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Original Article

Investigation of the Effects of Acute and Chronic PTZ Model Epilepsy in Rats Exposed to Neonatal Hyperoxia on *Bdnf, Ngf, Cyt c, Bax,* and *Bcl-2* Gene Expression Levels in the Brain

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Abstract

Objective: The aim of this study was to investigate the relationship between acute and chronic epilepsy that may occur in adulthood, gene expression levels, and the possible mechanism of neuronal loss in rats exposed to hyperoxia in the postnatal period.

Methods: The study was started with 12 female rats (mother rat). Two main groups were formed: six control and six hyperoxia groups. At the end of the experiment, brain tissue samples were collected and *Bdnf, Ngf, Cyt c, Bax*, and *Bcl-2* gene expressions were studied by quantitative polymerase chain reaction. *Bax* (Bcl-2 associated X-protein) and *Cytochrome (Cyt) c* gene expression levels were found to be significantly higher in the hyperoxia-epilepsy groups, especially in the male group, than in the other groups ($p<0.05$).

Results: While the *Ngf* gene expression level increases significantly in females due to epilepsy, it is independent of hyperoxy ($p<0.05$). *Bdnf* gene expression levels were found to be affected by hyperoxia in both males and females ($p<0.05$). In our study, a significant increase in *Bax* and *Cyt c* gene expression levels was observed in the neonatal hyperoxia and epilepsy group.

Conclusion: It is thought that this increase in gene expression levels molecularly supports neuronal loss, but the related pathways will be better clarified with further studies.

Keywords: Hyperoxia, neonatal hyperoxia, epilepsy, gene expression

INTRODUCTION

Preterm infants are relatively early to face hyperoxia because of their early delivery from the intrauterine environment and become more vulnerable to hyperoxic stress because of their insufficient antioxidant defense mechanisms.[1] Because they are susceptible to deterioration caused by reactive oxygen species (ROS), their endogenous radical scavenging systems are not fully mature.[2] In the sensitive period of brain development, supraphysiological oxygen therapy affects the developmental processes because of hyperoxia. The toxic effects of hyperoxia on the brain have been demonstrated in both experimental and clinical studies.[2,3]

Although individuals exposed to hyperoxia in the neonatal period may be prone to epilepsy, its mechanism has not yet been clarified. There are studies showing that hyperoxic brain damage in developed models causes widespread apoptosis and a decrease in the number of neurons in various regions of the brain because of increased oxidative stress and decreased activation of neurotrophin pathways. In the clinical setting, up to 50% of surviving premature infants exhibit cognitive deficits or behavioral problems in the later stages of development.[4-6]

Development of the mammalian brain is a dynamic process that includes structural and functional maturation processes. The evolution of the brain is characterized by neuronal cell development and proliferation, migration, glial cell proliferation, axonal and dendritic growth, synaptogenesis, and myelination of axons.[7] Although neuronal migration processes are usually completed in extremely preterm infants born at the limit of viability (about the 24th week of gestation), glial cell maturation, growth, and connection formation are still ongoing processes.[4,8] Neuronal electrical activity is strongly dependent on metabolic factors such as mitochondrial development, cerebral vascular density and blood flow, maturation of glucose utilization systems, and cytochrome oxidase activity.[9,10]

With the rapid development of neonatology in recent years, oxygen therapy has become the most important measure in the rescue and treatment of newborns and preterm infants. Studies have shown that prolonged exposure to hyperoxia in neonates can cause hyperoxic lung damage, hyperoxic retinopathy, hypoxic ischemic encephalopathy, and ultimately lead to worsening of the child's condition, lowering survival and quality of life, easily causing other diseases that can affect adulthood. Hyperoxic brain injury is common and more severe, particularly in very low birth weight and preterm infants. Studies have shown that high oxygen concentrations can affect the brain and reduce weight.[11]

Brain damage is more common in preterm newborns than in term newborns for various reasons, such as developmental and genetic weaknesses and different exposure to adverse perinatal environments.[12,13] However, the mechanisms of neonatal brain injury have not been fully understood so far.[14] Studies have proven that glutamate excitotoxicity is one of the main mechanisms of preterm-related brain injury.[15,16] To prevent the accumulation of extracellular glutamate, the brain relies on rapid uptake by sodium-dependent glutamate transporters such as excitatory amino acid transporters (EAATs) and vesicular glutamate transporters (VGLUTs).[17,18] In addition, γ-aminobutyric acid (GABA) is another important component of the balance between excitation and inhibition and plays an important role in different processes associated with brain development.[19] Zhao et al.[14] found hyperoxia-induced glutamate accumulation in the immature cerebrum and cerebellum of newborn rats. In the same study, increased oxidative stress and decreased expression of glutamate transporters, including EAATs and VGLUTs, within 2 weeks of hyperoxia threat contributed to impaired glutamate homeostasis in the rat brain. Under physiological conditions, astrocytes are necessary for the uptake of synaptic glutamate, thereby preventing neuronal hypersynchronization and glutamatergic excitotoxicity. However, reactive glia can promote neuronal apoptosis. Experimental models have shown the upregulation of the Bcl-2 protein in the sclerotic hippocampus in both neuronal and glial cells.[20-22] In addition, Bcl-xL has shown a positive correlation with seizure frequency in an amygdala-triggered seizure model, indicating an antiapoptotic response after recurrent seizures as a possible attempt to prevent neuronal loss.[23] One of the main biological functions of anti-apoptotic Bcl-2 proteins is to prevent the disruption of mitochondrial integrity. Members of the pro-and anti-apoptotic Bcl-2 family of proteins are expressed throughout the central nervous system during embryonic and adult life.[24] Regarding pro-apoptotic Bax-like proteins, Bax is widely expressed in the brain,[25] and Bax was the first Bcl-2 homologous gene that was found to act as an apoptosis executor. The Bax protein is expressed in various tissues as multiple alternative splicing variants that are normally localized in the cytosol or loosely attached to the mitochondria. Indeed, Bax expression in HeLa cells resulted in increased ER Ca^{2+} loading followed by Ca^{2+} release from ER, an increase in mitochondrial Ca^{2+} loading, and strengthening of mitochondrial Ca^{2+} responses, consequently triggering apoptosis.[26] These results agree with previous studies that reported that Bax/Bak overexpression facilitates the transfer of Ca^{2+} from the ER to mitochondria, makes mitochondria sensitive to absorb more Ca^{2+}, and thus induces cell death.[27,28] Bax also regulates dynamic Ca^{2+} signaling between the ER and cytosol in cortical neurons, regardless of its classical function in the mechanism of apoptotic cell death or its proposed involvement in the decoupling of mitochondrial PTP.[29] Emerging studies show that pro-and anti-apoptotic members of the Bcl-2 protein family not only modulate the mitochondrial pathway of apoptosis but also have important 'day-time' activities. These functions include the regulation of neuronal Ca^{2+} homeostasis and mitochondrial energy.[30] This studyaimed to investigate the susceptibility of rats exposed to postnatal high oxygen to convulsions and epilepsy in adulthood and the molecular mechanisms of this susceptibility. For this purpose, an acute and chronic epilepsy model was created in adulthood of rats exposed to hyperoxia during the neonatal period. In the case of epilepsy, which has any convulsive effects of exposure to hyperoxia in the newborn period, the expression levels of some proteins known to be responsible for neuronal loss in the brain were determined to obtain information about degeneration and regeneration in the brain, and some genes that are effective in degeneration were studied. At the same time, the differences between males and females, in this case, were considered.

MAIN POINTS

- Premature babies face hyperoxia relatively early due to inadequate antioxidant defense mechanisms and are more vulnerable to hyperoxic stress, exhibit cognitive deficits or behavioral problems in later stages of development, and become prone to epilepsy.
- Determination of different gene expression levels by applying an experimental epilepsy model in later stages of development in rats exposed to hyperoxia in the postnatal period may provide an understanding of the possible mechanisms of neuron loss.
- *Bdnf, Ngf, Cyt c, Bax,* and *Bcl-2* gene expression levels molecularly support neuron loss, but the relevant pathways will be better elucidated in future studies.

METHODS

Experimental Animals

The experimental animals used in the study were obtained from the Bolu Abant İzzet Baysal University Experimental Animals Application and Research Center. Until the start of the study and during the study, the animals will be kept in the Experimental Animals Application and Research Center for 12 h in a light/dark environment with a relative humidity of 60-70% and will be fed ad libitum. All experimental animals have been treated based on the guiding principles approved by the Animal Ethical Committee of Bolu Abant İzzet Baysal University, and all treatments comply with the recommendations provided in the Declaration of Helsinki (registration number: 2020/23, date: 26.08.2020). All experimental animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals. This study was started with 12 mother rats. Six of them were in the control group and others were in the hyperoxy group. Wistar albino female rats (n=12) was deposited by taking vaginal smears and determining the estrus period. The joining day was considered as day 0 of pregnancy. In the control groups, mother rats (n=6) and offspring (n=24). In the groups with hyperoxia, mother rats (n=6) and offspring (n=24) were subjected to controlled constant oxygen exposure with a concentration of 80% and a current rate of 4 L/h until the postnatal 5th day. When the rats were two months old, an acute and chronic pentylenetetrazole (PTZ) epilepsy model was created. Experimental Groups: It was composed of 2 main groups: control and hyperoxy. Control female (CF), control acute epilepsy female (CAEF), control chronic epilepsy female (CCEF), control male (CM), control acute epilepsy male (CAEM), control chronic epilepsy male (CCEM), and

hyperoxy female (HF), hyperoxy acute epilepsy female (HAEF), hyperoxy chronic epilepsy female (HCEF), hyperoxy male (HM), hyperoxy acute epilepsy male (HAEM), and hyperoxy chronic epilepsy male (HCEM) in a total of 12 subgroups. Four animals were used in each group. After the experiment, brain tissues of all animals were taken and *Cytochrome c, Bax, Bcl-2, Bdnf*, and *Ngf* gene expressions were determined by real-time polymerase chain reaction (RT-PCR). One-way analysis of variance was used for the statistical analysis of the results, and it was determined whether there was a difference between the groups and from which group this difference originated with the post-hoc LSD test. A value of $p<0.05$ was considered significant.

An acute and chronic PTZ model (kindling model) was created in 2-month-old female and male offspring. The animals were then decapitated, and their brains were removed. The cerebellum was separated, and the left hemisphere was stored in RNAase DNAase free tubes at -80 until gene expression was studied (Figure 1).

Creation of a Chronic PTZ Model of Epilepsy

To establish a chronic experimental epilepsy model, PTZ [35 mg/kg/intraperitoneally (i.p.)] was injected i.p. three times a week (Monday, Wednesday, and Friday) until the seizure behavior of the animals was observed. Severity of seizures was observed as stage 3 and stage 4 according to the Racine scale. For the Kindling model, 13 doses of 35 mg/kg were applied on Mondays, Wednesdays, and Fridays, 3 days a week. Stages 4 and 5 were observed according to the racing scale in the last three applications consecutively, and it was accepted that the ignition pattern was formed. It was evaluated according to the racing scale in both acute and chronic models (Figure 1).

Creation of an Acute PTZ Model of Epilepsy

A single dose of PTZ (50 mg/kg/i.p.) was administered to establish an acute experimental epilepsy model. It was evaluated according to the racing scale in both acute and chronic models (Figure 1).

Severity of seizures was scored as follows:

Stage 0: No response

Stage 1: Ear and facial twitching

Stage 2: Myoclonic body jerks without an upright position

Stage 3: Myoclonic tremors in theupright position with clonic forefoot convulsions

Stage 4: Tonic-clonic seizures

Stage 5: General tonic-clonic seizures and loss of postural control.

Q-PCR Method

To detect changes in gene expression levels, total mRNA was isolated, cDNA synthesis was performed, and quantitative RT-PCR (qRT-PCR) experiments were performe.

RNA isolation: For RNA isolation from tissue samples, 1 mL of Trizole solution was added to a 50-mg tissue sample and homogenized. The tubes were incubated at room temperature (Tm) for 5 minutes, then 200 µL chloroform was added, and the mixture was manually shaken for 15 s. The tubes were maintained at room Tm for 3 minutes, centrifuged at 12,000 g, and at 4 °C for 15 min. The transparent-colored upper phase was taken into a new tube and 500 µL of 100% isopropanol was added. After incubation at room Tm for 10 min, the tubes were centrifuged for 10 min at 12,000 g and 4 °C. At this stage, the RNA in the sample formed a white precipitate at the bottom of the tube. The liquid in the tube was removed, taking care not to touch this precipitate, and the RNA precipitate was washed with 1 mL of 75% ethanol and centrifuged at 7500 g and 4 °C for 5 min. The resulting RNA was dissolved with 20-50 µL of DEPC-ddH_2O, and its concentration was measured.

cDNA synthesis: For each sample, 1 µg of RNA, 2 µL of oligo dT, and DEPC-ddH_2O were mixed with a final volume of 8 µL and incubated for 5 min at 70 °C. After 10 µL of 2X reaction buffer and 2 µL of reverse transcriptase enzyme were added, the samples were incubated for 1 h at 42 °C and 5 min at 80 °C. The cDNA samples were stored at -20 °C.

qRT-PCR: Primers that bind with high specificity to the target gene regions to be tested for RT-PCR experiments were designed. The oligo design was performed using the Amplify program, and its properties such as melting Tm and primary-dimer formation were studied using the same program. To ensure that the selected primers did not bind to other unwanted regions (unspecific) in the genome, the primers were selected from the exon-intron junction regions. However, the specificity of the primers was confirmed using in silico PCR using the UC Genome Browser. To investigate the level of mRNA expression, 1 µL of cDNA, 1 µL of primer mixture (10 µM, forward+reverse), 10 µL of 2X SYBR Green, and 8 µL of ddH_2O were added to each qRT-PCR reaction. The following program was used for the reaction:

95 °C for 5 min, [95 °C for 15 sec, 60 °C for 30 sec, 72 °C for 30 sec] x 40, 72 °C for 5 min

Analysis of the qRT-PCR results: Normalization with a housekeeping gene such as GAPDH was performed to prevent differences between samples and possible pipetting errors during the detection of mRNA expression levels. The analysis was performed using the ddCt[31] method using the following equation:

ddCt=Ct (target gene) Ct (housekeeping gene)

Target gene expression = 2^(- ddCt)

Statistical Analysis

Statistical analysis of the results determined whether there was a difference between the groups with One-way analysis of variance, and it was determined from which group this difference originated with the post hoc test. The LSD test was used as a post hoc test, and differences with a p value of 0.05 were considered significant (Table 1).

RESULTS

Nerve growth factor (Ngf) gene expression levels were evaluated, and no significant difference was found between female control and hyperoxy (control a CF) and HF, CAEF and HAEF, CCEF and HCEF groups (Figure 2a). In addition, *Ngf* gene expression levels

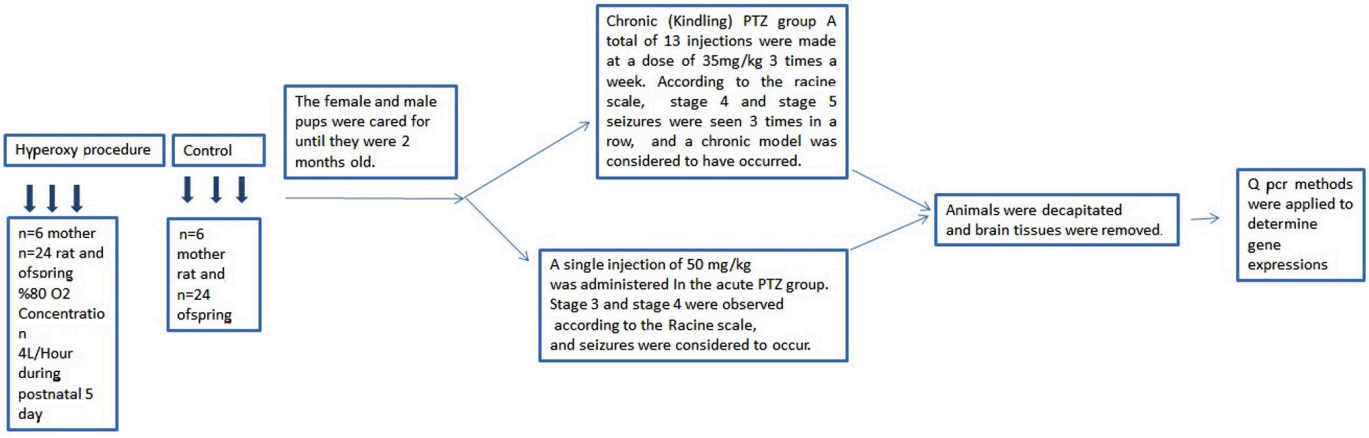

Figure 1. Experimental procedure
PTZ: Pentylenetetrazole

Table 1. Experimental groups

	Control (n=24)	Hyperoxy (n=24)
Female	**CF;** Healthy female rat (n=4)	**HF;** Female rat exposed to hyperoxy (n=4)
Acute female	**CAEF;** Healthy female acute PTZ created (n=4)	**HAEF;** Hyperoxy exposed female rat acute PTZ was established (n=4)
Chronic female	**CCEF;** Healthy female chronic PTZ created (n=4)	**HCEF;** Hyperoxy exposed female rat chronic PTZ was established (n= 4)
Male	**CM;** Healthy male rat (n=4)	**HM;** Hyperoxy exposed male rat acute PTZ was established (n=4)
Acute male	**CAEM;** Healthy male acute PTZ created (n=4)	**HAEM;** Hyperoxy-exposed female rat acute PTZ was induced (n=4)
Chronic male	**CCEM;** Healthy male chronic PTZ established (n=4)	**HCEM;** Hyperoxy exposed male rat chronic PTZ was established (n=4)

CF: Control female, CAEF: Control acute epilepsy female, CCEF: Control chronic epilepsy female, CM: Control male, CAEM: Control acute epilepsy male, CCEM: Control chronic epilepsy male, HF: Hyperoxy female, HAEF: Hyperoxy acute epilepsy female, HCEF: Hyperoxy chronic epilepsy female, HM: Hyperoxy male, HAEM: Hypeoxy acute epilepsy male, HCEM: Hyperoxy chronic epilepsy male, PTZ: Pentylenetetrazole

in the control group were evaluated among themselves, and *Ngf* gene expression levels were found to be significantly higher in the CAEF and CCEF groups than in the CF group ($p<0.05$) (Figure 2a). Then, *Ngf* gene expression levels in the hyperoxy group were evaluated among themselves. *Ngf* gene expression levels were found to be significantly higher in the HAEF and HCEF groups than in the HF group ($p<0.05$) (Figure 2a).

When the *Ngf* gene expression levels in males were evaluated, there was no significant difference between the CM and HM groups ($p<0.05$). There was no difference in Nfg levels in males when no convulsant substance was administered (Figure 2b).

The *Ngf* gene expression level was significantly lower in the CAEM and CCEM groups than in the CM group ($p<0.05$) (Figure 2b). *Ngf* gene expression levels were significantly higher in the HCEM group than in the HM group.

No significant difference was found between the CF group without epilepsy and the HF group in terms of *Ngf* gene expression levels. There was no significant difference between the CM and CF groups in the male groups without epilepsy. When the CF and CM groups were compared, the *Ngf* gene expression level was found to be higher in the CM group ($p<0.05$) (Figure 2a, 2b).

Brain-derived neurotrophic factor (Bdnf) gene expression levels were compared between the CF and HF groups, and there was no statistically significant difference. When the control group was evaluated within itself, the *Bdnf* gene expression level in the CAEF group was significantly higher than that in the CF and CCEF groups $p<0.05$ (Figure 3a).

The male groups were compared. The CM and HM groups were compared, and the Bdnf level was found to be significantly higher in the HM group. The control group is evaluated among themselves, there is no significant difference. *Bdnf* gene expression level was found to be significantly higher in HAEM and HCEM groups compared to HM group. The male and female groups were compared in terms of Bdnf, and there was no significant difference ($p<0.05$) (Figure 3b).

The *Cyt c* gene expression level in the CF group was significantly lower than that in the HF group ($p<0.05$) (Figure 4a).

There was no statistically significant difference in terms of *Cyt c* gene expression levels between the CAEF and HAEF groups ($p<0.05$). The *Cyt c* gene expression level in the HCEF group was significantly higher than that in the HF and HAEF groups ($p<0.05$) (Figure 4a).

In the male groups, the *Cyt c* gene expression level was significantly higher in the acute and chronic epilepsy groups in hyperoxia-treated rats ($p<0.05$) (Figure 4b).

When female and male groups were compared, *Cyt c* gene expression levels were found to be significantly higher in male acute and chronic epilepsy groups than in female acute and chronic epilepsy groups ($p<0.05$) (Figure 4a, 4b).

