedroom floor in a prone position. The third individual was witnessed to die during the daytime when watching TV. At autopsy, no significant toxicological or anatomical findings were revealed, except bite marks on the tongue and lips for two cases. Periorbital bruise was found in the third individual, indicating potential injury possibly caused by epileptic seizure prior to death. Two cases were presented with mild cardiac abnormalities, such as focal myocarditis and mild coronary atherosclerosis of the left anterior coronary artery. These two subjects also had detectable AEDs at the time of death, both in a sub-therapeutic range.

Basic characteristics and autopsy findings of the three cases categorized as SUDEP are summarized in Table 1.

3.2. Results of the literature search

The described search strategy yielded 16 publications, including 7 retrospective studies, 3 case-control studies, 5 case reports, and 1 prospective study, claiming 385 SUDEP cases in total (15–30). These studies were mostly from the USA (9/16). Among the 385 SUDEP cases, the male-to-female ratio was 227:158, similar to the cases reported in this study. Cases from the published literature are concentrated in the age group of 10–40 years, with the age range from 8 months to 83 years. Information on these studies and their detailed findings are documented in Table 2.

3.3. Major neurological, cardiac, and pulmonary findings for the SUDEP cases

We then pooled our three cases with those publications, yielding a total of 388 cases (Table 3). After a full review of all these cases, we found that the cerebral edema/congestion and old traumatic injury were the most common symptoms of neurological pathology, accounting for 60 (15.5%) and 58 (14.4%) cases, respectively. Brain sclerosis, brain atrophy, cortical/vascular malformation, and old cerebrovascular infarction were the consequent changes, claiming 32 (8.3%), 14 (3.7%), 12 (3.1%), and 12 cases (3.1%), respectively. Intracranial tumors were found in 8 cases (2.1%). A total of 183 (47.2%) cases had none or unclarified neurological pathology.

Interstitial fibrosis, myocyte disarray/hypertrophy, and mild atherosclerotic coronary artery disease were the most common symptoms of cardiac pathology, documented in 49 (12.7%), 18 (4.6%), and 15 cases (3.8%), respectively. Myocyte vacuolization was documented in 5 cases (1.3%). Other findings presented in five cases (1.3%) included focal myocarditis in one case, arteriolar wall thickening in one case, focal myocardial infarct adjacent to the bundle of His in one case, and cardiomegaly in two cases. A total of 299 (63.6%) cases had none or unclarified cardiac pathology.

Though it is non-specific to SUDEP and probably a result of any death, pulmonary congestion/edema comprised the most common finding of pulmonary pathology, documented in 247 cases (55.9%). Focal intra-alveolar hemorrhage was documented in two cases (0.5%). A total of 141 cases (36.1%) had none or unclarified pulmonary pathology.

4. Discussion

Sudden unexpected death in epilepsy was considered the leading cause of death in patients with epilepsy. By the common definition, the patients die suddenly with no anatomical or toxicological cause of death found, namely the negative postmortem examination. Diagnosis of SUDEP remains a difficult task for forensic pathologists (7, 31). Nevertheless, there is often a spectrum of pathological abnormalities among SUDEP individuals, including neuro- and cardiopulmonary pathological changes. To uncover such pathological changes, the present study pooled cases from our forensic center with literature-reported SUDEP cases and studied the neurological, cardiac, and pulmonary findings from a forensic autopsy perspective. The investigation yielded a total of 388 cases. All the SUDEP cases died at an early age (mostly 10–40 years), with the male-to-female ratio as 229:159. Neurological changes were the most common postmortem findings associated with SUDEP. Interstitial fibrosis, myocyte hypertrophy, and mild coronary artery atherosclerosis were the most common symptoms of primary cardiac pathology, and non-specific pulmonary edema/congestion was the major pulmonary finding.

The common neuropathological findings include mild degrees of cerebral edema or congestion, traumatic brain lesions, hippocampal sclerosis, vascular malformations, low-grade neoplasms, cerebellar atrophy, and cortical malformations (32, 33). The range of pathologies encompasses those commonly encountered in surgical epilepsy series, but no significant difference was shown in the frequency of neuropathological findings between the SUDEP cases and living patients with epilepsy (34). From a histological point of view, the most common finding was related to acute hypoxic neuronal changes, that is, eosinophilic neuronal changes, occurring in 55% of the SUDEP cases, most often in the hippocampus and also sometimes in the cortical and subcortical regions. Epilepsy-related acute hypoxic neuropathology in the brainstem may also contribute to the progression of epilepsy and eventually lead to brainstem dysfunction and cause SUDEP. These acute changes were more frequent when a seizure occurred 24 h before death, the body was in a prone position, or brain swelling was present (32–35). It is worth noting that, although many lesions may be identified, either grossly or microscopically, some epilepsy-related pathologies required specific immunohistochemistry to confirm the diagnosis (32, 33). In addition, published data suggested that neuropathology was very heterogeneous. The diverse neurological findings and heterogeneous data may reflect the non-standard method for conducting a brain examination across forensic institutes. It is thus important to highlight a standardized, widely occupied protocol for brain examination in suspected cases of SUDEP. The Royal College of Pathologists of United Kingdom (UK) issued guidelines in 2006 on autopsy practice in epilepsy death, suggesting that pathologists should have information on epilepsy, including seizure control, treatments, and the circumstantial evidence surrounding the death; a neuropathologist should be involved in the interpretation of brain pathology; and a case should be made for whole brain fixation and examination. A higher detection of intracerebral pathology was noted by examination of the whole fixed brain and examination of all essential regions microscopically compared with other methods (35). These guidelines are useful in forensic practice and may be

TABLE 1 Basic characteristics and autopsy findings of the three sudden unexpected deaths in epilepsy (SUDEP) cases.

	Age	Sex	Etiology detail for epilepsy	Medical history	Circumstances of death	Autopsy findings at postmortem	Postmortem toxicology
1	19	Male	Childhood-onset epilepsy (since 6–8 years old)	He had collapsed and lost consciousness 2 weeks before death. On a regular carbamazepine treatment	Unwitnessed, found dead in the morning in his bed, in a prone position	**Macroscopy:** tongue bite mark	Sub-therapeutic (carbamazepine)
						Microscopy: pulmonary congestion, minimal focal myocarditis, no remarkable neuropathological abnormality	
2	36	Male	Unknown	He was a current smoker and had chronic seizure disorder with EEG positivity. Lists of AEDs medication unknown	Unwitnessed, found dead in the morning on his bedroom floor, in a prone position	**Macroscopy:** lip bite mark.	Sub-therapeutic (valproic acid)
						Microscopy: pulmonary congestion, atherosclerosis of the left anterior descending coronary artery (Grade 1) without acute or old myocardial ischemia, without remarkable neuropathological abnormality	
3	34	Female	Unknown	She had a history of hypertension and chronic seizure disorder with EEG positivity. List of cardiovascular and AEDs medication unknown	Witnessed, had a witnessed seizure when watching TV during the daytime, Cardiopulmonary Resuscitation failed	**Macroscopy:** periorbital bruise, and resuscitation marks	Negative
						Microscopy: cerebral edema, and mild myocardial fibrosis, without typical pathological changes of hypertensive heart disease	

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TABLE 2 Summary of the postmortem findings from the autopsy study series.

	References (region)	Type of study	Patients Number of cases (*N*), gender, age range	Autopsy findings (neurological, cardiac, and pulmonary pathologies)	Postmortem AED levels
1	Terrence et al. (15), USA	Retrospective study	SUDEP cases underwent postmortem examination during the calendar years 1978 and 1979 recorded by the Allegheny Country Coroner's Office, Pittsburgh, PA *N* = 8 (4 males, 4 females, age range: 9–31 years)	**Neuropathology:** 2 with cerebral edema, 1 with old trauma to the brain. **Cardiac pathology:** no detail. **Pulmonary pathology:** all with pulmonary congestion/ edema	Sub-therapeutic: 2; Therapeutic: 3; Negative: 3
2	Leestma et al. (16), USA	Prospective study	SUDEP cases undergoing examination of the brain by the Medical Examiner of Cook Country (Chicago, IL) in the year 1983 *N* = 60 (46 males, 14 females, age range: 8 months—83 years)	**Neuropathology:** 24 with old contusions or traumatic brain injury (including old contusion, old penetrating injury, chronic subdural hematoma, and meningeal fibrosis), 7 with Ammon's horn sclerosis, 6 with hydrocephalus, 4 with cortical malformation, 4 with diffuse cerebellar degeneration, 1 with arteriovenous malformation, 1 with cerebral hemiatrophy, 1 with brain tumor, 8 with old cerebrovauclar accident; 14 with cerebral edema. **Cardiac pathology:** 9 with mild to moderate atherosclerosis; 2 with myocardial fibrosis. **Pulmonary pathology:** 42 with pulmonary congestion/edema	Sub-therapeutic: 51; Therapeutic: 3
3	Earnest et al. (17), USA	Retrospective study	"Sudden Unexplained Death Syndrome" cases identified in autopsy reports of persons with epilepsy from the Coroner's office of Denver Country and four adjacent counties from Jan 1982 through June 1987 *N* = 44 (28 males, 16 females, age range: 3–58 years)	**Neuropathology:** 14 with cerebral edema. **Cardiac pathology:** 5 with micro-focal interstitial fibrosis. **Pulmonary pathology:** 38 with pulmonary congestion/edema	Sub-therapeutic: 35; Therapeutic: 3; No detail: 6
4	Natelson et al. (18), USA	Case-control study	SUDEP cases underwent autopsy with a careful pathologic evaluation of the hearts *N* = 7 (5 males 2 females, age range: 12–44 years)	**Neuropathology:** 1 with communicating hydrocephalus. **Cardiac pathology:** 5 with myocyte vacuolization, 4 with interstitial fibrosis. **Pulmonary pathology:** no detail	Therapeutic: 2; No detail: 5
5	Antoniuk et al. (19), Brasil	Retrospective study	Cases recognized as SUDEP among deaths registered between Jan 1990 to July 1999 that underwent postmortem examination at the medico-legal institute of Crutibit-Brazia *N* = 20 (14 males, 6 females, age range: 17–47 years)	**Neuropathology:** 7 with cerebral edema. **Cardiac pathology:** no detail; **Pulmonary pathology:** 8 with pulmonary edema, 1 with pulmonary hemorrhage	No details
6	Shields et al. (20), USA	Case-control study	SUDEP cases between 1996–2000 underwent gross examination of the brain by either a forensic pathologist or consulting neuro-pathologist. Focusing on neuro-pathological findings in SUDEP cases *N* = 70 (38 males, 32 females, age range: 16–71 years)	**Neuropathology:** 19 with traumatic event (including contusions, gliosis, necrosis, cystic encephalomalacia, gunshot wound, and previous craniotomy site), 9 with cortical atrophy, 10 with cerebellar atrophy, 2 with venous hemangioma, 1 with leptomeningeal varix, 1 with tumor. **Cardiac pathology:** no details. **Pulmonary pathology:** 56 with pulmonary edema/congestion	Sub-therapeutic:29; Therapeutic: 13; Supra-therapeutic: 1; Negative: 27
7	Swallow et al. (21), UK	Case report	A case of 18-year-old boy died of SUDEP with postmortem examination from The Welsh Epilepsy Unit, University Hospital of Wales	Autopsy showed extensive cerebral edema and infarction, lungs were heavily congested and oozed edema, no details of cardiac pathology	No detail
8	Simona et al. (22), Denmark	Case-control study	Autopsy cases of SUDEP individuals during the period Jan 1998 to Sep 2000 by the department of Forensic Medicine, Aarhus University *N* = 15 (6 males, 9 females, age range: 14–58 years)	**Neuropathology:** no detail. **Cardiac pathology:** 6 with multiple foci of varying degrees of fibrosis in the myocardium. **Pulmonary pathology:** no detail	Sub-therapeutic: 7; therapeutic: 2; supra-therapeutic: 1; negative: 5

(Continued)

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TABLE 2 (Continued)

	References (region)	Type of study	Patients Number of cases (*N*), gender, age range	Autopsy findings (neurological, cardiac, and pulmonary pathologies)	Postmortem AED levels
9	Pollanen et al. (23), Canada	Retrospective study	SUDEP cases identified from March 2005 through May 2010 in the Provincial Forensic Pathology Unit, Toronto, Ontario, Canada *N* = 24 (11 males, 13 females, age range: 19–65 years)	**Neuropathology:** 1 with hippocampal sclerosis, 4 with old cortical contusions, 1 with gliotic scar, 1 with neuronal migration disorder. **Cardiac pathology:** no detail. **Pulmonary pathology:** no detail	Sub-therapeutic OR negative: 15; therapeutic: 8; supra-therapeutic: 1; unknown: 1
10	Zhuo et al. (24), USA	Retrospective study	Forensic autopsy SUDEP cases from 2007 to 2009 at the Office of the Chief Medical Examiner in the State of Maryland *N* = 74 (43 males, 31 females, age range: 14–63 years)	**Neuropathology:** 10 with traumatic lesions, 5 with malformation of cortical development, 4 with cerebellar atrophy, 3 with vascular malformation, 4 with acute focal subarachnoid hemorrhage, 2 with hippocampal gliosis, 2 with cerebral and hippocampal atrophy, 2 with remote infarcts, 3 with brain tumor, 1 with capillary angioma, 1 with acute hypoxic-ischemia change of hippocampi, 1 with cerebellar sclerosis, 1 with tuberous sclerosis. **Cardiac pathology:** 7 showed moderate ventricular hypertrophy; 3 had mild atherosclerotic coronary artery disease, 22 showed varying degrees of focal fibrosis in the myocardium. **Pulmonary pathology:** 52 with pulmonary congestion/edema	Sub-therapeutic: 19; therapeutic: 6; supra-therapeutic: 1
11	Ryvllin et al. (25), USA	Retrospective study/survey	SUDEP cases with cardiorespiratory arrests between Jan 2008 and Dec 2009 from epilepsy monitoring units Located in Europe, Israel, Australia, and New Zealand *N* = 16 (7 males, 9 females, age range: 19–62 years)	**Neuropathology:** 2 with hippocampal atrophy, 2 with encephalitis, 1 with tumor. **Cardiac pathology:** 1 with sub-endocardial fibrosis, 1 with coronary atherosclerosis. **Pulmonary pathology:** 3 with mild pulmonary edema	No details
12	Hashimoto et al. (26), Japan	Case report	A female student aged 19 died of SUDEP with postmortem examination from Department of Forensic Medicine, Graduate school of medicine, the University of Toyko	Brain edema, interstitial fibrosis, areteriolar wall thickening, and pulmonary edema with alveolar hemorrhage	No detail
13	Esen Melez et al. (27), Turkey	Retrospective study	Cases died of SUDEP in patients with a prior diagnosis of epilepsy, referred to the Ministry of Justice of Forensic Medicine in Istanbul between 2007 and 2011 *N* = 40 (21 males, 19 females, age range: 1–60 years)	**Neuropathology**: brain edema in 24. **Cardiac pathology**: hypertrophy in 11 cases, fibrosis in 9 cases. **Pulmonary pathology:** pulmonary edema in 37	Negative 21; Active 17 (without the details of concentration); Not performed 2
14	Neff and Lin (28), USA	Case report	A case of SUDEP of a 11-year-old girl with postmortem examination from Department of Laboratory Medicine and Pathology, Mayo Clinic	With focal myocardial infarct adjacent to bundle of His. No details of the neuro- and pulmonary pathology	Sub-therapeutic
15	Afandi et al. (29), Indonesia	Case report	A case of SUDEP of a 14-year-old boy with postmortem examination from Forensic Medicine and Medico-legal Studies Department, Faculty of Medicine, University Riau	With global cerebral edema and infarction, pulmonary edema, without remarkable change in the heart	Negative
16	Jordan et al. (30), USA	Case report	Three cases (a 33-year-old man, a 40-year-old man, and a 17-year-old girl) of SUDEP underwent postmortem examination from Department of Pathology, Wake Forest School of Medicine, Winstonsalem	**Neuropathology:** 1 with Schizencephaly, 1 with brain tumor, 1 with focal dysplasia. **Cardiac pathology:** 1 with coronary atherosclerosis, 2 with cardiomegaly. **Pulmonary pathology:** 1 with pulmonary edema	No details

10.3389/fneur.2023.1126652

TABLE 3 Major autopsy findings at the brain, the heart, and the lungs for sudden unexpected death in epilepsy (SUDEP) cases.

Anatomic location	Pathology	*N* (total = 388)	Percentage (%)
Brain	Cerebral edema/congestion	60	15.5%
	Old traumatic brain injury/contusion	58	14.9%
	Encephal-atrophy (cortical/cerebral/cerebellar atrophy or cerebral degeneration)	32	8.3%
	Cortical/vascular malformations	14	3.7%
	Brain sclerosis (hippocampal sclerosis/cerebellar sclerosis/tuberous sclerosis/cerebral sclerosis)	12	3.1%
	Old cerebrovascular infarction	12	3.1%
	Intracranial tumors	8	2.1%
	Hydrocephalus	7	1.8%
	Encephalitis	3	0.8%
	Venous hemanioma	3	0.8%
	Others (acute hypoxic-ischamic changes 1, schizencehaly 1, focal dysplasia 1, glial scar 1, leptomeningeal varix 1, acute subarachnoid hemorrhage 4)	9	2.3%
	Non or unclarified	183	47.2%
Heart	Interstitial fibrosis	49	12.7%
	Myocyte disarray/hypertrophy	18	4.6%
	Mild atherosclerosis coronary artery disease	15	3.8%
	Myocyte vacuolization	5	1.3%
	Others (focal myocarditis 1, arteriolar wall thickening 1, focal myocardial infarct adjacent to bundle of His 1, cardiomegaly 2)	5	1.3%
	None or unclarified	299	63.6%
Lung	Pulmonary edema	247	55.9%
	Focal pulmonary hemorrhage	2	0.5%
	None or unclarified	141	36.1%

recommended to be applied across different institutes to execute a uniform brain examination protocol.

Pulmonary pathology in SUDEP mainly consists of pulmonary edema/congestion and less often focal pulmonary hemorrhage. The two cases showing focal pulmonary hemorrhage may reflect asphyxia right before death due to SUDEP. Edema and pulmonary congestion are common autopsy findings of various cardiogenic or neurogenic deaths, including SUDEP (36). Confounding factors, such as postmortem interval, resuscitation with chest compression, and intravenous fluid administration as well as premorbid cardiopulmonary disease, may potentially contribute to the development of pulmonary edema (37). Therefore, pulmonary edema was a non-specific finding and may be of little association with SUDEP.

At the cardiac level, interstitial fibrosis, myocyte disarray/hypertrophy, myocyte vacuolization, and mild atherosclerotic coronary artery disease were mostly described in the literature. The increased frequency of cardiac findings in SUDEP may relate to the effects of seizures or psychotropic medications on the cardiac tissue (12, 38). Some studies argued that the presence and severity of cardiac pathology are not higher among SUDEP cases compared to age- and sex-matched controls who died from sudden cardiac death or trauma or to patients with epilepsy who died from causes other than SUDEP (39). From the forensic perspective, unremarkable coronary artery atherosclerosis, focal myocardial inflammatory infiltrates, isolated myocyte disarray, and idiopathic cardiac fibrosis were occasionally encountered by forensic pathologists, especially in case of sudden unexplained death. These changes, the so-called non-diagnostic autopsy findings, were insufficient to meet diagnostic criteria for known pathologies and insufficient to accord as causes for sudden death (40, 41). Although these subtle cardiac pathological findings could not prove the cause of death, a routine systemic histological examination of the myocardium, even in those who appear to be SUDEP cases, is warranted to monitor the significance of these unexpected findings. Furthermore, as regards the cardiac pathology among patients with epilepsy or SUDEP individuals, there is a need to obtain more information about the cardiorespiratory function in patients with epilepsy, which may contribute to better interpretation, and possibly, prevent SUDEP through interventions such as cardioprotective drugs and effective respiratory therapy.

Another important finding of the present study pertains to the varied postmortem levels of AEDs, ranging from the supra-therapeutic level to zero, mostly at sub-therapeutic concentration or being negative. Out of the 388 cases, 218 cases (56.2%) were at sub-therapeutic or negative levels of AEDs. Low levels of AEDs in postmortem blood have been proposed as a strong predictor of SUDEP, for low AED levels reflecting an inadequate dosage or non-compliance before death (42). However, some studies argued that detecting sub-therapeutic AED levels at autopsy has limited

value in determining the cause of death, due to uncertainties in the correlation of postmortem whole blood levels with antemortem serum levels and the definition of a therapeutic level (43). In a recently published study comparing 13 SUDEP cases with 18 non-SUDEP forensic autopsy cases, the authors also found no significant difference between the two groups of cases with regard to the use of AEDs when considering postmortem toxicological results (44). Though the connection between sub-therapeutic or absent AEDs and the occurrence of SUDEP remains to be debated, the adjuvant use of antipsychotic drugs serving as an underlying mechanism of SUDEP received widespread attention. Psychiatric comorbidity is common in patients with epilepsy, and antipsychotic drugs may be prescribed more commonly among patients with epilepsy than in the general population (45). Antipsychotics could induce cardiac side effects, including heart rate changes, blood pressure alterations, and more severe and fatal issues, such as QTc prolongation, congestive heart failure, and even sudden unexpected death (46) *via* directly binding to cardiac CB1R (47) or disturbing spliceosome signaling (48). Therefore, it is necessary to evaluate the safety and efficiency of antipsychotic medications among patients with epilepsy, and their potential contribution toward SUDEP.

Our study has several limitations. First, due to a lack of information on individual race, we were unable to determine whether neurological, cardiac, and pulmonary pathologies have racial differences or not. Second, due to technical limitations, we failed to obtain sufficient information on patients' medical records, such as the clinical type of seizure. Collaborative studies involving both clinical physicians and forensic pathologists would be more helpful in illustrating the characteristics of SUDEP.

5. Conclusion

In all, we systemically analyzed the neurological, cardiac, and pulmonary pathology for SUDEP using cases from both our forensic center and literature resources. Neuropathology was the most common change for such cases. While all these changes do not explain the cause of death, our study might pave the way for understanding the pathogenesis of SUDEP and the interpretation of death. The present study also highlighted the standard examination of the vital organs in circumstances of such cases.

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 Ethical Review Board at the School of Basic Medical Science, Gannan Medical University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

FY and YC were involved in the conception and design of the study and wrote the original draft. FZ, YY, and LZ were involved in the forensic data collection, literature search, and data analysis. All authors have read and agreed to the published version of the manuscript.

