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Original Article

Evaluation of Plasma Melatonin Levels in Children With Afebrile and Febrile Seizures

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ABSTRACT

BACKGROUND: Melatonin modulates central nervous system neuronal activity. We compared the melatonin levels of patients with febrile and afebrile seizures during and after seizure with those of healthy controls. **METHODS:** We enrolled 59 individuals with afebrile and febrile seizures (mean age, 6.09 ± 4.46 years) and 28 age-, sex-, and weight-matched healthy children. Melatonin levels were measured near the time of a seizure (0 to 1 hour) and at 12 and 24 hours post-seizure, and control melatonin levels were measured from a single venous blood sample. **RESULTS:** Plasma melatonin levels increased during seizures in the study group ($P < 0.001$). Post-seizure plasma melatonin levels were significantly lower in the study group than in the control group ($P < 0.05$). Plasma melatonin levels did not differ between patients with afebrile seizures who had and had not used antiepileptic drugs. Daytime (8 AM to 8 PM) and nighttime (8 PM to 8 AM) post-seizure melatonin levels were not significantly different. **CONCLUSIONS:** Melatonin levels were lower in pediatric patients prone to seizures than in healthy children and increased during seizures. Further research is needed to test the role of melatonin in the pathophysiology and treatment of epilepsy.

Keywords: antiepileptic, epilepsy, febrile seizure, melatonin

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Introduction

Epilepsy is the most common chronic childhood neurological condition, with an estimated prevalence of 0.5%-1%.[1] In contrast, febrile seizures typically occur in otherwise normal children between ages one month and five years of age.[1] Febrile seizures have a prevalence of 2% to 5% and are the most common type of seizure in children.[2,3]

Excitatory neurotransmitters such as glutamate and aspartate are believed to underlie the initiation and spread of epileptic attacks in the mammalian brain.[4] This hypothesis is supported by evidence showing that inhibitory neurotransmitters, such as glycine and gamma amino butyric acid (GABA), prevent epileptic attacks by hyperpolarizing cell membranes.[5] Moreover, nitric oxide (NO) may act as a proconvulsant or anticonvulsant depending on the dose,[6] and a decrease in NO levels has an anticonvulsant effect.[7]

The importance of the pineal gland and pineal indoleamines for the regulation and stabilization of electrical activity in the central nervous system is well established.[8] The pineal gland is a neuroendocrine organ that synthesizes and secretes melatonin in a circadian pattern such that plasma levels are low during the day and high at night.[9]

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Melatonin has anticonvulsant, sedative, and hypnotic effects in mammals.[10-13] This hormone suppresses neuronal excitation by inhibiting glutamate[14-17] and neuronal nitric oxide synthase activity and decreasing NO production[18,19] via increased cell membrane permeability of chlorine through Gamma-Aminobutyric Acid a (GABAa)–chloride channels.[20,21]

We investigated the relationship between a possible anticonvulsant effect and plasma levels of melatonin. Therefore, we measured melatonin levels in patients with afebrile and febrile seizures during and after seizures and compared them with melatonin levels in a healthy control group.

Materials and Methods

Participants

A total of 59 patients with seizures (29 afebrile and 30 febrile) from the Department of Pediatrics, Gülhane Medical Faculty were enrolled in the study. None of the patients with febrile seizures had a history of antiepileptic treatment. Of the patients with afebrile seizure, 11 had received antiepileptic medication; the remaining 18 presented with their first afebrile seizure and had received no previous antiepileptic treatment. We divided the patients according to whether their seizure occurred during the day (8 AM to 8 PM) or night (8 PM to 8 AM). The control group consisted of 28 age- and sex-matched healthy children.

We obtained information concerning age, sex, and weight from all participants in addition to their medical records, along with information about antiepileptic medication usage, additional systemic diseases, and family history. Patients who reported regular use of certain medications and experienced seizures caused by intracranial hemorrhage, electrolyte imbalance, or a systemic disease were excluded from the study. This study was conducted according to the principles expressed in the Declaration of Helsinki, and ethical approval was obtained from the Gülhane Medical Faculty ethics committee. Written informed consent was obtained from all participants and their families.

Sample collection and measurement

We measured melatonin levels in venous blood samples obtained from patients during the first hour and at 12 and 24 hours following seizures. The melatonin levels of the control group were measured from a single venous blood sample obtained between noon and 2 PM. The samples were centrifuged for 5 minutes at 3500 rpm, and the serum and plasma were aliquoted, labeled, and maintained at −80°C until analysis.

Plasma melatonin levels were measured in the Gülhane Medical Faculty Department of Medical Biochemistry High Performance Liquid Chromatography (HPLC) Laboratory using an Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, CA). We used Phenomenex Inertsil C18 HPLC columns, 5 μ particle diameter, 150 × 4.6 mm dimensions (Phenomenex, Torrance, CA), and an ODS-2 reversed phase column with a mobile phase consisting of sodium acetate/acetonitrile (75 mM sodium acetate/acetonitrile [72:28, v/v; pH: 5]).