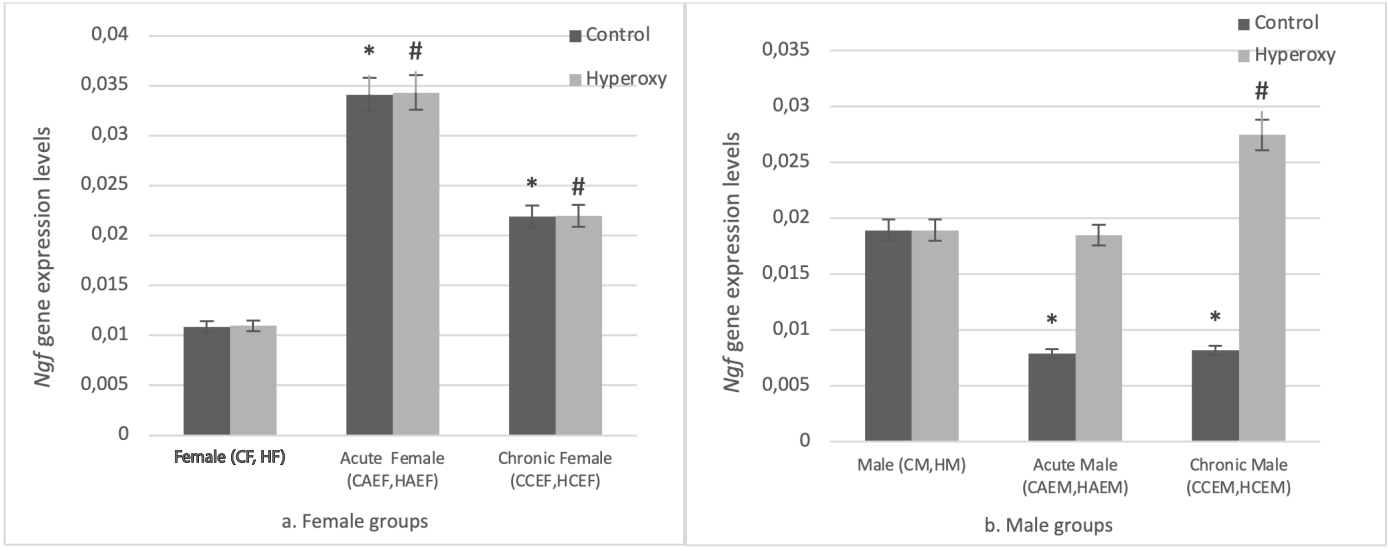

Figure 2. a) *Ngf* gene levels in brain in female groups *p<0.05 compare with control female groups, #p<0.05 compared with control hyperoxia female groups. **b)** *Ngf* gene expression levels in brain in male groups *p<0.05 compare with control male #p<0.05 compare with other with all hyperoxia groups
CF: Control female, CAEF: Control acute epilepsy female, CCEF: Control chronic epilepsy female, CM: Control male, CAEM: Control acute epilepsy male, CCEM: Control chronic epilepsy male, HF: Hyperoxy female, HAEF: Hyperoxy acute epilepsy female, HCEF: Hyperoxy chronic epilepsy female, HM: Hyperoxy male, HAEM: Hypeoxy acute epilepsy male, HCEM: Hyperoxy chronic epilepsy male

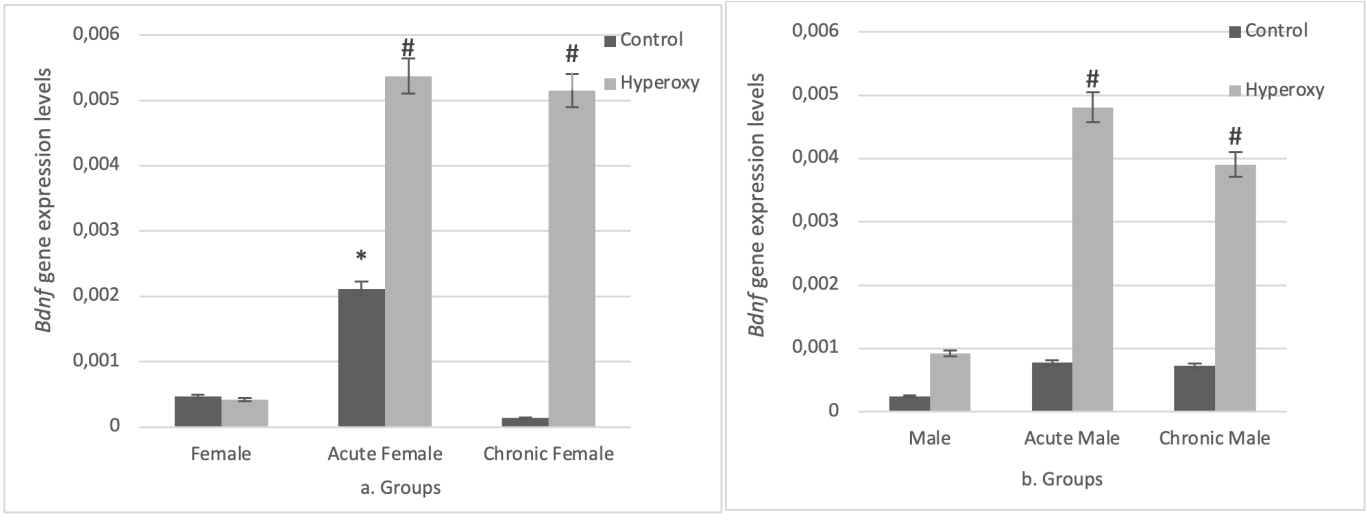

Figure 3. a) *Bdnf* gene expression levels in the brain in females *p<0.05 compared with control female groups, #p<0.05 compared with control hyperoxia female. **b)** *Bdnf* gene expression levels in the brain in males p<0.05 compared with other with all hyperoxia groups

There was no significant difference in *Bax* gene expression levels between the CF and HF groups.

When the control groups were evaluated among themselves, there was no significant difference between the groups. In the hyperoxy group, *Bax* gene expression levels were significantly higher in both acute and chronic epilepsy than in the HF group.

Both acute and chronic epilepsy *Bax* gene expression levels were significantly increased in the teeth exposed to hyperoxy compared with the control groups. According to this result, it was found that epilepsy developed in females exposed to hyperoxia, and the level of *Bax* gene expression in their brains increased. Together with hyperoxia, epilepsy had the effect of increasing the level of *Bax* gene expression ($p<0.05$) (Figure 5a).

Bax gene expression levels were significantly higher in the HM group than in the CM group ($p<0.05$).

When the HM group was evaluated among themselves, no significant difference was observed between the groups. When the CM group was compared among themselves, there was no significant difference between the groups. When the hyperoxia and control groups were compared, it was found that the number of male rats exposed to hyperoxia was significantly higher than that of the control group in all groups ($p<0.05$) (Figure 5b).

The *B-cell lymphoma 2 (Bcl-2)* gene expression level in the CAEF and CCEF groups was significantly higher than that in the CF group ($p<0.05$) (Figure 6a).

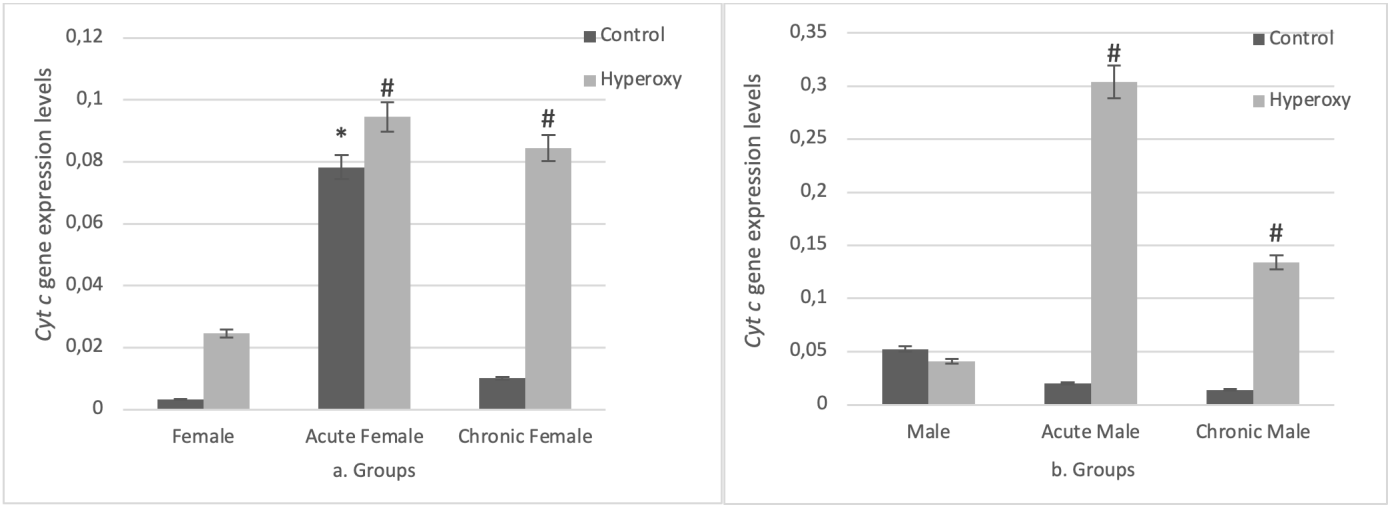

Figure 4. a) *Cyt c* gene expression levels in brain in female *p<0.05 compared with control female groups, #p<0.05 compared with control hyperoxia female. **b)** *Cyt c* gene expression levels in the brain in males p<0.05 compared with other with all hyperoxia groups

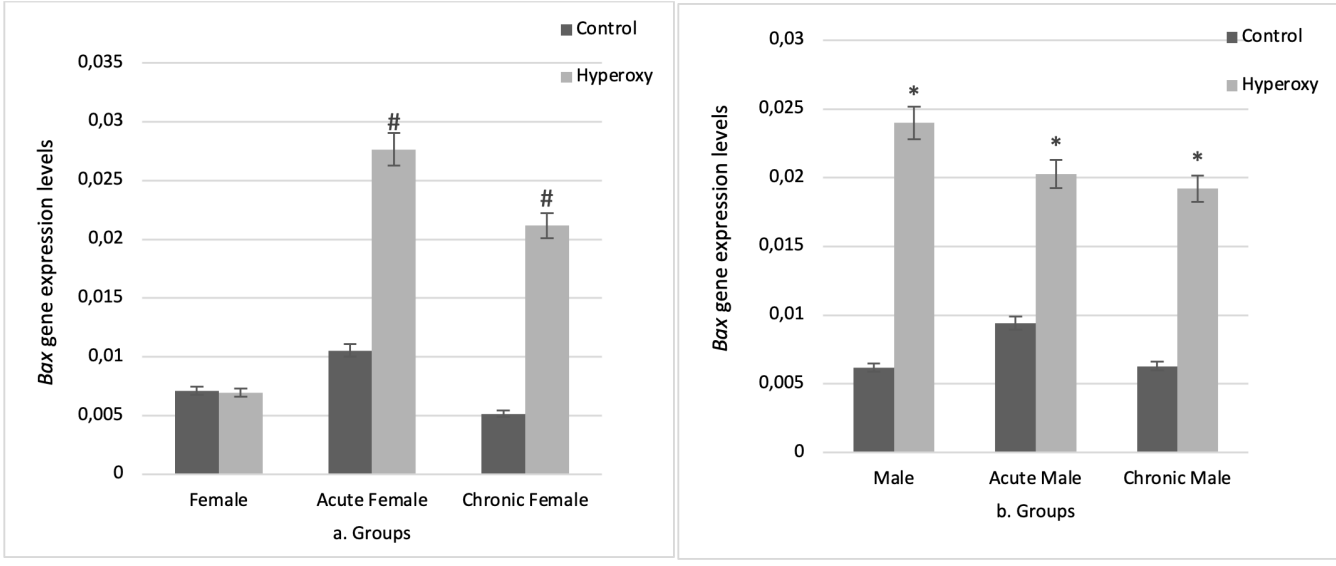

Figure 5. a) *Bax* gene expression levels in the brain in females, #compare with control hyperoxia female. **b)** *Bax* gene expression levels in the brain in males *compared with control groups.

The *Bcl-2* gene expression level was evaluated, and there was no significant difference between HF and CF. The *Bcl-2* gene expression level in the CAEF group was significantly higher than that in the CCEF and CF group (p<0.05) (Figure 6a). The *Bcl-2* gene expression level was found to be significantly higher in the HAEF group than in the CAEF group (p<0.05) (Figure 6a). The *Bcl-2* gene expression level was also found to be significantly higher in the HCEF group than in the CCEF group (p<0.05) (Figure 6a). It was found that *Bcl-2* gene expression levels increased in the acute and chronic epilepsy groups in females exposed to hyperoxia.

The HM group was evaluated among themselves, and no significant difference was observed between the groups. When the CM group was compared among themselves, there was no significant difference between the groups. When the hyperoxia and control groups were compared, it was found that the number of male rats exposed to hyperoxia was significantly higher than that of the control group in all groups (p<0.05) (Figure 6b).

DISCUSSION

Thanks to the developing technologies in the medical field, the birth and survival rates of preterm infants are constantly increasing. It is estimated that approximately 15 million preterm infants are born every year in the world,[32] of which have led to an increase in problems with the brain due to premature birth. Until now, many factors involved in hyperoxia have been associated with brain damage.[33] Oxygen has become an important treatment approach for these patients because atmospheric oxygen therapy concentrations significantly improve the neonatal hypoxic state. However, high amounts of oxygen[34] can stimulate the production of several active oxygen substances. In infants, especially preterm infants, the immune and antioxidant defense system is not adequately developed; therefore, preterm infants are more vulnerable to these substances.[35]

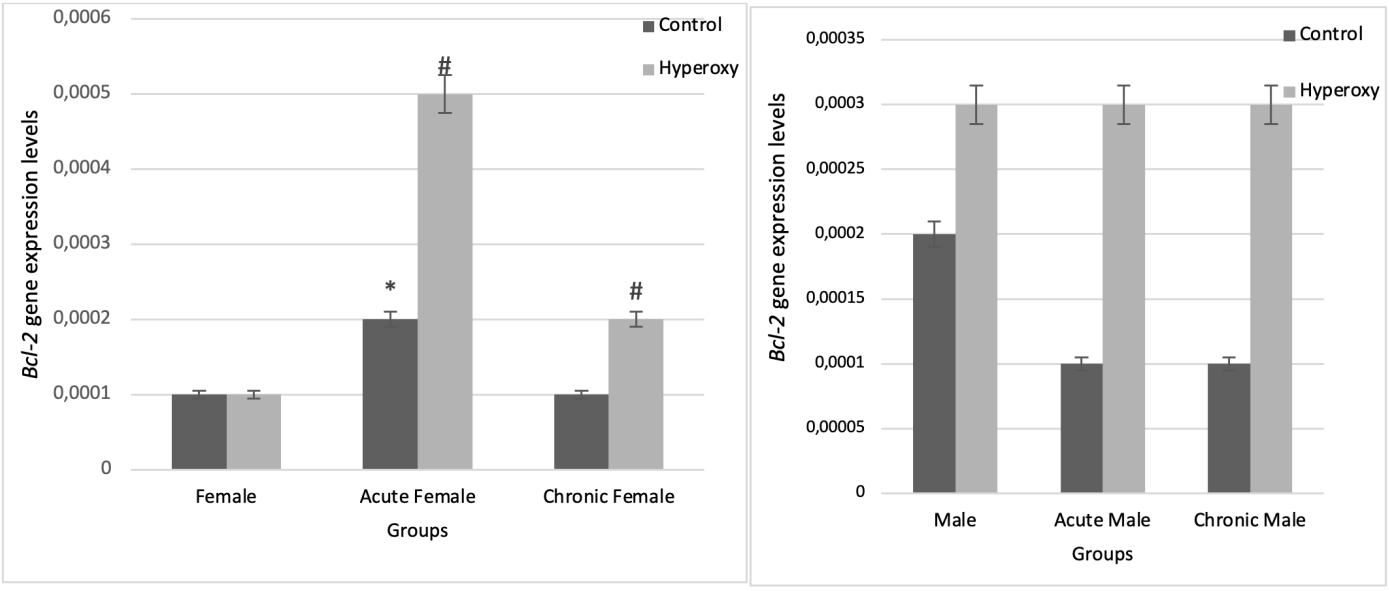

Figure 6. a) *Bcl-2* gene expression levels in brain in female groups *p<0.05 compare with control female groups, #p<0.05 compared with control hyperoxia female groups. **b)** *Bcl-2* gene expression levels in the brain in male

Oxidative stress indicates an imbalance between the formation of free radicals and resistance against oxidative substances, which leads to tissue damage.[36] Considering that preterm newborns exhibit higher levels of oxidative stress because of their inability to face the oxygen-rich environment when they are born, many studies have shown that oxidative stress has an important role in the formation and development of brain damage. During this condition, the body is unable to effectively eliminate the oxidative stress reaction, which produces ROS and leads to apoptosis.[34,37]

Epilepsy is defined as excessive and synchronous excitation of neurons. Its general mechanism is defined as a decrease in inhibitory neurotransmitters (GABA) and an increase in the number of excitatory neurotransmitters. Energy and mitochondria are essential in this mechanism. This is because ATP is required for neurons to be stimulated. During a seizure, the amount of ATP decreases, which causes intracellular calcium to pass to the mitochondria. In mitochondria, the amounts of anti- and pro-apoptotic protein increase. Mitochondrial permeability increases; the apoptosome, consisting of the triad of Cytochrome c, procaspase-9, and apaf 1 forms.[38] These trigger the formation of caspase-9. Caspase-9 leads to the formation of caspase-3, and cell death occurs. Caspase-6 is also found in the brain, which causes neuronal cell death and is more effective than caspase-3. Calcium enters this pathway directly through the endoplasmic reticulum (ER, Ca), and caspase-12 is formed if this pathway is activated. If the inflammation pathway is activated, caspase-8 is also formed Caspase-12 and caspase-8 can stimulate caspase-3, -6, and -7, or altogether (Caspase-8, Caspase-9, Caspase-12) can cause a stimulus, and irreversible cell death occurs.[20]

Ngf and Bdnf are important for the survival, maintenance, and regeneration of certain neuronal populations in the brain. Depletion of these neurotrophic factors is associated with disease pathology and symptoms and is considered a potential therapeutic approach for neurodegenerative diseases.[39] Bdnf was found to be high in the serum of epilepsy patients in direct proportion to the severity of the disease.[40] Studies have shown that Bdnf injection into the temporal and hippocampal brain regions of mice causes seizures, and when Bdnf transcription is blocked, seizures are almost completely eliminated.[41] Similarly, in our study, it was observed that *Bdnf* and *Ngf* gene expression increased and increased significantly, especially with hyperoxia, in the epilepsy group.

According to this result, the increase in the *Ngf* gene expression level in females is independent of hyperoxia. In this study, it was found that the increase in the *Ngf* gene expression level in females was related to epilepsy. Unlike in females, *Ngf* gene expression level increased with the combined effect of hyperoxia and epilepsy in males.

Oxidative stress indicates an imbalance between the formation of free radicals and resistance against oxidative substances, which leads to tissue damage.[36] Considering that preterm newborns exhibit higher levels of oxidative stress because of their inability to face the oxygen-rich environment when they are born, many studies have shown that oxidative stress has an important role in the formation and development of brain damage. During this condition, the body is unable to effectively eliminate the oxidative stress reaction, which produces ROS and leads to apoptosis.[34,37]

Bax and Bcl-2 are Bcl-2 family proteins that are key factors in the regulation of intrinsic apoptosis. Specifically, Bax is a pro-apoptotic protein, whereas Bcl-2 is an anti-apoptotic protein. Bcl-2 proteins are key regulators of the intrinsic apoptotic pathway. Each member of this family contains one or more Bcl-2 homology (BH) domains, BH1-BH4.[42] Bcl-2 family proteins are critical regulators of apoptosis for inhibiting or promoting cell death via the intrinsic pathway of apoptosis.[43] Bax is an important pro-apoptotic protein of the intrinsic pathway. Bax moves into mitochondria by inducing the release of Cyt c into the cytoplasm. Finally, Cyt c activate caspase 9, which destroys caspase 3. Bcl-2, an anti-apoptotic protein, can bind to Bax by inhibiting the release of Cyt c.[44] Studies involving Bax expression in temporal lobe epilepsy are controversial. Several studies have shown an increase in Bax, whereas others have shown similar immunostaining compared with control samples. Bcl-2 and

active caspases are overexpressed in both the neuronal and glial cytoplasm of the sclerotic hippocampus.[20,21,44]

When looking at the *Cyt c* gene expression level in general, it can be seen that the Cyt c level in males is significantly higher than that in females. According to this result, it has been shown that the *Cyt c* gene expression level is higher in cases of acute or chronic epilepsy in male individuals exposed to hyperoxia.