Funding

This study received funding from the open project of the Key Laboratory of Forensic Pathology, Ministry of Public Security (No. GAFYBL201903), the Science and Technology Project of Jiangxi Provincial Department of Education (No. GJJ160979), the Research Project of Gannan Medical University (No. XN201924), and the open project of the Key Laboratory of Prevention and Treatment of Cardiovascular and Cerebrovascular Diseases of Ministry of Education, Gannan Medical University (No. XN201810).

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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frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 23 February 2023
DOI 10.3389/fneur.2023.1139521

OPEN ACCESS

EDITED BY
Beixu Li,
Shanghai University of Political Science and Law, China

REVIEWED BY
Kang Wang,
Nanjing Medical University, China
Yiwu Zhou,
Huazhong University of Science and Technology, China
Zhengdong Li,
Academy of Forensic Science, China

*CORRESPONDENCE
Zhifang Yang
✉ yangzf@sumhs.edu.cn
Yehui Lv
✉ lvyh_15@sumhs.edu.cn

SPECIALTY SECTION
This article was submitted to Epilepsy, a section of the journal Frontiers in Neurology

RECEIVED 07 January 2023
ACCEPTED 30 January 2023
PUBLISHED 23 February 2023

CITATION
Tong F, Lin J, Zeng Z, Wang Q, Yang Z and Lv Y (2023) Sudden unexpected death in epilepsy: A bibliometric overview.
Front. Neurol. 14:1139521.
doi: 10.3389/fneur.2023.1139521

Sudden unexpected death in epilepsy: A bibliometric overview

Fang Tong[1,2], Jian Lin[2,3], Zixuan Zeng[1], Qi Wang[4], Zhifang Yang[1,2]* and Yehui Lv[1,2]*

[1]School of Basic Medical Sciences, Shanghai University of Medicine and Health Sciences, Shanghai, China, [2]Institute of Wound Prevention and Treatment, Shanghai University of Medicine and Health Sciences, Shanghai, China, [3]Chongming Hospital Affiliated to Shanghai University of Medicine and Health Sciences, Shanghai, China, [4]Department of Adult Internal Medicine, Hubei Maternity and Child Health Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

The mechanism of sudden unexpected death in epilepsy (SUDEP) is elusive and many questions remain unanswered. Autopsy is generally unhelpful in providing evidence for the cause of death, as pathological changes may be on the molecular level. Although histopathological examination occasionally demonstrates pathology such as vascular malformation, old traumatic injury, and tumor, in most cases of SUDEP, the examination is negative. We examined the current status of SUDEP research by performing a bibliometric analysis of studies in the Web of Science Core Collection database published between 2002 and 2022. Our aim was to demonstrate areas of interest and frontiers of SUDEP research. A total of 1803 papers were included in the analysis. The number of published papers focused on SUDEP has been increasing since 2002. Main areas of interest include clinical manifestations, prevalence, treatment, and underlying mechanisms. Research teams from the United States and Europe are leading the way in SUDEP research, while Asia trails behind. Future studies regarding the mechanism and neuropathology of SUDEP are warranted.

KEYWORDS

SUDEP, epilepsy, bibliometric analysis, research interest, English literature, autopsy rate

1. Introduction

Public interest in sudden unexpected death in epilepsy (SUDEP) has increased in recent years (1). Autopsy findings do not distinguish SUDEP from non-SUDEP deaths in individuals with epilepsy (2). Although pathologic brain examination can occasionally identify epilepsy-related pathology such as vascular malformation, old traumatic brain injury, and brain tumor, most SUDEP cases have negative findings.

The annual incidence of SUDEP is ~1.2 in 1,000 adult patients with epilepsy (3). Previously, the incidence in children was estimated to be 0.22 per 1,000 patient-years (4, 5); however, the latest data suggests the rate is similar to that in adults (6). In a study of 1,086 SUDEP cases in the United States, males comprised 63.2% of cases and the median age at death was 39 years. Death was unwitnessed in 83.2% of cases and 77.1% occurred at home. Interestingly, ~50% of victims were found in bed and 42.4% were in the prone position (3). The incidence of epilepsy in children, adolescents and young adults has decreased over the last few decades because of improvements in medical care, sanitation, and control of infectious diseases (7). In contrast, incidence of epilepsy is increasing in the elderly because of its association with age-related diseases such as stroke and neurodegenerative disorders and recent increases in life expectancy (7). Approximately 10 million people are suffering from epilepsy in China (8), however, the detection rate of SUDEP is rarely reported.

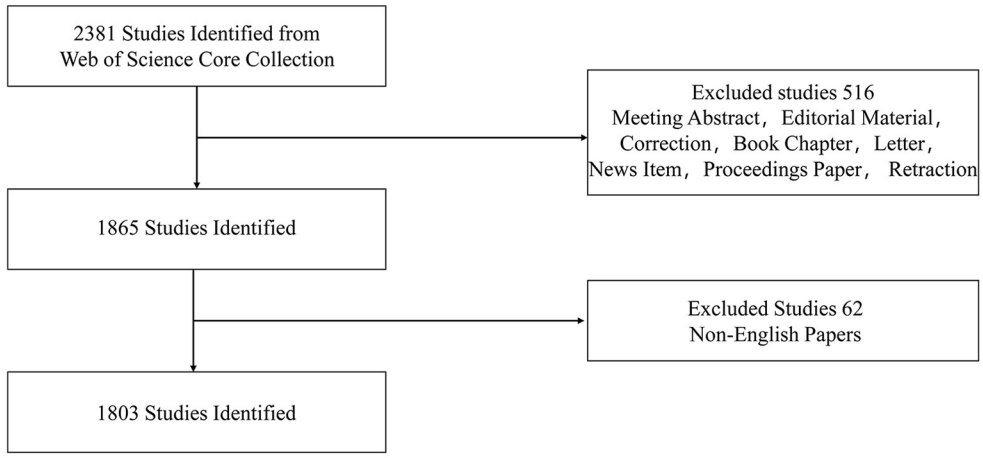

FIGURE 1
Flow chat for article selection.

Known mechanisms of SUDEP include persistent seizures, brain stem dysfunction, and cardiorespiratory inhibition; however, the cause of death in most cases is unexplained (9, 10). Underlying disease, fever, traumatic brain injury, drug withdrawal, infection, and metabolic insults have been reported as factors related to SUDEP. Convulsive seizure is thought to be a common risk factor. Progressive bradycardia occurs at the onset of convulsive seizure, followed by terminal apnea along with terminal asystole (11). In a mouse model of epilepsy, lesions in the ventrolateral medulla were associated with respiratory suppression in fatal seizure (12).

The diagnosis of SUDEP is challenging because no clear diagnostic criteria have been established. In the practice of legal medicine, SUDEP is a diagnosis of exclusion. Toxicology testing should be performed (2). Other potential causes of sudden death including coronary heart disease and stroke must be also eliminated. In many cases, SUDEP does not directly lead to death, however, it can give rise to accidental falls and trauma as well as drowning. The prevalence of SUDEP is underestimated (13). Previous studies have demonstrated that only ~30% of SUDEP cases are reported as SUDEP, seizure, or epilepsy (4, 14). Prevention of SUDEP-related death is imperative and has attracted worldwide concern. Primary care physicians can act to decrease epilepsy-related risks, most of which are non-neurological and preventable (1, 15).

Questions regarding the pathology, etiology, and mechanisms of SUDEP warrant further study. Here, we present a bibliometric analysis of the SUDEP literature published over the last two decades.

2. Materials and methods

2.1. Database and search strategy

We searched the Web of Science Core Collection databases, including the Science Citation Index and Social Science Citation Index, on October 1, 2022 to identify studies regarding SUDEP published from 2002 to 2022. The search strategy was (TS=sudden unexpected death in epilepsy OR TS=SUDEP) OR (TS=sudden unexpected death AND TS=epilepsy).

2.2. Eligibility criteria

Mechanism and cohort studies associated with SUDEP and published in the English language were eligible for inclusion. Publication types included original articles, reviews, meeting papers, and online publications. Meeting abstracts, editorial materials, corrections, book chapters, letters, news items, proceedings papers, and retractions were excluded. The selection process is shown in Figure 1.

2.3. Data analysis

CiteSpace version 5.8.2 (https://sourceforge.net/projects/citespace/postdownload) and Graphpad Prism 9 were used for data analysis after initial screening. If we did not assure the article classification of subtopics based on the metadata, the full text was read in detail for manual classification. The following indicators were chosen for analyzing research trends in SUDEP: top 10 countries and institutions for publishing SUDEP articles, top 10 journals for publishing SUDEP articles, top 10 funding sources for SUDEP studies, top 10 most cited papers and authors, and top 25 keywords. Visual maps and tables related to these indicators were created using CiteSpace.

3. Results

Two thousand three hundred eighty-one studies were identified and evaluated. Five hundred sixteen papers which did not meet criteria were excluded. Sixty-two non-English papers were also removed. Finally, 1,803 were included in

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TABLE 1 Annual publication and citation numbers.

Year	Publication number	Citation number
2002	23	0
2003	21	44
2004	25	115
2005	22	141
2006	34	317
2007	24	459
2008	32	523
2009	58	880
2010	58	1,150
2011	75	1,355
2012	67	1,451
2013	88	2,226
2014	75	2,220
2015	105	2,935
2016	135	4,137
2017	122	3,693
2018	154	4,582
2019	155	5,693
2020	181	6,708
2021	235	9,178
2022	113	6,857
	h-index	100
	Self-Citation	20,639
	Sum	54,714
	Citation per paper	30.35

the bibliometric analysis. Original articles (1,384, 76.761%) were most common, followed by review papers (n = 389, 21.575%), meeting papers (n = 65, 3.605%), and online publications (n = 5, 0.277%).

3.1. Growth trends of annual publication and citation number

The growth trends of annual publication and citation numbers are shown in Table 1 and Figure 2. The number of annual publications related to SUDEP drastically increased with slight fluctuation over the last 20 years. Only 23 SUDEP papers were published in 2002, while 235 were published in 2021. One hundred fourteen articles had been published in 2022 prior to October 1. The citation number also increased from 0 in 2002 to 9178 in 2021, which paralleled the annual publication growth trend.

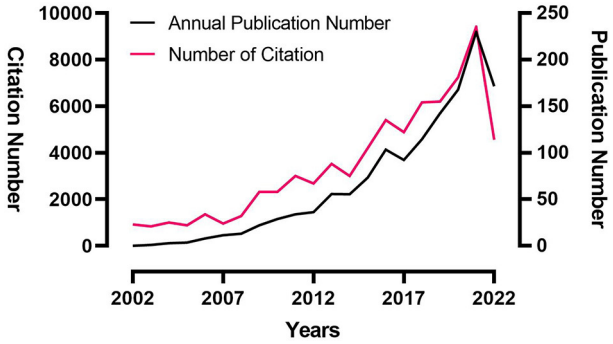

FIGURE 2
Growth trends in annual publication and citation numbers.

TABLE 2 Top 10 countries and institutions which published the highest number of SUDEP articles.

Rank	1803 publications included in this study			
	Country	Count[a]	Institutions	Count
1	USA	790	University of London	179
2	England	275	University College London	156
3	Canada	139	Harvard University	100
4	Germany	119	New York University	90
5	Netherlands	109	University of California System	88
6	Brazil	104	Harvard Medical School	71
7	Italy	104	Universidade Federal De São Paulo Unifesp	68
8	China	104	Mayo Clinic	65
9	Australia	103	Columbia University	63
10	France	89	University of Melbourne	61

[a]The total count of countries is 2,785.

3.2. Main countries/regions and institutions

A total of 95 countries/regions and 2,198 institutions contributed to 1,803 scientific articles. The countries and institutions which published the highest number of SUDEP articles from 2002 to 2022 are shown in Table 2. The United States and England were the top two countries with 790 and 275 articles, respectively. Canada, Germany, the Netherlands, Brazil, Italy, China, Australia, and France contributed 119, 109, 104, 104, 104, 103, and 89 papers, respectively during the 20 years. The top two institutions, the University of London (n = 179) and University College London (n = 156), were both in England. Most of the other institutions in the list were from the United States, including Harvard University (n = 100), New York University (n = 90), University of California System (n = 88), Harvard Medical School (n = 71), Mayo Clinic (n = 65), and Columbia University (n = 63). Network maps of countries and institutions are shown in

FIGURE 3
Network map of countries engaged in SUDEP research.

FIGURE 4
Network map of institutions engaged in SUDEP research.

TABLE 3 Top 10 funding agencies for SUDEP research.

Rank	Funding agency	Number	Percentage (%)
1	United States Department of Health Human Services, USA	340	18.857
2	National Institutes of Health, USA	333	18.469
3	National Institute of Neurological Disorders Stroke, USA	224	12.424
4	UCB Pharma SA, Belgium	68	3.771
5	Eisai Co., Ltd, Japan	65	3.605
6	National Institute for Health and Care Research, UK	63	3.494
7	Conselho Nacional De Desenvolvimento Cientifico E Tecnologico, Brazil	52	2.884
8	European Commission, EU	48	2.662
9	Fundacao De Amparo A Pesquisa Do Estado De São Paulo, Brazil	46	2.551
10	GlaxoSmithKline, UK	42	2.329

TABLE 4 Core journals which published the highest number of SUDEP papers.

Rank	Journal	Country	Count	Self-citation rate (%)	5-IF
1	Epilepsia	USA	252	9.8	7.478
2	Epilepsy Behavior	USA	232	20.6	3.504
3	Seizure European Journal of Epilepsy	England	116	10.5	3.892
4	Epilepsy Research	Netherlands	95	5.7	3.820
5	Neurology	USA	60	5.6	11.602
6	Frontiers in Neurology	Switzerland	60	6.2	4.321
7	Epileptic Disorders	Netherlands	26	2.1	9.162
8	Arquivos De Neuro Psiquiatria	Brazil	25	7.2	1.805
9	Epilepsia Open	USA	16	7.6	2.544
10	Pediatric Neurology	USA	15	11.8	3.536
11	American Journal of Forensic Medicine and Pathology	USA	14	4.2	0.939

Figures 3, 4. The top 10 countries and institutions had considerable cross-references to each other.

3.3. Primary funding agencies

Among the 1,803 analyzed papers, 340 (18.875%) were funded by the United States Department of Health and Human Services. Three hundred thirty-three (18.469%) and 224 (12.424%) were supported by the National Institutes of Health and National Institute of Neurological Disorders and Stroke of the United States. The top three funding agencies supported approximately half of the SUDEP studies. Other studies were sponsored by UCB Pharma SA (Belgium), Eisai Co., Ltd (Japan), National Institute for Health and Care Research (UK), Conselho Nacional De Desenvolvimento Cientifico E Tecnologico (Brazil), European Commission (EU), Fundacao De Amparo A Pesquisa Do Estado De São Paulo (Brazil), and GlaxoSmithKline (UK). Further details are presented in Table 3.

3.4. Predominant journals

The SUDEP articles analyzed were published in 436 journals. Six journals published more than 50 papers during the 20-year period, and three published more than 100. According to Bradford's Law, 11 journals were considered core journals in the field of SUDEP (Table 4). Eight hundred and eleven papers were published in the top 11 journals, accounting for 50.5% of all. Among all the core journals, *American Journal of Forensic Medicine and Pathology* was the only one associated with forensic medicine. While the top 10 journals in the list were all specialized journals of neurology and the top three were epilepsy-specific. The impact factor of the listed journals ranged from 0.939 to 11.802.

3.5. Impactful articles and authors

Eight papers were cited more than 400 times and three more than 500. The most-cited paper was "Incidence and mechanisms of cardiorespiratory arrest in epilepsy monitoring units (MORTEMUS): a retrospective study," which was published

TABLE 5 Top 10 impactful SUDEP papers.

Rank	Most cited articles					
	First author	Title	Journal	Year	Number of citations	Citations per year
1	Ryvlin, P	Incidence and mechanisms of cardiorespiratory arrests in epilepsy monitoring units (MORTEMUS): a retrospective study (11)	LANCET NEUROLOGY	2013	611	61.1
2	Thurman, DJ	Standards for epidemiologic studies and surveillance of epilepsy (16)	EPILEPSIA	2011	598	49.8
3	Duncan, JS	Adult epilepsy (17)	LANCET	2006	557	32.8
4	Sander, JW	The epidemiology of epilepsy revisited (18)	CURRENT OPINION IN NEUROLOGY	2003	554	27.7
5	Devinsky, O	Cannabidiol in patients with treatment-resistant epilepsy: an open-label interventional trial (19)	LANCET NEUROLOGY	2016	503	71.9
6	Moshe, SL;	Epilepsy: new advances (20)	LANCET	2015	486	60.8
7	Tomson, T	Sudden unexpected death in epilepsy: current knowledge and future directions (21)	LANCET NEUROLOGY	2008	467	33.6
8	Thijs, RD	Epilepsy in adults (22)	LANCET	2019	444	111.0
9	Stecker, EC	Population-based analysis of sudden cardiac death with and without left ventricular systolic dysfunction - Two-year findings from the Oregon sudden unexpected death study (23)	JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY	2006	349	20.5
10	Laxer, KD	The consequences of refractory epilepsy and its treatment (24)	INTERNATIONAL EPILEPSY & BEHAVIOR	2014	241	34.4

in Lancet Neurology in 2013 and cited 611 times. The journals which published the top 10 most-cited articles included Lancet Neurology, Epilepsia, Lancet, Current Opinion in Neurology, Journal of the American College of Cardiology, and International Epilepsy & Behavior. The most-cited papers are listed in Table 5. The impact of the 742 authors who published SUDEP research is visualized in Figure 5, which demonstrates the co-occurrence among authors. The impact of an author is positively associated with the name size exhibited in the figure. Orrin Devinsky, Josmir W Sander, Daniel Friedman, Fulvio A Scozar, and Esper A Cavalheiro, were the top impactful authors.

3.6. Top keywords and analysis

Keywords were extracted from the SUDEP articles and 25 burst words were exported after CiteSpace analysis. Keywords with the strongest citation burst are listed in Figure 6. Burst words significantly changed over the 20-year period. "Unexplained death" and "sudden death" were the top two strongest keywords in the list, emerging from 2002 to 2010 and 2002 to 2011, respectively, with burst intensities of 28.75 and 23.2. The third-ranked keyword was "risk factor" with a strength of 18.67.

Clustering analysis on the co-occurrence of keywords is shown in Figure 7. The figure demonstrates many important issues of co-occurrence such as sudden unexpected death, seizure, unexplained death, children, risk factor, heart rate variability, temporal lobe epilepsy, and mechanism.

4. Discussion

The results from this bibliometric analysis demonstrate that SUDEP attracted growing research attention from 2002 to 2022. The number of SUDEP articles published in 2021 was 10 times higher than that published in 2002. The burst of papers is partly related to increasing support from official agencies. National Institutes of Health funding for SUDEP increased by 10,000-fold between 2009 and 2016 (3), and the number has increased by 2.5-fold from 2016 to 2022 (25). United States funding agencies supported the most SUDEP research, followed by the United Kingdom, Japan, European Union, and Brazil. Chinese funding agencies did not make it into the top 10, which is unparallel to their greater support for other fields of research (26).

From 2002 to 2022, the United States and European countries were the predominant drivers in SUDEP research. However,

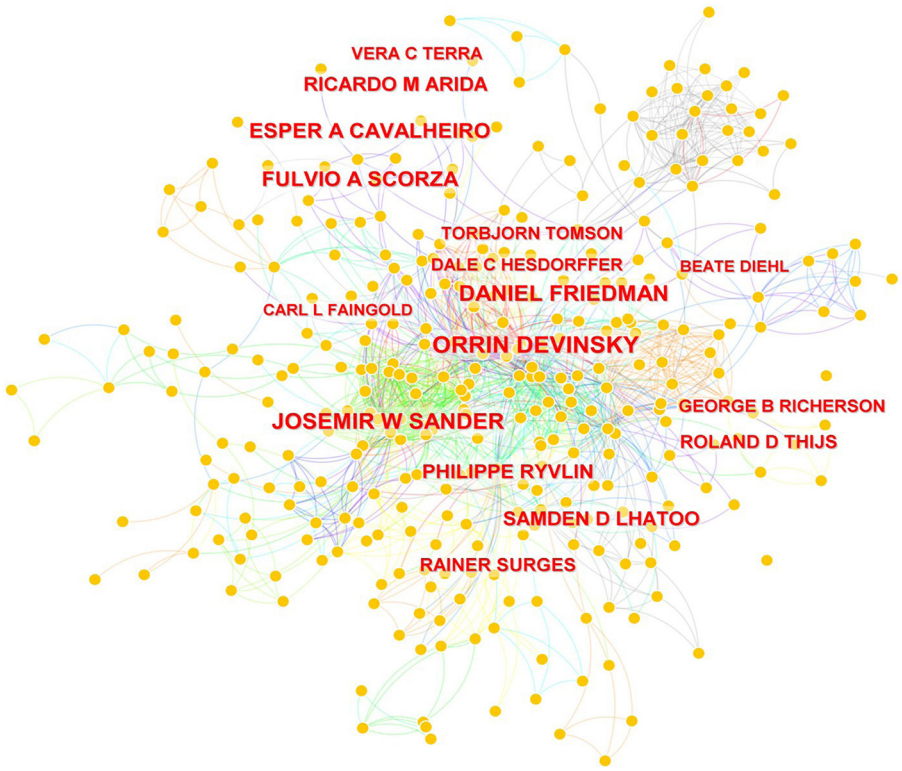

FIGURE 5
Network map of active authors contributing to SUDEP research.

developing countries including China and Brazil also participate. Both countries contributed 104 articles over the 20-year period examined and rank in the top 10. Although institutions from the United States published the highest number of articles in the period, the top two institutions are located in the United Kingdom and three institutions from the top 10 are in London. Institutions from the United States account for six positions in the top 10. An institution from Brazil, Universida de Federal de São Paulo, published 68 papers in the 20-year period and is ranked seventh; this was the only developing country institution in the top 10. Surprisingly, institutions from the European Union were absent from the top 10.

Our results also demonstrate that the most important journals publishing SUDEP research are Epilepsia and Epilepsy Behavior. Epilepsia published the highest number of articles, accounting for 14%. The top 10 journals combined published 49.7% of all SUDEP papers. Most journals publishing SUDEP articles focus on neurology and some specialize in epilepsy or seizure. Diagnosis of SUDEP is extremely difficult in the practice of forensic medicine and should be paid great attention. *American Journal of Forensic Medicine and Pathology* is one of the core journals of SUDEP according to the Bradford's Law, publishing 14 papers regarding SUDEP in the two decades. We hope that more practitioners in forensic medicine can take part in the research of SUDEP.