Statistical analysis

The distribution characteristics of the variables were taken into account for the between-group comparisons. The Wilcoxon test was used to assess non-normally distributed dependent variables, and the Kruskal-Wallis or Mann-Whitney *U* tests were used for the independent variables. Pearson correlation test was used to assess intervariable correlations. *P* values <0.05 were deemed to indicate statistical significance.

Results

Patient characteristics

We enrolled 59 patients (31 males and 28 females) with a mean age of 6.09 years (range, 8-16 years). The healthy control group comprised 16 males and 12 females with a mean age of 7.27 years (range, 1-16 years). The control and study groups were age and weight matched (Table 1).

Plasma melatonin levels

Plasma melatonin levels during the first hour after the seizure were significantly higher than those at 12 and 24 hours post-seizure in the patients ($P < 0.001$). The patient melatonin levels at 0 to 1 hour were not significantly different from those of the control group ($P = 0.97$); however, the 12- and 24-hours post-seizure melatonin concentrations were significantly lower than those of the controls ($P = 0.02$ for both; Table 2).

Plasma melatonin levels in patients with afebrile seizure according to antiepileptic drug use

The comparison of melatonin levels at 0 to 1 hour in patients with afebrile seizures revealed no significant difference between those who had and had not received antiepileptic medication ($P = 0.38$), suggesting that previous antiepileptic treatment had no effect on plasma melatonin levels.

Comparison of plasma melatonin levels during daytime and nighttime seizures

Comparing the melatonin levels at 0 to 1 hour in individuals with daytime (8 AM to 8 PM) and nighttime (8 PM to 8 AM) seizures, the time of day had no significant effect on melatonin levels in patients with afebrile ($P = 0.10$) or febrile seizures ($P = 0.27$). We also compared the plasma melatonin levels in the afebrile and febrile seizure groups at 0 to 1 hour and found no significant difference in melatonin levels between groups according to the time of seizure (daytime, $P = 0.98$; nighttime, $P = 0.83$). The comparison of control group melatonin levels with those of the daytime and nighttime seizure groups at 0 to 1 hour revealed no significant differences ($P = 0.41$ and 0.34, respectively; Table 3).

Discussion

Although the anticonvulsant effect of melatonin is not fully understood, the hormone has been shown to inhibit excitatory neurotransmission and to activate inhibitory neurotransmitters.[18,19] Antiepileptic drugs, such as valproic

TABLE 1.
Demographic Characteristics of the Study and Control Groups

	Patients ($n = 59$)	Controls ($n = 28$)	*P* Value
Age (years)	6.09 ± 4.46	7.27 ± 4.21	0.62
Weight (kg)	25.53 ± 14.20	28.78 ± 17.30	0.85
Sex (M/F)	31/28	16/12	0.22

TABLE 2.
Comparison of Plasma Melatonin Levels at 0-1, 12, 24 hours in the Study and Control Groups

Study Group ($n = 59$)	Melatonin (pg/mL) (Mean ± SD)		
		Control Group ($n = 28$)	*P* Value
0-1 hour*	28.30 ± 12.41	32.58 ± 22.71	0.97
12 hours post seizure*	19.10 ± 9.30		0.02
24 hours post seizure*	18.09 ± 7.71		0.02

* $P < 0.001$: Comparison of plasma melatonin levels between 0-1 hour and 12 hours post seizure, and 24 hours post seizure in the study group.

acid and benzodiazepine, modulate GABAa/benzodiazepine receptor function, and melatonin acts to enhance GABAa/benzodiazepine receptor binding.[14,22] Moreover, the anticonvulsant effect of melatonin may be mediated through its action on the cerebral Na-K pump. Experimental models have shown that decreased cerebral Na+/K(+)-ATPase activity may cause epileptic seizures,[20,23,24] and melatonin may modulate sodium pump function by increasing Na+/K(+)-ATPase activity.[20] Previous studies have shown that melatonin decreases NO production and neuronal excitation by inhibiting neuronal nitric oxide synthase,[18,19] and that kynurenic acid formed as a by-product of melatonin metabolism is a robust endogenous anticonvulsant.[25]

In our study, plasma melatonin concentrations increased during and immediately after seizure but decreased 12 and 24 hours post seizure. The elevation in melatonin levels suggests that the pineal gland increased melatonin release during seizure, which, in turn, activated a temporary endogenous anticonvulsant. Moreover, the temporary increase in plasma melatonin, with its sedative effect, may underlie the tendency to sleep in the postictal period.[26] Molina-Carballo et al.[27] reported that melatonin levels in 54 children with afebrile and febrile seizures significantly increased during seizure and returned to normal values in the postseizure follow-up periods. The authors postulated that the changes in hormone levels were related to neuronal hyperexcitation and/or seizure activity.