According to our study, while hyperoxia did not cause any effect in acute epilepsy, it was an important factor in females, and in chronic epilepsy, it was observed that the Cyt c level increased significantly. While there was no difference with the control group in acute epilepsy in hyperoxia-exposed female rats, the *Cyt c* gene expression level in chronic epilepsy was significantly increased compared with the chronic CF group. Cyt c gene expression levels increased significantly in both acute and chronic epilepsy in male rats exposed to hyperoxia compared with the control.

Study Limitations

The aim of this study was to investigate the relationship between acute and chronic epilepsy that may occur in adulthood, gene expression levels, and the possible mechanism of neuronal loss in rats exposed to hyperoxia in the postnatal period. However, due to lack of budget, the limitations of this study are the inability to verify protein and the inability to stain Bdnf, Ngf, Cyt c, Bax, and Bcl-2 in the tissue by immunohistochemistry, and the inability to perform TUNEL staining.

CONCLUSION

As a result, if any form of epilepsy develops in adulthood in males and females exposed to hyperoxia, high *Bax* gene expression levels in females with chronic epilepsy and high *Cyt c* gene expression levels in male individuals can be accepted as evidence of neuronal loss. In addition, neuronal loss may occur by different mechanisms in males and females. Ngf is higher in females exposed to hyperoxia, and neuronal loss in females may be less than that in males.

Ethics

Ethics Committee Approval: The study was approved by the Bolu Abant İzzet Baysal University of Animal Ethical Committee (registration number: 2020/23, date: 26.08.2020).

Informed Consent: Animal experiment.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: C.Ö., Ü.K., H.S., Concept: C.Ö., Design: C.Ö., Ü.K., Data Collection or Processing: C.Ö., Ü.K., H.S., Analysis or Interpretation: C.Ö., Ü.K., H.S., Literature Search: C.Ö., Ü.K., H.S., Writing: C.Ö., Ü.K., H.S.

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REFERENCES

1. Saugstad OD. Is oxygen more toxic than currently believed? *Pediatrics*. 2001;108(5):1203-1205. [Crossref]
2. Reich B, Hoeber D, Bendix I, Felderhoff-Mueser U. Hyperoxia and the Immature Brain. *Dev Neurosci.* 2017;38(5):311-330. [Crossref]
3. Yiş U, Kurul SH, Kumral A, et al. Hyperoxic exposure leads to cell death in the developing brain. *Brain Dev*. 2008;30(9):556-562. [Crossref]
4. Volpe JJ. Perinatal brain injury: From pathogenesis to neuroprotection. *Ment Retard Dev Disabil Res Rev*. 2001;7(1):56-64. [Crossref]
5. Hoehn T, Felderhoff-Mueser U, Maschewski K, et al. Hyperoxia causes inducible nitric oxide synthase-mediated cellular damage to the immature rat brain. *Pediatr Res*. 2003;54(2):179-184. [Crossref]
6. Felderhoff-Mueser U, Bittigau P, Sifringer M, et al. Oxygen causes cell death in the developing brain. *Neurobiol Dis*. 2004;17(2):273-282. [Crossref]
7. Stiles J, Jernigan TL. The basics of brain development. *Neuropsychol Rev.* 2010;20(4):327-348. [Crossref]
8. Back SA. Perinatal White Matter Injury:The Changing Spectrum Of Pathology And Emerging Insights Into Pathogenetic Mechanisms. *Ment Retard Dev Disabil Res Rev.* 2006;12:129-140. [Crossref]
9. Khazipov R, Luhmann HJ. Early patterns of electrical activity in the developing cerebral cortex of humans and rodents. *Trends Neurosci*. 2006;29(7):414-418. [Crossref]
10. Karbowski J. Approximate invariance of metabolic energy per synapse during development in mammalian brains. *PLoS One*. 2012;7(3):e33425. [Crossref]
11. Goren B, Cakir A, Sevinc C, et al. Uridine treatment protects against neonatal brain damage and long-term cognitive deficits caused by hyperoxia. Brain *Res*. 2017;1676:57-68. [Crossref]
12. Parikh P, Juul SE. Neuroprotection strategies in preterm encephalopathy. *Semin Pediatr Neurol*. 2019;32:100772. [Crossref]
13. Volpe JJ. Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. *Lancet Neurol*. 2009;8(1):110-124. [Crossref]
14. Zhao Y, Liang L, Liu G, Liu Y, Zheng H, Dai L. The effects of short time hyperoxia on glutamate concentration and glutamate transporters expressions in brain of neonatal rats. *Neurosci Lett*. 2021;758:136013. [Crossref]
15. Pregnolato S, Chakkarapani E, Isles AR, Luyt K. Glutamate transport and preterm brain injury. *Front Physiol*. 2019;10:417. [Crossref]
16. Tehse J, Taghibiglou C. The overlooked aspect of excitotoxicity: Glutamate-independent excitotoxicity in traumatic brain injuries. *Eur J Neurosci*. 2019;49(9):1157-1170. [Crossref]
17. Magi S, Piccirillo S, Amoroso S, Lariccia V. Excitatory amino acid transporters (Eaats): Glutamate transport and beyond. *Int J Mol Sci*. 2019;20(22):5674. [Crossref]
18. Vigneault É, Poirel O, Riad M, et al. Distribution of vesicular glutamate transporters in the human brain. *Front Neuroanat*. 2015;9:23. [Crossref]
19. Behuet S, Cremer JN, Cremer M, Palomero-Gallagher N, Zilles K, Amunts K. Developmental Changes of Glutamate and GABA Receptor Densities in Wistar Rats. *Front Neuroanat*. 2019;13:100. [Crossref]
20. Henshall DC, Simon RP. Epilepsy and apoptosis pathways. *J Cereb Blood Flow Metab*. 2005;25(12):1557-1572. [Crossref]
21. Engel T, Henshall DC. Apoptosis, Bcl-2 family proteins and caspases: The ABCs of seizure-damage and epileptogenesis? *Int J Physiol Pathophysiol Pharmacol*. 2009;1(2):97-115. [Crossref]
22. Murphy B, Dunleavy M, Shinoda S, et al. Bcl-w protects hippocampus during experimental status epilepticus. *Am J Pathol*. 2007;171(4):1258-1268. [Crossref]
23. Henshall DC, Clark RS, Adelson PD, Chen M, Watkins SC, Simon RP. Alterations in bcl-2 and caspase gene family protein expression in human temporal lobe epilepsy. *Neurology*. 2000;55(2):250-257. [Crossref]
24. Lindsten T, Zong WX, Thompson CB. Defining the role of the Bcl-2 family of proteins in the nervous system. *Neuroscientist*. 2005;11(1):10-15. [Crossref]
25. Krajewski S, Mai JK, Krajewska M, Sikorska MJ, Mossakowski MJ, Reed JC. Upregulation lschemia of Bax Protein Levels in Neurons following. *J Neurosci*. 1995;15(10):6364-6375. [Crossref]
26. Chami M, Prandini A, Campanella M, et al. Bcl-2 and bax exert opposing effects on Ca2+ signaling, which do not depend on their putative pore-forming region. *J Biol Chem*. 2004;279(52):54581-54589. [Crossref]

27. Nutt LK, Chandra J, Pataer A, et al. Bax-mediated Ca2+ mobilization promotes cytochrome c release during apoptosis. *J Biol Chem*. 2002;277(23):20301-20308. [Crossref]
28. Nutt LK, Pataer A, Pahler J, et al. Bax and Bak promote apoptosis by modulating endoplasmic reticular and mitochondrial Ca2+ stores. *J Biol Chem*. 2002;277(11):9219-9225. [Crossref]
29. D'Orsi B, Kilbride SM, Chen G, et al. Bax regulates neuronal Ca2+Homeostasis. *J Neurosci*. 2015;35(4):1706-1722. [Crossref]
30. D'Orsi B, Mateyka J, Prehn JHM. Control of mitochondrial physiology and cell death by the Bcl-2 family proteins Bax and Bok. *Neurochem Int*. 2017;109:162-170. [Crossref]
31. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*. 2001;25(4):402-408. [Crossref]
32. Howson CP, Kinney MV, McDougall L, Lawn JE; Born Too Soon Preterm Birth Action Group. Born too soon: preterm birth matters. *Reprod Health*. 2013;10(Suppl 1):1. [Crossref]
33. Panfoli I, Candiano G, Malova M, et al. Oxidative stress as a primary risk factor for brain damage in preterm newborns. *Front Pediatr*. 2018;6:369. [Crossref]
34. Ozsurekci Y, Aykac K. Oxidative Stress Related Diseases in Newborns. *Oxid Med Cell Longev*. 2016;2016:2768365. [Crossref]
35. Kang L, Dong W, Ruan Y, Zhang R, Wang X. The Molecular Mechanism of Sirt1 Signaling Pathway in Brain Injury of Newborn Rats Exposed to Hyperoxia. *Biol Pharm Bull*. 2019;42(11):1854-1860. [Crossref]
36. Czerska M, Mikołajewska K, Zieliński M, Gromadzińska J, Wąsowicz W. Today's oxidative stress markers. *Med Pr*. 2015;66(3):393-405. [Crossref]
37. Thibeault DW. The precarious antioxidant defenses of the preterm infant. *Am J Perinatol*. 2000;17(4):167-181. [Crossref]
38. Liu Z, Sekito T, Spírek M, Thornton J, Butow RA. Retrograde signaling is regulated by the dynamic interaction between Rtg2p and Mks1p. *Mol Cell*. 2003;12(2):401-411. [Crossref]
39. Langhnoja J, Buch L, Pillai P. Potential role of NGF, BDNF, and their receptors in oligodendrocytes differentiation from neural stem cell: An in vitro study. *Cell Biol Int.* 2021;45(2):432-446. [Crossref]
40. Croll SD, Suri C, Compton DL, et al. Brain-derived neurotrophic factor transgenic mice exhibit passive avoidance deficits, increased seizure severity and in vitro hyperexcitability in the hippocampus and entorhinal cortex. *Neuroscience*. 1999;93(4):1491-1506. [Crossref]
41. Binder DK, Routbort MJ, Ryan TE, Yancopoulos GD, McNamara JO. Selective inhibition of kindling development by intraventricular administration of TrkB receptor body. *J Neurosci*. 1999;19(4):1424-1436. [Crossref]
42. Singh R, Letai A, Sarosiek K. Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins. *Nat Rev Mol Cell Biol*. 2020;20(3):175-193. [Crossref]
43. Orrenius S. Mitochondrial regulation of apoptotic cell death. *Toxicol Lett.* 2004;149(1-3):19-23. [Crossref]
44. Adams JM, Cory S. The Bcl-2 protein family: Arbiters of cell survival. *Science*. 1998;281:1322-1326. [Crossref]

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Original Article

Electrocardiographic Evaluation in Patients Receiving Lamotrigine Monotherapy/Duotherapy

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Abstract

Objective: Despite its widespread use and safety data, the cardiac safety of lamotrigine was brought into question in October 2020 when the U.S. Food and Drug Administration issued a safety warning about its cardiac side effects. Here, we investigated whether there are differences in electrocardiogram (ECG) findings between epilepsy cases receiving lamotrigine monotherapy and those receiving duotherapy.

Methods: Patients older than 16 years who were followed up with a diagnosis of epilepsy and receiving lamotrigine were retrospectively identified. Those receiving only lamotrigine and any second anti-seizure medication (ASM) in addition to lamotrigine were included in the study, and those receiving more than two ASMs were excluded. Eligible patients were asked to apply to any health institution and have an ECG performed. Heart rate, PR distance, QRS duration, QT duration, corrected QT value, and Tp-Tend value were calculated manually, and ST-T changes were evaluated. Comparisons were made between patients receiving monotherapy and dootherapy and those receiving low-dose and high-dose lamotrigine.

Results: There were 19 patients receiving monotherapy and 11 receiving duotherapy. The ECG parameters of all other patients were within normal values. When ECG parameters were compared between patients receiving monotherapy and those receiving duotherapy, no significant differences were found in heart rate, PR distance, QRS duration, QT duration, QTc duration, Tp-Tend duration, and presence of ST-T changes. When the patients were divided into low-dose and high-dose lamotrigine groups, there were no significant differences in the ECG parameters between these two groups.

Conclusion: The relationship between the use of lamotrigine and cardiac conduction problems in patients with epilepsy has attracted the attention of physicians since its introduction into clinical practice. Although our results did not indicate a significant relationship, there is still a need to determine the risk groups and clarify the pathophysiology of lamotrigine-related arrhythmia through genotype- and phenotype-related studies.

Keywords: Cardiac conduction disorders, cardiac side effect, electrocardiography, lamotrigine, neurology

INTRODUCTION

Lamotrigine has been widely used as an anti-seizure medication (ASM) since 1994.[1] It acts by modulating voltage-gated sodium channels. Accordingly, lamotrigine prevents the pathologically continuous, high-frequency, repetitive firing of voltage-gated sodium channels and reduces presynaptic glutamate release. This effect inhibits the glutamate-mediated action potential. Thus, it suppresses neuronal hyperexcitability and prevents seizure occurrence.[2,3]

Despite its widespread use and safety data, the cardiac safety of lamotrigine was brought into question in October 2020 when the U.S. Food and Drug Administration (FDA) issued a safety warning about the cardiac side effects of lamotrigine. This warning was rooted in the fact that lamotrigine exhibits class IB antiarrhythmic activity at therapeutically relevant concentrations through the inhibition of human cardiac sodium channels with rapid onset and offset kinetics and strong voltage dependance Although it did not slow ventricular conduction (widen QRS) in healthy individuals in a thorough QT study, it could slow ventricular conduction (widen QRS) and induce proarrhythmia, including sudden death, in patients with structural heart disease or myocardial ischemia.[4,5] Therefore, this warning addressed the cardiac risk group, stating that therapeutic levels of lamotrigine in individuals with any structural or functional heart disease might cause life-threatening arrhythmias. The term “structural or functional heart diseases” indicates heart valve diseases, heart failures, a history of cardiac ischemia, cardiac conduction disorders (second- and third-degree heart block), ventricular arrhythmias, congenital heart diseases, Brugada syndrome, and similar channelopathies, along with any coronary artery disease risk factors without a history of cardiac disease.

It has also been reported that the simultaneous use of a different sodium channel blocker increases this effect.[6] It was emphasized that this effect is not observed in healthy individuals but may occur in those with any of the above-mentioned cardiac disorders, which can be considered a risk factor for arrhythmia.[4]

Thereafter, the International League Against Epilepsy and American Epilepsy Association established the Task Force on the Cardiac Effects of Lamotrigine and released an advisory for healthcare professionals regarding the use of lamotrigine and monitoring cardiac side effects. The study group listed their recommendations under two headings. First, the presence of asymptomatic heart disease would be very rare in individuals under 60 years of age, and in the absence of cardiovascular risk factors such as diabetes, hypertension, hyperlipidemia, and smoking, the risk would be negligible. Therefore, electrocardiography (ECG) before lamotrigine treatment is recommended only in patients younger than 60 yearsof age. Because the risk of asymptomatic cardiac conduction disorders increases in patients over 60 years of age, ECG is recommended before starting lamotrigine in all patients in this group and should be repeated after increasing to the target dose if necessary. In addition, for patients currently receiving lamotrigine therapy, ECG and cardiology consultation should be performed in cases of sudden syncope or presyncope with loss of muscle tone if there is no clear vasovagal/orthostatic cause.[7]

A standard 12-lead ECG provides prognostic information about current and future cardiac events through parameters such as heart rate, PR distance, QT duration, corrected QT value (QTc), Tp-Tend value, and ST-T changes. In many studies, it has been observed that a short PR distance increases all-cause mortality and undesirable cardiovascular events.[8] A prolonged QRS duration usually indicates the presence of changes in the myocardium due to underlying heart disease and is generally associated with decreased ejection fraction or enlarged left ventricular volumes.[9] Heart rate-QTc is the classical method for assessing cardiac repolarization time. A prolonged QTc interval is associated with a higher risk of death in patients with coronary heart disease and in the general population.[10] The Tp-Tend value is the interval from the peak of the T wave at the end of the T wave and is considered a distribution index of ventricular repolarization. Prolonged Tp-Tend interval has been associated with arrhythmia, sudden cardiac death, and increased cardiovascular mortality.[11]

Considering the current but unclear report of cardiac adverse events of lamotrigine, we aimed to investigate whether there are significant changes in the ECG findings of epilepsy cases receiving lamotrigine monotherapy versus myotherapy and low-dose versus high-dose lamotrigine therapy.

MAIN POINTS

- Lamotrigine use may result in Brugada-like cardiac conduction problems.
- Increased neuronal sodium channel expression in cardiac tissue was revealed to cause Brugada-like conduction disorders by increasing the affinity of lamotrigine for cardiac sodium channels in epileptic mice.
- Post-lamotrigine upregulation of pathogenic sodium channel proteins in cardiac tissue may explain this side effect in humans.
- There is a need to determine the risk groups and clarify the pathophysiological mechanism through genotype- and phenotype-related studies.

METHODS

Epilepsy cases followed up in a tertiary healthcare center were included in the study. The İstanbul Training and Research Hospital Local Ethical Committee approved the study (decision no: 266, date: 13.10.2023), and a written patient consent form was obtained. Patients older than 16 years who were receiving lamotrigine treatment were retrospectively identified by examining their medical records. Among them, patients receiving lamotrigine monotherapy or lamotrigine myotherapy with any second ASM were included in the study, whereas patients receiving more than two ASMs were excluded.

There were 400 patients receiving lamotrigine therapy; among them, 100 patients met the inclusion criteria. These patients were contacted by phone and informed about the study. Patients who gave consent were asked to apply to any health institution and have a 12-lead ECG performed and delivered to our center. Thirty had an ECG and were included in the study among these 100 patients. The ECG (paper speed 25 mm/sec and amplitude 10 mm/mV) results of all participants were examined by the same cardiologist. Heart rate, PR distance, QRS duration, QT duration, QTc value, and Tp-Tend value were calculated manually, and ST-T changes were evaluated. The Tp-Tend value was determined as the distance from the peak of the T wave at the end of the T wave. The calculated QT duration was corrected using the Bazett formula. In addition, branch blocks, atrioventricular conduction disorders, ST changes greater than 1 mm, T wave changes, and Brugada patterns on ECGs were investigated.[12,13]

Thirty patients were included in the study, and the following data were collected:

i. Demographics, medical history, and family history of sudden cardiac death.

ii. Seizure onset age, seizure type, epilepsy etiology, daily dose of lamotrigine, duration of lamotrigine use, and additional drugs.

iii. Heart rate, PR interval, QRS width, QT distance, QTc, Tp-Tend distance, and ST change on ECG.

Statistical Analysis

Data analyses were performed using the GraphPad Prism 8.4.3 software statistical package. The data distribution was not normal. We compared demographic and clinical data between patients receiving monotherapy and dootherapy and low-dose and high-dose lamotrigine. We used the chi-square test for qualitative data. A p value ≤ 0.05 was considered significant.

RESULTS

A total of 30 patients participated in the study. The demographic and clinical data of the study groups are presented in Table 1. The mean age of the patients was 31.6±8.5 years [minimum-maximum (min-max): 16-48 years]. With the exception of one patient who had hypothyroidism, none of the patients had any known chronic disease or smoking history that could be a cardiovascular risk factor.

The daily lamotrigine dose for the entire patient group was 200±116.8 mg. There were 19 patients receiving monotherapy,

and the daily dose of lamotrigine for this group was 207.9±106.7 mg. The number of patients receiving myotherapy was 11, and the daily dose of lamotrigine was 228±152 mg for this group. The ASMs used in myotherapy were valproic acid, carbamazepine, levetiracetam, and pregabalin. The duration of lamotrigine use for the entire patient group was 6.8±4.6 years (min-max: 2-18 years). This period was 6.16±3.7 years (min-max: 2-12 years) for patients receiving monotherapy and 7.9±5.7 years (min-max: 2-18 years) for patients receiving dootherapy. There was no significant difference between the two groups regarding the mean lamotrigine dose and duration of use.

When the ECG parameters were evaluated, one patient, whose daily lamotrigine dose was 300 mg/day, had prolonged QT, QTc, and Tp-Tend durations. This patient was consulted by a cardiologist. Her repeated ECG was normal; therefore, the changes in the first ECG were attributed to reversible causes, such as electrolyte imbalance, rather than lamotrigine itself. The ECG parameters of all other patients were within normal values. When ECG parameters were compared between patients receiving monotherapy and duotherapy, no significant differences were found in the heart rate, PR distance, QRS duration, QT duration, QTc duration, Tp-Tend duration, and presence of ST-T changes (Table 2). Finally, the patients were divided into low-dose and high-dose lamotrigine groups; nevertheless, there were no significant differences in the ECG parameters between these two groups (Figure 1).