The most impactful article was "Incidence and mechanisms of cardiorespiratory arrests in epilepsy monitoring units (MORTEMUS): a retrospective study," written by Ryvlin et.al. They retrospectively collected and analyzed data from all cardiorespiratory arrests associated with SUDEP in numerous epilepsy monitoring units in Europe, Israel, Australia, and New Zealand (11). They proposed improving supervision at night to prevent immediate cardiorespiratory dysfunction induced by unpredictable seizures (11). The second most-cited paper was a guideline, 'Standards for epidemiologic studies and surveillance of epilepsy' published in Epilepsia. In this article (16), Thurman et.al. discussed the following issues: (1) conceptual and operational definitions of epilepsy, (2) data resources and recommended data elements, and (3) methods and analyses appropriate for epidemiologic studies or epilepsy surveillance. The paper is instructional for designing studies with different purposes and provides variant methods for data retrieval based on the particular needs of different study teams.

Keyword frequency can reflect study interests within a particular research field. According to our results, the hotspots in SUDEP research predominantly focus on mortality, epidemiology, and clinical manifestations. The results demonstrated in the list of burst keywords are in accordance with the hotspots figure. Most research did not pay attention to the etiology of SUDEP. Issues such as disease mechanism and neuropathology were not sufficiently studied during the period, which hinders a deeper understanding of the disease. Although postmortem examinations are likely to be unhelpful in SUDEP cases, occasionally they can provide interesting findings. In one case report (27), a capillary telangiectasia was observed in the hippocampus in a patient who died of unexplained seizures, implying that hippocampal pathology could induce fatal epilepsy. Some study teams start to

FIGURE 6
Top 25 keywords with the strongest citation burst.

study SUDEP at the molecular level in the near 10 years. Indeed, keywords of *de novo* mutation and sodium channel emerged in 2015, which suggests that research on SUDEP is reaching the deep-water zone. Overall, the etiology of SUDEP includes a series of different factors and cannot be explained by a single theory. Despite the low detection rate, we still recommend performing systematic pathological examination in all suspected cases of SUDEP. Furthermore, brain tissue, cerebrospinal fluid, and serum should be stored in a special SUDEP tissue bank to enable further studies at the molecular level.

We also propose that the Chinese government increase SUDEP funding and increase the autopsy rate in SUDEP cases. A better understanding of SUDEP can prolong life expectancy and enhance quality of life. The low autopsy rate is hindering China from making progress in SUDEP as well as other fields of research. Developed countries pay greater attention to autopsies for all deaths: in 2019, the autopsy rate in the Commonwealth of Nations and the World Health Organization European Region was 43.4 and 25.9%, respectively (28). In the United States, the autopsy rate was 50% in the 1940s but drastically declined to 8% in 2018 (29). Only 1.6% of all deaths in Japan were autopsied in 2014, the lowest among developed countries (30). Unfortunately, the percentage in China is even lower—from 1990 to 2011, the autopsy rate in large teaching hospitals in China was <1% and some hospitals performed no autopsies (31). Therefore, we can speculate that the overall autopsy rate in China is extremely low. If China increased the national autopsy rate to 10%, they would be able to make considerable contributions to SUDEP research.

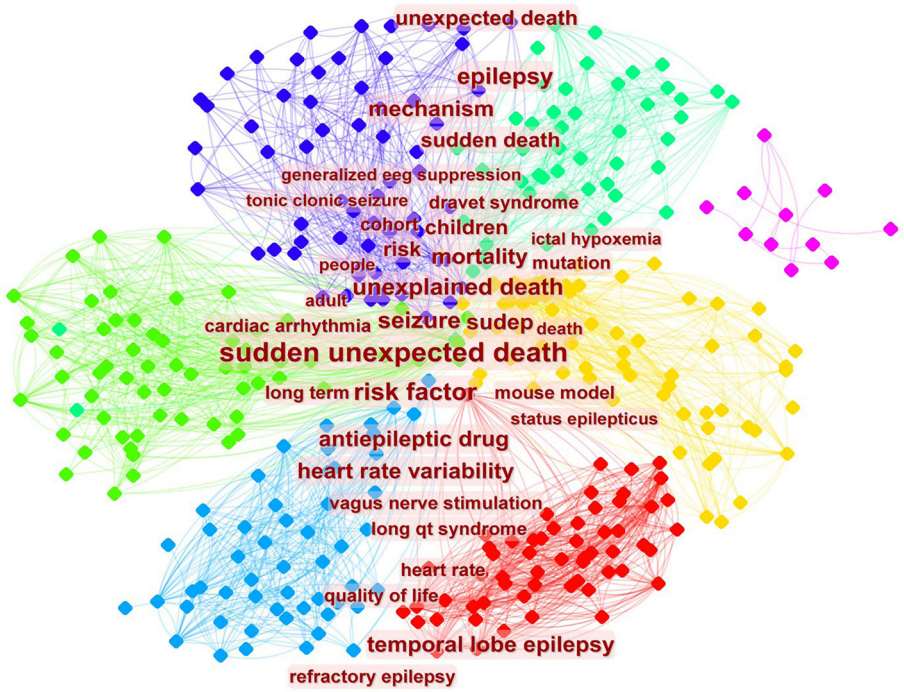

FIGURE 7
Co-occurrence map of keywords.

5. Conclusion

The number of published papers focused on SUDEP has been increasing since 2002. Current study interests involve clinical manifestations, prevalence, treatment, and underlying mechanisms. Research teams from the United States and Europe are leading the way in SUDEP research, while Asia trails behind. Systematic pathological examination could increase the understanding of SUDEP. Tissue banks of SUDEP could be established to enable further studies at the molecular level. Studies which focus on mechanisms and neuropathology are warranted to improve our understanding of this disease.

Data availability statement

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

Author contributions

FT, YL, and ZY designed the framework of the review and drafted the manuscript. FT, ZZ, JL, ZY, YL, and QW provided supervision and contributed to manuscript writing and editing. All authors have read and approved the latest version of the manuscript.

Funding

This work was funded by the Shanghai Sailing Plan (21YF1418800), Innovative Foundation Project for Students of Shanghai University of Medicine and Health Sciences (202210262058 and A3-0200-22-309009-190), and Discipline Construction Projects of Institute of Wound Prevention and Treatment, Shanghai University of Medicine and Health Sciences (HXXM-22-08-004).

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.

The reviewer YZ declared a shared affiliation with the author QW to the handling editor at the time of review.

Publisher's note

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frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 13 March 2023
DOI 10.3389/fneur.2023.1147576

OPEN ACCESS

EDITED BY
Likun Wang,
Affiliated Hospital of Guizhou Medical University, China

REVIEWED BY
Josef Halamek,
Institute of Scientific Instruments (ASCR), Czechia
Daniela Marino,
San Donato Hospital, Italy

*CORRESPONDENCE
Berj L. Bardakjian
✉ berj.bardakjian@utoronto.ca

SPECIALTY SECTION
This article was submitted to Epilepsy, a section of the journal Frontiers in Neurology

RECEIVED 18 January 2023
ACCEPTED 21 February 2023
PUBLISHED 13 March 2023

CITATION
Gravitis AC, Tufa U, Zukotynski K, Streiner DL, Friedman D, Laze J, Chinvarun Y, Devinsky O, Wennberg R, Carlen PL and Bardakjian BL (2023) Ictal ECG-based assessment of sudden unexpected death in epilepsy. *Front. Neurol.* 14:1147576. doi: 10.3389/fneur.2023.1147576

Ictal ECG-based assessment of sudden unexpected death in epilepsy

Adam C. Gravitis[1], Uilki Tufa[1], Katherine Zukotynski[2,3], David L. Streiner[4], Daniel Friedman[5], Juliana Laze[5], Yotin Chinvarun[6], Orrin Devinsky[5], Richard Wennberg[7], Peter L. Carlen[1,7] and Berj L. Bardakjian[1,3]*

[1]Institute of Biomedical Engineering, University of Toronto, Toronto, ON, Canada, [2]Department of Radiology, McMaster University, Hamilton, ON, Canada, [3]Department of Electrical and Computer Engineering, University of Toronto, Toronto, ON, Canada, [4]Faculty of Health Sciences, McMaster University, Hamilton, ON, Canada, [5]Grossman School of Medicine, New York University, New York, NY, United States, [6]Department of Medicine, Phramongkutklao Royal Army Hospital, Bangkok, Thailand, [7]Department of Medicine (Neurology), University of Toronto, Toronto, ON, Canada

Introduction: Previous case-control studies of sudden unexpected death in epilepsy (SUDEP) patients failed to identify ECG features (peri-ictal heart rate, heart rate variability, corrected QT interval, postictal heart rate recovery, and cardiac rhythm) predictive of SUDEP risk. This implied a need to derive novel metrics to assess SUDEP risk from ECG.

Methods: We applied Single Spectrum Analysis and Independent Component Analysis (SSA-ICA) to remove artifact from ECG recordings. Then cross-frequency phase-phase coupling (PPC) was applied to a 20-s mid-seizure window and a contour of −3 dB coupling strength was determined. The contour centroid polar coordinates, amplitude (alpha) and angle (theta), were calculated. Association of alpha and theta with SUDEP was assessed and a logistic classifier for alpha was constructed.

Results: Alpha was higher in SUDEP patients, compared to non-SUDEP patients ($p < 0.001$). Theta showed no significant difference between patient populations. The receiver operating characteristic (ROC) of a logistic classifier for alpha resulted in an area under the ROC curve (AUC) of 94% and correctly classified two test SUDEP patients.

Discussion: This study develops a novel metric *alpha*, which highlights non-linear interactions between two rhythms in the ECG, and is predictive of SUDEP risk.

KEYWORDS

epilepsy, sudden unexpected death in epilepsy, cross-frequency coupling, ECG, signal processing, risk assessment, non-linear interaction in cardiac rhythms

1. Introduction

SUDEP is the sudden death of a person with epilepsy without known cause, which typically occurs after a convulsive seizure in sleep and accounts for 1 in 5 cases of epilepsy-related mortality (1–3). SUDEP often follows a generalized tonic-clonic seizure (GTCS) and results from brainstem dysfunction that impairs arousal, respiration and cardiac processes, where brainstem dysfunction may be related to suppression of activity due to spreading depolarization or other mechanisms (4–8).

Risk factors for SUDEP include increased frequency or recent history of seizure, especially tonic-clonic seizures, sub-therapeutic anti-seizure medication (ASM) levels, and lack of supervision during sleep (4). A third of epilepsy patients are not fully controlled by ASMs and many suffer ASM-related adverse effects (9). A biomarker for SUDEP could alert clinicians to recommend nocturnal monitoring or alternative treatments such as neuromodulation therapy or surgical resection (10).

Analysis of electrocardiogram (ECG) recordings may provide a biomarker of SUDEP [e.g., ventricular conduction abnormalities (11) or decreased heart rate variability (12)] but is limited by muscle-induced artifact among other issues (11–13). Single Spectrum Analysis and Independent Component Analysis (SSA-ICA) have been shown to remove artifacts from electroencephalogram (EEG) signals with simulated artifacts (14). Cross-frequency Phase-Phase Coupling (PPC) reduces sensitivity to high-amplitude noise and has been shown to be physiologically relevant for analysis of neural signals (15).

The objective of our study was to use SSA-ICA to remove artifacts from ictal ECG recordings, and assess PPC features to predict SUDEP risk. The metrics *alpha* and *theta* were derived from the PPC of a 20 s mid-seizure window.

2. Materials and methods

2.1. Study description

Our series included 9 definite-SUDEP (16) patients (sudden, unexpected death of a patient without relevant comorbidities, in which postmortem examination, including toxicology, does not reveal a cause of death other than epilepsy) and 12 non-SUDEP patients with drug-resistant focal (temporal or extratemporal lobe) epilepsy, undergoing presurgical evaluation. Patients were not on ASMs at the time of their epilepsy monitoring unit (EMU) EEG recording. The ECG recordings were acquired using the Natus/Xltek EEG system, 2-lead recordings, active electrode placed in left supraclavicular region, reference electrode on left mastoid. Apart from their definite-SUDEP designation, the following data were not available in this retrospective study: simultaneous video EEG, sleep/wakefulness states, other medications, MRI findings, or non-epilepsy medical history.

TABLE 1 Table of patients.

Patient	Classification	Age at recording	Sex	ECG sampling rate (Hz)	# Of GTC seizures	# Of non-generalized seizures	GTCS duration range (s)	Non-generalized sz duration range (s)
1	Non-SUDEP	31	M	200	-	3	-	65–142
2	Non-SUDEP	28	M	200	-	2	-	109–232
3	Non-SUDEP	21	M	256	-	1	-	109
4	Non-SUDEP	52	F	500	-	1	-	162
5	Non-SUDEP	41	M	512	7	2	27–225	43–60
6	Non-SUDEP	35	F	512	1	-	32	-
7	Non-SUDEP	19	M	512	3	1	62–133	124
8	Non-SUDEP	62	F	512	2	6	65–84	58–79
9	Non-SUDEP	42	F	512	1	1	87	20
10	Non-SUDEP	39	F	512	-	6	-	31–115
11	Non-SUDEP	38	M	512	-	4	-	21–68
12	Non-SUDEP	28	M	512	5	3	62–110	55–75
13	SUDEP	49	M	250	-	1	-	127
14	SUDEP	30	M	250	1	-	127	-
15	SUDEP	26	F	512	-	1	-	63
16	SUDEP	13	M	256	2	1	86–186	76
17	SUDEP	21	F	256	-	1	-	241
18	SUDEP	34	M	500	-	1	-	284
19	SUDEP	43	F	512	6	2	107–394	90–129
20	SUDEP (test patient)	30	F	500	4	-	61–99	-
21	SUDEP (test patient)	47	M	200	4	3	40–53	84–371

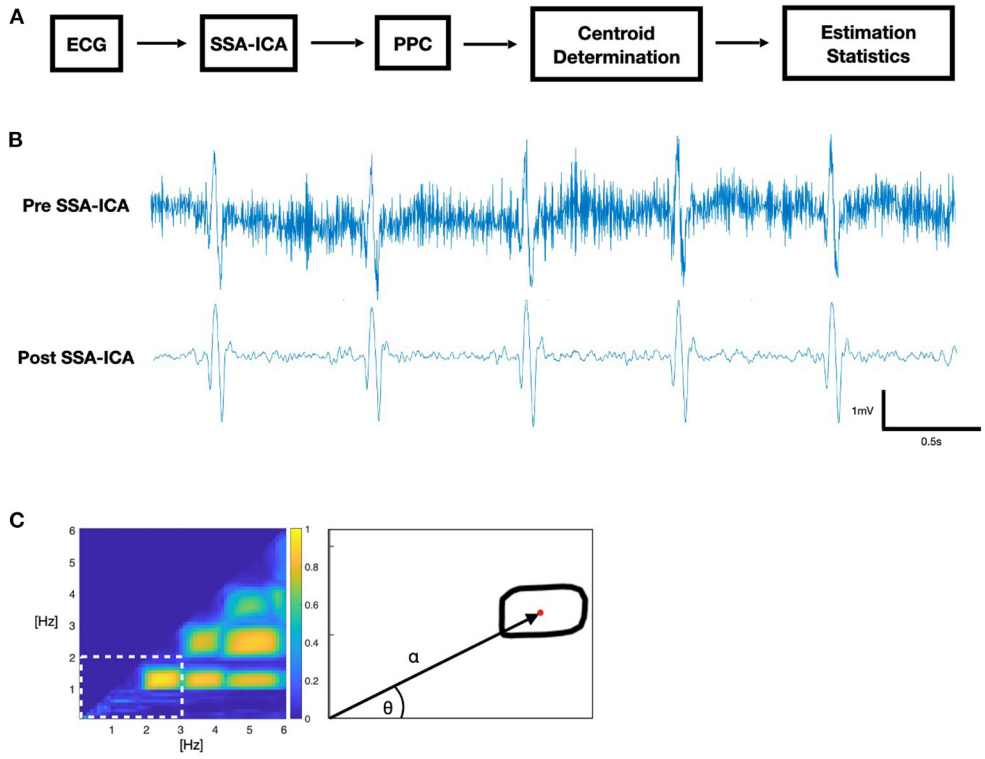

FIGURE 1
(A) Block diagram of ECG data analysis. **(B)** Sample ictal ECG. **(C)** Phase-phase comodulogram (PPC) feature extraction: polar coordinates *alpha* and *theta* of the contour centroid (red).

Two of the definite-SUDEP patients who succumbed to SUDEP more than 8 years after their last simultaneous EEG and ECG recordings were reserved as test cases (patients 20, 21); the remaining SUDEP patients died within 3 years of their last available recordings. Patients categorized as non-SUDEP did not die within 10 years of their last available recordings. Concurrent EEG and ECG recordings were obtained from the patients through the consortium formed by the Toronto Western Hospital, the New York University (NYU) Comprehensive Epilepsy Center, and the Phramongkutklao Royal Army Hospital (Table 1). Ictal (seizure) durations were identified from EEG scalp electrode recordings by board-certified neurologists/electroencephalographers.

2.2. Ethics approval and patient consent statement

The institutional review boards of the consortium approved the study protocol and all patients gave informed consent.

2.3. Data analysis

A 4-step process was used (Figure 1): (1) Applying SSA-ICA to the ictal ECG; (2) Using PPC to generate a comodulogram; (3) Analyzing the −3 dB contour of coupling power to extract the *centroid* in polar coordinates; (4) Training a logistic classifier for risk assessment. Computations were performed on the Niagara supercomputer at the SciNet HPC Consortium using Python 3.9.

2.3.1. Step 1: Artifact removal

Recordings were down sampled to the lowest sample rate in the dataset, 200 Hz. We applied SSA-ICA to remove artifacts, using a 250 samples (1.25 s) window size, decomposed into 15 components. Since a residual DC-level, or drift, in the signal after the application of SSA-ICA presents as low-frequency artifact, we applied a 0.5 Hz high-pass filter to prevent ECG "baseline wander" from appearing as strongly phase-coupled artifacts in our PPC (17).

2.3.2. Step 2: PPC comodulogram

A comodulogram representing the PPC of frequencies was calculated from the phase values of a continuous wavelet transformation using a morlet wavelet with center frequency of 0.8125 Hz. Phase values were compared by phase locking value (PLV) at each combination of frequencies between 0.1 and 6 Hz, using 0.1 Hz steps, where the PLV is an established measure of phase coherence (18). We used a time-averaged *n:m* PLV (19) for values of n, $m = 1, 2, 3 \ldots 30$, where $m > n$. The comodulogram indicates maximum coupling (1.0) when two frequencies within the same signal are phase-locked with

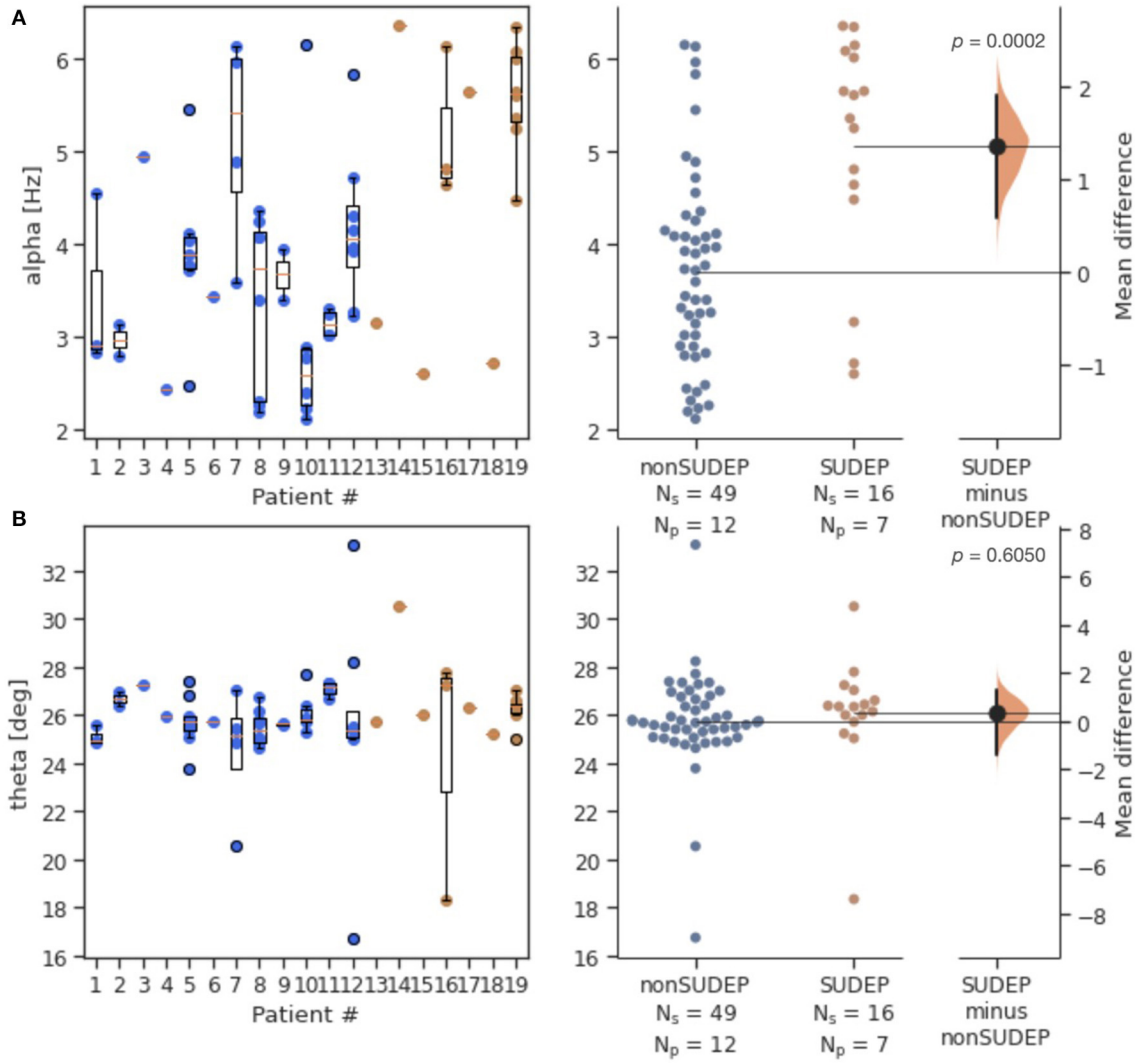

FIGURE 2
Estimation statistics of both generalized and non-generalized seizures, comparing mid-seizure 20s windows for SUDEP vs. non-SUDEP populations. Population sizes indicated by N_s (number of seizures), and associated N_p (number of patients). *Alpha* metric **(A)** and *theta* metric **(B)** of the contour centroid. Blue dots represent seizures from non-SUDEP patients. Orange dots represent seizures from SUDEP patients. Pink horizontal bars are mean values by patient.

respect to the quotient m/n, and no coupling (0.0) when there is no phase coherence between them. SSA-ICA was applied to the entire recording, and PPC analysis was performed for a 20 s window at the midpoint of the seizure, as identified by the electroencephalographer.

2.3.3. Step 3: Contour centroid

The region of interest (ROI) for further analysis was determined by selecting the lowest-frequency contour formed by the −3 dB threshold of maximum coupling power and determining the half-power point contour using the −3 dB threshold of the *local* maximum coupling within the region where the centroid (α, θ) of this ROI is the first moment of area in polar coordinates, based on Hall (20).

2.3.4. Step 4: Logistic classifier

Binary logistic regression was used to assess SUDEP risk of the seizures. Propensity scores obtained from training set alpha values were used to create a validation receiver operating characteristic (ROC) curve by varying the classification threshold from 0 to 1. An optimal propensity score threshold was selected for accuracy. Patients were then classified as SUDEP vs. non-SUDEP based on the classification of the majority of their seizures.

3. Results

Alpha was significantly elevated in SUDEP compared to non-SUDEP patients ($p < 0.0001$), while there were no significant differences for theta ($p = 0.6050$). Figure 2 shows both the estimation statistics and the per-patient box plots. Estimation

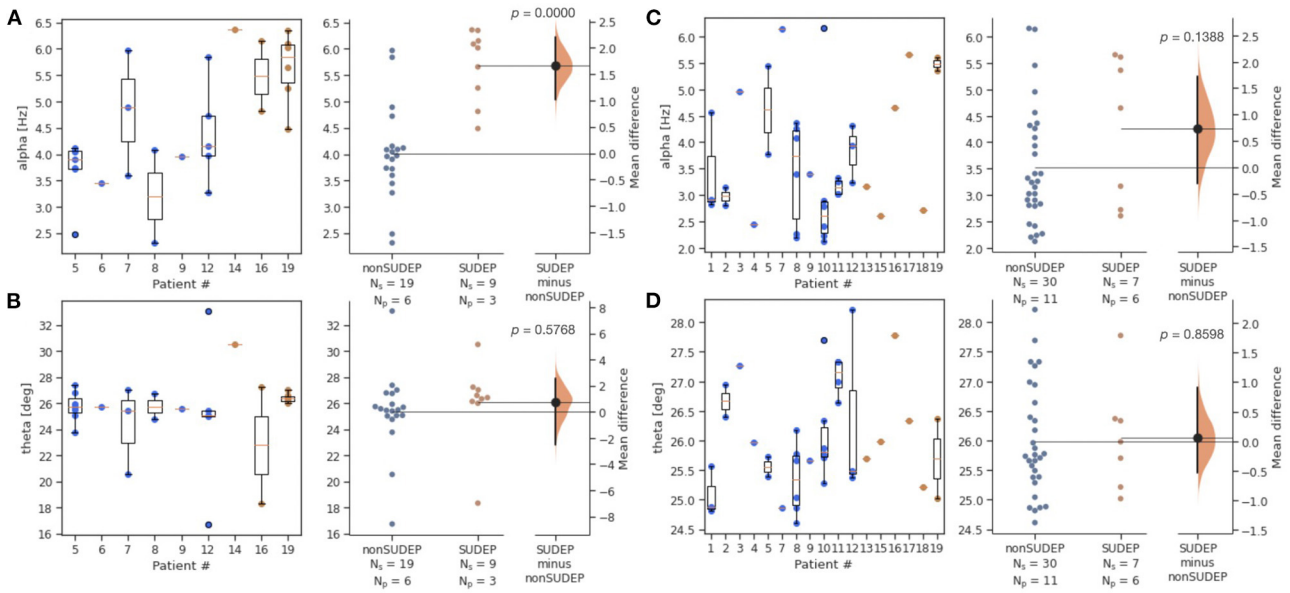

FIGURE 3
Estimation statistics for *alpha* and *theta* metrics, by two seizure types (GTCS and non-GTCS). Patient number is consistent across all figures, however not all patients have both types of seizures. N_s is number of seizures, N_p is number of patients. Blue dots represent seizures from non-SUDEP patients. Orange dots represent seizures from SUDEP patients. Pink horizontal bars are mean values by patient. **(A)** *Alpha* for GTCS only. **(B)** *Theta* for GTCS only. **(C)** *Alpha* for non-GTCS only. **(D)** *Theta* for non-GTCS only.

statistics were used to plot the overall difference between SUDEP and non-SUDEP populations. The use of estimation statistics decreases overreliance on *p*-values alone (21), and explicitly shows the effect size, range of results, and number of data points. Reported *p*-values use the Wilcoxon rank-sum test (22). Box plots show per-patient results, with one column per patient, and one data point seizure. All measures resulted in wide ranges of results on a per-patient basis, attributable to differences between seizures of a given patient. Figure 3 presents results separated by seizure type. The most significant differences are in Figure 3A, the alpha metric for GTCS ($p < 0.0001$). The theta metric was insignificant for both types of seizures.

The two SUDEP patients with recordings most distant from death were reserved for testing a classifier (patients 20, 21). Figure 4 compares *alpha* and *theta* measures for GTCS in this test set against those of non-SUDEP patients. The results are consistent with those of the training set of SUDEP GTCS seizures, where *alpha* ($p < 0.002$), but not *theta*, was significant.

For clinical application, a logistic classifier was trained on *alpha* for all the GTCS. The resulting ROC curve of training data had an area under the curve (AUC) of 94% (Figure 5). When optimizing for accuracy, the resulting F1 score is 80%, with a classifier threshold of 0.305. Each seizure was categorized as SUDEP (1.0) or non-SUDEP (0.0) relative to this threshold, and the mean patient classification scores are plotted. Error bars reflect standard deviation of each population. Both test patients are classified as SUDEP by this system.

4. Discussion

We describe a previously unreported PPC-based ECG metric to assess SUDEP risk using patients' GTCS features. Clinical application of this method requires only a 20-s mid-seizure ECG window for PPC analysis and *alpha* estimation. This study assesses a patient's risk of SUDEP by logistic classification using the *alpha* metrics of the majority of their seizures.

Two SUDEP test patients were withheld from classifier training. Ictal ECG recordings from the test patients 20 and 21 were acquired 10 and 8 years prior to SUDEP, respectively. Both patients resulted in mean classification scores of 0.75 (Figure 5D), thereby correctly assessed as high risk of SUDEP.

A 2010 matched-pair case-control study evaluated an extensive set of ECG features for SUDEP prediction (23). The study investigated features including peri-ictal heart rate (HR), heart rate variability (HRV), corrected QT interval (QTc), and postictal HR recovery, concluding these were not significant predictors of SUDEP. We therefore aimed to develop a novel ECG metric not derived from existing features to successfully assess SUDEP risk.

Surges et al. suggest that ECG features of SUDEP (maximal ictal HR, postictal HR recovery) identified by Nei et al. (24) can be attributed to a higher prevalence of secondarily GTCS in SUDEP patients (23, 25, 26). There were significant differences in HR and HRV between GTCS and non-GTCS, however a GTCS-only subpopulation was not investigated. A comparison between these parameters and the metrics developed in this study would be beneficial for a larger population of low-artifact ictal ECG recordings.

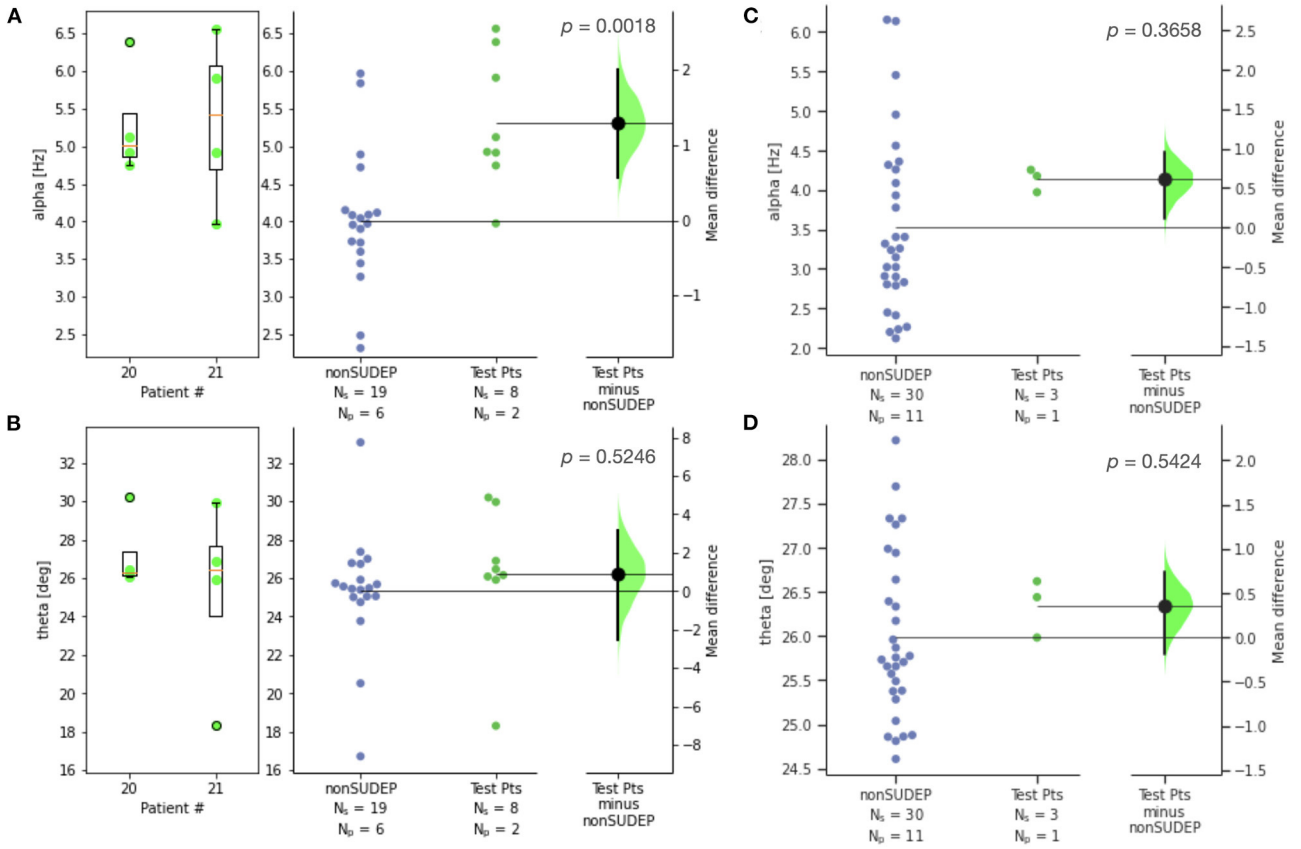

FIGURE 4
Estimation statistics for **(A)** *alpha* and **(B)** *theta* metrics for GTCS of two SUDEP patients reserved for testing, compared to those of non-SUDEP patients; **(C)** *alpha* and **(D)** *theta* metrics for non-GTCS of SUDEP patient reserved for testing (patient 21), compared to non-SUDEP patients. Patient number is consistent across all figures. N_s is number of seizures, N_p is number of patients. Blue dots represent seizures from non-SUDEP patients. Green dots represent seizures from SUDEP patients reserved for testing. Pink horizontal bars are mean values by patient.

The assessment developed in this study controls for seizure type by considering only GTCS. The *alpha* metric in this study stands as a novel ECG feature, without derivation from previously reported ECG metrics. The *alpha* metric highlights non-linear interactions between different rhythms in the ECG. The set of ECG features used in cardiac investigations should be expanded to include this metric. Our results were obtained in ictal states only, due to limitations of the dataset in this retrospective study. Prospective studies will include sufficient pre- and post-ictal data, and explore the *alpha* metric in the context of the cardiac syncytium.

We chose a logistic regression to produce a *propensity score* (27) for each seizure from the cohorts available for this study (Figure 5B). Propensity scores can overcome an inability to pair-match in observational studies. The optimal propensity score threshold was used to categorize patients as SUDEP, or non-SUDEP, based on the classification of the majority of their seizures.

SSA-ICA was successful in decreasing artifacts throughout the recording. ECG artifacts often overlapped with frequency ranges of interest and other blind source separation methods such as ensemble empirical mode decomposition (EEMD) were less suitable under these conditions. Phase-amplitude coupling (28), where the phase of a low frequency rhythm modulates the amplitude of a higher frequency rhythm, has been used in the development of EEG-based biomarkers (29). However, PPC may be more appropriate for ECG-based biomarkers as the phases of cardiac waves are of particular interest and more robust measures than their absolute amplitudes. Measures of phase-phase coherence such as PLV are used to detect synchronization in noisy systems (19). PLV measures the cyclic relative phases between two signals. Higher artifact recordings necessitated lengthier time windows for strong coherence results. This study used a 20-s time-averaged PLV, which resulted in higher-contrast comodulograms and clearer −3 dB thresholds than were obtained for shorter durations. An artifact-tolerant measure based on PPC would enhance investigations of other cardiac-related unexplained death syndromes, such as sudden infant death syndrome (SIDS) (30), among others, and may provide an accessible measure of seizure severity.

A natural extension of this work is the application of this methodology to pre-ictal, post-ictal (29, 31) and inter-ictal ECG recordings in patients with epilepsy. Further, investigation of the PPC comodulograms could isolate the effect of waveform variability. Also, a multivariate approach incorporating EEG, respiration, and ECG would improve the risk assessment of SUDEP. A detailed analysis of the *alpha* metric would clarify its relationship with cardiac function. Analysis of a larger cohort of SUDEP patients would enhance the statistical power of these

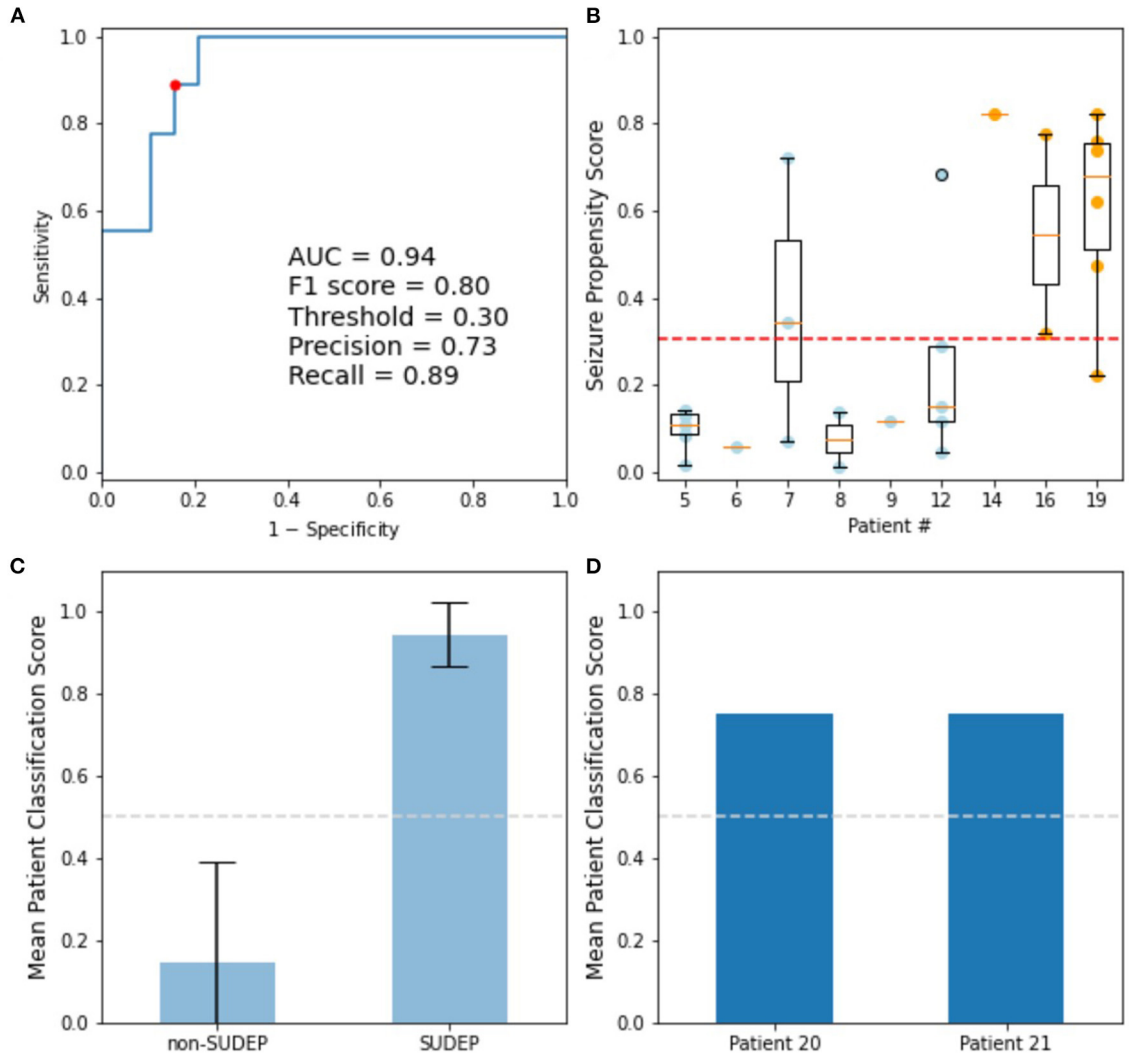

FIGURE 5
Logistic classifier trained on *alpha* determined from PPCs of GTCS. **(A)** ROC curve of training data: Accuracy optimized point (red), used to determine classifier decision threshold. **(B)** Training seizure propensity scores by patient; blue dots are non-SUDEP, orange dots are SUDEP patients. **(C)** Mean training patient classification scores, with standard deviation shown. **(D)** Mean patient classification scores for GTCS of two eventual SUDEP patients not included in training set.

conclusions and more rapidly lead to clinical application of this work.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: The anonymized datasets used in this study are available upon request. They are not publicly available due to institutional restrictions associated with the original data acquisition protocols. Requests to access these datasets should be directed to BB, berj.bardakjian@utoronto.ca.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committees associated with the Toronto Western Hospital, the New York University (NYU) Comprehensive Epilepsy Center, and the Phramongkutklao Royal Army Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Funding

BB acknowledges grant support by the Natural Sciences and Engineering Research Council of Canada (NSERC), and EpLink—the Epilepsy Research Program of the Ontario Brain Institute. The Ontario Brain Institute is an independent non-profit corporation, funded partially by the Ontario Government. The opinions, results

and conclusions are those of the authors and no endorsement by the Ontario Brain Institute is intended or should be inferred. Computations were performed on the Niagara supercomputer at the SciNet HPC Consortium. SciNet was funded by the Canada Foundation for Innovation; the Government of Ontario; Ontario Research Fund—Research Excellence; and the University of Toronto. Finding A Cure for Epilepsy and Seizures (FACES) supported the collection and processing of NYU Langone Epilepsy Center data.

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

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2023.1147576/full#supplementary-material

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frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 24 March 2023
DOI 10.3389/fneur.2023.1129395

OPEN ACCESS

EDITED BY
Bin Tu,
Columbia University,
United States

REVIEWED BY
Ana Suller Marti,
Western University,
Canada
Brad Kamitaki,
Rutgers Robert Wood Johnson University Hospital,
United States
Nicole Odom,
Dartmouth Hitchcock Medical Center,
United States

*CORRESPONDENCE
Yassine Lamrani
✉ yassine.lamrani@umontreal.ca

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Epilepsy, a section of the journal Frontiers in Neurology

RECEIVED 21 December 2022
ACCEPTED 03 March 2023
PUBLISHED 24 March 2023

CITATION
Lamrani Y, Tran TPY, Toffa DH, Robert M, Bérubé A-A, Nguyen DK and Bou Assi E (2023) Unexpected cardiorespiratory findings postictally and at rest weeks prior to SUDEP. *Front. Neurol.* 14:1129395. doi: 10.3389/fneur.2023.1129395

Unexpected cardiorespiratory findings postictally and at rest weeks prior to SUDEP

Yassine Lamrani[1]*, Thi Phuoc Yen Tran[1], Dènahin Hinnoutondji Toffa[1,2], Manon Robert[1], Arline-Aude Bérubé[3], Dang Khoa Nguyen[1,2,3†] and Elie Bou Assi[1,2†]

[1]University of Montreal Hospital Research Center (CRCHUM), Montreal, QC, Canada, [2]Department of Neuroscience, University of Montreal, Montreal, QC, Canada, [3]Division of Neurology, University of Montreal Hospital Center (CHUM), Montreal, QC, Canada

Introduction: Mechanisms underlying sudden unexpected death in epilepsy (SUDEP) are unclear, but autonomic disorders are thought to play a critical role. However, those dysfunctions have mainly been reported in the peri-ictal context of generalized tonic–clonic seizures. Here, we explored whether heart rate variability (HRV), heart rate (HR), and breathing rate (BR) changes could be observed perictally during focal seizures with or without impaired awareness as well as interictally to assess the risk of SUDEP. We report the case of a 33-year-old patient with drug-resistant bilateral temporal lobe epilepsy who died at home probably from an unwitnessed nocturnal seizure ("probable SUDEP").

Methods: Ictal and interictal HRV as well as postictal cardiorespiratory analyses were conducted to assess autonomic functions and overall SUDEP risk. The SUDEP patient was compared to two living male patients from our local database matched for age, sex, and location of the epileptic focus.

Results: Interictal HRV analysis showed that all sleep HRV parameters and most awake HRV parameters of the SUDEP patient were significantly lower than those of our two control subjects with bitemporal lobe epilepsy without SUDEP ($p < 0.01$). In two focal with impaired awareness seizures (FIAS) of the SUDEP patient, increased postictal mean HR and reduced preictal mean high frequency signals (HF), known markers of increased seizure severity in convulsive seizures, were seen postictally. Furthermore, important autonomic instability and hypersensitivity were seen through fluctuations in LF/HF ratio following two seizures of the SUDEP patient, with a rapid transition between sympathetic and parasympathetic activity. In addition, a combination of severe hypopnea (202 s) and bradycardia (10 s), illustrating autonomic dysfunction, was found after one of the SUDEP patient's FIAS.

Discussion: The unusual cardiorespiratory and HRV patterns found in this case indicated autonomic abnormalities that were possibly predictive of an increased risk of SUDEP. It will be interesting to perform similar analyses in other SUDEP cases to see whether our findings are anecdotal or instead suggestive of reliable biomarkers of high SUDEP risk in focal epilepsy, in particular focal with or without impaired awareness seizures.