DISCUSSION

In this study, ECG parameters were used to evaluate the cardiac side effects of lamotrigine, revealing that there was no significant conduction disturbance associated with lamotrigine.

Although the FDA warning regarding the safety of cardiac side effects of lamotrigine is relatively recent, the information that sodium channel blockers can cause cardiac arrhythmia has long been known since studies had investigated lamotrigine-related cardiac conduction disorders before the FDA's warning. In 1994, the year lamotrigine was launched, Steinhoff et al.[14] followed up patients receiving lamotrigine with ECG recordings before and under medication. Interestingly, this study was designed following the observation of a patient with typical chest pain and repolarization disorder on ECG under lamotrigine treatment. However, they did not find a relationship between increased risk of cardiac side effects and lamotrigineuse.

Table 1. Demographic and clinical data of the patient population

Characteristic	Mean±SD
Age, year	31.6±8.5
Seizure onset, year	15.3±7.1
Sex, F (n, %)	25 (86%)
Epilepsy type	
- Generalized	17
- Focal	13
Antiseizure medication	
- Lamotrigine monotherapy	19
- Duotherapy	11
Family history of sudden cardiac death	0/30

SD: Standard deviation, F: Female

Table 2. Comparison of ECG characteristics of patients receiving lamotrigine monotherapy and duotherapy

Parameter	Monotherapy (n=19)	Duotherapy (n=11)	p value
	Mean±SD	Mean±SD	
Heart rate, beat/min	85.2±12.7	77.7±10.3	0.11
PR interval, ms	146.2±21.2	139.9±23.3	0.46
QRS duration, ms	83.6±9.8	83.5±10.9	0.99
QT duration, ms	351.2±28.6	364.8±24.7	0.20
QTc duration, ms	412.8±30.3	415.5±31.4	0.82
Tp-Tend duration, ms	80.7±12.4	82.9±14.9	0.67
ST-T change	0	0	

SD: Standard deviation, ECG: Electrocardiogram

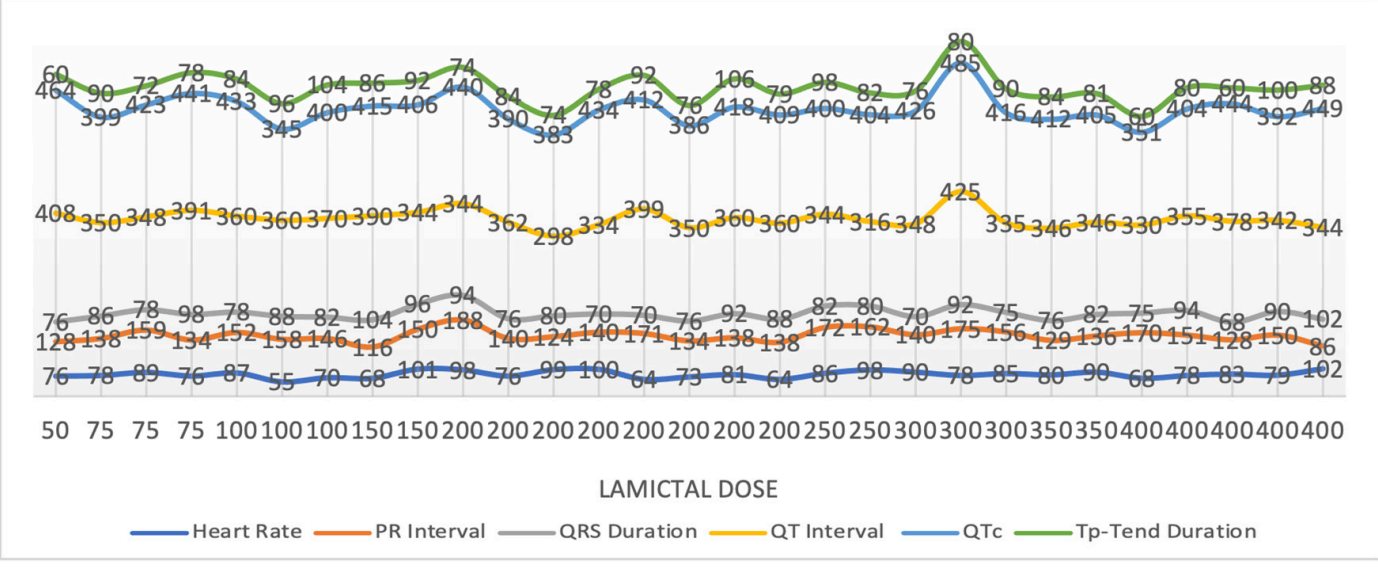

Figure 1. Electrocardiographic parameters of the study group according to lamotrigine dose

In a study on the effects of lamotrigine on ECG in healthy individuals, the drug was initiated with a daily dose of 100 mg and increased to 400 mg, and an average prolongation of the PR distance of 5 ms (normal range: 120-200 ms) was observed, which was negligible.[15] The researchers concluded that the maximum effect of lamotrigine on PR was reached at certain doses and did not increase in a dose-dependent manner because the prolongation of the PR distance was similar at medium and high doses. Furthermore, when QT and QTc durations were examined in the same population, lamotrigine at therapeutic doses did not cause prolonged QT and QTc.[5] Both studies were conducted in healthy young people. In another study conducted in patients over 65 years of age, ECG was performed before and at the 40th week of treatment. Heart rate, QTc, and QRS duration were examined, and no significant ECG changes were observed.[16]

Another concern raised by lamotrigine-related cardiac side effects is that it may be associated with sudden unexpected death in epilepsy (SUDEP). Although the exact cause of SUDEP is unknown, it has been associated with cardiac arrest resulting from respiratory failure in the peri-ictal period.[17] Concomitant cardiac arrhythmia is a risk factor for SUDEP.[18] These findings raise the question of whether the adverse side effects of lamotrigine on cardiac conduction are related to SUDEP. However, there are no definitive findings in the literature showing that any specific antiepileptic drug poses a greater risk for SUDEP than others. Current literature data indicate that SUDEP rates in lamotrigine use are similar to those in other studies.[19]

Although follow-up studies of the cardiac side effects of lamotrigine at therapeutic doses have not been associated with ECG changes indicative of cardiac conduction disturbance or SUDEP, case reports of lamotrigine overdose-related cardiac conduction disturbances and cardiac arrest cannot be disregarded, prompting researchers to further investigate this issue. In the first case report, changes suggestive of Brugada syndrome were detected on ECG of a female patient whose serum lamotrigine level was within a toxic range.[20] Diagnostic ECG changes for Brugada syndrome were observed after the procainamide challenge test. Following lamotrigine withdrawal, ECG was normal after repeated procainamide tests. The authors concluded that toxic doses of lamotrigine may affect cardiac sodium channels, causing drug-related Brugada syndrome.

In a case series, two of nine cases with lamotrigine overdose had prolongation of QRS and QTc on ECG, which was explained by cardiac sodium channel blockage associated with lamotrigine overdose.[21] Although sodium channel blockers, such as phenytoin and carbamazepine, might cause Brugada-type ECG changes, especially in those receiving polytherapy, the reports of patients receiving lamotrigine monotherapy are significantly higher than those of patients receiving other sodium channel blocker ASMs.[22-26] In addition, two of these reports observed ECG changes similar to those in patients with type 1 Brugada syndrome receiving therapeutic doses of lamotrigine.[27,28]

Since the warning in 2020, there have been numerous efforts to examine these side effects in population-based cohorts and systematic reviews. However, sufficient evidence could not be established yet.[29-32] Considering these findings, there is no significant evidence of an increased risk of cardiac arrhythmia with lamotrigine, according to both the literature and our study results. However, the presence of cases receiving therapeutic lamotrigine levels and with Brugada syndrome-like ECG changes cannot be disregarded. At this point, two questions need to be answered:

1) Why does this effect come to the fore with lamotrigine rather than with other sodium channel blockers?

2) Who are the high-risk groups?

Evidence that may answer the first question has come from animal studies. In 2012, Biet et al.[33] studied sodium channel isoforms responsible for producing electrical impulses in the cardiac tissue of canine cardiac muscle. They showed that sodium channels in canine cardiac muscle tissue comprised not only the cardiac isoform NaV 1.5 but also noncardiac isoforms, including neuronal ones. The authors suggested that the overexpression of noncardiac isoforms may be associated with arrhythmias with a prolonged QT interval. Subsequently, in a study published in 2015, it was shown that neuronal sodium channel isoforms were upregulated in the heart tissue and brain in epileptic mice compared with the control group.[34] Finally, following the FDA's warning in 2020, increased neuronal sodium channel expression in the cardiac tissue of epileptic mice was revealed to cause Brugada-like conduction disorders by increasing the affinity of lamotrigine for cardiac sodium channels.[35] The authors showed a decrease in lamotrigine-related cardiac excitability in epileptic mice, whereas there was no significant change in healthy mice, despite receiving the same drug. Although it is unclear whether these findings are valid for humans, they may partially shed light on the issue in question.

Regarding the second question on at-risk individuals, Brugada syndrome needs to be examined, given that cardiac conduction disorders and Brugada syndrome cases come to the fore with lamotrigine. Brugada syndrome is a rare, inherited disease that increases the risk of sudden cardiac death and arrhythmias despite a structurally normal heart. Diagnosis is based on a distinctive ECG finding spontaneously or after the administration of a sodium channel blocker (challenge test).[36] This cardiac conduction disorder, first described in 1992, was first associated with the *SCN5A* gene encoding the cardiac sodium channel. Today, at least 18 loci are associated with the complex polygenic inheritance of the condition.[37] There are reports of family members with the same genetic mutation but without the phenotype,[38] which suggests that lamotrigine may cause ECG changes by revealing phenotypic features with an additive impact. Therefore, if the use of lamotrigine might be arrhythmogenic in individuals with mutations that are not reflected in the phenotype, it seems reasonable to check for any changes that indicate a conduction disorder by performing ECG before the treatment. If the mechanisms proven by animal experiments are also valid for humans, post-lamotrigine upregulation of pathogenic sodium channel proteins in cardiac tissue might be another explanation for this side effect. In addition, genetic examinations in patients who develop cardiac side effects upon lamotrigine treatment are likely to shed light on the current uncertainties and the common pathophysiological mechanism causing Brugada syndrome and epilepsy.

Study Limitations

The most significant limitation of our study is the inadequate number of patients. However, our results are not different from those in the above-mentioned more comprehensive studies conducted with a similar methodology.

CONCLUSION

The relationship between the use of lamotrigine in patients with epilepsy and Brugada-like cardiac conduction problems has attracted the attention of physicians since the drug was introduced into clinical practice; however, it has been under the microscope only since 2020, following FDA warning. There is still a need to determine the risk groups and clarify the pathophysiological mechanism through genotype- and phenotype-related studies.

Ethics

Ethics Committee Approval: The İstanbul Training and Research Hospital Local Ethical Committee approved the study (decision no: 266, date: 13.10.2023).

Informed Consent: Written patient consent form was obtained.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.H.S., B.G.T., S.N.Y., Concept: S.N.Y., Design: S.N.Y., Data Collection or Processing: M.H.S., B.G.T., Ö.S.S., Analysis or Interpretation: Ö.S.S., Literature Search: M.H.S., B.G.T., Ö.S.S., Writing: M.H.S., S.N.Y.

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REFERENCES

1. Goa KL, Ross SR, Chrisp P. Lamotrigine: A Review of its Pharmacological Properties and Clinical Efficacy in Epilepsy. *Drugs*. 1993;46(1):152-176. [Crossref]
2. Macdonald RL, Kelly KM. Antiepileptic drug mechanisms of action. *Epilepsia*. 1995;36(Suppl 2):S2-S12. [Crossref]
3. Rambeck B, Wolf P. Lamotrigine clinical pharmacokinetics. *Clin Pharmacokinet*. 1993;25(6):433-443. [Crossref]
4. Lamictal. Full prescribing information. GlaxoSmithKline, Research Triangle Park, NC 27709, USA. Available from: https://gskpro.com/content/dam/global/hcpportal/en_US/Prescribing_Information/Lamictal/pdf/LAMICTAL-PI-MG.PDF. (last accessed March 2023). Available from: https://gskpro.com/content/dam/global/hcpportal/en_US/Prescribing_Information/Lamictal/pdf/LAMICTAL-PI-MG.PDF [Crossref]
5. Dixon R, Job S, Oliver R, et al. Lamotrigine does not prolong QTc in a thorough QT/QTc study in healthy subjects. *Br J Clin Pharmacol*. 2008;66(3):396-404. [Crossref]
6. United States Food and Drug Administration. Drug Safety and Availibility. Studies show increased risk of heart rhythm problems with seizure and mental health medicine lamotrigine (Lamictal) in patients with heart disease. Last Accessed Date: 02 March 2023. [Crossref]
7. French JA, Perucca E, Sander JW, et al. FDA safety warning on the cardiac effects of lamotrigine: An advisory from the Ad Hoc ILAE/AES Task Force. *Epilepsia Open*. 2021;6(1):45-48. [Crossref]
8. Holmqvist F, Thomas KL, Broderick S, et al. Clinical outcome as a function of the PR-interval-there is virtue in moderation: data from the Duke Databank for cardiovascular disease. *Europace*. 2015;17(6):978-985. [Crossref]
9. Brenyo A, Zaręba W. Prognostic significance of QRS duration and morphology. *Cardiol J*. 2011;18(1):8-17. [Crossref]
10. Crow RS, Hannan PJ, Folsom AR. Prognostic significance of corrected QT and corrected JT interval for incident coronary heart disease in a general population sample stratified by presence or absence of wide QRS complex: the ARIC Study with 13 years of follow-up. *Circulation*. 2003;108(16):1985-1989. [Crossref]
11. Rosenthal TM, Masvidal D, Abi Samra FM, et al. Optimal method of measuring the T-peak to T-end interval for risk stratification in primary prevention. *Europace*. 2018;20(4):698-705. [Crossref]
12. Moss AJ. Measurement of the QT interval and the risk associated with QTc interval prolongation: a review. *Am J Cardiol*. 1993;72(6):23B-25B. [Crossref]
13. Kusumoto FM. Noninvasive Electrocardiography in Clinical Practice. *Chest*. 2002;121(4):1383. [Crossref]
14. Steinhoff BJ, Stodieck SR, Tiecks FP, Wedel R, Polatschek B. Cardiac side effects and ECG changes with lamotrigine?--A clinical study. *Schweiz Arch Neurol Psychiatr (1985)*. 1994;145(3):8-12. [Crossref]
15. Dixon R, Alexander S, Brickel N. Effect of lamotrigine on the PR interval in healthy subjects. *Br J Clin Pharmacol*. 2011;71(6):961-962. [Crossref]
16. Saetre E, Abdelnoor M, Amlie JP, et al. Cardiac function and antiepileptic drug treatment in the elderly: a comparison between lamotrigine and sustained-release carbamazepine. *Epilepsia*. 2009;50(8):1841-1849. [Crossref]
17. Langan Y, Nashef L, Sander JW. Case-control study of SUDEP. *Neurology*. 2005;64(7):1131-1133. [Crossref]
18. Nashef L, Hindocha N, Makoff A. Risk factors in sudden death in epilepsy (SUDEP): the quest for mechanisms. *Epilepsia*. 2007;48(5):859-871. [Crossref]
19. Walczak T. Do antiepileptic drugs play a role in sudden unexpected death in epilepsy? *Drug Saf*. 2003;26(10):673-683. [Crossref]
20. Strimel WJ, Woodruff A, Cheung P, Kirmani BF, Stephen Huang SK. Brugada-like electrocardiographic pattern induced by lamotrigine toxicity. *Clin Neuropharmacol*. 2010;33(5):265-267. [Crossref]
21. Moore PW, Donovan JW, Burkhart KK, Haggerty D. A case series of patients with lamotrigine toxicity at one center from 2003 to 2012. *Clin Toxicol (Phila)*. 2013;51(7):545-549. [Crossref]
22. Ishizue N, Niwano S, Saito M, et al. Polytherapy with sodium channel-blocking antiepileptic drugs is associated with arrhythmogenic ST-T abnormality in patients with epilepsy. *Seizure*. 2016;40:81-87. [Crossref]
23. Rodrigues R, Amador P, Rassi L, et al. Brugada pattern in a patient medicated with lamotrigine. *Rev Port Cardiol*. 2013;32(10):807-810. [Crossref]
24. Chavez P, Casso Dominguez A, Herzog E. Evolving Electrocardiographic Changes in Lamotrigine Overdose: A Case Report and Literature Review. *Cardiovasc Toxicol*. 2015;15(4):394-398. [Crossref]
25. Leong KM, Seligman H, Varnava AM. Proarrhythmogenic effects of lamotrigine during ajmaline testing for Brugada syndrome. *HeartRhythm Case Rep*. 2017;3(3):167-171. [Crossref]
26. Sotero FD, Silva CS, Cunha N, Franco A, Pimentel J. Lamotrigine-induced Brugada syndrome: A rare adverse event. *Seizure*. 2022;94:7-9. [Crossref]
27. Banfi P, Coll M, Oliva A, et al. Lamotrigine induced Brugada-pattern in a patient with genetic epilepsy associated with a novel variant in SCN9A. *Gene*. 2020;754:144847. [Crossref]
28. Omer H, Omer MH, Alyousef AR, Alzammam AM, Ahmad O, Alanazi HA. Unmasking of Brugada syndrome by lamotrigine in a patient with pre-existing epilepsy: A case report with review of the literature. *Front Cardiovasc Med*. 2022;9:1005952. [Crossref]
29. Bunschoten JW, Husein N, Devinsky O, et al. Sudden Death and Cardiac Arrythmia With Lamotrigine: A Rapid Systematic Review. *Neurology*. 2022;98(17):e1748-e1760. [Crossref]
30. Restrepo JA, MacLean R, Celano CM, Huffman JC, Januzzi JL, Beach SR. The assessment of cardiac risk in patients taking lamotrigine; a systematic review. *Gen Hosp Psychiatry*. 2022;78:14-27. [Crossref]
31. Ingleby-Talecki L, van Dijkman SC, Oosterholt SP, et al. Cardiac sodium channel inhibition by lamotrigine: In vitro characterization and clinical implications. *Clin Transl Sci*. 2022;15(8):1978-1989. [Crossref]
32. Christensen J, Trabjerg BB, Dreier JW. Cardiac morbidity and mortality associated with the use of lamotrigine. *Epilepsia*. 2022;63(9):2371-2380. [Crossref]
33. Biet M, Barajas-Martínez H, Ton AT, Delabre JF, Morin N, Dumaine R. About half of the late sodium current in cardiac myocytes from dog

ventricle is due to non-cardiac-type Na(+) channels. *J Mol Cell Cardiol*. 2012;53(5):593-598. **[Crossref]**

34. Biet M, Morin N, Lessard-Beaudoin M, et al. Prolongation of action potential duration and QT interval during epilepsy linked to increased contribution of neuronal sodium channels to cardiac late Na+ current: potential mechanism for sudden death in epilepsy. *Circ Arrhythm Electrophysiol*. 2015;8(4):912-920. **[Crossref]**
35. Contreras Vite JA, Vega Valle C, Biekeu Mbem H, Boivin SM, Dumaine R. Epilepsy-Induced High Affinity Blockade of the Cardiac Sodium Current INa by Lamotrigine; A Potential for Acquired Arrythmias. *Pharmaceuticals (Basel)*. 2022;15(10):1208. **[Crossref]**
36. Gourraud JB, Barc J, Thollet A, Le Marec H, Probst V. Brugada syndrome: Diagnosis, risk stratification and management. *Arch Cardiovasc Dis*. 2017;110(3):188-195. **[Crossref]**
37. Li KHC, Lee S, Yin C, et al. Brugada syndrome: A comprehensive review of pathophysiological mechanisms and risk stratification strategies. *Int J Cardiol Heart Vasc*. 2020;26:100468. Erratum in: *Int J Cardiol Heart Vasc*. 2020;32:100699. **[Crossref]**
38. Korlipara H, Korlipara G, Pentyala S. Brugada syndrome. *Acta Cardiol*. 2021;76(8):805-824.

Archives of Epilepsy

Original Article

How Much is SUDEP Known by Patients by Epilepsy?

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Abstract

Objective: Sudden unexpected death in epilepsy (SUDEP) is the most common cause of death in patients with epilepsy. The issue of informing every epilepsy patient and/or their relatives about SUDEP remains controversial. We evaluated the level of knowledge about SUDEP in patients with epilepsy.

Methods: Patients were asked whether they knew about seizure risks and wanted to be informed about seizure risks, whether they had heard of SUDEP before and if they did, from whom or where they had heard about it, and seizure triggers. In addition, the patient's relatives were asked about what to do or not to do during the seizure.