KEYWORDS

epilepsy, SUDEP, focal seizures, HRV, autonomic changes

1. Introduction

Sudden unexpected death in epilepsy (SUDEP) refers to the sudden, unexpected, witnessed or unwitnessed, nontraumatic and nondrowning death in patients with epilepsy (1). SUDEP is not only the leading epilepsy-related cause of death (2), but also the second neurological cause of total potential life-years lost, only behind stroke (3). Mechanisms underlying SUDEP are still not fully understood but cardiorespiratory and autonomic dysfunctions in the postictal phase are generally thought to play an important role (4). Identified risk factors for SUDEP include generalized tonic–clonic seizures (GTCS), temporal lobe epilepsy, nocturnal seizures, and prone sleeping position (4). A history of GTCS and presence of GTCS in the last year were associated with a 10- and 27-fold increase in SUDEP risk, respectively (5). In recent years, numerous papers have been published regarding autonomic changes in GTCS and detection of this subtype of seizure (6); those findings have given hope to people with epilepsy (PwE) suffering from GTCS that quicker interventions during seizures and better prevention of SUDEP is possible, even though no quality evidence is present to support the claim that increased knowledge, detection and diagnosis of GTCS have led to decreased SUDEP mortality (7). To the contrary, little research has been conducted regarding peri-ictal changes in focal with impaired awareness seizures (FIAS) and their link to SUDEP. In this work, we retrospectively analyzed the interictal (awake and sleep) and peri-ictal heart rate variability (HRV), as well as peri-ictal heart rate (HR) and breathing rate (BR) of FIAS of a patient who suffered from a "probable SUDEP" 3 weeks following presurgical investigation at our epilepsy monitoring unit. The goal was to determine whether autonomic changes could be seen in between or during FIAS, and assess whether these changes, if seen, could be considered as potential markers for an increased risk of SUDEP.

2. Methods

This study was performed in accordance with the guidelines set forth by our local Institutional Review Board regarding patient consent. Written consent was obtained from next of kin.

2.1. Patients

The SUDEP patient's medical chart was reviewed to retrieve clinical information, neuroimaging findings, and video-electroencephalography (VEEG) results. We also included a control group of two living male patients from our local database matched for age, sex, and location of the epileptic focus (confirmed by continuous video-surface EEG).

2.2. Interictal and peri-ictal HRV analyses

Electrocardiogram (ECG) recordings of the SUDEP patient and the living control group, acquired during VEEG monitoring, were used to retrospectively analyze HRV. In order to study *interictal* HRV, six 5-min awake segments and six 5-min sleep stage II (N2) segments were selected following recommendations by Myers et al. (8) for HRV analysis in patients with epilepsy: (1) at least 8 h after the last tonic–clonic seizure; (2) at least 1 h after the last known electroencephalographic seizure, and (3) at least 1 h before the subsequent seizure. For *peri-ictal* HRV analysis, electroclinical seizures which lasted more than 2 min with a reliable R peak detection on ECG recordings were chosen; the minimal 2 min duration established here is often used for ultra-short term HRV analysis and represents the minimal window for root mean square of successive RR interval differences (RMSSD) analysis in athletes (9). The preictal and postictal periods were, respectively, 6 and 18 min in duration, each divided into three and nine 2-min epochs (18 min were used here vs. 17 min for the cardiorespiratory analysis described below to allow us to have nine epochs of equal duration, 2 min in this case). The interictal and peri-ictal ECG data were exported from the VEEG recording system (Nihon Koden) into European data format (EDF) files. The Brain Vision Analyzer 2.1 commercial software was initially used to identify R peaks from ECG data, then visually inspected to detect and manually correct artifacts, missed beats, or ectopic beats. Ectopic beats were removed from the recordings and replaced by an interpolated R-R interval. The Kubios HRV 3.2.0 software was used to calculate the standard HRV parameters, including the RMSSD, low-frequency power (LF), high-frequency power (HF), and the LF/HF ratio (10). The Wilcoxon Signed-Rank statistical test was used to assess whether a statistically significant difference of HRV metrics existed between the patient with SUDEP and the controls. Bonferroni correction was applied to account for multiple comparisons. The significance level (alpha) using this correction was set at $\alpha = 0.0125$.

2.3. Cardiorespiratory analysis

A postictal cardiorespiratory analysis was conducted on selected seizures of the SUDEP patient and of the control group using the BR and HR as measured with respiratory bands embedded within the Hexoskin smartwear (Carré Technologies Inc., Montreal, Canada), which was worn by the patients during their stay at the epilepsy monitoring unit. Inclusion criteria for analyzed seizures were the following: (1) FIAS that lasted more than 45 s, (2) without evolution to bilateral convulsive seizures. These inclusion criteria, applied to the active control group, were used to ensure that seizures of similar duration and type (FIAS) to those of the SUDEP patient were used; minimum seizure duration for the SUDEP patient was 58 s. The postictal period was defined as the 17-min period after seizure termination. Postictal duration was based on the MORTEMUS study, where SUDEP was seen up to 17 min postictally (4). Apnea and hypopnea were defined as a complete absence of respiratory movements for ≥10 s and as a reduction of ≥50% in ventilation with signs of arousal, respectively, based on guidelines from the 2017 American Academy of Sleep Medecine Manual (11).

It is important to note that for the analyses that were performed here, we did not consider that the control group had "0 risk of SUDEP." As they are still alive at this moment, SUDEP risk will be present until they suffer from a non-SUDEP death. However, we based our analysis on the principle that the controls, when compared to the SUDEP case, were less at risk of SUDEP at the same age range because they were still alive. This allowed us to infer that findings in the control group could reasonably be compared with the SUDEP case and suggest hypotheses based on those findings.

3. Results

3.1. Case presentation

A 33-year-old male, with drug-resistant epilepsy since age 22 years, was admitted to our epilepsy monitoring unit for presurgical assessment. He reported having 2–3 FIAS per month without or with an aura (visual flashes, dizziness). Focal to bilateral tonic–clonic seizures (FBTCS) occurred once per year on average, with two of them evolving into status epilepticus in the context of nonadherence to anti-seizure medication (ASM). His cardiac history was unremarkable apart from a transient increase in the length of the ECG P wave which was observed at the emergency department after two episodes of FBTCS in 2011 and 2016. This anomaly spontaneously resolved on the third-day control ECG for both episodes. Other ECGs were normal.

Upon his admission to our unit, the neurological examination was normal. Neuropsychological evaluation showed significant impairment of both verbal and non-verbal memories. VEEG recordings revealed bilateral temporal interictal epileptiform discharges with left predominance. Twelve FIAS (eight during sleep and four while awake) and two electrical seizures (both while awake) were recorded, with a left temporal onset in eight and right temporal in three; three were non-lateralized. One seizure occurred prior to medication withdrawal (brivaracetam 200 mg/day, extended-release carbamazepine 1,200 mg/day and clobazam 20 mg/day), one after brivaracetam was weaned, 10 after both brivaracetam and carbamazepine had been weaned, and two while off all antiseizure medications. No postictal generalized EEG suppression patterns were observed. Brain MRI identified left mesial temporal sclerosis and a small right anterior temporal cavernous angioma. Positron emission tomography scan revealed left anterior temporal hypometabolism. Ictal single emission computed tomography showed an activation in the left anterior temporal lobe which extended to the inferior insula. At day 12, the patient was discharged with lacosamide (200 mg/day), extended-release carbamazepine (1,200 mg/day), and clobazam (10 mg/day). Lacosamide was only started upon discharge. At the 2-week follow up, the patient reported no seizures. Three weeks after his discharge, the patient died during the night, at home, alone. A coroner's investigation, without autopsy, concluded that the most probable cause of death was an epileptic seizure. His death fulfilled the criteria for a "probable SUDEP" (1).

3.2. Presentation of controls

The two control patients were aged 34 and 24 years old, respectively. Both lived with their family and were not married; it is uncertain whether they shared a room with a family member. Duration of epilepsy was 20 and 6 years, respectively. Both controls had around 3–5 FIAS seizures per month, with a history of FBTCS in the last year. Both have had nocturnal seizures (albeit not exclusively) though none were recorded during their stay in the EMU. The first control patient had a congenital infarct in the right middle cerebral artery territory. The second control patient had non-lesional bitemporal lobe epilepsy confirmed by VEEG recordings. The first control patient was taking levetiracetam (4 g/day), valproic acid (1,250 mg/day), and topiramate (300 mg/day) while the second was on eslicarbazepine (1,000 mg/day), topiramate (200 mg/day), and clobazam (30 mg/day).

3.3. Interictal HRV

Table 1 shows awake and sleep HRV values of the SUDEP patient compared to control subjects with bitemporal lobe epilepsy (biTLE) matched for age and sex. All awake HRV parameters except LF/HF values were significantly lower for the SUDEP patient as compared to those of the control subjects ($p < 0.0125$). Similarly, during N2 stage sleep, all parameter values of the SUDEP patient except LF were significantly lower than those of the control subjects ($p < 0.0125$ for RMSSD, HF, and LF/HF).

TABLE 1 Awake and sleep HRV analysis results.

HRV metrics	Awake state		
	SUDEP Patient—Median (IQR)	Non-SUDEP biTLE patients (2)—Median (IQR)	Value of *p*
RMSSD (ms)	33.3 (27.0–36.3)	66.0 (44.7–78.5)	0.0027*
LF (ms^2)	942.9 (601.4–1318.4)	2961.7 (1867.0–1983.0)	0.0014*
HF (ms^2)	215.3 (152.0–254.2)	1187.7(451.1–2500.4)	0.0011*
LF/HF	5.1 (2.7–6.9)	3.3 (2.3–4.9)	0.1252
	Sleep state (N2 sleep stage)		
	SUDEP patient - Median (IQR)	**Non-SUDEP biTLE patients (2) - Median (IQR)**	**Value of *p***
RMSSD (ms)	28.9 (26.4–31.8)	85.8 (68.9–99.9)	*0.0003**
LF (ms^2)	918.4 (506.4–1462.8)	1709.1 (1086.5–3,168)0.0	0.0329
HF (ms^2)	255.4 (106.3–269.5)	2161.2 (1555.8–2897.6)	0.0003*
LF/HF	6.6 (2.3–11.9)	0.98 (0.5–1.8)	0.0013*

biTLE, bitemporal lobe epilepsy; HRV, heart rate variability; RMSSD, root mean square standard deviation; HF, high frequency; LF, low frequency; REM, rapid eye movement; IQR, interquartile range; SD, standard deviation; *statistically significant, after Bonferroni correction.

3.4. Peri-ictal HRV

For peri-ictal HRV analyses, the SUDEP patient had five FIAS that met our inclusion criteria (duration of >2 min and with clearly identifiable R peaks), of which, four were diurnal (seizures 1, 3–5), and one was nocturnal (seizure 2). Five seizures of the control group were also selected to analyze peri-ictal HRV (three FIAS and two FBTCS). None of the control patients had nocturnal seizures during their VEEG. In addition to FIAS, two FBTCS were selected from controls to compare changes seen in FIAS of the SUDEP patient to FBTCS from controls. This allowed us to determine whether FIAS of the SUDEP patient were more severe from an autonomic standpoint, and therefore evaluate if FIAS could be used as reliable markers of SUDEP risk, similarly to FBTCS in the current literature. Figure 1 shows HRV changes during preictal, ictal, and postical periods of the five FIAS from the SUDEP patient. Figure 2 shows HRV changes during preictal, ictal, and postical periods of the five seizures of the two living control subjects. Table 2 shows pre-ictal, ictal, and post-ictal results for the HRV analysis comparing the SUDEP case and controls.

3.4.1. Heart rate

For all seizures of both groups, we observed the same pattern of an ictal increase in HR followed by a decrease in the postictal period. HR decreased after seizure termination in both the SUDEP patient and controls, but mean postictal HR was higher than preictal mean HR in both groups (Table 2).

3.4.2. Root mean square of successive differences between normal heartbeats

The SUDEP patient had preictal RMSSD values that were 59.92–75.24% lower than those of the controls (Table 2), with a statistically significant difference being noted in preictal RMSSD values between the two groups ($p < 0.05$). In the SUDEP patient, a 21.97% increase in ictal RMSSD was seen when compared to preictal median RMSSD. In controls,

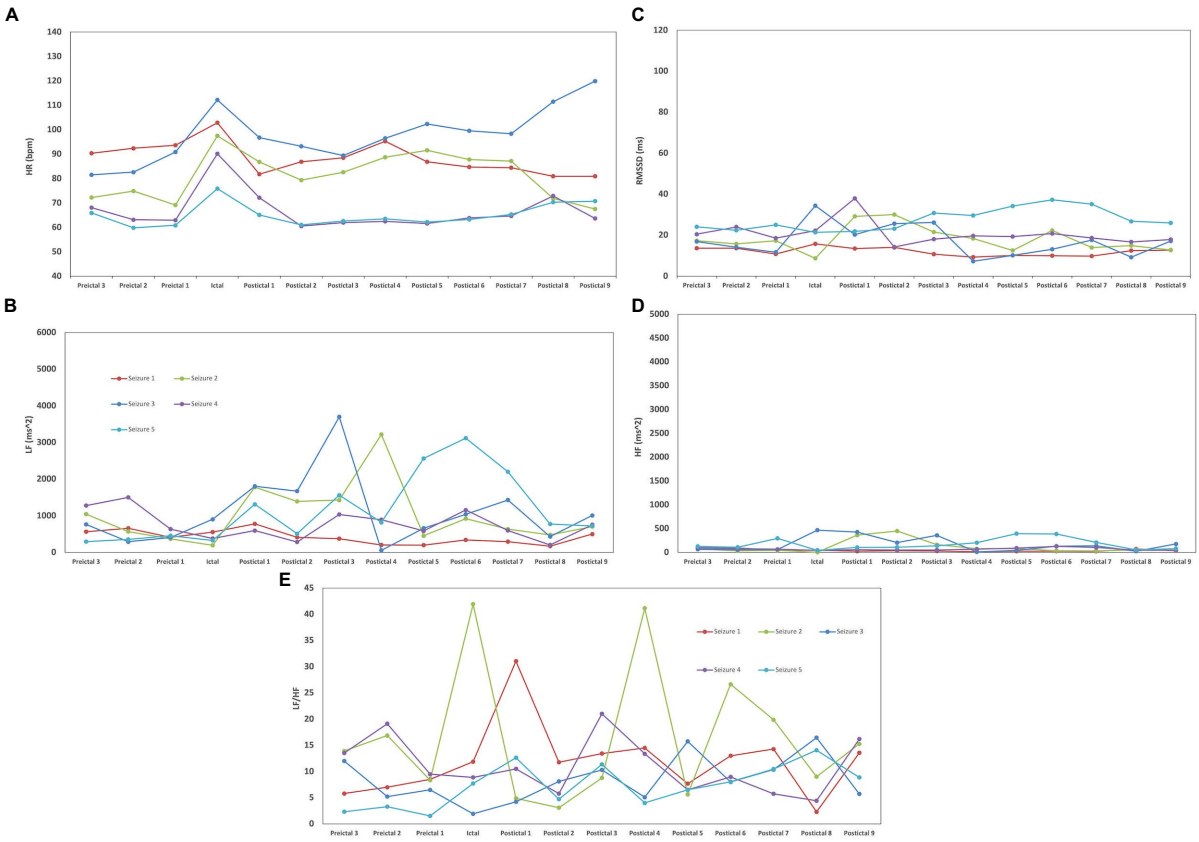

FIGURE 1
Heart rate variability (HRV) changes during preictal, ictal, and postical periods of five focal with impaired awareness seizures (FIAS) for the sudden unexpected death in epilepsy (SUDEP) patient. **(A)** For all seizures of both groups, we observed the same pattern of an increase in HR during the seizure and a decrease in postical HR when compared to the preictal state. In seizures 2 and 3, postictal mean HR was increased when compared to preictal mean HR (19.05–20.17%). **(B)** Delayed LF postical recuperation was seen, particulary in the nocturnal seizure of the SUDEP patient. In all seizures of the SUDEP patient, LF continued to increase in the first postictal period (39.8–819%) when compared to ictal LF. **(C)** In the SUDEP patient, a 21.97% increase in ictal RMSSD was seen when compared to preictal median RMSSD. Preictal RMSSD were 59.92–75.24% lower than those of the controls, with a statistically significant difference being noted in preictal RMSSD values between the two groups ($p < 0.05$). **(D)** HF values were 92.45–97.18% lower than those of the controls. **(E)** LF/HF ratio was increased in all epochs in the SUDEP case when compared to controls; there was a significant difference in the postictal LF/HF ratio between the SUDEP patient and controls ($p < 0.05$). In seizure 1 of the SUDEP patient, a pattern of important increase (161.24%) and decrease (62.05%) was seen between the ictal period and second postictal period. In seizure 2 of the SUDEP patient, a similar pattern was observed, with LF/HF ratio being increased by 401.40% during the seizure before decreasing by 88.31% during the 1st postictal epoch. A similar increase in LF/HF (368.33%) and decrease (86.33%) was seen between the third and fifth postictal periods. No similar variations were noted in the other three seizures of the SUDEP patient.

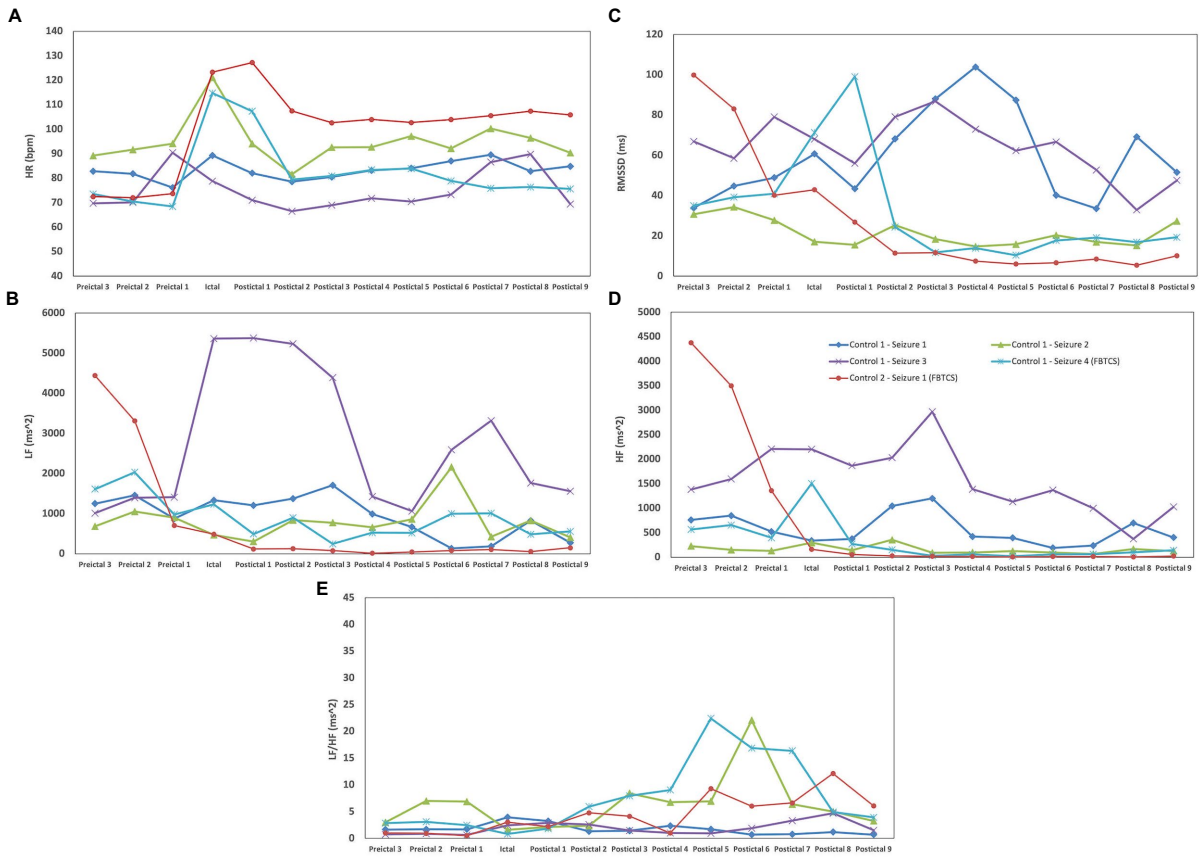

FIGURE 2
HRV changes during preictal, ictal, and postical periods in five seizures of two controls with biTLE. **(A)** For all seizures of both groups, we observed the same pattern of an increase in HR during the seizure and a decrease in postical HR when compared to the preictal state. **(B)** In 4/5 seizures of the control group, postictal LF recuperation was seen immediately after seizure termination; in 1/5 seizures of the control group (seizure 3 of control #1), LF postictal recuperation was only seen 8min following seizure termination. **(C)** In controls, RMSSD generally increased during seizures, followed by an important decrease in postictal. **(D)** No postictal mean HF increase was noted in both controls when compared to preictal HF. **(E)** In seizure 2 of control #1, an important and rapid increase (218.33%) and decrease (71.07%) in LF/HF was seen between the fifth and seventh postictal periods. No similar variations were noted in the other four seizures of the controls.

RMSSD increased during seizures (22.37%), followed by an important decrease when compared to the median preictal value (−137.45%).

3.4.3. Low frequency

Preictal LF values were higher in controls than in the SUDEP case but these differences were not statistically significant after Bonferroni correction ($p > 0.0125$). Also, in all seizures of the SUDEP patient, LF postictal recuperation was not seen immediately in the first postictal epoch, particulary for the nocturnal seizure; LF continued to increase in the first postictal period (39.8–819%) when compared to ictal LF (Figure 1). On the other hand, in all seizures of both controls, LF postictal recuperation was seen immediately after the first postictal epoch, with no continued increase in LF following seizures (Figure 2).

3.4.4. High frequency

The SUDEP patient had preictal HF values that were 92.45–97.18% lower than those of the controls, with this difference being statistically significant ($p < 0.05$). In 2/5 seizures (seizures 2 and 3 of Figure 1) of the SUDEP patient, postictal HF was increased when compared to preictal HF (75.98–120.92%), but mean postictal HF of all combined seizures was not increased when compared to preictal HF. In both controls, the postictal mean HF was not increased when compared to preictal HF.