Results: We included 80 patients with epilepsy in the study, of which 45 were female (56.2%) and 35 were male (44.8%). Twenty-five (31.2%) patients stated that they had not received any information about epilepsy. Only nine (11.2%) patients stated that they heard about SUDEP, six of them learned from the internet, three from a doctor, two of them said it happened to their relatives, and 69 (86.2%) patients thought that epilepsy patients should definitely be informed about this issue.

Conclusion: Our findings indicate that the level of knowledge about SUDEP among epilepsy patients is quite low. Even though learning SUDEP caused uneasiness in patients, it was observed that patients wanted to learn this information. More efforts should be made to inform patients with epilepsy about epilepsy and its risks and SUDEP.

Keywords: Relatives, seizure risk, Sudden unexpected death in epilepsy, survey

INTRODUCTION

Epilepsy is one of the most common neurological diseases affecting approximately 0.6-1% of the world's population.[1] Epilepsy patients may die unexpectedly without structural or pathological etiology, and this condition is called sudden unexpected death in epilepsy (SUDEP). SUDEP is the most common cause of death in people with chronic epilepsy.[2] The incidence of SUDEP varies between 0.3 and 9.3 per 1000 people per year in population-based studies.[3] Frequent seizures, especially a history of generalized tonic-clonic seizures (GTCS), nocturnal seizures, lack of nighttime supervision, male gender, use of multiple anti-seizure medication (ASM), long epilepsy duration, and being diagnosed with epilepsy at a young age are thought to increase the risk of SUDEP.[4]

Recent studies have shown that knowing about potential risks can prevent accidents, injuries, and therefore the occurrence of SUDEP, as well as increase drug compliance and awareness of triggering factors.[5] Therefore, training and information given to patients and their relatives are of critical importance. However, the issue of informing every epilepsy patient and/or their relatives about SUDEP remains controversial. In addition, studies conducted under current conditions have pointed out that the rate of physicians discussing SUDEP with patients and their relatives varies between 12% and 30%, and the rate of physicians who never mention SUDEP varies between 7% and 10%, and cultural differences may play a role in attitudes toward counseling about SUDEP.[6-8]

We aimed to evaluate the level of knowledge about SUDEP and seizure triggers in patients with epilepsy who applied to a tertiary epilepsy center in Turkey, as well as the level of knowledge of patient relatives about behaviors that should or should not be performed during seizures.

METHODS

Between July 2022 and August 2022, patients with epilepsy and their relatives were asked questions about SUDEP and seizures through questionnaires prepared in the University of Health Sciences Turkey, Antalya Training and Research Hospital Epilepsy Outpatient Clinic. The Ethics Committee of University of Health Sciences Turkey, Antalya Training and Research Hospital approved this cross-sectional study (decision no: 14/53, date: 28.07.2022).

Patients and Their Relatives

Patients over the age of 18 years who do not have mental retardation and their relatives were included in the study. Patients without relatives during the outpatient clinic control were not included in the study. An informed consent form was obtained from all patients and their relatives.

Questionnaire

The demographic data of the prepared questionnaires were filled in from their files by the neurologist who followed up. Patients were asked whether they knew about seizure risks (possible answer yes/no), whether they wanted to be informed about seizure risks (possible answer: yes/no), whether they had heard of SUDEP before (possible answer: yes/no), and if they did, from whom or where they had heard (possible answer: doctor/internet/relative/other). Afterwards, the patients were told about SUDEP and asked how they felt learning SUDEP (possible answer: angry/shock/nervous/calm/confidence/courage). Then, the question "Do you think SUDEP should be explained all patients with epilepsy?" was asked. (Possible answer: yes/no). The patients were then asked about seizure triggers (such as adherence to ASM and sleep patterns).

In addition, the patient's relatives were asked about what to do or not to do during the seizure (such as trying to open the mouth during the seizure, trying to control the patient's limbs, trying to feed them).

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences 25.0 software. Categorical variables are described as percentages, and continuous variables are described using mean±standard deviation. Means for continuous variables were compared using independent group t-tests when the data were normally distributed. Categorical variables were analyzed by chi-square and p values of 0.05 or below were considered statistically significant.

MAIN POINTS

- Sudden unexpected death in epilepsy (SUDEP) is the most common cause of death in patients with epilepsy.
- Knowing about potential risks can prevent accidents, injuries, and therefore the occurrence of SUDEP, and increase drug compliance and awareness of triggering factors.
- Our findings indicated that the level of knowledge about SUDEP among patients with epilepsy was very low (18.7%). In addition, even though learning SUDEP caused uneasiness in patients, it was observed that patients wanted to learn this information.

RESULTS

Of the 80 epilepsy patients included in the study, 45 were female (56.2%) and 35 were male (44.8%), with a mean age of 37.6±12.8 years and a mean frequency of seizures of 10.9±8.6 (most frequently every day, seizure-free for at least 3 years). Twenty-six patients (32.5%) had GTCSs ranging from 2 per month to 1 per year while using the available ASM. Twelve patients (15%) had nocturnal seizures. Demographic data of the patients are presented in Table 1.

Twenty-five (31.2%) patients stated that they had not received information about epilepsy and its risks before, whereas 69 (86.2%) patients reported that they wanted to know more. Only nine (11.2%) patients stated that they heard about SUDEP, six of them learned about it from the internet, three from a doctor, and two of them said it happened to their relatives. When asked how learning SUDEP felt after the SUDEP briefing, 36 (45%) patients stated that they felt anxious, 20 (25%) patients stated that they felt safe, five (6.25%) patients said that they felt shocked, and one (1.25%) patient said that they felt angry. When the patients were asked whether SUDEP should be discussed with all epilepsy patients, 11 (13.7%) patients answered no, while 69 (86.2%) patients thought that epilepsy patients should definitely be informed about this issue. The questions asked the patients about SUDEP are summarized in Table 2.

Considering the relationship between age, gender, educational status, frequent GTCS, presence of nocturnal seizures, and long epilepsy duration in patients with knowledge about SUDEP, no statistical significance was observed in our study (p=0.181, 0.095, 0.076, 0.070, 0.110, 0,092 respectively).

Table 1. Demographic data of the patients

Characteristics	n	Percent (%)
Gender		
Male	32	40
Female	48	60
Marital status		
Married	51	63.7
Single	29	36.2
Level of education		
Illiterate	2	2.5
Primary education	32	40
High school	28	35
University	18	22.5
Epilepsy type		
Focal	55	68.7
Generalized	20	25
Unknown	5	6,2
GTCS presence	26	32.5
Number of anti-seizure medications		
Monotherapy	55	68.7
2	15	18.7
3 and above	10	12.5

GTCS: Generalized tonic-clonic seizures

In addition, when the relationship between age, seizure frequency, and GTCS frequency was examined in patients who answered yes to the question of whether SUDEP should be explained to epilepsy patients, no statistically significant correlation was observed (p=0.173, p=0.774, p=0.675, respectively), whereas statistical significance was observed with long epilepsy duration (p=0.035).

Likewise, in the questionnaires, patients were asked about seizure triggers and the patient's relatives were asked about the dos and don'ts during seizures, and these answers are summarized in Table 3.

DISCUSSION

Our findings indicated that the level of knowledge about SUDEP among patients with epilepsy was very low (18.7%). When the literature is examined, it is observed that these rates vary between 14 % and 34% in accordance with our study.[5] In a previous study conducted in North America, SUDEP awareness was associated with increased level of education, long epilepsy duration, and being followed by an epileptologist.[9] In another follow-up study conducted in Germany, an inverse correlation was observed between SUDEP awareness and age.[5] In our study, no correlation was observed between epilepsy awareness and age, gender, frequent GTCS, presence of nocturnal seizures, and long epilepsy duration, but it was noteworthy that all our patients who had knowledge about SUDEP were under 40 years of age.

One of the most important reasons for this low awareness of SUDEP detected in the literature and in our study is that most neurologists exclude informing patients and their relatives about SUDEP in their daily medical practice. In a previous study in which multicenter and pediatric neurologists participated, it was questioned whether physicians gave information about SUDEP to their patients and/or their relatives, and it was observed that 2% of physicians informed all their patients and 8% frequently informed them.[10] In a similar study conducted in Canada, 6.8% of the neurologists who participated in the study stated that they always provided information, while 11.6% of the physicians stated that they never provided information.[8] It was stated that the reason for the tendency to not give information by physicians was the anxiety it would cause on patients, and indeed, the first reaction detected in these studies was fear and anxiety between 40 % and 60%.

During our study, when patients were asked if they wanted to have information about the risks of epilepsy, 86.2% of the patients said they wanted to be informed. All patients were told about SUDEP, and when asked whether SUDEP should be explained to all epilepsy patients, 89 patients (86.2%) stated that they should be explained. When asked how they felt, 45% of the patients stated that they felt anxious. The conclusion we can draw from this is that learning about the presence of SUDEP may indeed cause anxiety in some patients. However, despite this anxiety, a high majority of patients stated that SUDEP should be explained.[7,8,10]

In our study, in support of other studies, although 45% of the patients who received detailed information about SUDEP stated that they felt anxious, despite the feeling of uneasiness, the majority of patients (86.2%) stated that all patients should be informed about this issue. In addition, in several previous studies, the effects on patients were investigated months after SUDEP was explained, and it has been shown that it did not have significant effects on the quality of life of patients or their relatives.[11,12]

The practice guidelines of the American Academy of Neurology and the American Epilepsy Society also recommend that clinicians should warn adult patients with epilepsy to talk about SUDEP and exercise due care. However, to regress the anxiety that will occur in patients, it is recommended to inform the patients that SUDEP

Table 2. Questions asked to the patients about SUDEP

Questions	Answers	n (%)
Have you ever heard about epilepsy and its risks?	Yes	60 (75)
	No	20 (25)
Would you like to know more?	Yes	69 (86.2)
	No	11 (13.7)
Have you heard of SUDEP before?	Yes	15 (18.7)
	No	65 (81.2)
From whom?	Doctor	3 (20)
	Internet	6 (40)
	Other	6 (40)
When?	When the first diagnosis is made	4 (26.6)
	When seizures become frequent	4 (26.6)
	Other	7 (46.7)
How did you feel when you learned of the existence of SUDEP?	Angry	1 (1.2)
	Shock	5 (6.2)
	Anxious	36 (45)
	Calm	16 (20)
	Safe	20 (25)
	Courage	2 (2.5)
Should all patients be told about SUDEP?	Yes	67 (83.7)
	No	13 (16.2)

SUDEP: Sudden unexcepted death in epilepsy

Table 3. Questions asked to the patients and the relatives

To the patients	Answers	n (%)
Epilepsy patients should sleep 7-7.5 hours if possible. Insomnia is a trigger for seizures	Yes	77 (96.3)
	No	3 (3.7)
A low level of anti-seizure medication for any reason is an important seizure trigger (adherence to the medications, medications change)	Yes	61 (76.2)
	No	19 (23.7)
Some antibiotics and medications can trigger seizures	Yes	50 (62.5)
	No	30 (37.5)
To the relatives	**Answers**	**n (%)**
It is correct to try to open one's mouth during a seizure to make it easier to breathe	Yes	25 (31.3)
	No	55 (68.7)
Is it right to block or try to stop the movement of the patient's limbs during a seizure?	Yes	14 (17.5)
	No	66 (82.5)
It is recommended to give water, food and even coffee to speed up the recovery of the patient after the seizure.	Yes	3 (3.8)
	No	77 (96.2)
Any convulsive seizure lasting longer than 5 minutes is indicative to call the emergency services.	Yes	72 (90)
	No	8 (10)

typically affects 1 in 1000 adult patients with epilepsy per year, and therefore 999 patients are not affected.[13]

In a recent multicenter study, it was observed that there was no increase in the depression and anxiety levels of the patients after SUDEP information, and it was argued that SUDEP training should be included in standard epilepsy training. In addition, in this study, it was observed that compliance with ASM increased with SUDEP training.[14]

As observed in our study, the majority of patients who have information about SUDEP access this information mostly from the internet. It is obvious that with the increase in internet use, the young population gains more information. Therefore, in our opinion, it will be more reliable for patients to access this information from the doctor instead of having information from the internet and will increase the patient's trust in the doctor. We believe that this will also increase the patient's compliance with ASM.

In addition, in our study, we received a significant amount of incorrect answers to the questions we asked the patients about seizure triggers and the questions we asked the relatives of the patients about the situations that should not be done during the seizure. Explaining SUDEP effectively to patients and their relatives can help them pay more attention to seizure triggers (such as lifestyle, sleep hygiene, regular use of ASM) and to draw attention to what the patient's relatives can do during the seizure.

Study Limitations

Our prospective study has several limitations. Although our study was conducted in an epilepsy center where high-level refractory epilepsy patients were followed, many patients with refractory seizures were excluded from the study because of mental retardation. Therefore, in our study population, there were relatively few patients with refractory epilepsy. In addition, all questions were asked to the patients in the presence of their relatives. The patients may not have expressed their feelings clearly because they are afraid of their relatives or in order not to disturb them. Furthermore, because the study was conducted in a tertiary hospital, patients with more frequent and severe seizures were more likely to be more common than the generalpopulation. Therefore, it is difficult to generalize the results to a larger epilepsy population.

CONCLUSION

In conclusion, our findings indicate that the level of knowledge about SUDEP among epilepsy patients is quite low. In addition, even though learning SUDEP caused uneasiness in patients, it was observed that patients wanted to learn this information. Therefore, more efforts should be made to inform patients with epilepsy about epilepsy and its risks and SUDEP.

Ethics

Ethics Committee Approval: The Ethics Committee of University of Health Sciences Turkey, Antalya Training and Research Hospital approved this cross-sectional study (decision no: 14/53, date: 28.07.2022).

Informed Consent: Written informed consent forms were obtained from all patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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References

1. Fiest KM, Sauro KM, Wiebe S, et al. Prevalence and incidence of epilepsy: a systematic review and meta-analysis of international studies. *Neurology*. 2017;88(3):296-303. [Crossref]
2. Shankar R, Donner EJ, McLean B, Nashef L, Tomson T. Sudden unexpected death in epilepsy (SUDEP): what every neurologist should know. *Epileptic Disord*. 2017;19(1):1-9. [Crossref]
3. Asadi-Pooya AA, Trinka E, Brigo F, et al. Counseling about sudden unexpected death in epilepsy (SUDEP): a global survey of neurologists' opinions. *Epilepsy Behav*. 2022;128:108570. [Crossref]
4. Sveinsson O, Andersson T, Mattsson P, Carlsson S, Tomson T. Clinical risk factors in SUDEP: a nationwide population-based case-control study. *Neurology*. 2020;94(4):419-429. [Crossref]
5. Schwab C, Wadle NE, Knake S, et al. Patients' knowledge about epilepsy-related risks, morbidity, and mortality: A multicenter cohort study from Germany. *Epilepsy Behav*. 2021;124:108343. [Crossref]
6. Morton B, Richardson A, Duncan S. Sudden unexpected death in epilepsy (SUDEP): don't ask, don't tell. *J Neurol Neurosurg Psychiatry*. 2006;77(2):199-202. [Crossref]
7. Vegni E, Leone D, Canevini MP, Tinuper P, Moja EA. Sudden unexpected death in epilepsy (SUDEP): a pilot study on truth telling among Italian epileptologists. *Neurol Sci*. 2011;32(2):331-335. [Crossref]
8. Friedman D, Donner EJ, Stephens D, Wright C, Devinsky O. Sudden unexpected death in epilepsy: knowledge and experience among U.S. and Canadian neurologists. *Epilepsy Behav*. 2014;35:13-18. [Crossref]
9. Kroner BL, Wright C, Friedman D, et al. Characteristics of epilepsy patients and caregivers who either have or have not heard of SUDEP. *Epilepsia*. 2014;55(10):1486-1494. [Crossref]
10. Strzelczyk A, Zschebek G, Bauer S, et al. Predictors of and attitudes toward counseling about SUDEP and other epilepsy risk factors among Austrian, German, and Swiss neurologists and neuropediatricians. *Epilepsia*. 2016;57(4):612-620. [Crossref]
11. Gayatri NA, Morrall MC, Jain V, Kashyape P, Pysden K, Ferrie C. Parental and physician beliefs regarding the provision and content of written sudden unexpected death in epilepsy (SUDEP) information. *Epilepsia*. 2010;51(5):777-782. [Crossref]
12. Tonberg A, Harden J, McLellan A, Chin RF, Duncan S. A qualitative study of the reactions of young adults with epilepsy to SUDEP disclosure, perceptions of risks, views on the timing of disclosure, and behavioural change. *Epilepsy Behav*. 2015;42:98-106. [Crossref]
13. Harden C, Tomson T, Gloss D, et al. Practice Guideline Summary: Sudden Unexpected Death in Epilepsy Incidence Rates and Risk Factors: Report of the Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology and the American Epilepsy Society. *Epilepsy Curr*. 2017;17(3):180-187. [Crossref]
14. Wadle NE, Schwab C, Seifart C, et al. Prospective, longitudinal, multicenter study on the provision of information regarding sudden unexpected death in epilepsy to adults with epilepsy. *Epilepsia*. 2023;64(2):406-419. [Crossref]

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Original Article

Surgical Results in Temporal Lobe Epilepsies Due to Structural Lesions

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Abstract

Objective: Temporal lobe epilepsy (TLE) is the most common localization-related epilepsy syndrome in adults. The aim of this study was to determine the long-term efficacy of epilepsy surgery in patients with TLE with focal lesions and to evaluate the predictive factors for seizure-free status after surgery.

Methods: Among 109 patients aged more than 17 years, 26 cases with a postoperative follow-up period of at least 2 years and who underwent anterior temporal lobectomy and lesionectomy were included in the study. Each patient was evaluated with a detailed history, video-electroencephalography (EEG), neuroimaging, and postsurgical outcomes according to Engel classification to predict postsurgical seizure freedom.

Results: Patients with chronic TLE (n=26) associated with structural lesions were included in the study. According to Engel's classification, the seizure freedom rate was found to be 92.3% in the first year and 80.8% in the second year after surgery. At the postoperative 2nd year, demographic parameters, disease duration before surgery, mean age of patients, presence of focus to bilateral tonic-clonic seizure, EEG, video EEG monitoring, clinical lateralization, scanning results, surgical technique, and histopathological diagnosis did not demonstrate a significant difference between the seizure-free (Engel's class I) and non-seizure-free groups (Engel's class II, III, IV) ($p>0.05$).

Conclusion: Refractory epilepsy surgery for temporal lobe tumors often offers complete seizure freedom. Complete surgical excision of the epileptogenic region is of great importance for achieving seizure-freeness.

Keywords: Engel classification, epilepsy surgery, temporal lobe epilepsy

INTRODUCTION

Temporal lobe epilepsy (TLE) is the most common localization-related epilepsy syndrome in adults. Previous reports have shown that only 8-23% of TLE patients achieve complete remission with antiepileptic drugs (AEDs) and that surgical treatment is highly superior to medical treatment in these patients.[1,2] Approximately 10-30% of TLE cases are associated with brain tumors, the majority of which are low-grade tumors.[3] The frequency and variety of epileptic seizures vary depending on the tumor localization and histopathological type. Accordingly, low-grade tumors are more epileptogenic than high-grade tumors. Among the most common causes of drug-resistant epilepsy are developmental brain lesions, particularly glioneuronal tumors, cortical developmental anomalies, and focal cortical dysplasias, and surgical treatment is recommended in these patients.[3] The main goal is to provide seizure control.[4]

The aim of this study was to determine the long-term efficacy of epilepsy surgery in patients with TLE with focal lesions and to evaluate predictive factors in terms of post-surgical seizure-free (SF) status.

METHODS

Patients who were diagnosed with medically refractory TLE and underwent standard anterior temporal lobectomy (ATL) between 2010 and 2015 at the Gazi University Medical Faculty Epilepsy Center were retrospectively evaluated. Among 109 patients aged more than 17 years, 26 cases with a postoperative follow-up period of at least 2 years and who underwent ATL and lesionectomy were included in the study. The same examination protocols were applied to all patients before surgery. The present study was approved by the Institutional Ethical Board of the Gazi University Faculty of Medicine (decision no: 49, date: 25.01.2016) and performed in accordance with the ethical standards

laid down in the 1964 Declaration of Helsinki. In preoperative examinations, detailed clinical and medical history and physical and neurological examinations of the patients were performed first. Subsequently, all cases were monitored with scalp electrodes using a 32-channel electroencephalography (EEG), an international 10-20 electrode system, and anterior temporal electrodes. Patients were monitored until they had enough typical seizures. In EEG, interictal epileptiform discharges were accepted as unilateral if they were seen at a rate of 80% and above in one temporal lobe. Temporal lobe localization and right/left lateralization of the patients were determined by correlation with ictal clinical signs and ictal and interictal EEG. Magnetic resonance imaging (MRI) was performed using 1.5- or 3-T thin-sliced epilepsy protocols, including axial and sagittal T1-weighted, axial and coronal T2-weighted, oblique coronal fluid-attenuated inversion recovery perpendicular to the long axis of both hippocampi, and three-dimensional inversion recovery. All images were evaluated by experienced neuroradiologists.

Fluorodeoxyglucose/positron emission tomography (FDG-PET) was performed on all patients before surgery. PET images were acquired using the Discovery ST Camera (GE Medical Systems). Experienced nuclear medicine specialists evaluated the images with respect to the presence of regional hypometabolism, which is an expected abnormal finding in the epileptogenic zone. Psychiatric and neuropsychological evaluations were performed in all cases before surgery, and no psychiatric disorder with contraindications for surgery was found in any patient. Neuropsychological evaluation was given to all patients by a neuropsychologist in the form of a battery before surgery. All patients underwent a WADA test or functional MRI to obtain information about hemispheric lateralization of language and memory functions and make an opinion on possible postoperative deficits. The results of the pre-operative evaluation protocols were discussed in the multi-disciplinary council, and if the clinical semiological findings, interictal and ictal EEG, neuroimaging, and neuropsychological examinations were compatible with each other and localized to a single focus, a surgical decision was made and the surgical technique was determined. The surgical procedure was performed by the same surgeon in all patients in the neurosurgery department of our hospital. The surgery was tailored and guided by the involvement of the hippocampus, mesial temporal structures, and tumor size. If the tumor involved the hippocampus and mesial temporal structures, ATL with amygdala hippocampectomy was performed; if not, only lesionectomy was performed. Patients with other mesial temporal lesions underwent lesion resection and removal of the mesial temporal structures and surrounding cortex. The World Health Organization (WHO) definition of primary brain neoplasms was used for pathological diagnosis.[5] In the postoperative period, the patients were examined by the same epileptology at the 2nd and 6th months, and then once a year, and were evaluated in terms of seizure status and AED use. In accordance with the AED cut-off protocol of our clinic, the drug treatment of all patients was continued for 6 months after surgery, and at the end of the 6th month, the AEDs of the patients who were SF were gradually reduced to one drug. The second drug was also reduced, and it was planned to discontinue all drugs at the end of the second year. The Engel seizure classification was used in the evaluation of postoperative outcomes (class I: free of disabling seizures, class II: rare disabling seizures, class III: worthwhile improvement, class IV: no worthwhile improvement).[6] For the analysis, the SF group (Engel I) was compared with the group with seizures (Engel II, III, IV). The seizure state of the cases was followed up at 6 months, 1 and 2 years, and once a year thereafter. Finally, all factors of both presurgical workup and postsurgical outcomes were compared to predict the possibility of seizure freedom. The following data were matched to postsurgical Engel classifications to determine the predictive value of postsurgical seizure freedom: age at seizure onset, duration of epilepsy, etiology, seizure classification, interictal-ictal EEG findings, MRI, PET, age at surgery, type of surgery, and pathological results.

MAIN POINTS

- Resective surgery provides excellent results for intractable epilepsy.
- Surgery for refractory tumoral temporal lobe epilepsy offers complete seizure freedom. In majority, provided the complete epileptogenic zone is excised.
- Early successful surgical intervention will minimize adverse medical, behavioral, and psychosocial consequences of long-standing refractory epilepsy.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 21.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Descriptive statistics were expressed as mean, standard deviation, median, minimum, and maximum values. Pearson's chi-square test and Fisher's exact chi-square test were used in the analysis of categorical variables. Non-parametric tests (Mann-Whitney U test and Kruskal-Wallis test) were used in the analysis of continuous variables because the sample size was less than 30. The Spearman's correlation test was used to evaluate the relationship between two continuous variables. The statistical significance level was set as $p<0.05$.

RESULTS

A total of 26 patients with chronic TLE associated with structural lesions included in the study were analyzed. The mean age of the patients was 32.31±7.72 (range, 18-49) years, and the mean disease duration before surgery was 17.38±8.34 (range, 4-38) years. The demographic and clinical characteristics of the study group are presented in Table 1. The basic characteristics, seizure semiology, lesion characteristics, and postoperative findings of the patients were compared between the SF (Engel I) and non-SF (NSF) (Engel II, IV) groups. Regarding the risk factors present in patients, history of febrile convulsion (FC) was present in 38.5%, difficult birth, consanguineous marriage and family history of epilepsy was noted in 23.1%, infection history of the central nervous system (CNS) was found in 11.5%. The mean time they had with seizures was 17.38±8.34 years, and the median was 18 (4-38) years. Focal impaired awareness seizures were in all patients examined. Seizures in 69.2% of the patients were of the focus to bilateral tonic-clonic seizure (FBTCS) type. At the postoperative 2nd year, demographic parameters, disease duration before surgery, mean age of patients, presence of FBTCS, EEG, video EEG monitoring (VEM), and clinical lateralization did not demonstrate a significant difference between the SF (Engel's class I) and NSF groups (Engel's class II, III, IV) ($p>0.05$) (Table 1).

Table 1. Demographic and clinical characteristics in SF and NSF patients at the 2nd year after surgery for Engel's outcome

	SF group (n=21)	NSF group (n=5)	p
	n (%*)	n (%*)	
Age (n=26) (32.31±7.72)			
Mean±SD	31.38±7.01	36.20±10.16	0.308
Median (min-max)	31 (18-44)	36 (23-48)	
Gender (n=26)			
Female (n=14)	11 (52.4)	3 (60.0)	1.000[a]
Male (n=12)	10 (47.6)	2 (40.0)	
Lateralization (n=26)			
Right (n=18)	14 (66.7)	4 (80.0)	1.000[a]
Left (n=8)	7 (33.3)	1 (20.0)	
Age of onset (year) (n=26) (14.92±7.50)			
Mean±SD	14.43±6.84	17.00±10.56	0.900
Median (min-max)	16 (1-24)	15 (7-34)	
Duration of epilepsy (year) (n=26) (17.38±8.34)			
Mean±SD	16.95±7.19	19.20±13.10	0.659
Median (min-max)	18 (6-32)	21 (4-38)	
Risk factors			
Febrile seizure (n=10)	10 (47.6)	0	0.121[a]
Trauma (n=6)	6 (28.6)	0	0.298[a]
Dystocia (n=6)	6 (28.6)	0	0.298[a]
Consanguineous marriage (n=6)	4 (19.0)	2 (40.0)	0.558[a]
Family history (n=6)	5 (23.8)	1 (20.0)	0.998[a]
CNS infection (n=3)	1 (4.8)	2 (40.0)	0.085[a]
FIAS	21 (100)	5 (100)	
FIAS frequency			
1-10 seizure	6 (28.6)	2 (40.0)	0.628[a]
11-30 seizure	15 (71.4)	3 (60.0)	
FBTCS	6 (28.6)	2 (40.0)	0.628[a]
FBTCS frequency			
1-10 seizure	13 (86.7)	3 (100)	0.998[a]
11-35 seizure	2 (13.3)	0	
EEG			
Left temporal	14 (66.7)	2 (40.0)	
Right temporal	6 (28.6)	2 (40.0)	0.395
Bitemporal	1 (4.8)	1 (20.0)	
VEM IEDs			
Left temporal	13 (61.9)	2 (40.0)	
Right temporal	8 (38.1)	2 (40.0)	0.103
Bitemporal	0	1 (20.0)	
VEM ictal			
Left temporal	13 (61.9)	2 (40.0)	
Right temporal	8 (38.1)	2 (40.0)	0.103
Bitemporal	0	1 (20.0)	
Clinical lateralization			
Left	11 (52.4)	2 (40.0)	
Right	7 (33.3)	2 (40.0)	0.877
Non-lateralized	3 (14.3)	1 (20.0)	

*Column percentage; SD: Standard deviation; [a]Fisher's exact test.
CNS: Central nervous system, FIAS: Focal impaired awareness seizure, FBTCS: Focal to bilateral tonic-clonic seizure, EEG: Electroencephalography, VEM: Video EEG monitoring, IEDs: Interictal epileptiform discharges, SF: Seizure-free, NSF: Non-SF, min-max: Minimum-maximum

According to the Engel classification of the cases at the 6th month, 1st, and 2nd years after the operation, at the 6th month follow-up, 96.2% of the patients were Engel's class I and 3.8% were Engel's class II. In the first year of follow-up, 92.3% of the patients were Engel's class I and 7.7% were Engel's class II. In the second-year follow-up, 80.8% of the patients were Engel's class I and 19.2% were Engel's class II. There were no patients with Engel's class III and IV in the 6th month, 1st, and 2nd year follow-ups (Table 2).

Preoperative MRI, interictal EEG, and long-term VEM studies were performed on all 26 patients included in the study, and FDG-PET (84.6%) was performed on all patients except 4 patients. PET is not performed in these patients because VEM, EEG, and MRI results can best localize the epileptogenic focus and do not require additional examination. Considering the MRI appearances of the patients, one patient's MRI result was normal. Mesial temporal sclerosis (MTS) was found in 42.3% (n=11) of the patients, tumors were present in 30.8% (n=8), vascular lesions were seen in 15.4% (n=4), and developmental anomalies were found in 7.7% (n=2). No statistically significant difference was found between the patients with and without seizures in the second postoperative year in terms of lateralization and pathological appearance on MRI (p=0.632, p=0.775, respectively) (Table 3). In terms of surgical approach, 50.0% of patients underwent ATL and amygdalohippocampectomy (AH), 38.5% underwent ATL + lesionectomy and AH, 11.5% underwent lesionectomy + AH. When we evaluated the postoperative pathology results, low-grade tumor was found in 42.3% of the patients, cortical dysplasia in 38.5%, cavernous hemangioma in 11.5%, and high-grade tumor in 7.7%. No statistically significant difference was found between the patients with and without seizures in the second postoperative year in terms of the type of surgery and pathology results (p=0.774 p=1.00, respectively) (Table 3).

DISCUSSION

In this study, after a 2-year follow-up of lesional TLE patients, the SF rate was 92.3% in the first year and 80.8% in the second year. This is consistent with the results of previous studies on the positive outcome of surgery in lesional epilepsy.[7,8]

Although there are various opinions about the adequate postoperative follow-up time to determine the prognosis, the general trend is that at least two years of follow-up is sufficient to determine the outcome of the seizure after surgery.[9] For example, Panda et al.[10] observed that 79% of the patients were completely SF at a mean follow-up of 4 years. In the study by Ravat et al.,[11] 85.29% of the patients were SF (Engel's class I) in a mean follow-up of 62 months.

Table 2. Postoperative sixth month, first and second year surgical results

(n=26)	Seizure-free group (Engel's class I)	Non-seizure-free group
	n (%)	n (%)
Sixth month	25 (96.2)	1 (3.8)
First year	24 (92.3)	2 (7.7)
Second year	**21 (80.8)**	**5 (19.2)**

%: percentage

Table 3. Distribution of radiological imaging, type of operation and pathology results of patients at the 2nd year after surgery for Engel's outcome

	Seizure-free group (n=21)	Non-seizure-free group (n=5)	
	n (%*)	n (%*)	p
MRI lateralization			
Left	12 (57.1)	2 (40.0)	
Right	8 (38.1)	3 (60.0)	0.632
Normal	1 (4.8)	0	
MRI			
Mesial temporal sclerosis	9 (42.8)	2 (40.0)	
Tumor	7 (33.3)	1 (20.0)	
Vascular lesion	3 (14.3)	1 (20.0)	0.775
Developmental anomaly	1 (4.8)	1 (20.0)	
Normal	1 (4.8)	0	
Operation type			
ATL + AH	11 (52.4)	2 (40.0)	
ATL+ lesionectomy + AH	8 (38.1)	2 (40.0)	0.774
Lesionectomy + AH	2 (9.5)	1 (20.0)	
Pathology result			
Tumor	11 (57.9)	2 (50.0)	1.000[a]
Cortical dysplasia	8 (42.1)	2 (50.0)	

*Column percentage, [a]Fisher's exact test.
MRI: Magnetic resonance imaging, ATL: Anterior temporal lobectomy, AH: Amygdalohippocampectomy

Age and duration of epilepsy during surgery are undoubtedly one of the most important factors in terms of patient quality of life. In this study, we found that the mean age and duration of epilepsy of the patients were not effective on surgical outcomes. Most studies reported that a younger age of onset and long epilepsy duration were associated with poor surgical outcomes.[12,13] It has been shown that there is a delay in the referral of patients with focal epilepsy to epilepsy centers, and similar to our study, the average time from the onset of epilepsy to surgery is 20 years.[14] The long delay before surgery may be due to the anxiety of complications such as possible post-operative disability and cognitive deficits, delayed referral of possible candidates, and long waiting lists in specialized hospitals.[15] Considering the studies evaluating prognostic factors for surgical results, no clear feature was specified in predicting the outcome. When the relationship between FC and MTS is investigated, there is a strong relationship between these two conditions, although there is no definite evidence that FC is a risk factor for MTS. In addition to studies showing that FC has a positive predictive value on surgical outcome,[16,17] many studies did not show any independent effect of this factor on surgical outcome despite high SF rates,[8,18] consistent with our study. Similarly, we could not find a relationship between other factors such as perinatal complications, developmental delay, FCs, family history of seizures, history of head trauma and CNS infection, and seizure-freeness after surgery.

The most common appearance in the MRI of the patients was MTS, and the second most common was tumor. MRI results of one patient were normal. No statistically significant difference was found between patients with and without seizures in the second postoperative year in terms of lateralization and pathological appearance on MRI. While MRI can detect 80% of patients with MTS, it has 100% diagnostic value in lesions consistent with the mass. In studies using MRI techniques, it has been reported that an accurate diagnosis can be made at a rate of 80-90% when the findings are compared with the pathological findings.[19] Hippocampal sclerosis (HS) is the most common pathology detected on MRI in patients with drug-resistant TLE. Other pathological MRI findings include tumors, vascular malformations, malformations of cortical development, and evidence of distant trauma, post-infection, or ischemic injury. However, approximately 30% of patients with electroclinical evidence of drug-resistant TLE have normal MRI scans on visual inspection (MRI-negative TLE). This causes an inherent difficulty in identifying the epileptogenic site in these patients. However, with advanced localization techniques, these cases are usually suitable for surgical resection.[20] In the study, there was no significant relationship between MRI results (in order of frequency, MTS, tumor, vascular lesion, developmental anomaly, normal appearance) and second-year SF rates. Hu et al.[21] reported that there was no significant relationship between MRI results and Engel classification.

Postoperative seizure-freeness may be due to etiology and pathological findings.[9] When patients are evaluated in terms of pathology results, the most common causes according to the WHO classification are grade 1-2 tumors and cortical dysplasia. In a histopathological series of approximately 10,000 brain tissue samples obtained during epilepsy surgery, HS (36.4%) was the most common histopathological diagnosis. Brain tumors were the second most common histopathological diagnosis, occurring in 23.6% of the samples, and ganglioglioma was the most common (10.4%). Dysembryoplastic neuroepithelial tumors were the second most common tumor type (5.9%). The most common location of these tumors was the temporal lobe. One year after surgery, 68.4% of patients with tumors (79.9% of children and 63.5% of adults) were SF.[14]

Although we found a significant relationship between pathology results and SF rates in our study, the underlying pathology causing seizures affects surgical results. de Tisi et al.[22] and Bien et al.[23] showed in their studies that the resection of benign tumors and HS is associated with good surgical outcome. In another study, Mehvari Habibabadi et al.[24] reported that they could not find a significant relationship between pathology results and Engel classification. In a large, multicenter, cohort study of 9147 patients undergoing epilepsy surgery in 18 European countries, the proportion of patients with Engel class 1 was 68% 2 years after surgery, compared with 78% of patients with low-grade epilepsy-related neuroepithelial tumors. It has been emphasized that the histopathological diagnosis is an important and independent determinant of the outcome.[15]

Three types of surgical strategies were applied to the patients in this study. Half of the patients underwent ATL + AH, approximately 40% underwent ATL + lesionectomy + AH, and the rest underwent lesionectomy + AH. When these surgical procedures and postoperative SF rates were compared, no significant relationship was found. Several studies have reported satisfactory seizure control in lesional mTLEs, particularly in tumors, when the lesion is completely resected, although the extent of resection is more limited than that in conventional ATL.[25,26] It has been reported that this method is advantageous in terms of various complications that may occur with conventional ATL.[27] In addition, there are

studies in the literature that support better seizure outcome with anterior mesiotemporal resection compared with lesionectomy in temporal neoplastic lesion.[28,29] In a recent study by Raiyani et al.,[30] it was argued that for lesions located in the mesial temporal lobe, anteromesial temporal resection and lesionectomy provide a better seizure outcome than lesionectomy alone. As can be seen, there is no consensus in the literature on surgical procedures for epilepsy. In the clinic where the study was conducted, surgical treatment is decided with a multidisciplinary approach, considering many factors such as the location of the lesion, MRI, comorbid conditions of the patient, and socio-cultural status.

Study Limitations

The main limitation of our study is the small sample size because we only included patients with lesional TLE. Another limitation is that our patients were limited to a short follow-up period. Simultaneously, the fact that the choice of surgical procedures mainly reflects our surgical strategy at that time can be considered among the limitations.

CONCLUSION

Surgery for refractory epilepsy in temporal lobe tumors often offers complete seizure freedom. Complete surgical excision of the epileptogenic region is of great importance for achieving seizure-freeness. Early successful surgical intervention will minimize the adverse medical, behavioral, and psychosocial consequences of long-standing refractory epilepsy.

Ethics

Ethics Committee Approval: The study was approved by Institutional Ethical Board of Gazi University Faculty of Medicine (decision no: 49, date: 25.01.2016).

Informed Consent: Informed consent was obtained from all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: T.G.D., İ.Y., G.K., Concept: M T.G.D., İ.Y., E.B., Design: T.G.D., İ.Y., E.B., G.K., Data Collection or Processing: T.G.D., G.K., Analysis or Interpretation: T.G.D., İ.Y., E.B., Literature Search: T.G.D., Writing: T.G.D.