3.4.5. LF/HF ratio

LF/HF ratio was increased in all epochs of the SUDEP case when compared to controls; there was a significant difference in the postictal LF/HF ratio between the SUDEP patient and controls ($p < 0.05$). In seizure 1 of the SUDEP patient, an initial pattern of important increase (161.24%) followed by decrease (62.05%) in LF/HF Ratio was seen between the ictal period and second postictal period. In seizure 2 of the SUDEP patient, a similar pattern was observed, with LF/HF ratio being increased by 401.40% during the seizure before decreasing by 88.31% during the first postictal epoch. A similar increase in LF/HF (368.33%) and decrease (86.33%) was seen between the third and fifth postictal periods. No similar variations were noted in the other three seizures of the SUDEP patient. In seizures of both controls, a similar pattern was only seen in seizure 2 of control subject #1 (see Figure 2), but with a late onset, between the fifth and seventh postictal epochs. All patterns mentioned above can be seen in Figures 1, 2.

TABLE 2 Peri-ictal HRV analysis results.

HRV metrics	SUDEP Patient—Median (IQR; n_seizures=5)	Non-SUDEP biTLE patients (2)—Median (IQR) (n_seizures=5)	*p* value
Pre-Ictal			
HR (bpm)	73.3 (72.6–75.6)	78.0 (76.7–81.5)	0.8337
RMSSD (ms)	17.5 (16.4–18.2)	43.6 (40.5–45.9)	0.0121*
LF (ms^2)	568.6 (478.1–696.0)	1262.0 (1118.0–1355.6)	0.0366
HF (ms^2)	76.5 (67.6–92.1)	566.9 (503.0–648.2)	0.0121*
LF/HF	7.2 (6.2–8.7)	2.2 (2.0–2.4)	0.0601
Ictal			
HR (bpm)	97.5 (90.2–102.9)	114.8 (89.3–121.1)	0.4009
RMSSD (ms)	21.4 (15.8–22.2)	60.7 (42.9–68.0)	0.0601
LF (ms^2)	378.7 (328.9–557.3)	1234.7 (486.4–1333.5)	0.0949
HF (ms^2)	42.6 (42.5996–46.9023)	337.1 (297.3–1506.2)	0.0601
LF/HF	8.9 (7.7–11.9)	2.4 (1.6–3.0)	0.0601
Post-Ictal			
HR (bpm)	78.2 (75.4–80.1)	81.2 (79.6–84.1)	0.6745
RMSSD (ms)	18.0 (14.6–20.8)	33.6 (26.5–39.8)	1.0000
LF (ms^2)	761.0 (521.0–1140.5)	965.6 (546.9–1414.0)	0.6745
HF (ms^2)	73.8 (47.7–117.2)	254.9 (215.9–404.0)	0.6745
LF/HF	9.5 (6.8–13.5)	3.4 (2.1–4.9)	0.0120*

biTLE, bitemporal lobe epilepsy; HRV, heart rate variability; RMSSD, root mean square standard deviation; HF, high frequency; LF, low frequency; REM, rapid eye movement; IQR, interquartile range; SD, standard deviation; *statistically significant, after Bonferroni correction.

3.5. Cardiorespiratory analysis

For the cardiorespiratory analysis, 14 seizures of the SUDEP patient and five seizures of the control group matched our inclusion criteria. Of those seizures, 13/14 of the SUDEP patient's seizures and 4/5 of the control group's seizures were unremarkable (no abnormal cardiorespiratory patterns seen during the postictal epochs) and thus are not described here. Figure 3 illustrates the delayed postictal cardiorespiratory recuperation observed following one of the SUDEP patient's seizures. The FIAS (lasting 90 s) was followed by immediate and simultaneous tachycardia (mean HR = 116 bpm) and hypopnea (mean BR = 9.9 rpm) for 15 and 12 s, respectively. This was immediately followed by an abrupt decrease in HR resulting in bradycardia for 10 s (mean HR = 59.4 bpm) and more severe hypopnea for 202 s (mean BR = 6.4 rpm); in comparison, the patient's HR and BR at rest were 74 bpm and 15 rpm, respectively. In one of our control's seizures, a similar pattern of simultaneous hypopnea and bradycardia was observed, with BR and HR between 10–6 rpm and 60–58 bpm, respectively, for 8 s (between 279 and 288 s postictally). However, the onset of this abnormal pattern was seen more than 4 min following seizure termination (Figure 3).

4. Discussion

4.1. SUDEP risk factors

The SUDEP patient had three main risk factors for SUDEP, namely a history of GTCS, the presence of nocturnal seizures and the fact that he was sleeping alone. A recent study showed that the risk of SUDEP increased 15- and 5-fold in patients with nocturnal GTCS in the last year of observation and in patients living alone, respectively (5). Other SUDEP risk factors present in this patient were the following: refractory epilepsy, temporal lobe epilepsy, and a history of GTCS (5). Temporal lobe epilepsy has been reported to increase the severity of postictal hypoxemia when compared to extratemporal lobe epilepsy (12). It has also been shown to induce abnormal and sustained brainstem and autonomic activation when compared to frontal lobe epilepsy, mainly in the postictal phase (13), potentially leading to parasympathetic hyperactivity, and thus increased SUDEP risk (14). Furthermore, this patient had biTLE, with a left temporal onset predominance (eight left temporal vs. three right temporal). In a recent study, left temporal lobe epilepsy (L-TLE), when compared to right temporal lobe epilepsy (R-TLE) showed increased parasympathetic activity (15). L-TLE predominance therefore could have put the patient at an increased risk of elevated peri-ictal parasympathetic tone, and thus SUDEP. Furthermore, presence of R-TLE also suggests a potential risk of sympathetic overdrive, which can lead to autonomic disturbances and imbalances when coupled with L-TLE. Ultimately, biTLE leads to risk of autonomic disturbances related to important variations in sympathetic and parasympathetic activity.

4.2. Awake and sleep HRV findings

During resting awake time and N2 sleep stage, RMSSD, LF, and HF were all reduced in the SUDEP patient when compared to age and

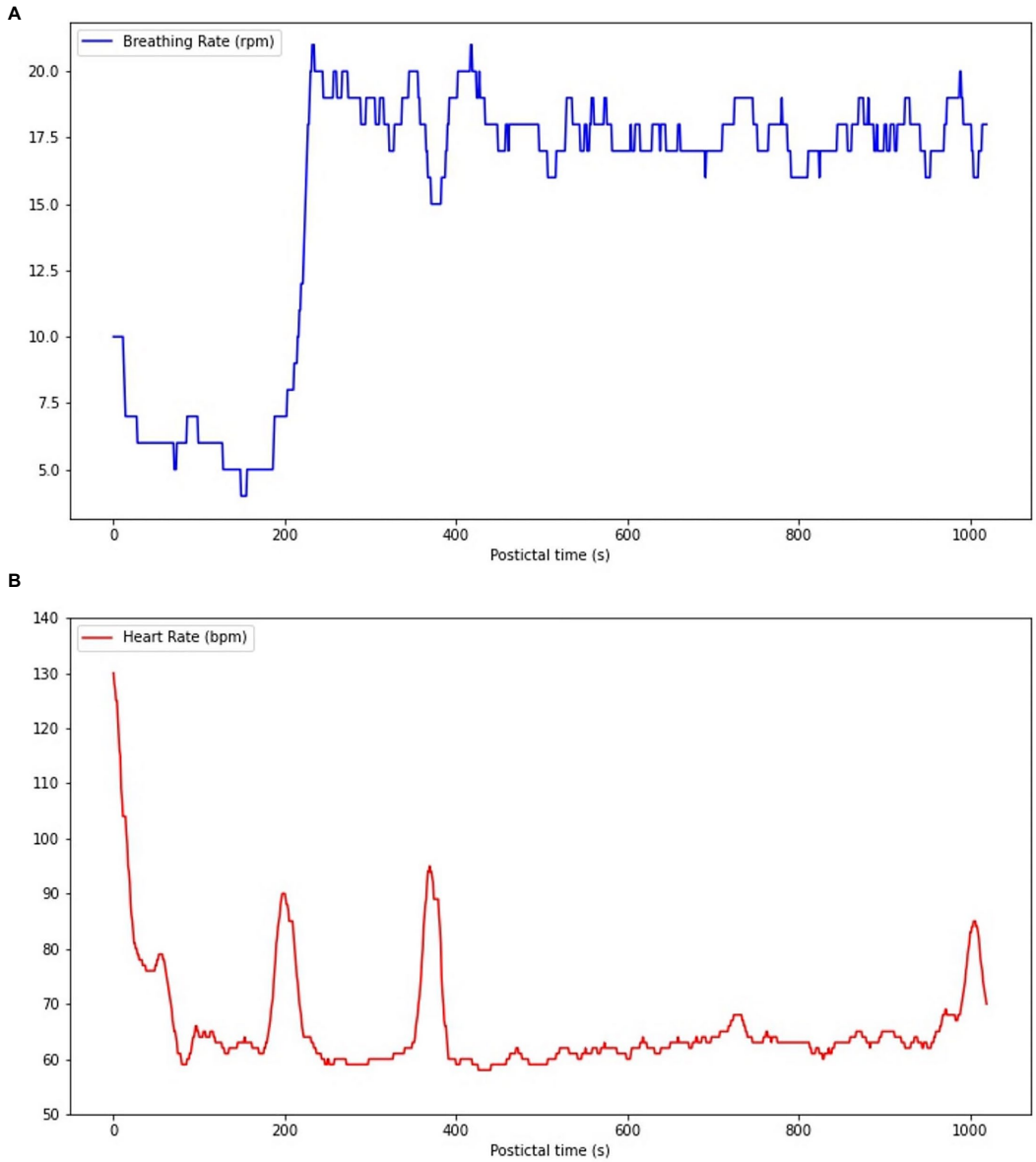

FIGURE 3
Cardiorespiratory functions during the postictal phase following one of the SUDEP patient's FIAS. **(A)** A hypopnea (mean BR=9.9rpm) immediately follows seizure termination for 12s, with BR decreasing to 4rpm. **(B)** A tachycardia (mean HR=116bpm) is present following seizure termination for 15s, but HR rapidly decreases to 59bpm, resulting in a bradycardia for 10s (mean HR=59.4bpm).

sex-matched controls with biTLE. Reduced short-term LF is currently considered as a valid biomarker for sudden cardiac death (16) and was associated with SUDEP in a recent study on 31 SUDEP cases and 56 living epileptic controls (17), while increased HF at rest was associated with longer survival and was potentially cardioprotective in SUDEP in this same study (17). HF and RMSSD reflect parasympathetic activity to the heart (vagal drive) (18), and an increase in vagal tone was found to be cardioprotective (19). This is further supported by the fact that a 25% reduction of SUDEP events was seen in patients with refractory epilepsy who were treated with adjunctive vagus nerve stimulation (VNS) therapy when compared to patients with refractory epilepsy not treated by VNS (20). However, while vagal tone can be considered as cardioprotective, SUDEP cases in VNS patients have been reported; while no hypothesis has been proposed to why VNS therapy does not eliminate SUDEP, this is most probably due to the inability of VNS to prevent complete recurrence of FBTCS, the main SUDEP risk factor (5). Also, many deaths resulting from SUDEP are due to pillow suffocation, as this is purely mechanical and not related to autonomic and physiological functions (18).

Increased RMSSD at rest was seen in high level athletes during high training loads in preparation for competitions (19). Increased awake and sleep RMSSD and increased sleep LF were seen in moderately trained individuals when compared to sedentary individuals (20). Lower HF, LF, and RMSSD, as seen in our patient, were seen in patients with chronic refractory epilepsy when compared to healthy controls; in the same study, RMSSD was negatively correlated with SUDEP-7 risk (21). This suggests that people with chronic refractory epilepsy, such as the SUDEP patient, do not have

the cardiovascular adaptations to training and efforts, from frequent seizures in this case, seen in moderately and highly trained athletes. Those cardiovascular adaptations would protect the patient from a potential cardiopulmonary breakdown resulting from the consequences of intensive and sustained ictal manifestations of a FBTCS that would lead to important oxygen demand, and thus desaturation. This non-adaptative cardiovascular state could have put this patient at risk in the context of an intense and brief physical and cerebral effort, as seen in a FBTCS. Therefore, the findings mentionned above are in line with our results, which suggest that the SUDEP patient, with reduced HF, RMSSD, and LF at rest, could potentially have been at an increased risk of SUDEP; he might not have had the expected cardioprotective adaptation to his epilepsy. Based on this analysis, this patient could have had autonomic dysfunctions, possibly due to abnormalities in the brainstem's cardiorespiratory regulation centers. The transient anomalies of the length of P-waves observed after two episodes of FBTCS (see *Case presentation*) possibly reflect the increased cardiorespiratory vulnerability in this patient.

4.3. Peri-ictal HRV findings

In two seizures (seizures 2 and 3) of the SUDEP patient, postictal mean HF and HR were increased when compared to preictal mean HF and HR. Increased postictal mean HR (vs. preictal HR) and reduced preictal mean HF (vs. postictal HF) in GTCS are both associated with increased GTCS severity (22). In our SUDEP patient, this pattern was seen with FIASs and not GTCSs, suggesting that the SUDEP patient's seizures were of an important severity. One could hypothesize that if those patterns are seen in FIAS, which result in less oxygen and energy consumption when compared to FBTCS, any FBTCS in this patient would be this much more severe. In all seizures of the SUDEP patient, LF continued to increase in the first postictal period when compared to ictal LF, while this was not seen in both controls. LF is believed to represent cardiac sympathetic drive to the heart (23). Our patient had an overall increased sympathetic activity seen through all epochs, with an LF/HF ratio that was significantly increased postictally when compared to controls ($p < 0.05$). The sudden sympathetic activity increase following a seizure and the overall increased sympathetic activity through all epochs could suggest that an autonomic shift toward an abnormally increased sympathetic drive, particularly in the postictal period, is occurring which could lead to cardiorespiratory breakdown by increased and sustained workload and oxygen demand, and thus severe dyshomestoasis, and ultimately SUDEP. However, sympathetic activity could also counteract parasympathetic activity, which inhibits cardiorespiratory functions, therefore acting as a potential protective mechanism against depressed vital functions, and thus SUDEP. In two seizures of the SUDEP patient (one nocturnal and one awake), an important increase in LF/HF ratio (368.33–401.40%) was seen followed by an important decrease (86–33-88.31%) after 4 min. LF/HF ratio is believed to represent the autonomic balance between parasympathetic (LF and HF) and sympathetic (LF) activity (23). A rapid increase in LF/HF ratio would suggest an increased and hyperactive sympathetic system while a rapid decrease in LF/HF ratio would suggest an increased and hyperactive parasympathetic activity. The pattern seen in two seizures of our patient, a rapid sympathetic activity increase followed by rapid parasympathetic activity increase, suggests an autonomic vulnerability and hypersensitivity that could lead to severe dyshomeostasis and cardiorespiratory imbalance. The protective effect of sympathetic activity discussed above is therefore less valuable when it precedes parasympathetic activity, as the latter is not countered; cardiorespiratory functions are therefore inhibited after being greatly stimulated, leading to severe imbalance. Variability, especially in LF/HF ratio, can be seen between the five analyzed seizures of the SUDEP patient. Three of the five seizures had a left temporal onset with contralateral spread, one had an unknown onset with bilateral implication and the last one had a right temporal onset with contralateral spread. Therefore, the onset foci are not consistent throughout those seizures, which could explain the difference in HRV parameter variations. As mentionned above, R-TLE and L-TLE have different effects on the autonomic system, with L-TLE showing increased sympathetic activity (15) (Figure 4).

Ultimately, those pericital HRV findings suggest that the SUDEP patient's FIAS were autonomically more severe and intense when compared to those of other patients with epilepsy. This increased risk of autonomic imbalance might have put him at a higher risk of adverse postictal events. We presume that a more intense seizure, such as a FBTCS occurring in sleep, could have triggerred a fatal autonomic deterioration which led to his SUDEP.

On the other hand, the SUDEP patient had significantly lower preictal RMSSD and HF values when compared to both controls. In a study by Jepessen et al. (2014), a 25-year-old patient who died of SUDEP had increased preictal parasympathetic activity preceding his GTCS when compared to 11 GTCS from 11 male patients with epilepsy with a median age of 30.5 years (24). Although our study yielded opposing results, other HRV parameters seen in our patient and mentioned above suggest that the SUDEP patient presented autonomic dysfunctions frequently encountered in other cases of SUDEP. Therefore, the peri-ictal HRV pattern seen in our patient reinforces the idea that no clear variation in HRV parameters can be identified as a general biomarker of increased SUDEP risk in patients with epilepsy. However, the general tendency in our analyses suggests that this patient had abnormal HRV patterns, which further supports our suggestion that he was at an increased risk of SUDEP.

4.4. Postictal cardiorespiratory findings

Following symptomatic seizures, tachypnea and tachycardia are usually observed as adaptative transient cardiorespiratory hyperactivity to enable a fast recovery from the ictal oxygen debt (25). Even in a suboptimal adaptation scenario, any postictal decrease of HR should be compensated by a larger increase of BR, and vice versa. However, one of our patient's FIAS was followed by severe hypopnea and an abrupt HR decrease shortly after seizure termination (Figure 2). Such an unexpected cardiorespiratory pattern carries a risk of severe and potentially fatal postictal acid–base imbalance (26). This postictal phase resembles, to a lesser extent, the pattern of a neurovegetative breakdown that is known to classically lead to SUDEP, which consists of an initial tachypnea followed by apnea and bradycardia and ending with terminal apnea and asystole (4). The paradoxical cardiorespiratory postictal pattern observed may result from postictal dysfunction of respiratory and cardiovascular centers

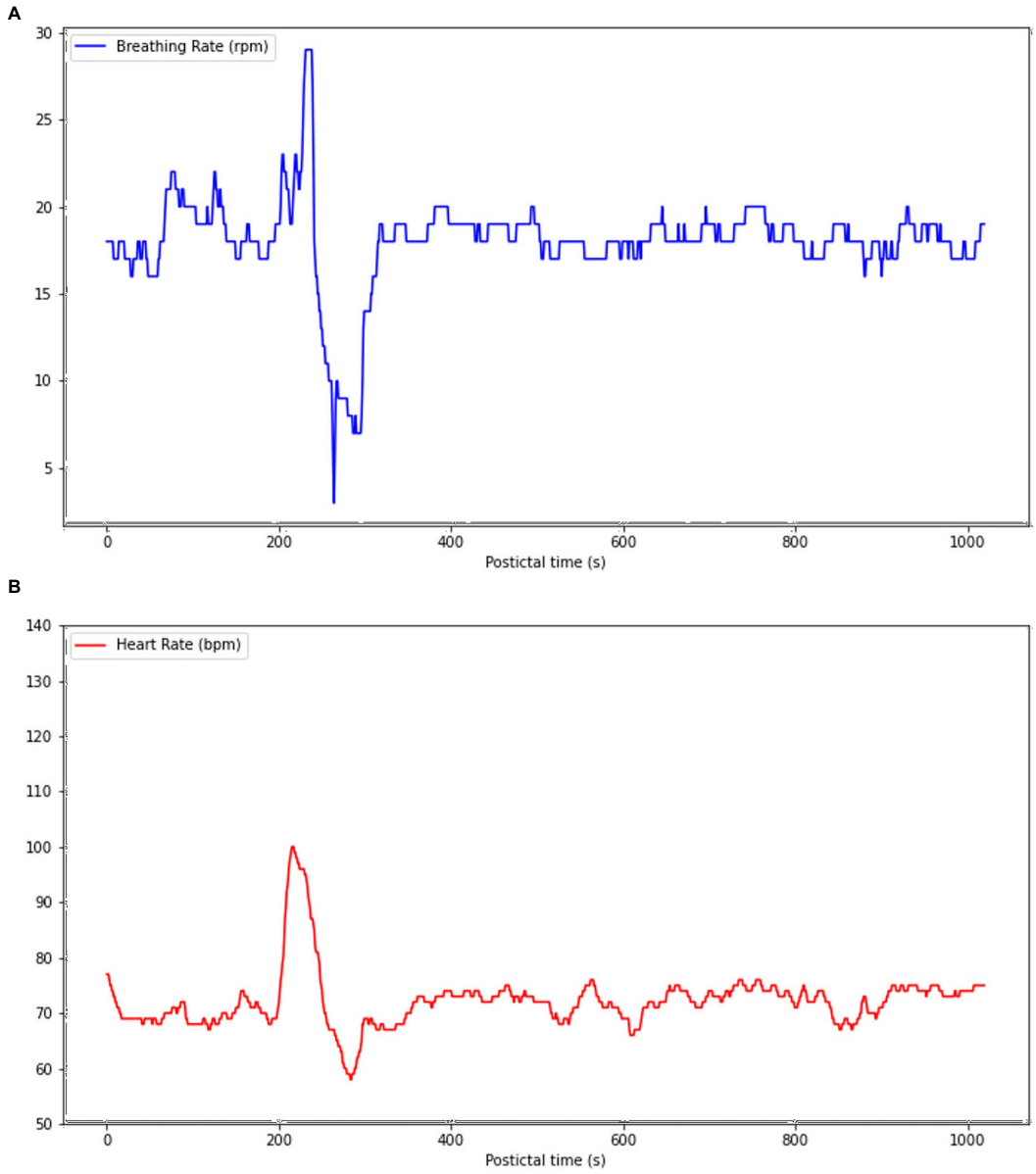

FIGURE 4
Cardiorespiratory functions during the postictal phase following one control's focal seizure with awareness (FAS). **(A)** Hyperpnea is present 200s following seizure termination, but BR rapidly decreases to 6rpm, resulting in hypopnea 279s following seizure termination. **(B)** A tachycardia is present simultaneously to hyperpnea, but HR rapidly decreases simultaneously to BR to a rhythm of 58bpm 279s, resulting in abnormal and simultaneous bradycardia and hypopnea.

of the brain stem (27). A study by Park et al. revealed that the cardiac arrythmogenic threshold is lowered when oxygen saturation decreases <90%, therefore suggesting that important ictal and postictal hypoxemia could facilitate the onset of fatal arrythmias such as ventricular tachycardia and sinus arrest (28). Following a FBTCS, one could hypothesize that such an abnormal and disorganized cardiorespiratory pattern can lead to severe dyshomeostasis or SUDEP. A similar pattern was observed in one of our control's seizures (see Figure 3), but onset of the abnormal pattern was seen more than 4 min following seizure termination while it was seen immediatly after seizure offset in reported SUDEP cases, making it is less likely that those cardiorespiratory changes were directly caused by seizure dyshomeoastasis.