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REFERENCES

1. Kumlien E, Doss RC, Gates JR. Treatment outcome in patients with mesial temporal sclerosis. *Seizure*. 2002;11(7):413-417. [Crossref]
2. Schmidt D, Stavem K. Long-term seizure outcome of surgery versus no surgery for drug-resistant partial epilepsy: a review of controlled studies. *Epilepsia*. 2009;50(6):1301-1309. [Crossref]
3. Hennessy MJ, Elwes RD, Honavar M, Rabe-Hesketh S, Binnie CD, Polkey CE. Predictors of outcome and pathological considerations in the surgical treatment of intractable epilepsy associated with temporal lobe lesions. *J Neurol Neurosurg Psychiatry*. 2001;70(4):450-458. [Crossref]
4. Giulioni M, Marucci G, Martinoni M, et al. Epilepsy associated tumors: Review article. *World J Clin Cases*. 2014;2(11):623-641. [Crossref]
5. Kleihues P, Burger PC, Scheithauer BW. Definitions and explanatory notes. *Histological Typing of Tumours of the Central Nervous System*. 1993:11-51. [Crossref]
6. Engel J, Van Ness PC, Rasmussen TB, Ojemann LM. Outcome with respect to epileptic seizures. In: Engel J., Jr (ed). Surgical treatment of the epilepsies, ed 2nd. *Raven Press*. 1993:609-621. [Crossref]
7. Mohan M, Keller S, Nicolson A, et al. The long-term outcomes of epilepsy surgery. *PLoS One*. 2018;13(5):e0196274. [Crossref]
8. Mehvari Habibabadi J, Moein H, Jourahmad Z, et al. Outcome of epilepsy surgery in lesional epilepsy: Experiences from a developing country. *Epilepsy Behav*. 2021;122:108221. [Crossref]
9. Nagarajan L, Lee M, Palumbo L, et al. Seizure outcomes in children with epilepsy after resective brain surgery. *Eur J Paediatr Neurol*. 2015;19(5):577-583. [Crossref]
10. Panda S, Radhakrishnan VV, Radhakrishnan K, Rao RM, Sarma SP. Electro-clinical characteristics and postoperative outcome of medically refractory tumoral temporal lobe epilepsy. *Neurol India*. 2005;53(1):71-72. [Crossref]
11. Ravat S, Iyer V, Muzumdar D, et al. Clinical characteristics, surgical and neuropsychological outcomes in drug resistant tumoral temporal lobe epilepsy. *Int J Surg*. 2016;36(Pt B):436-442. [Crossref]
12. Lee YJ, Lee JS. Temporal lobe epilepsy surgery in children versus adults: from etiologies to outcomes. *Korean J Pediatr*. 2013;56(7):275-281. [Crossref]
13. Haque OJ, Mandrekar J, Wyatt K, et al. Yield and Predictors of Epilepsy Surgery Candidacy in Children Admitted for Surgical Evaluation. *Pediatr Neurol*. 2015;53(1):58-64. [Crossref]
14. Blumcke I, Spreafico R, Haaker G, et al. Histopathological Findings in Brain Tissue Obtained during Epilepsy Surgery. *N Engl J Med*. 2017;377(17):1648-1656. [Crossref]
15. Lamberink HJ, Otte WM, Blümcke I, et al. Seizure outcome and use of antiepileptic drugs after epilepsy surgery according to histopathological diagnosis: a retrospective multicentre cohort study. *Lancet Neurol*. 2020;19(9):748-757. [Crossref]
16. Capraz IY, Kurt G, Akdemir Ö, et al. Surgical outcome in patients with MRI-negative, PET-positive temporal lobe epilepsy. *Seizure*. 2015;29:63-68. [Crossref]
17. Elsharkawy AE, Alabbasi AH, Pannek H, et al. Long-term outcome after temporal lobe epilepsy surgery in 434 consecutive adult patients. *J Neurosurg*. 2009;110(6):1135-1146. [Crossref]
18. Asadi-Pooya AA, Nei M, Rostami C, Sperling MR. Mesial temporal lobe epilepsy with childhood febrile seizure. *Acta Neurol Scand*. 2017;135(1):88-91. [Crossref]
19. Carne RP, O'Brien TJ, Kilpatrick CJ, et al. MRI-negative PET-positive temporal lobe epilepsy: a distinct surgically remediable syndrome. *Brain*. 2004;127(Pt 10):2276-2285. [Crossref]
20. Muhlhofer W, Tan YL, Mueller SG, Knowlton R. MRI-negative temporal lobe epilepsy-What do we know? *Epilepsia*. 2017;58(5):727-742. [Crossref]
21. Hu WH, Ge M, Zhang K, Meng FG, Zhang JG. Seizure outcome with surgical management of epileptogenic ganglioglioma: a study of 55 patients. *Acta Neurochir (Wien)*. 2012;154(5):855-861. [Crossref]
22. de Tisi J, Bell GS, Peacock JL, et al. The long-term outcome of adult epilepsy surgery, patterns of seizure remission, and relapse: a cohort study. *Lancet*. 2011;378(9800):1388-1395. [Crossref]
23. Bien CG, Raabe AL, Schramm J, Becker A, Urbach H, Elger CE. Trends in presurgical evaluation and surgical treatment of epilepsy at one centre from 1988-2009. *J Neurol Neurosurg Psychiatry*. 2013;84(1):54-61. [Crossref]
24. Mehvari Habibabadi J, Moein H, Basiratnia R, et al. Outcome of lesional epilepsy surgery: Report of the first comprehensive epilepsy program in Iran. *Neurol Clin Pract*. 2019;9(4):286-295. [Crossref]
25. Giulioni M, Galassi E, Zucchelli M, Volpi L. Seizure outcome of lesionectomy in glioneuronal tumors associated with epilepsy in children. Seizure outcome of lesionectomy in glioneuronal tumors associated with epilepsy in children. *J Neurosurg*. 2005;102(3 Suppl):288-293. [Crossref]

26. Phi JH, Kim SK, Cho BK, et al. Long-term surgical outcomes of temporal lobe epilepsy associated with low-grade brain tumors. *Cancer*. 2009;115(24):5771-5779. [Crossref]
27. Hill SW, Gale SD, Pearson C, Smith K. Neuropsychological outcome following minimal access subtemporal selective amygdalohippocampectomy. *Seizure*. 2012;21(5):353-360. [Crossref]
28. Giulioni M, Rubboli G, Marucci G, et al. Seizure outcome of epilepsy surgery in focal epilepsies associated with temporomesial glioneuronal tumors: lesionectomy compared with tailored resection. *J Neurosurg*. 2009;111(6):1275-1282. [Crossref]
29. Jooma R, Yeh HS, Privitera MD, Gartner M. Lesionectomy versus electrophysiologically guided resection for temporal lobe tumors manifesting with complex partial seizures. *J Neurosurg*. 1995;83(2):231-236. [Crossref]
30. Raiyani V, Singh S, Sardhara J, et al. Seizure Outcome after Lesionectomy With or Without Concomitant Anteromedial Temporal Lobectomy for Low-Grade Gliomas of the Medial Temporal Lobe. *Asian J Neurosurg*. 2021;16(3):518-524. [Crossref]

Archives of Epilepsy

Case Report

Epilepsy Spectrum Associated with *PRRT2* Variants: Case Presentations

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Abstract

Variations in the *PRRT2* gene have been shown to cause a variety of diseases, including benign familial infantile epilepsy (BFIE) and paroxysmal kinesigenic dyskinesia (PKD). Next-generation sequencing techniques have allowed the broadening of this disease spectrum. In this study, we aimed to present patients with epilepsy who were shown to have *PRRT2* variants in our clinic. The characteristics of 13 patients with epilepsy, including two families with *PRRT2* variants and one patient with a sporadic homozygous variant, were reviewed by screening the epilepsy archive. P.R217Pfs∗8 variation was detected in patients of our first family with both BFIE and PKD diseases. This family was included in the article in which this gene was first described in 2012. In the first generation there were 3 patients with BFIE, in the second generation there were 2 patients with BFIE-PKD and one patient with BFIE. The second family had only BFIE. In this family, the c.604_607del (p.Ser202HisfsTer26) variation was detected in the *PRRT2* gene in the index case. In this phenotypically homogeneous family, BFIE was present in all 3 generations. Although the seizures remitted, electroencephalography abnormalities continued for 2 years in our index case. Migration of the epileptogenic focus to the posterior of the hemispheres over time is an interesting observation. Our sporadic case was a patient with a diagnosis of juvenile absence epilepsy, and a homozygous c.67G>A;p.(Glu23Lys) variant was detected in this patient. Findings in *PRRT2*-associated epilepsy patients show the importance of next-generation sequencing techniques. It indicates that different epilepsy phenotypes can be seen in variations associated with a single gene. With better recognition of epilepsy associated with *PRRT2* gene variants, which are considered as synaptopathy, it will be possible to switch from current symptomatic treatments to therapeutic options targeting specific pathophysiological changes.

Keywords: Synaptopathy, benign familial infantile epilepsy, paroxysmal kinesigenic dyskinesia, *PRRT2*

INTRODUCTION

Genetic causes are important in many systemic and neurological diseases. The process, which started with family studies previously, is now progressing much faster with the developments in gene sequencing technologies and bioinformatics. Thus, our knowledge and treatment options are increasing. It becomes clear over time that genetic etiology plays a major role in epilepsy, movement disorders, and migraine.[1] It was first determined that variants in the *PRRT2* gene cause paroxysmal kinesigenic dyskinesia (PKD), which is a distinct phenotype, through genetic linkage analysis and whole-exome sequencing.[2] In the following period, it was shown that the same gene causes benign familial infantile epilepsy (BFIE).[3] Today, the spectrum of *PRRT2*-related diseases has expanded beyond these entities and has included several different diseases, such as neurodevelopmental disorders and hemiplegic migraine. There are not many publications from our country about variants in this gene.

Our aim in this study was to present epilepsy patients in our clinic with variants detected in the *PRRT2* gene and to review the developments in the spectrum of this gene.

CASE PRESENTATIONS

In this article, we will present two families with a *PRRT2* variant and a sporadic case with a homozygous variant in the same gene, followed up in our clinic for epilepsy for years.

Family 1

In the first family diagnosed with BFIE and PKD together, ENST00000358758.12:c.649dup; p.(Arg217ProfsTer8) pathogenic variation (ClinVar: VCV000065758.86) was detected in five patients in 2012. This family was included in a article in which the *PRRT2* gene was first described.[4] In the first generation, three patients had BFIE that resolved spontaneously without the use of medication. Two patients in the second generation had BFIE-PKD, and one patient had BFIE only (Figure 1). All patients had BFIE, and generalized convulsive seizures were observed with daily frequency, starting at the age of 8-10 months. There was moderate cyanosis but no fever. The seizures were controlled with low-dose phenobarbital and ended between the ages of 2 and 4 years, after which the drug treatment was discontinued. In two patients in the second generation, PKD was observed after BFIE, which started at the age of 4-8 years. Involuntary movements in the extremities were observed, triggered by sudden and rapid movements lasting less than 1 min, and without loss of awareness. The movements were unilateral in one patient and bilateral in the other sister.

These involuntary movements were controlled with carbamazepine 100 mg/day. Seizure frequencies and PKD severities vary among patients. Neurological examination and cranial magnetic resonance imaging (MRI) of all patients were normal, and electroencephalography (EEG) examinations of the two index patients showed no significant features other than nonspecific theta paroxysms.

Family 2

The patients from the second family had only BFIE. In the index case, pathogenic ENST00000358758.12:c.604_607del in the *PRRT2* gene; p.(Ser202HisfsTer26) variation (ClinVar: rs1064793851) was detected. The members of this phenotypically more homogeneous family experienced seizures that started at 4-6 months of age and were controlled with phenobarbital (Figure 2). Although the seizures stopped, the EEG abnormality in our index case continued for 2 years (Figures 3a, 3b, 3c). The patient's older sister had a history of similar seizures, which stopped immediately with treatment. Seizures seen in his mother, aunt, maternal uncle, and maternal grandfather ended without medication. Neurological examination and cranial MRI were normal.

Sporadic Case

Our sporadic case was a female patient who was diagnosed with juvenile absence epilepsy and had a generalized convulsion once. EEG showed nonspecific generalized paroxysms and photosensitivity. A homozygous but possibly benign c.67G>A;p.(Glu23Lys) variant (ClinVar: rs140383655) was detected in the patient whose parents were relatives (Figure 4).

MAIN POINTS

- Findings in *PRRT2*-associated epilepsy patients show the importance of next-generation sequencing techniques.
- The *PRRT2* gene is expressed in the central nervous system, especially in the cerebral cortex, basal ganglia and cerebellum.
- BFIE, PKD, and PKD/IC form the basis of the spectrum of *PRRT2*-associated paroxysmal disorders.

DISCUSSION

The *PRRT2* gene is located on chromosome 16p11.2 and encodes the PRRT2 protein, which consists of 340 amino acids. The *PRRT2* gene is expressed in the central nervous system, particularly in the cerebral cortex, basal ganglia, and cerebellum.[2,3] In animals, the expression of PRRT2 has been examined in the developing nervous system. Accordingly, it has been shown that there is a significant increase in gene expression in the early postnatal period and a decrease in adulthood.[5] Age-related changes in gene expression may be important in explaining the onset and termination of symptoms in patients at certain age intervals.

At the cellular level, the PRRT2 protein is localized in axons, especially in glutamatergic synapses, and is associated with the GRIN1A glutamate receptor, which is a member of the AMPA receptor family.[4] It can be thought that variants in this gene are related to the etiopathogenesis of the seizures in our cases. PRRT2 interacts with the synaptic t-SNARE protein SNAP25 (Synaptosomal-Associated Protein), indicating its role in the synaptic vesicle mechanism and neurotransmitter release. Subsequent studies have shown that PRRT2 also interacts with other synaptic proteins involved in neurotransmitter release, such as VAMP2 (Vesicle Associated Membrane Protein 2) and synaptotagmin (Syt1 and 2).[6]

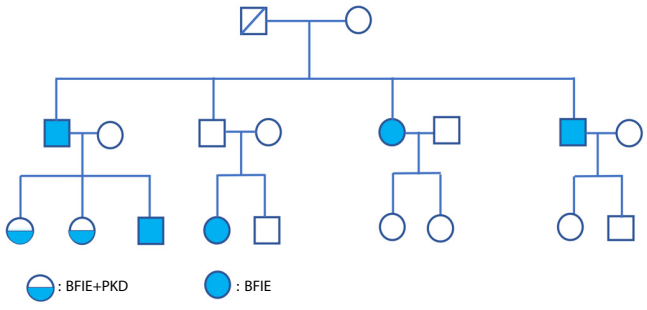

Figure 1. Family 1, in the first generation, 3 patients with the p.R217Pfs*8 variation had only BFIE, which ended spontaneously without the use of medication, while BFIE-PKD coexistence was noted in 2 patients in the second generation, and the presence of BFIE in one patient
BFIE: Benign familial infantile epilepsy, PKD: Paroxysmal kinesigenic dyskinesia

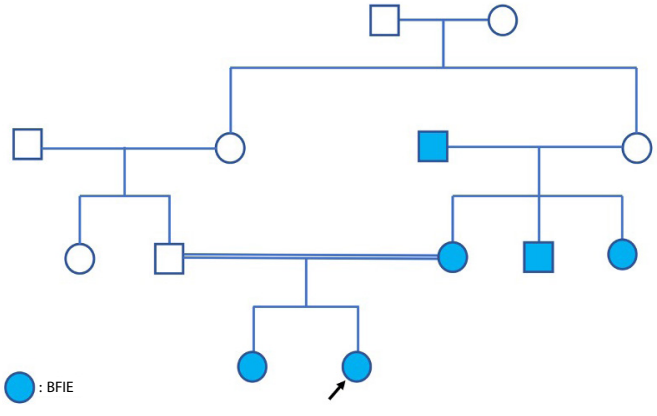

Figure 2. Family 2 was phenotypically homogeneous, with members in three generations having BFIE
BFIE: Benign familial infantile epilepsy

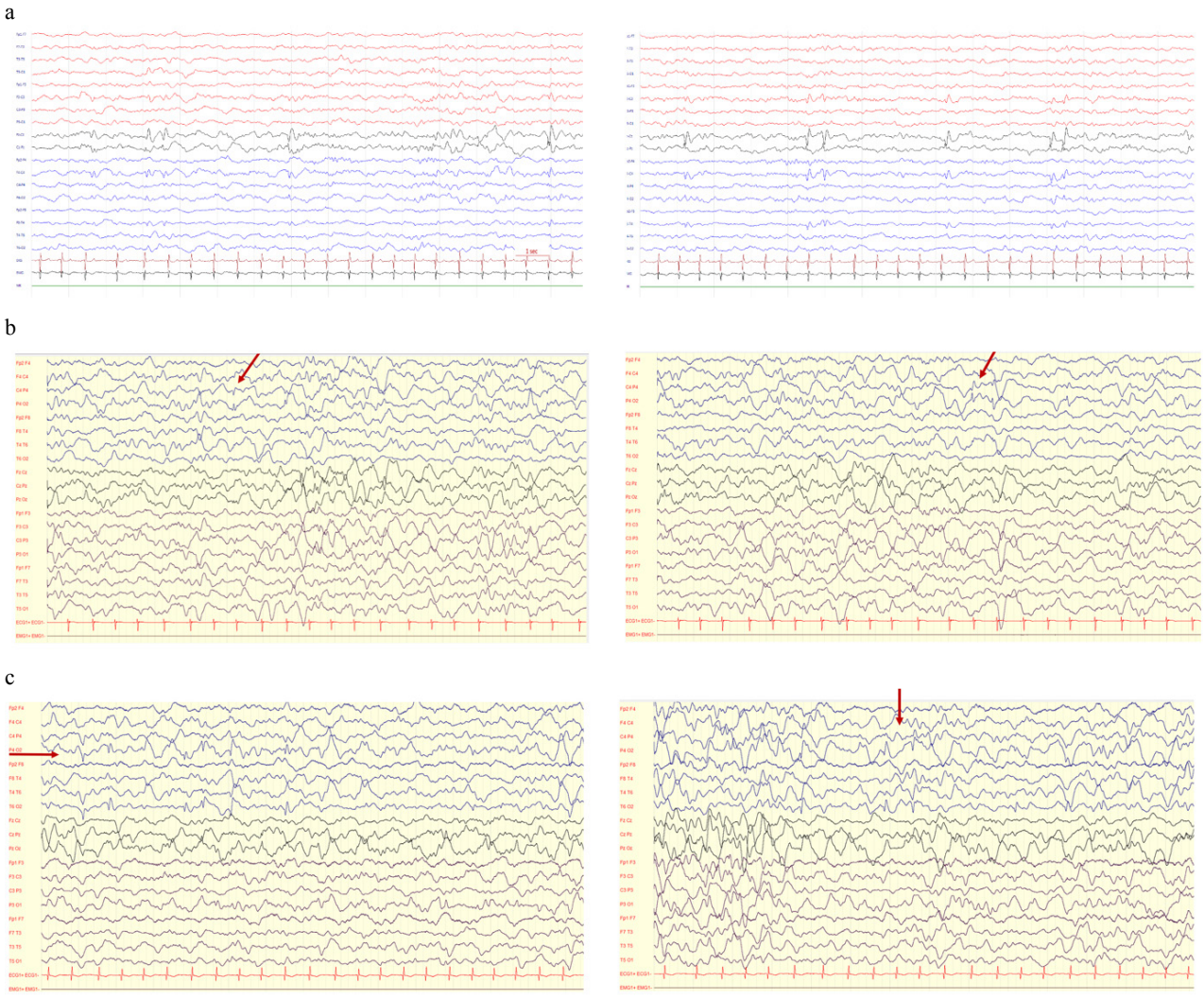

Figure 3. a) Family 2, interictal EEG of the index case shows the presence of epileptogenic foci in the centro-parieto-temporal regions, prominent in the midline and on the right (at the age of 2.5). b) The interictal EEG examination of family 2-index case shows low-amplitude spike-wave activity in the right parietal region during sleep (at the age of 3.5 years). c) Family 2-index case's interictal EEG examination shows epileptiform activity in the right occipital region during sleep. Although the patient's seizures ended over time, the migration of epileptogenic foci towards the posteriors of the hemispheres was impressive (at the age of 4)
EEG: Electroencephalography

SNAP25 is expressed predominantly in neurons and neuroendocrine cells and is extensively involved in presynaptic terminals. SNAP25 regulates calcium-triggered exocytosis in synaptic vesicles by three mechanisms. SNAP25 acts as a t-SNARE protein, plays a role in the molecular mechanism required for synaptic vesicle fusion, contributes to endocytosis at synapses, and negatively regulates voltage-gated channels. Therefore, the inactivation of SNAP25 results in increased activity in glutamatergic neurons. Therefore, impairment or decrease in the function of PRRT2, which interacts with SNAP25, may lead to changes in synaptic vesicle release and neuronal hyperexcitability.[7]

Recent studies have shown that PRRT2 also causes negative modulation of voltage-gated Nav1.2 and Nav1.6 channels.[8] Studies on induced pluripotent stem cell-derived neurons from homozygous patients and neurons from *PRRT2* knockout mice have shown an increase in sodium currents.[8] This causes spontaneous firing when suprathreshold, high-frequency stimulation is applied to neurons. The abnormal firing was completely reversed when *PRRT2* was regained by the cells. Beyond synaptic dysfunction, the impairment in cellular excitability caused by the negative modulation of Na + channels may explain the paroxysmal character of *PRRT2*-related disorders and the effectiveness of molecules acting on the sodium channel.[2,8]

In the review by Ebrahimi-Fakhari et al.[9], patients with 70 different *PRRT2* variations were analyzed. According to this review, 5.5% of the variations were *de novo*, whereas 87.1% were familial. It was highlighted that almost 80% of the patients had the pathogenic c.649dupC; p. Arg217Profs*8 variation, which was also found in the first family in our study. When the variations in the *PRRT2* gene were classified according to their types, it was understood that approximately three quarters of them caused premature stop codons due to missense or frameshift variations. Such variations, including ENST00000358758.12:c.649dupC, may lead to a decrease in mRNA stability or a rapidly degradable protein product.[10]

BFIE, PKD, and PKD/IC form the basis of the spectrum of *PRRT2*-associated paroxysmal disorders (Figure 5).

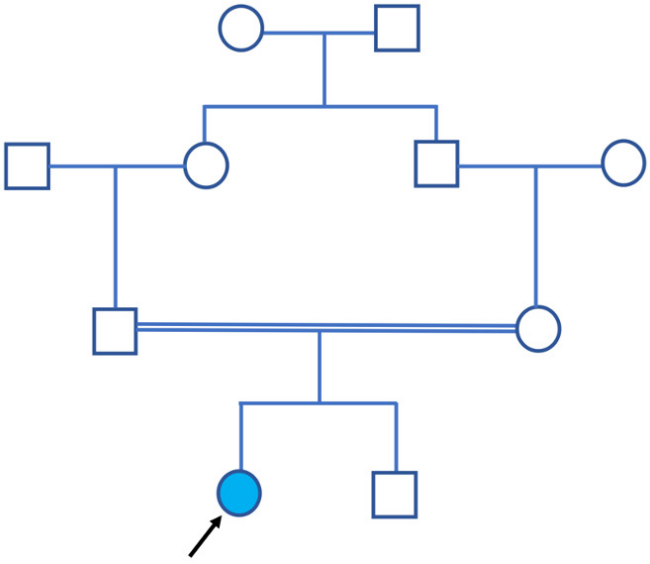

Figure 4. Our sporadic case who was diagnosed with juvenile absence epilepsy and had generalized tonic-clonic convulsion only once. Homozygous but possible benign c.67G>A;p.(Glu23Lys) variant was detected in this case

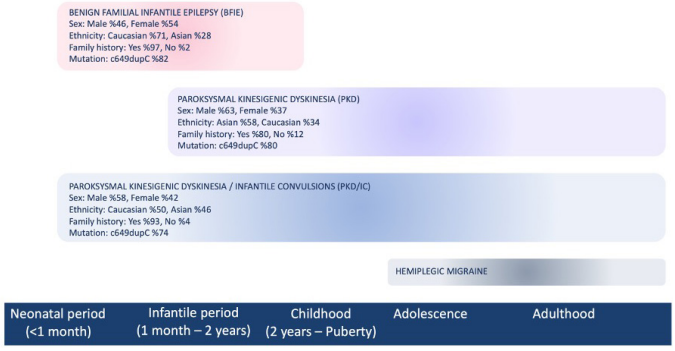

Figure 5. Clinical spectrum of *PRRT2*-associated paroxysmal disorders according to age of onset
BFIE: Benign familial infantile epilepsy, PKD/IC: Paroxysmal kinesigenic dyskinesia/infantile convulsions

BFIE, which we diagnosed in our cases, is a self-limiting seizure disorder characterized by nonfebrile seizures that usually begin between 3 and 12 months of age and end at 2 years of age. BFIE-associated seizures usually occur as focal-onset seizures with impaired awareness or generalized tonic-clonic seizures. The focal features observed in our EEG samples are noteworthy. Interictal neurological examination and MRI are normal, as in our cases. Ictal EEG usually shows parieto-occipital epileptic activity, which then becomes generalized.[11]

In most cases, seizures respond easily to antiseizure medications, with remission rates over 90%. Complete remission in our patients is consistent with the literature. Levetiracetam was found to be significantly ineffective compared with phenobarbital and valproate. While sodium channel blockers such as carbamazepine and oxcarbazepine have a positive effect, the effectiveness of levetiracetam was found to be insufficient.[12]

Although PKD is rare, with a prevalence of 1:150,000, it is still the most common paroxysmal movement disorder. The movement disorder, which would later be defined as paroxysmal PKD, was first described by Shuzo Kure in 1892.[13] Bruno et al.[14] defined the diagnostic criteria for PKD in 2004. These criteria were identified as the kinesigenic trigger for the attacks, short duration of attacks (<1 minute), no loss of consciousness or pain during attacks, exclusion of other organic diseases and normal neurological examination, control of attacks with phenytoin or carbamazepine (if tried), and age at onset between 1 and 20 years (if no family history of PKD).

As typically observed in our cases, symptoms begin between the ages of 5 and 15. Clinically, PKD is characterized by unilateral or bilateral hyperkinetic movements triggered by sudden voluntary movements, such as starting to walk, getting up from a chair, or being startled. Involuntary movements involving one or more extremities may be dystonic contractions of the extremities or may be choreoathetoid or ballistic.

Most patients experience bilateral attacks, the upper limbs are most commonly affected, and the attack lasts approximately 30 s on average. It does not occur during sleep, and there is no loss of consciousness, pain, or weakness. Interictal examination, EEG, and MRI are normal, and no long-term neurological sequelae have been reported. Carbamazepine at low doses (50-200 mg/day) is usually sufficient, and a complete response is observed in approximately 95% of the cases, as observed in our cases.

It has been observed in our cases and in the literature that *PRRT2* variation can cause BFIE alone, PKD alone, or a combination of PKD and BFIE. In addition, the age of onset and disease severity can vary significantly.[4] Patients may have additional symptoms such as mental retardation, attention deficit and hyperactivity disorder, absence epilepsy, migraine, paroxysmal nonkinesigenic dyskinesia, and episodic ataxia.[15] Therefore, it was thought that the homozygous variant detected during research in our sporadic case might be related to epilepsy.

Confirmation of *PRRT2* variations in patients with typical infantile seizures may reassure parents that seizures are probably self-limiting. Early genetic diagnosis can help provide appropriate advice and education to families about the possibility of PKD later in life; thus, it can facilitate early diagnosis and treatment.

There is a need for research on the relationship between *PRRT2* variations and other seizure types and whether they lower the epilepsy threshold. In addition to nonfebrile and febrile seizures, cases of generalized tonic-clonic seizures, absence, nonconvulsive seizures, Dravet syndrome, West syndrome, and Rolandic epilepsy have also been reported.

In summary, although current data have clearly shown the relationship between *PRRT2* variations and BFIE and PKD, further research is needed to obtain information about its role in other types of epilepsy.

CONCLUSION

Findings in *PRRT2*-associated disorders demonstrate the importance of next-generation gene sequencing techniques and indicate that different phenotypes can be observed in single gene-related variations. The spectrum of *PRRT2*-associated disorders is likely to be broader than we currently know. The identification of *PRRT2* variants as the genetic cause of several diseases is an important starting point for a better understanding of the molecular

mechanisms underlying paroxysmal diseases. Comprehensive genetic and phenotypic characterization will elucidate a broad phenotypic spectrum and pave the way for personalized treatments.

Ethics

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.B., Concept: B.B., Design: B.B., S.T., Data Collection or Processing: B.B., S.A.U.İ., S.T., N.B., Analysis or Interpretation: B.B., S.A.U.İ., S.T., N.B., Literature Search: B.B., Writing: B.B., S.T.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Biesecker LG, Green RC. Diagnostic clinical genome and exome sequencing. *N Engl J Med*. 2014;370(25):2418-2425. [Crossref]
2. Chen WJ, Lin Y, Xiong ZQ, et al. Exome sequencing identifies truncating mutations in PRRT2 that cause paroxysmal kinesigenic dyskinesia. *Nat Genet*. 2011;43(12):1252-1255. [Crossref]
3. Heron SE, Grinton BE, Kivity S, et al. PRRT2 mutations cause benign familial infantile epilepsy and infantile convulsions with choreoathetosis syndrome. *Am J Hum Genet*. 2012;90(1):152-160. [Crossref]
4. Lee HY, Huang Y, Bruneau N, et al. Mutations in the gene PRRT2 cause paroxysmal kinesigenic dyskinesia with infantile convulsions. *Cell Rep*. 2012;1(1):2-12. [Crossref]
5. Michetti C, Castroflorio E, Marchionni I, et al. The PRRT2 knockout mouse recapitulates the neurological diseases associated with PRRT2 mutations. *Neurobiol Dis*. 2017;99:66-83. [Crossref]
6. Valente P, Castroflorio E, Rossi P, et al. PRRT2 Is a Key Component of the Ca(2+)-Dependent Neurotransmitter Release Machinery. *Cell Rep*. 2016;15(1):117-131. [Crossref]
7. Xu J, Luo F, Zhang Z, et al. SNARE proteins synaptobrevin, SNAP-25, and syntaxin are involved in rapid and slow endocytosis at synapses. *Cell Rep*. 2013;3(5):1414-1421. [Crossref]
8. Fruscione F, Valente P, Sterlini B, et al. PRRT2 controls neuronal excitability by negatively modulating Na+ channel 1.2/1.6 activity. *Brain*. 2018;141(4):1000-1016. [Crossref]
9. Ebrahimi-Fakhari D, Saffari A, Westenberger A, Klein C. The evolving spectrum of PRRT2-associated paroxysmal diseases. *Brain*. 2015;138(Pt 12):3476-3495. [Crossref]
10. Wu L, Tang HD, Huang XJ, et al. PRRT2 truncated mutations lead to nonsense-mediated mRNA decay in Paroxysmal Kinesigenic Dyskinesia. *Parkinsonism Relat Disord*. 2014;20(12):1399-1404. [Crossref]
11. Callenbach PM, de Coo RF, Vein AA, et al. Benign familial infantile convulsions: a clinical study of seven Dutch families. *Eur J Paediatr Neurol*. 2002;6(5):269-283. [Crossref]
12. Döring JH, Saffari A, Bast T, et al. Efficacy, Tolerability, and Retention of Antiseizure Medications in *PRRT2*-Associated Infantile Epilepsy. *Neurol Genet*. 2022;8(5):e200020. [Crossref]
13. Kato N, Sadamatsu M, Kikuchi T, Niikawa N, Fukuyama Y. Paroxysmal kinesigenic choreoathetosis: from first discovery in 1892 to genetic linkage with benign familial infantile convulsions. *Epilepsy Res*. 2006;70(Suppl 1):174-184. [Crossref]
14. Bruno MK, Hallett M, Gwinn-Hardy K, et al. Clinical evaluation of idiopathic paroxysmal kinesigenic dyskinesia: new diagnostic criteria. *Neurology*. 2004;63(12):2280-2287. [Crossref]
15. Delcourt M, Riant F, Mancini J, et al. Severe phenotypic spectrum of biallelic mutations in PRRT2 gene. *J Neurol Neurosurg Psychiatry*. 2015;86(7):782-785. [Crossref]

Archives of Epilepsy

Case Report

Trigeminal Neuralgia Associated with Vagus Nerve Stimulation: A Case Presentation and Literature Review

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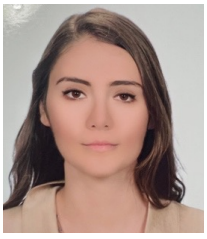

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Abstract

Vagus nerve stimulation (VNS) is an effective therapeutic option that is widely used worldwide in drug-resistant epilepsy cases. Because it is a surgical procedure, some complications may develop with VNS implantation. Although VNS-related pain symptoms have been reported, VNS-related trigeminal neuralgia is an unexpected and rather rare side effect. This report presents a case of trigeminal pain as an adverse effect of VNS. A patient with drug-resistant epilepsy undergoing VNS treatment developed pain synchronously with stimulation in his left upper and lower jaw and teeth. Pain occurred on the day of stimulation's current intensity (SCI) increase. The sudden disappearance of pain with decreasing SCI suggested that trigeminal pain was related to VNS. Because it is a rare side effect, trigeminal pain may not be regarded as a VNS-related side effect and may lead to unnecessary examinations. Being a rapidly reversible side effect, recognizing it and reducing SCI is crucial. VNS stimulation paradigms on nociception are still largely unknown, and it will be an important step to elucidate the important impact of VNS in pain modulation.

Keywords: Epilepsy, nociception, side effects, trigeminal pain, vagus nerve stimulation

INTRODUCTION

Approximately 35% of patients with epilepsy have seizures refractory to antiseizure drugs.[1] For patients who are not candidates for epilepsy surgery, vagus nerve stimulation (VNS) is indicated. VNS is widely used worldwide as an approved add-on therapy in drug-resistant epilepsy cases. Previous reports have shown that 50% of patients responded to VNS with a reduction in epileptic seizures.[2,3] Being a surgical procedure, some complications that involved surgical and technical procedures may develop with VNS implantation. Lead fracture or disconnection and stimulator malfunction are hardware difficulties that lead to VNS complications. Wound infections, wound hematoma, transient bradycardia, lower facial weakness, and vocal cord palsy are surgery-related adverse events of VNS. The most reported stimulation-related side effects were voice alteration/hoarseness, dyspnea, cough, neck pain, headache, pharyngeal paresthesia or pain, and dysphagia. These side effects develop after an increase in stimulation and disappear spontaneously over some time or after a reduction in stimulation.

Here we report a patient treated with VNS who developed trigeminal pain associated with an increase in stimulation current intensity (SCI), which is an extremely rare condition.

CASE PRESENTATION

A 21-year-old mild mentally retarded man with drug-resistant epilepsy has been following in our clinic since 2015. He had seizures since he was 1 year old. He had been diagnosed with hemimegalencephaly with typical MRI features, such as gray matter heterotopia, causing volume increase in the left hemisphere and shifting from left to right. His parents had refused epilepsy surgery at his younger age. He had focal onset seizures with impaired awareness and a motor component that involved oral automatism, ipsilateral head and eye version to the right, and asymmetric tonic limb posturing that could sometimes progress to bilateral tonic-clonic seizures. He was implanted with a VNS in 2016. He did not show a good response with the 1.25 mA SCI reached in the 6th month after implantation. More than 80% reduction in seizure frequency was achieved with 2 mA SCI reached at 1 year after implantation. Four years after VNS implantation, the patient was admitted to the intensive care unit (ICU) with status epilepticus. At that time, he was treated with levetiracetam on 4000 mg/day,

topiramate on 600 mg/day, and clobazam on 30 mg/day. Thiopental infusion was administered until his seizures stopped. Due to not stopping his focal seizures after the initiation of lacosamide at 200 mg/day, phenobarbital was added to his treatment, and the dose was gradually increased to 400 mg/day. His seizures became infrequent, and he recovered consciousness 10 days after the thiopental infusion was stopped. On the last day of his stay in the ICU, the SCI was increased from 2.0 mA to 2.5 mA, and he was transferred from the ICU to the neurology unit. Within the same day, he began to complain of intense shooting pain in his left upper and lower jaw and teeth. The pain recurred many times throughout the day and lasted for seconds. At first, it was suspected that it could be a dental cause of the pain, but a dental examination did not reveal any pathological findings. His mother noticed that the pain appeared with the stimulation on and disappeared with the stimulation off. Because the pain is synchronized with the impulses, it was understood that the pain was related to stimulation. SCI was decreased from 2.5 mA to 2 mA, and his pain was relieved immediately.

DISCUSSION

Dysesthesias such as pain and tingling in the throat and neck region, which are known to be directly related to VNS stimulation, have been reported.[4,5] There has even been a report of VNS-related chest pain thought to be caused by activating the cardiac visceral nociceptive component of the vagus nerve.[6] In addition to the pain referred to regions innervated by the vagus nerve, there have been a small number of cases[7-9] who suffered from trigeminal pain as an adverse effect of VNS. Besides being a rare condition, it is underdiagnosed because of reasons such as the inability of some patients to mention their discomforts, adaptation of mild pain, and trigeminal pain not occurring immediately after SCI increase.[9]

The important role of VNS in the modulation of pain has been demonstrated in many studies over several years. The nucleus of the solitary tract, which is the first relay station for most sensory fibers of the vagal nerve, and the spinal trigeminal nucleus, the second-order nociceptive neuron that receives ipsilateral projections from the vagal nerve, play a prominent role in the pathophysiology of headache. Vagal afferent stimulation modifies pain-modulating brain structures through these pathways. Several non-human studies have demonstrated that low-intensity VNS induces a pronociceptive effect, whereas high-intensity VNS induces an antinociceptive effect.[10] There have been human studies with controversial results showing that patients implanted with a VNS device respond to vagal stimulation with both a decrease[11,12] and an increase[13] in the pain threshold during experimentally induced pain. Moreover, the VNS intensities used in humans (0.25-2.75 mA) were much higher than those used in animals. Similar to the published cases of VNS-related trigeminal pain, the VNS intensity that caused the development of pain in our case (2.5 mA) was within the range of the intensities used in the above-mentioned studies. Although stimulation paradigms favoring pro-or antinociceptive effects of VNS in humans are yet to be clarified[14], individual differences in pain perception change in response to various VNS device parameters should be another consideration to be assessed.[12]

MAIN POINTS

- Trigeminal pain is a rare side effect after vagus nerve stimulation (VNS) implantation.
- VNS-associated trigeminal neuralgia is a reversible side effect associated with a reduction in stimulation current intensity.

The pain in the jaw and tooth region of our patient developed after the increase in SCI, similar to previous cases.[7-9] However, the occurrence of pain differed from these previous cases in that it occurred on the day of SCI augmentation. Time to pain onset from the last augmentation was 2 months in the case reported by Shih[7] and 2 weeks and 2 months in two cases reported by Timarova and Šteňo.[8] Only one of the 3 cases reported by Carius and Schulze-Bonhage[9] developed trigeminal pain symptoms a few days after the last augmentation, and the rest developed after 2 weeks and 1 month. We can associate the early onset of pain in this study with the barbiturates added to his antiseizure medication during his ICU admission. In addition to controlling seizures, barbiturates are involved in pain modulation at the central nervous system (CNS) level by activating the GABA-A receptor. It has been suggested that with the stimulation of GABAergic (inhibitory) neurons associated with the pain-modulating structures that constitute the descending inhibitory system at the upper levels of the CNS, painful inputs from the periphery may increase, and thus phenobarbital-related hyperalgesia may occur.[15]

CONCLUSION

Although it is a rare side effect, both patients with VNS and their relatives should be informed about VNS-related trigeminal pain. Because trigeminal pain under VNS stimulation is a rapidly reversible adverse effect, treating physicians must recognize and reduce the stimulation current.

Ethics

Informed Consent: Consent form was filled out by a participant.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: G.Z., A.Ç., Concept: G.Z., G.G., F.E., Design: G.Z., G.G., F.E., A.S., Data Collection or Processing: G.Z., F.E., Analysis or Interpretation: G.Z., G.G., F.E., Z.B.G., A.Ç., A.S., Literature Search: G.Z., G.G., Z.B.G., A.Ç., A.S., Writing: G.Z., Z.B.G.

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REFERENCES

1. Behr C, Goltzene MA, Kosmalski G, Hirsch E, Ryvlin P. Epidemiology of epilepsy. *Rev Neurol (Paris)*. 2016;172(1):27-36. [Crossref]
2. Englot DJ, Chang EF, Auguste KI. Vagus nerve stimulation for epilepsy: a meta-analysis of efficacy and predictors of response. *J Neurosurg*. 2011;115(6):1248-1255. [Crossref]
3. Casazza M, Avanzini G, Ferroli P, Villani F, Broggi G. Vagal nerve stimulation: relationship between outcome and electroclinical seizure pattern. *Seizure*. 2006;15(3):198-207. [Crossref]
4. Ergene E, Behr PK, Shih JJ. Quality-of-Life Assessment in Patients Treated with Vagus Nerve Stimulation. *Epilepsy Behav*. 2001;2(3):284-287. [Crossref]

5. Galbarriatu L, Pomposo I, Aurrecoechea J, et al. Vagus nerve stimulation therapy for treatment-resistant epilepsy: a 15-year experience at a single institution. *Clin Neurol Neurosurg.* 2015;137:89-93. **[Crossref]**
6. Nichols JB, McCallum AP, Khattar NK, et al. Pseudoanginal chest pain associated with vagal nerve stimulation: A case report. *BMC Neurol.* 2020;20(1):144. **[Crossref]**
7. Shih JJ, Devier D, Behr A. Late onset laryngeal and facial pain in previously asymptomatic vagus nerve stimulation patients. *Neurology.* 2003;60(7):1214. **[Crossref]**
8. Timarova G, Šteňo A. Late-onset jaw and teeth pain mimicking trigeminal neuralgia associated with chronic vagal nerve stimulation: Case series and review of the literature. *BMC Neurol.* 2017;17(1):113. **[Crossref]**
9. Carius A, Schulze-Bonhage A. Trigeminal pain under vagus nerve stimulation. *Pain.* 2005;118(1-2):271-273. **[Crossref]**
10. Ren K, Randich A, Gebhart GF. Vagal afferent modulation of a nociceptive reflex in rats: involvement of spinal opioid and monoamine receptors. *Brain Res.* 1988;446(2):285-294. **[Crossref]**
11. Ness TJ, Fillingim RB, Randich A, Backensto EM, Faught E. Low intensity vagal nerve stimulation lowers human thermal pain thresholds. *Pain.* 2000;86(1-2):81-85. **[Crossref]**
12. Borckardt JJ, Kozel FA, Anderson B, Walker A, George MS. Vagus nerve stimulation affects pain perception in depressed adults. *Pain Res Manag.* 2005;10(1):9-14. **[Crossref]**
13. Ness TJ, Randich A, Fillingim R, Faught RE, Backensto EM. Left vagus nerve stimulation suppresses experimentally induced pain. *Neurology.* 2001;56(7):985-986. **[Crossref]**
14. Latremoliere A, Woolf CJ. Central Sensitization: A Generator of Pain Hypersensitivity by Central Neural Plasticity. *J Pain.* 2009;10(9):895-926. **[Crossref]**
15. Yokoro CM, Pesquero SM, Turchetti-Maia RM, Francischi JN, Tatsuo MA. Acute phenobarbital administration induces hyperalgesia: pharmacological evidence for the involvement of supraspinal GABA-A receptors. *Brazilian J Med Biol Res.* 2001;34(3):397-405. **[Crossref]**

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