4.5. Effects of medication on SUDEP risk

While in general, patients with epilepsy taking ASMs have a reduced SUDEP risk compared to non-treated patients (5), we recognize that some ASMs have potential cardiorespiratory effects. For example, patients with epilepsy on carbamazepine had reduced parasympathetic activity compared to other ASMs, which may pose a risk of SUDEP (29). In a cohort study, 10,241 patients with epilepsy under enzyme-inducing and non-enzyme-inducing ASM were found to be at an increased risk of cardiovascular events when compared to non-epileptic controls (32). Furthermore, in a recent study on transverse brainstem slices from mice, carbamazepine and lamotrigine were found to impair the gasping response during hypoxia, but not

during normoxia, suggesting a possible role of those specific sodium channel blocker ASMs in worsening postictal hypoxia (33), and therefore probably increasing SUDEP risk.

4.6. Limitations

Our case report has four main limitations: (1) sudden death occurred a week after his epilepsy monitoring unit stay and not during it, hence the lack of data during the dramatic event (31); (2) the patient did not have a FBTCS during VEEG monitoring, thus the lack of data during a convulsive seizure. Any of those elements would have provided a better understanding of autonomic disorders associated with more severe seizures; (3) the small size of this study increases the risk of sampling bias. We performed a selection based on patient availability in our database (seizures recorded during their stay in the EMU) and resemblance to the case (age, sex, and bTLE). Therefore, the selected patients might not represent the general epileptic population; (4) the control patients did not have nocturnal seizures during their stay in the EMU. Thus, we were not able to compare the nocturnal seizures from the SUDEP patient and the controls, which limits the extent of our conclusions. Finally, it should be noted that, for the cardiorespiratory analysis, HR and BR signals were acquired using a wearable device (Hexoskin) instead of regular medical devices (plethysmography and ECG). The Hexoskin was used because it allowed us to continuously measure breathing rhythm and heart rhythm in patients. Main advantages were that it allowed for continuous and automatic recording of physiological signals, and it was less obtrusive for patients. The Hexoskin Smartwear has been previously validated for the acquisition of reliable measurements of these signals (30). Also, acquiring experience in the use of such smartwear in the EMU could potentially lead us to eventually promote its use in an outpatient setting, which may provide new data to increase our understanding of SUDEP.

5. Conclusion

This case report shows intriguing cardiorespiratory and HRV features recorded at rest and postictally following a FIAS, weeks prior to SUDEP, that are suggestive of autonomic imbalance, and ultimately of increased SUDEP risk. Although the value of our observations is subject to confirmation by larger studies, it opens up new avenues of research regarding biomarkers of SUDEP risk. If our findings are verified by further studies, these features could help clinicians better assess SUDEP risk even with focal with or without impaired awareness seizures not evolving to bilateral tonic–clonic seizures at the epilepsy monitoring unit.

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 Research Ethics Board of the University of Montreal Hospital Research Center. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

DN, EB, and MR contributed to conception of the study. MR, EB, and YL organized the database. YL and TT performed the statistical analysis. YL wrote the first draft of the manuscript. YL, EB, DT, TT, and DN wrote the sections of the manuscript. A-AB, DN, and EB read, and approved the submitted version. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) (grant #: 415079), and the Institute for Data Valorization (IVADO) (grant #: 51628 and 3927033406).

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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frontiers | Frontiers in Neurology

TYPE Mini Review
PUBLISHED 26 April 2023
DOI 10.3389/fneur.2023.1169003

OPEN ACCESS

EDITED BY
Beixu Li,
Shanghai University of Political Science and Law, China

REVIEWED BY
Tiantong Yang,
China University of Political Science and Law, China
Ningguo Liu,
Academy of Forensic Science, China

*CORRESPONDENCE
Qiang Wang
✉ 956740870@qq.com

SPECIALTY SECTION
This article was submitted to Epilepsy, a section of the journal Frontiers in Neurology

RECEIVED 18 February 2023
ACCEPTED 20 March 2023
PUBLISHED 26 April 2023

CITATION
Sun D and Wang Q (2023) The application of SUDEP in forensic diagnosis: a mini review. *Front. Neurol.* 14:1169003. doi: 10.3389/fneur.2023.1169003

The application of SUDEP in forensic diagnosis: a mini review

Daming Sun and Qiang Wang*

Forensic Science Center, East China University of Political Science and Law, Shanghai, China

In the epilepsy population, the risk of sudden death from epilepsy is rare but is ~24 times greater than the risk of sudden death from other causes. Sudden unexpected death in epilepsy (SUDEP) has been widely recognized in clinical studies. Despite its significance as a cause of death, SUDEP is rarely used in forensic practice. This review focuses on the forensic characteristics of SUDEP, analyzed the reasons for its underuse in forensic practice, and illustrated the prospect of establishing uniform diagnostic criteria for sudden unexpected death in epilepsy and molecular anatomy in aiding forensic diagnosis.

KEYWORDS

epilepsy, medical examiner, forensic diagnosis, molecular anatomy, SUDEP

Introduction

The methodology used in epidemiological studies and the type of epilepsy population assessed have a significant impact on the incidence of SUDEP. Disparities in SUDEP diagnostic criteria often affect the results. In the epilepsy population, the risk of sudden death from epilepsy is rare but is ~24 times greater than the risk of sudden death from other causes (1).

Sudden unexpected death in epilepsy is defined as a sudden, unexpected, witnessed or unwitnessed death, in a non-traumatic or non-drowning manner, with or without evidence of a seizure, excluding documented status epilepticus, in which there is no toxicological or anatomical cause of death (2). The risk of premature death is two to three times higher in a person with epilepsy than in the general population (3). There are three types of causes of death in people with epilepsy as follows: those unrelated to the disease; those brought on by the disease; and those caused directly by the disease (4, 5). The diagnosis of death related to epilepsy is mainly divided into two categories. The first type of death occurs in the state of seizures, and the other type is caused by seizures, such as traffic accidents caused by seizures in daily life, hospital treatment-related deaths, and even deaths resulting from seizures caused by fire (5, 6). There is growing evidence that SUDEP is one of the leading causes of death among people with epilepsy. This evidence demonstrates that it accounts for up to 17% of all deaths among this population (6).

Current situation of SUDEP

General characteristics of the deceased

According to the current study, young people are more likely to be affected by SUDEP (7, 8). Despite this being most likely, there has been no difference in the incidence of SUDEP in older adults. Evidence suggests that the incidence of SUDEP in older adults is underappreciated (9). A slight increase in the probability of dying from SUDEP was also observed in men compared to women (10).

Circumstances of death

It is evident from previous studies that the majority of SUDEP deaths occurred at home (11–15), suggesting that all these individuals died while performing routine activities at home (7). A retrospective analysis of 67 cases revealed that the scenes of the deaths were similar, with 58 of them (87%) being discovered in their homes. A total of 38 cases (57%) were found dead on beds or couches. In terms of death posture, the body was discovered in a prone position majority of the time (16). Notably, most of these deaths were witnessed by no one (10, 12, 17, 18).

Examination of corpses

There was evidence of recent seizure activity (tongue lacerations and contusions or superficial abrasions and contusions on the skin) in all cases (58%) that underwent autopsy. Visceral congestion and visceral edema were present in addition to pulmonary edema (11, 14, 19). Aside from structural brain lesions (17), aneurysms and tumors were the most common causes of epilepsy as determined by autopsy findings, followed by cerebrovascular malformations/aneurysms and brain tumors (10).

Toxic (drug) substance test

According to a review of studies on postmortem physical and chemical detection, antiepileptic drugs (AEDs) and conventional poisons were determined as the cause of death in patients with SUDEP. The detection of antiepileptic drugs in postmortem cadaveric blood is classified into three major categories as follows: the presence of one antiepileptic drug, the presence of two antiepileptic drugs, and the presence of more than two antiepileptic drugs (16), with phenytoin being the most prevalent AED (10). Although there have been some evidence that the use of antiepileptic drugs may contribute to SUDEP, it is still unclear whether using more than one drug increases the risk of death. Several studies have suggested that the use of three AEDs may increase the risk of SUDEP by a factor of 10 when compared with monotherapy (20). Another study stated that SUDEP can occur even when only one anticonvulsant is administered at the therapeutic levels (18). Several possible explanations for the high mortality associated with combined therapy include disease severity, increased toxicity, highly variable, unpredictable, and complex drug interactions between antiepileptic drugs, the precipitation of central apnea following onset, and postural asphyxia caused by sedation-inducing combination therapy (6). The aforementioned studies have also shown that there is still a long way to go to clarify the pathological mechanism of SUDEP.

Classification of cause of death

SUDEP is rarely used in forensic practice as a cause of death. According to a retrospective study of 104 epilepsy-related autopsy cases in Maryland, SUDEP was cited as the cause of death in only 7.7% of cases, while seizures or epilepsies were cited in 63.5% of cases (10). Additionally, another national survey of unexplained deaths caused by epilepsy revealed that, in most cases, SUDEP was not the medical examiner's preferred diagnosis on the death certificate, even when the cause of death could not be determined by autopsy and other possible causes of death had been excluded (21). It has been reported that SUDEP may be underreported on death certificates in Sweden and Wake County, North Carolina, with <30% of SUDEP cases listing SUDEP, seizures, or epilepsies as the cause of death (22).

Discussion

Forensic diagnostic criteria and identification points of SUDEP

Undoubtedly, SUDEP refers to a catastrophic death in people with epilepsy. Its incidence can have a substantial impact on an individual, their family, and society as a whole. Previous studies have primarily centered on risk factors. However, the underlying mechanisms and preventive measures are still unclear, and there is no evidence that these measures are effective (23). In addition, the incidence of SUDEP is also difficult to determine due to its dependence on the anatomy of the system for its diagnosis (24).

The following criteria must be satisfied to make a definitive diagnosis of SUDEP (23):

1. A person with epilepsy problems.
2. In the course of normal daily activities, the individual died (e.g., during sleep).
3. A sudden and unexpected death occurred within minutes of the accident.
4. There was no other medical condition that would predispose the individual to death.
5. Neither aspiration, suffocation, drowning, nor status epilepticus were known complications of the seizure episode that caused death.
6. Unknown cause of death.

An autopsy may not be able to identify the cause of death when all six criteria are met and a diagnosis of definite SUDEP is made (23). Probable SUDEP is considered when all six criteria are met but an autopsy is skipped. In addition, probable SUDEP is a very demanding diagnosis, which is because it generally serves as a substitute for an autopsy while also meeting the six diagnostic criteria listed above. If all six criteria are met even though an autopsy was not performed, SUDEP is considered probable. The possibility of SUDEP is taken into account when the death exhibits features of SUDEP, but an autopsy has not ruled out other causes of death. According to a retrospective analysis of nine SUDEP cases collected in China from January 2005 to June 2019, the following points should be considered when identifying SUDEP forensically. The following conditions were excluded as causes of death: epilepsy in young men; death while sleeping in prone or left lateral decubitus positions; substance abuse or mixed drug use before death; symptoms of asphyxia and urinary incontinence; unilateral hemorrhage in the neck muscle group and/or bilateral

pectoralis minor muscle hemorrhage; pathological manifestations of respiratory depression and acute cardiac dysfunction; injuries, poisoning, and other organic diseases. Despite the small amount of data, SUDEP forensic identification may be guided by the summary of identification experience (16).

The reasons for the low diagnostic rate of SUDEP

The uncertainty of SUDEP

It is not uncommon for medical examiners to be uncertain about the cause of death, as they must determine what they believe to be the most likely cause of death. Medical examiners usually perform an autopsy or forensic toxicology test when an autopsy cannot determine the cause of death. Medical examiners often neglect to determine whether epilepsy contributed to the death or was the cause of it (25).

SUDEP is rarely used as a cause of death in China because of the low autopsy rate of deaths. In most cases, patients with epilepsy are not autopsied due to a lack of consent from their families or the high cost of autopsies (21). A number of factors affect the rate of autopsy. The study showed that the family's acceptance of the autopsy and the cultural background of the individual are important factors. Families in some parts of China are reluctant to destroy the body of the deceased to show their respect for the deceased (26). Despite this, the study suggests that the autopsy rate in China will increase in the future as the use of autopsies becomes more popular and the need to clarify the cause of death increases. It is also possible to improve the certainty of medical examiners for SUDEP by collecting comprehensive medical history data of patients with epilepsy using big data technology and by collecting case-related information. As a result, SUDEP may be considered a more reliable cause of death to some extent.

Lack of awareness of SUDEP

SUDEP is not widely recognized among forensic pathologists, and/or forensic pathologists have differing philosophical opinions about how epilepsy-related deaths should be classified or labeled (27). Maryland medical examiners were surveyed to determine their acceptance of SUDEP. Based on the results of the survey, medical examiners preferred to use seizure disorder or epilepsy rather than a term that implies an uncertain cause when completing death certificates, despite the high rate of acceptance of SUDEP as a valid diagnosis (14/15 medical examiners) (10).

Pathology training appears to be useful in assisting medical examiners in diagnosing SUDEP when no cause of death was found at autopsy, according to a study on the awareness and use of SUDEP by coroners and medical examiners in urban and rural America (28). In recent years, forensic practice and scientific research have contributed to a more comprehensive understanding of SUDEP among neurologists (29) and medical examiners (28). However, compared to other causes of death, this improvement is still very low (30).

To improve the diagnosis of SUDEP as the cause of death, the study suggests that corresponding educational programs should be developed. A national SUDEP surveillance program and an international standard for the investigation and postmortem examination of SUDEP death scenes are also necessary to collect accurate statistics on SUDEP incidence (10).

Application prospects of molecular autopsy

In 2016, the development and application of molecular anatomy were facilitated by high-throughput sequencing technology. Genetic factors contribute to the pathogenesis of epilepsy, which has a high prevalence in the population (31). Epilepsy is a condition caused by a dysfunctional ion channel. Additionally, a number of causal genes have been identified for epilepsy. As a result of the use of a mouse epilepsy model, KCNA1 knockout mice exhibited seizures, arrhythmia, increased vagal tone, and premature death (32), which was later validated in a human SUDEP case (33). According to another study, there is also evidence that CNVs on chromosome 15 are associated with autism, epilepsy, and SUDEP. In cases of SUDEP without autism, changes to CNVs in chromosome 15 can modify the risk of SUDEP (34). Finally, the hyperpolarization-activated cyclic nucleotide-gated ion channels HCN1-4 have been implicated in cardiac arrhythmias as well as epilepsy as they generate the cation (Na+ and K+)–triggered I_h depolarizing current that facilitates the generation of action potentials and spontaneous rhythmic activity in neurons and pacemaking cardiomyocytes (35–40).

As a result of expanding the SUDEP gene profile, medical examiners can recognize molecular anatomy more quickly and accurately. The detection of high-risk genes can help medical examiners make a definite diagnosis of SUDEP to a certain extent. However, there are also some problems in the practical application of molecular anatomies, such as non-standard molecular anatomy procedures, insufficient ability to interpret data, and the high cost of genetic testing (41). Sudden death can be effectively solved using molecular anatomy (42). Even though molecular anatomy has some shortcomings in practical application, we believe that molecular anatomy can be further developed and continues to play a role in the diagnosis of SUDEP with the development of science and technology and the improvement of the system, as well as the increasing frequency of international cooperation and interdisciplinary dialog in human and experimental translational research on sudden death.

Conclusion

Despite reviewing the forensic examination of SUDEP, the authors were still unable to determine the typical characteristics of this disease. To establish the forensic diagnosis of SUDEP, a variety of indirect evidence must be presented. In practice, the presence of witnesses is a less common indication of SUDEP, but it is still a strong evidence of SUDEP.

The uncertainty of SUDEP is the reason for its low diagnostic rate. Nonetheless, there is a primary reason for solving this problem through systematic autopsy. The number of autopsies in China has to be increased as much as possible. Although molecular anatomy aids in the forensic diagnosis of SUDEP, there are a few limitations as well. Lack of awareness regarding this cause of death is another factor in the poor diagnosis rate of SUDEP. An

international standard SUDEP postmortem examination protocol and systematic education are required to further improve medical examiners' knowledge of SUDEP.

In summary, this review first summarized the forensic characteristics of SUDEP and the reasons for the low diagnostic rate of SUDEP. Finally, it has been emphasized that the establishment of international standards and the application of molecular anatomy techniques will benefit the application of SUDEP in forensic diagnosis.

Author contributions

QW performed the literature research, evaluated the literature, and wrote the manuscript. DS designed the study and participated in the writing and discussion of the results. All authors contributed to the manuscript 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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frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 29 June 2023
DOI 10.3389/fneur.2023.1231515

OPEN ACCESS

EDITED BY
Beixu Li,
Shanghai University of Political Science and Law, China

REVIEWED BY
Meng He,
Fudan University, China
Hui Li,
Academy of Forensic Science, China

*CORRESPONDENCE
Antonina Argo
✉ antonella.argo@unipa.it
Maria Puntarello
✉ maria.puntarello@community.unipa.it

RECEIVED 30 May 2023
ACCEPTED 12 June 2023
PUBLISHED 29 June 2023

CITATION
Argo A, Puntarello M, Malta G, Buscemi R, Scalzo G, Triolo V, Albano GD and Zerbo S (2023) The analysis of SUDEP forensic autopsies leading to preventable events. *Front. Neurol.* 14:1231515. doi: 10.3389/fneur.2023.1231515

The analysis of SUDEP forensic autopsies leading to preventable events

Antonina Argo[1]*, Maria Puntarello[1]*, Ginevra Malta[1], Roberto Buscemi[1], Giovanni Scalzo[1], Valentina Triolo[2], Giuseppe Davide Albano[1] and Stefania Zerbo[1]

[1]Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties, Section of Legal Medicine, University of Palermo, Palermo, Italy, [2]Policlinic Hospital, University of Palermo, Palermo, Italy

Introduction: The diagnosis of unexpected death by excluding non-natural causes, particularly in subjects with epilepsy, is a topic of interest and it is difficult to identify in the forensic field. Health professionals sometimes are faced with cases of sudden death, generally in young adults with a long history of epilepsy that require, for judicial purposes, an explanation in terms of cause and means to determine the death. SUDEP is an entity diagnosed by the exclusion of other causes that may have led to death, and then for forensic purposes, it requires particular attention and knowledge, and there is difficulty in identifying it. Our contribution aims to illustrate the scientific community pathological findings, medical history, and circumstantial evidence of four cases of sudden death in epileptic subjects.

Method: We illustrated four cases of judicial autopsies from the Institute of Forensic Medicine of Palermo, Italy; the purpose was to exclude the criminal intervention in determining the death as non-natural. The study of victims' medical history, the toxicological investigations, and the autopsy findings analyzed both from macroscopic and microscopic aspects have made it possible to highlight some findings that can be traced back to SUDEP despite the small sample of subjects studied.

Results: These presented findings of four SUDEP cases could help forensic pathologists in recognizing this entity, by highlighting its characteristics, and allowing for a pathological classification, also in relation to the use of drugs for epilepsy treatment and circumstances of death.

Discussion: To obtain a definite diagnosis of SUDEP, a complex investigation process is required in a multidisciplinary approach. Considering the literature review with criticism, it could allow health professionals to select the characteristics of epileptic patients at risk of sudden death. Processing human behaviors, molecular and histopathological findings of the autopsies, but also the physiological, and pathological human body system functions thanks to Artificial Intelligence, could be the key to explaining SUDEP mechanisms and the future results to prevent it.

KEYWORDS

SUDEP, epilepsy, forensics, autopsy, research, preventable events, artificial intelligence

Introduction

Sudden cardiac death and sudden unexpected epilepsy death are the two major causes of sudden unexplained deaths (1). SUDEP is defined as "sudden, unexpected, witnessed or unwitnessed, non-traumatic, and non-drowning death in patients with epilepsy with or without evidence of a seizure and excluding documented status epilepticus, in which postmortem examination does not reveal a toxicologic or anatomic cause of death," and it is classified into

definite, probable, and possible (2, 3). In definite SUDEP, an autopsy has confirmed the absence of an anatomical or toxicological cause of death (4). In probable SUDEP, an autopsy has not been done but the circumstances of death are strongly suggestive of SUDEP, while possible SUDEP describes a situation in which SUDEP cannot be excluded and should be considered among the explanations of death (5–7). Epilepsy is a common neurological disorder with population rates ranging from four to 10 per 1,000 people. It is characterized by seizures. More than 50 million people worldwide have epilepsy. Epileptic people have a 2–3 times higher risk of premature death than the general population, and the risk of sudden and unexpected death is approximately 24 timers higher (8, 9). Sudden and unexpected death in epilepsy (SUDEP) represents the main cause of premature deaths in young adults (between 20 and 40 years of age) suffering from epilepsy (10–12). It is more common in patients with poorly controlled generalized seizures (13). To date, the pathological mechanisms of SUDEP are unknown and unclear. Several studies indicate that tonic-clonic seizures can lead to transient respiratory arrest and apnea. The extent of oxygen desaturation is related to the convulsion's duration time, and it is associated with an increase in end-tidal carbon dioxide levels. Authors have shown that seizure activity can cause hypoventilation and, therefore, hypoxemia and hypercapnia (13). Suspected SUDEP's mechanisms also include changes in cardiovascular stability and baroreflex sensitivity during the interictal state. Seizure activity can also be associated with acute pulmonary edema from increased pulmonary vascular pressure and central apnea that result in fatal anoxia (14). This evidence suggests that a combination of acute cardiovascular and pulmonary events related to epileptic discharges may cause death (9, 15, 16). An elevated seizure frequency is a risk factor for SUDEP. An interesting study by F. Scorza et al. aims to share with the scientific community the possible correlation between aberrant neurogenesis of epileptic patients and seizure frequency. Based on these results, the aberrant neurogenesis could negatively influence the cardiovascular system of the patient with epilepsy, leading to cardiac abnormalities and, therefore, SUDEP (17). Several studies have identified that some drugs, antiepileptics, for example, could determine an arrhythmic death (18–20) or induce acquired long-QT syndrome (21). Although the histopathological findings in death related to SUDEP are unclear, Theodora A. Manolis tried to explain the possible mechanism of SUDEP, highlighting respiratory and cardiovascular dysfunction as potential mechanisms of sudden death in epileptic patients as well as the disruption of the central autonomic control in SUDEP (22). Recently, several studies using imaging with magnetic resonance and measurements of heart rate variability suggested that a dysfunction of the brainstem could increase SUDEP risk (23). Several postmortem studies reported that disorganization of the hippocampus and amygdala that appears with altered gray matter volumes on MRI has a role in the control of the autonomic nervous system (24) and may increase the risk of SUDEP (25). In addition, cardiovascular dysfunction plays an important role in the determination of SUDEP: in epileptic patients, we could highlight arrhythmias, bradyarrhythmias, or tachyarrhythmias related to epileptic drugs. The correlation between drugs (lamotrigine and carbamazepine) is discussed (26). This is an important topic to highlight because some epileptic patients take two or more different antiepileptic drugs to control seizures, and a lot of study demonstrates that polytherapy is a risk factor for SUDEP (23, 27). Variable compliance with antiepileptic drugs could be a potentially preventable cause of sudden unexpected death in epilepsy. To confirm the correlation between a low level of drugs and SUDEP during an autopsy, it is important to make hair analysis and blood/urine laboratory exams (28). Recent studies by the scientific community described a possible pathological mechanism of SUDEP related to some neurotransmitters, such as serotonin and adenosine. Adenosine is an inhibitory modulator of neuronal excitability; while adenosine increases neuronal activity, serotonin can modulate neuronal excitability, stimulating respiratory centers in response to hypercapnia. Indeed, MRI-based measurement showed that the brain volume of the medullary raphe (29), where serotonin is produced, was lost in patients that died of SUDEP. During and after seizures, the rise of adenosine is correlated to respiratory failure (30). Therefore, these neurotransmitters provide possible treatment targets for SUDEP (30). Lastly, genetic alterations were found in patients with SUDEP diagnosis (31). Germline loss-of-function mutations in DEPDC5 cause focal epilepsies and increase SUDEP risk (32). Emerging genetic research suggests a correlation between mutations in ion channel genes and familial LQTS and SUDEP (33) and variants of KCNQ1, KCNH2, and SCN5A genes (34–37). Patients with sodium channel mutations are predisposed to progress from mild cerebral edema to severe cerebral edema which may represent an additional contributing factor in the events leading to the sudden death of patients with epilepsy (38, 39). The complexity of the histopathological mechanisms of SUDEP is simply schematized in Figure 1.

Materials and methods

Our contribution aims to share with the scientific community pathological findings, medical history, and circumstantial evidence of four cases of sudden death in epileptic subjects. These are four cases at first judicial autopsies from the Palermo's Institute of Forensic Medicine in which the purpose was to exclude criminal intervention in determining the death as non-natural. The study of the patients medical history, the toxicological investigations, and the autopsy findings are analyzed from macroscopic, histopathological, and toxicologic findings. All forensic blood samples were screened for alcohol, drugs, and medicinal drugs. Ethanol was screened and quantified by headspace gas chromatography methods combinate with flame ionization detection. Illegal or medical drugs were screened by an immunological method with confirmation and quantification using gas-chromatography mass spectrometry.

Case 1

A male subject, aged 33, who was a prisoner. He was found in the early-morning hours unconscious and pulseless while asleep in bed. He featured a clenched jaw, the tongue clamped between his teeth, and he was drooling. In his medical history, there was the presence of psychiatric disorders and drug addiction but not epilepsy. He was taking 1,500 mg daily. The external examination showed a mantle-like congestion of the cranio-cephalic district, the tongue stapled between the teeth with a dental impression to the left of the tip, abrasions at the upper and lower gingival fornices, and subungual cyanosis. His brain's weight was 1,114 g. Macroscopically, an edematous encephalon with flattened circumvolutions and hemorrhagic punctuation was observed, which was also confirmed on histopathological examination. In the cerebellum, there were histological signs of

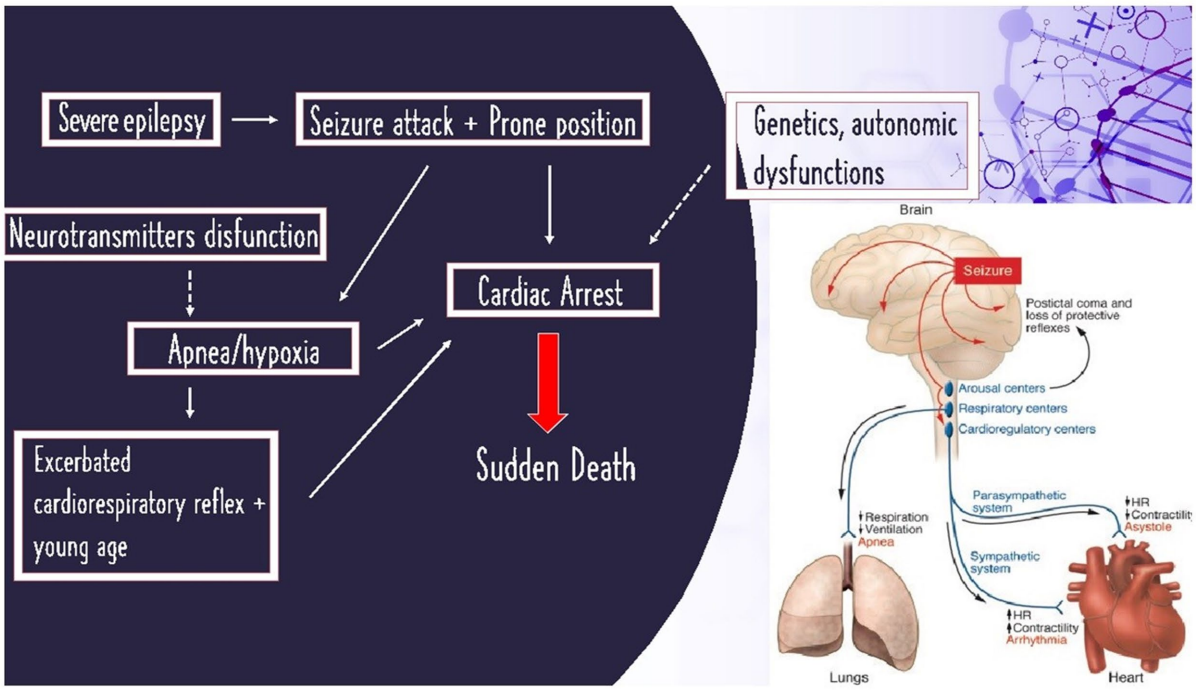

FIGURE 1
On the left: schematized histopathological mechanisms of SUDEP; on the right: image illustration of SUDEP from Friedman et al. (40).

hemorrhagic areas and neuronal necrosis, while in the bulb and medulla, evidence of neuronal edema alternating with areas of ischemic distress was detected. The lungs weighed 1.690 g; they were edematous with macroscopic and microscopic signs of acute pulmonary edema; the heart weighed 310 g and had diffused hemorrhagic petechiae at the pericardial and epicardial surfaces at the tip and posterior surface. Toxicological examinations showed the presence of valproic acid within the cut-off. The autopsy did not reveal an anatomic or toxicologic cause of death; there were no findings of criminal interventions, and signs of typical indirect seizures were found, which allowed for the diagnosis of SUDEP.

Case 2

A male subject, aged 41, with a positive medical history of epilepsy with tonic–clonic seizures and alcohol and drug addiction. He was found in the early-morning hours unconscious and pulseless while asleep in bed. He was taking 100 mg of phenobarbital daily. Upon external inspection, there was cranio-cephalic congestion; upon opening his mouth, there was the presence of a protruded and stapled tongue between the teeth and a foamy material inside it. Approximately 2 days earlier, he had a seizure with respiratory failure. His brain's weight was 1,115 g, his lungs weighed 1,550 g, and his heart weighed 350 g. Macroscopic examination showed brain edema, pulmonary congestion, and left ventricular hypertrophy. The left ventricular free wall measured 1.5 cm, while the right ventricular free wall was 0.5 cm. The septum measured 1.2 cm. Microscopically, significant cerebral and pulmonary edema was confirmed. Toxicological analysis showed positivity to barbituric and benzodiazepines within the cut-off. Microscopic and histopathological findings excluded anatomic or toxicological causes of death.

Case 3

A female subject, aged 67, with a positive medical history of epilepsy and psychiatric disorders. She was being treated with valproic acid of 1,500 mg daily and escitalopram. She was found in the afternoon dying. On reaching the emergency room, her death was noted. On external inspection, there were no indirect signs of seizures and evidence of cervical-cephalic congestion and subungual cyanosis. At autopsy, the leptomeninges appeared opaque; the encephalon was diffusely edematous, and its weight was 1,010 g. The heart weighed 350 g; it was flaccid to the touch, with evidence of hemorrhagic spiking at the free wall of the right atrium. Its transverse diameter was 10.5 cm, and its longitudinal diameter was 14 cm. The left ventricular free wall was 1.3 cm, the right ventricular free wall was 0.3 cm, and the septum measured 1.3 cm. The lungs were mildly edematous. Microscopically, massive cerebral edema and neuronal reduction of the bulb and medulla were confirmed. The lungs weighed 1,185 g and showed signs of blood stasis with areas of fibrosis and focal edema. All toxicological analyses were negative. Anatomic or toxicological cause of death was not found.

Case 4

A 16-year-old female subject with intellectual disability and epilepsy was a resident of a nursing facility. The patient was treated with valproic acid of 1,000 mg (morning and evening), Perampanel 10 mg (evening), and diazepam 10 drops (morning and evening). Early in the morning, the girl was found dead, and an autopsy was ordered. On external examination, cervical and encephalic congestion was noted. On examination of the organs, the brain appeared edematous, with flattened furrows and congestion of the

leptomeningeal vessels; the heart, weighing 375 g, showed the following thicknesses: the left ventricle was 1.4 cm, the interventricular septum was 1.1 cm, and the right ventricle was 0.8 cm; the lungs weighed 1.670 g and diffuse edema was evident on cutting surface. First-level toxicological screening tests were performed on blood matrix which showed positivity for benzodiazepines, which was compatible with the drugs in use. Intellectual disability worsened the girl's reflex response to seizures. Her clinical history was complicated by drug-resistance epilepsy. It required polytherapy drugs.

Discussion

SUDEP is the most common cause of death in epileptic patients. In forensic field making, a diagnosis of SUDEP is difficult (14, 41). As the diagnosis of SUDEP is also made by the exclusion of other causes of death, forensic pathologists must collect informations about the death scene, the circumstance of the death, and the victim's medical history. Recent clinical findings and symptoms before the death must be sought although prodromal symptoms are often non-specific (42). The type of drugs eventually taken by the victim could link with sudden death but the external examination by the forensic pathologist could highlight signs and findings that indirectly link to epilepsy, such as abrasions and/or ecchymosis in areas of accidental fall trauma (protruding areas of the face, extensor surfaces of limb joints, conjunctival petechiae, lacerations, or hemorrhagic infiltration of the tongue) (21). Generally, sudden death, especially in young people always requires a systematic forensic autopsy including toxicological analyses. Sudden unexpected death in epilepsy is an interesting topic for forensics. Indeed, a lot of studies and scientific literature come from retrospective postmortem studies carried out by schools of Legal Medicine (12). Luo Zhuo and his colleagues highlighted autopsy cases of SUDEP in the Office of Chief Medical Examiner, in the State of Maryland (41). From 2007 to 2009, they analyzed 104 cases of sudden unexpected deaths directly or indirectly caused by epilepsy or seizures. Their findings are similar to our cases: subjects' prevalence in age was between 21–50 years (41), death was especially during early-morning hours, on external examination there were indirect findings of seizure, and on microscopical findings, there were cerebral and pulmonary edema and neuronal necrosis. Our case history although small deviates from the literature because we had an equal incidence between male and female SUDEP victims. An interesting item to analyze to properly diagnose, even in forensic and histopathological fields, is the victims' risk factor for SUDEP (43, 44). Risk factors are today's study objects and not of unequivocal interpretation: the scientific community generally agrees on young subjects and male subjects, with epilepsy diagnosis at an early age and long illness duration, with tonic–clonic and nocturnal seizures and with antiepileptic polytherapy or epilepsy non-medication treatment. Alcohol and drug consumption increases SUDEP risk (6, 43, 44). For forensics, an interesting question is the relationship between seizures and sudden cardiac arrest: could the seizure be a trigger for sudden cardiac arrest? Often, forensic pathologists highlight indirect findings of seizures on the victims, but such evidence, although suggestive, is not absolutely linked to death during a seizure (45). This aspect is, indeed, non-clear in the scientific literature. E.C. Stecker and colleagues analyzed a population with epilepsy and sudden cardiac arrest; in 66% of epileptic patients, there was no relationship between seizures and sudden cardiac arrest (27). On the other hand, studies affirmed the claim otherwise (46, 47). By analyzing our findings, both macroscopical and histopathological signs and circumstantial details are consistent with the literature study (48–54): in fact, all four cases showed cerebral and pulmonary edema, and seizure was not directly seen by witnesses; in two of four cases, there were indirect signs of seizures, and an altered toxicological range of anti-epileptic drugs was not found. Three patients took monotherapy for epilepsy, while only one patient took polytherapy, and two victims were drug/alcohol addicted. There were no cardiac fibrosis findings in the histopathological study (55). The schematic representation of our findings is included below (Table 1).

A forensic autopsy could highlight SUDEP characteristics and facilitate SUDEP diagnosis by analyzing many aspects simultaneously: circumstantial details, death scene, external inspection, macroscopical and microscopical findings, and toxicological investigations. These elements make it possible to have a definite diagnosis of SUDEP. This process of diagnosis is complex and requires a multidisciplinary approach. When more than one pathological finding is found in a case of suspected SUDEP, making a differential diagnosis becomes extremely difficult. We aim to detect alterations that, with the support of further studies and numbers of SUDEP, can highlight specific features that can more easily be attributed to SUDEP. The autopsies' pathological findings alterations found that, if individually taken into consideration for critical appreciation without the contribution of circumstantial data, indirect signs of a seizure and medical history are not specific elements, which is why identifying a diagnosis of SUDEP is not easy. For this reason, in the future perspective, it would be desirable to identify markers that if dosed make the autopsy evidence more specific. In this regard, an important role could be played by heat shock protein 70 (HSP70), which is a molecular chaperone involved in the inflammatory response that is upregulated after the epileptic state. HSP70 has been described as an endogenous intracellular ligand of Toll-like receptor 4. It is released from damaged

TABLE 1 Macroscopic and histopathological findings in our SUDEP death diagnosis.

	Gender	Age	Epilepsy diagnosed	Polytherapy for epilepsy	Type of molecular therapy	Indirect signs of seizures	Death in bed	Cerebral/ pulmonary edema	Neuronal necrosis
Case 1	Male	32	No	No	Valproic Acid	Yes	Yes	Yes	Yes
Case 2	Male	41	Yes	No	Barbituric	Yes	Yes	Yes	No
Case 3	Female	67	Yes	No	Valproic Acid	No	Unknown	Yes	Yes
Case 4	Female	16	Yes	Yes	Valproic Acid perampanel diazepam	No	Yes	Yes	n.d.

tissues and activates immune cells after an epileptic seizure. The timing and mode by which HSP70 is released are unclear to date. There are not many human studies in the literature; something has been found in an animal study, indicating the overexpression of HSP70 immediately after seizure (56). Studying the immunohistochemical expression of HSP70 in the hippocampus, the parahippocampal cortex, parietal cortex, amygdala, and thalamus (areas most affected by neuronal damage during a seizure) could not only allow us to understand whether the timing of seizure is closely related to SUDEP, but in the forensic setting, positivity to HSP70 could bring important implications in the safety diagnosis of SUDEP by making macroscopic and histopathological evidence more specific. An additional benefit could be to make HSP70 a therapeutic target to limit the neuronal loss and inflammatory reaction control (4, 57–60). Automatically, this would reduce the frequency of seizures and could be a protective factor for SUDEP in patients most at risk. In the future perspective, with the advancement of artificial intelligence in the medical field, it might be useful to create algorithms that based on the clinical characteristics of patients can identify those most at risk of sudden cardiac death so as to attempt experimental pharmaceutical approaches (target HSP70) and apply closer monitoring, especially during the night, a time when, statistically, SUDEP occurs most frequently based on the present state of the heart (57). Recent studies have focused attention on wearable multimodal bracelets, among them Embrace and E4, that are based on the detection of electrodermal activity, motion sensors, plethysmography, and temperature to detect crisis and through signaling mechanisms alert is sent to the rescuers (61). The wristbands use machine learning mechanisms facilitated by the user's ability to report false alarms *via* the app (62, 63). The bracelets represent an evolution of audio-video monitoring during the night, an ambulatory method used for the study of nocturnal seizures (64). Other methods that could help healthcare providers identify seizures in the future could be evolutions of video-audio monitoring involving the use of previously programmed algorithms along with deep learning mechanisms (65, 66). The foundation of artificial intelligence is used to describe "machines" able to demonstrate cognitive functions that humans associate with other human minds such as learning and problem-solving. Machine learning is based on the compilation of a complex algorithm and software that mimics the human mind to decipher critical problems that include visual perception, decision-making, and speech recognition. Deep learning is similarly described as a class of artificial neural networks that learn in a supervised and unsupervised manner. To analyze real-world data, deep learning decomposes information into various abstraction levels. Each decomposition level corresponds to a neural network. Artificial intelligence can learn human behaviors in different areas. In medicine and particularly in the case of SUDEP, artificial intelligence and deep learning through supervised or unsupervised learning modes could allow us to act on two fronts. The first involves a study of the human activity of the epileptic subject to research the mechanisms underlying sudden cardiac death. For example, we could consider using deep learning to study the characteristics and variations of respiratory, cardiac, and nerve activity by decomposing each apparatus into various abstract sublayers. In this way, by monitoring all patients defined as "at risk," we could highlight the pathophysiological mechanisms of SUDEP that today are unclear. The interaction between the various systems of the human body in the determination

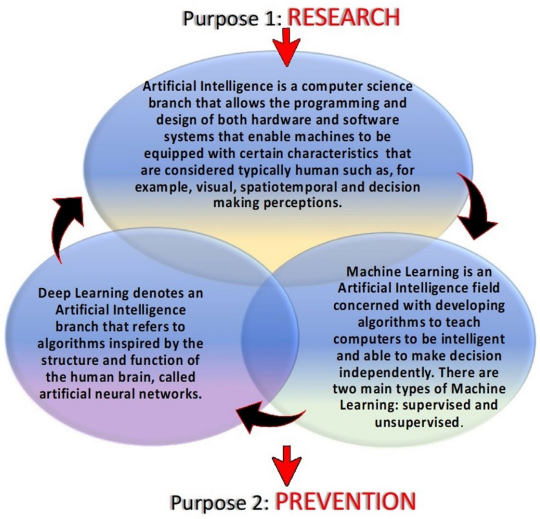

FIGURE 2
The use of artificial intelligence and its subtypes.

of sudden cardiac death in epileptic patients could be investigated by encouraging machine learning (67, 68). The second use of deep learning is closely related to the first: through machine learning resulting from research, extrapolated data can be applied for prevention purposes. The interaction of algorithms based on the study of respiratory activity (e.g., the number of breath acts, the depth of each breath, and the blood acid–base balance) but also the study of the various sub-levels of cardiac and nerve activity before, during, and after a seizure could lead to highlighting critical passages, a prelude to SUDEP, so as to promptly alert the rescuers and increase the likelihood of saving a patient as well as identifying which of them is actually most at risk, progressively limiting false alarms through the use of machine learning (Figure 2).

Processing human behaviors, molecular and histopathological findings of the autopsies, but also the physiological and pathological human body system functions thanks to Artificial Intelligence could be the key to explaining SUDEP mechanisms and the future results to prevent it.

Conclusion

Attention to SUDEP pathophysiology, the study of physiological changes detected in SUDEP victims, cardiac study with ECG monitorization, post-mortem instrumental investigations such as CT or MRI, the study of the genetic predisposition for SUDEP, and the study of interactions between antiepileptic drugs and SUDEP could lead to important implications in the knowledge of the illness but especially in preventing sudden cardiac death in epileptic patients. To assess the state of knowledge about SUDEP, the American Epilepsy Society and the Epilepsy Foundation convened a task force that had five goals: develop a position statement describing if, when, what, and how SUDEP should be discussed with patients, their families, and caregivers; design methods by which the medical and lay communities become aware of the risk of

SUDEP; recommend research directions in SUDEP; explore steps that organizations can take to perform large-scale, prospective studies of SUDEP to identify risk factors; identify possible preventive strategies for SUDEP (69). In the forensic field, often the purpose is excluding criminal intervention or non-natural death causes. SUDEP diagnosis is important, but with our small contribution, we aim to identify indirect and direct findings of SUDEP during autopsies that could facilitate SUDEP identification, knowledge, and prevention (70, 71). We aim to launch a hypothesis involving the collaboration of artificial intelligence and, in particular, deep learning and the study of HSP70 expression that could not only elucidate the pathophysiological mechanisms of SUDEP and its correction by seizures but also facilitate the autopsy diagnosis of SUDEP and intervene in the prevention of sudden unexpected death in epileptic patients.

Data availability statement

The datasets presented in this article are not readily available because of restriction on judicial data and privacy restrictions. Requests to access the datasets should be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the Policlinic Hospital "P. Giaccone". Written informed consent to participate in this study was provided by the patient/participants' legal guardian/next of kin.

Author contributions

AA, MP, and GM designed the framework of the review and drafted the manuscript. SZ, GA, RB, GS, VT, and AA provided supervision and contributed to manuscript writing and editing. All authors have read and approved the latest version of the manuscript.

Funding

This work was supported by University of Palermo, Eurostart 2021-22, cod. n. PRJ-1030. Title: "Tissue markers predictive of damage from substances of abuse and their correlation to preventable adverse cardiovascular events".

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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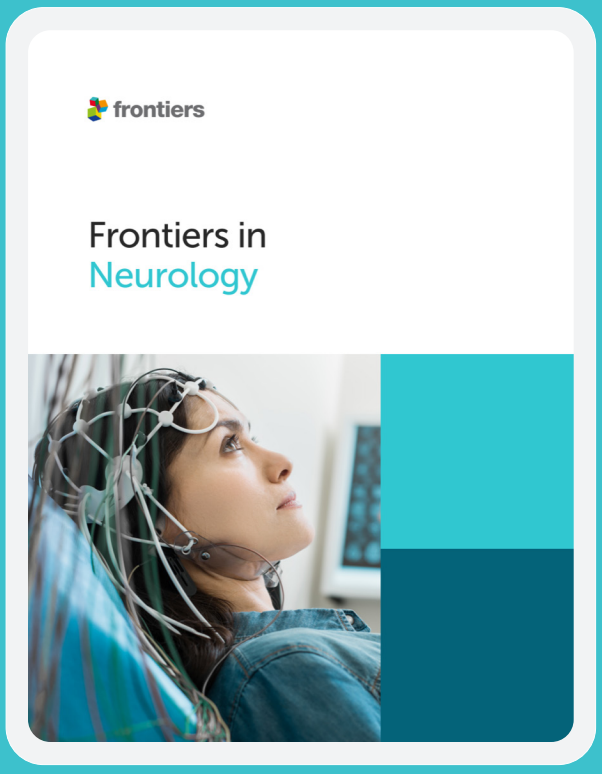

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