We found that plasma melatonin values 24 hours after seizure were lower in the study group than in the control group. Previous studies have shown that exogenous melatonin delivered via intravenous infusion has a short half-life (30 to 60 minutes).[28-31] Cavallo and Ritschel[31] measured urine 6-hydroxymelatonin sulfate, the primary melatonin metabolite in urine, and melatonin levels in serum and saliva following the infusion of intravenous melatonin in children and adults. They found that prepubertal children metabolized melatonin faster than the adults. Considering the short half-life of melatonin and the rapid metabolism characteristic of children, it is likely that melatonin returns to normal levels within hours of a seizure in children. Accordingly, we believe the values we obtained 24 hours post seizure reflected the basal melatonin levels of the patients. Thus we propose that the low 24-hour melatonin values compared with the control group indicate that basal melatonin levels are lower in patients with afebrile and febrile seizures than in healthy children, and that the temporary increase following a seizure is an anticonvulsant effect. It may be that the pineal gland plays a role in the inhibition of seizures by increasing melatonin release during an attack, thus allowing melatonin levels to reach normal values. In a similar study to ours, Guo and Yao[32] found that serum melatonin levels were significantly lower in children with epilepsy (8.66 ± 1.38 ng/L) and complex febrile seizure (14.91 ± 2.61 ng/L) compared with the control group (23.93 ± 2.01 ng/L). However, the authors found no significant difference in melatonin concentrations between children with simple febrile seizure (20.72 ± 2.54 ng/L) and those in the control group.

In our study, plasma melatonin concentrations immediately after seizure were not significantly different between the afebrile and febrile groups. Similarly, plasma melatonin levels did not differ between patients with afebrile seizures who received and did not receive antiepileptic drugs. These findings suggest that body temperature and antiepileptic drug use had no effect on the seizure-induced elevation of melatonin levels, suggesting that the increase was primarily related to the seizure itself. Muñoz-Hoyos et al.[33] investigated the effect of midazolam anesthesia on plasma melatonin levels in children. They found no significant difference between pre-midazolam and post-midazolam infusion melatonin levels and concluded that midazolam, which is an anticonvulsive agent, had no effect on melatonin release from the pineal gland. These results support our finding that anticonvulsant drugs have no effect on plasma melatonin levels.

Serum melatonin levels are lower in the daytime (10 to 20 pg/mL) and reach peak values between 2 am and 3 am (80 to 150 pg/mL) in healthy people.[34] Because the pineal gland production of melatonin increases at night, we expected melatonin levels to be higher in individuals who had nighttime seizures than in those who had daytime seizures. However, we found that the seizure-related changes in plasma melatonin levels were similar between daytime and nighttime seizures. Similarly, we found no difference in daytime melatonin levels between the control and study groups following either daytime or nighttime seizures; thus, daytime levels of melatonin in the control group were similar to those of patients after a nighttime seizure. Because melatonin levels are expected to increase at night, this finding suggests that the melatonin circadian rhythm is dysfunctional in patients with seizures.

TABLE 3.
Comparison of Groups' Daytime and Nighttime Plasma Melatonin Levels

	Daytime Seizure ($n = 32$)	Nighttime Seizure ($n = 27$)	Control Group ($n = 28$)	*P* Value
Melatonin (pg/mL) at 0-1 hour (Mean ± SS)	25.33 ± 11.06	32 ± 13.37	32.58 ± 22.71	0.41*
				0.34†

* Comparison between the daytime seizure and control groups.
† Comparison between the nighttime seizure and control groups.

Molina-Carballo et al.[27] reported an increase in melatonin concentrations during daytime and nighttime seizures; however, the nighttime levels were higher than those associated with daytime seizures. In a previous study, Molina-Carballo et al.[35] found that the melatonin circadian rhythm was altered by epileptic and febrile seizures; the natural melatonin peak, which occurs at 2 pm to 4 pm, shifted to noon to 2 pm in patients with epilepsy, and the circadian rhythm disappeared in patients with febrile seizures, such that the hormone peaks expected at night appeared during the day. Ardura et al.[36] found no significant changes in the circadian rhythm of salivary gland melatonin in children with afebrile and febrile seizures. However, the authors reported that the peak concentration of melatonin after febrile seizure was significantly lower than that of the control group. Thus the effect of seizures on the circadian rhythm of melatonin is not clear; however, our findings suggest that nighttime melatonin secretion is affected by afebrile and febrile seizures regardless of whether it results from circadian rhythm disruption. Further broad-based studies examining this question are needed.

The changes in melatonin release observed during convulsive seizures have prompted investigations into the efficacy of melatonin to reduce the frequency or inhibit seizures. Molina-Carballo et al.[37] administered melatonin replacement therapy in accordance with the circadian rhythm (20 mg in the morning and 100 mg at night) to children with severe myoclonic epilepsies and conducted a 2-year follow-up. The seizures were controlled during the follow-up period, and significant psychomotor improvement was observed with no side effects. In a recent similar study, Uberos et al.[38] treated 10 pediatric patients with drug-resistant epileptic disorders using a nightly dose of placebo for 1 week followed by a nightly 3-mg dose of melatonin for three months. Urine 6-sulfatoxymelatonin and plasma melatonin concentrations were measured following each treatment period. The authors reported that the plasma melatonin levels improved following treatment with the hormone, and the number of seizures decreased. In the clinic, toxicity following melatonin treatment is rare. Side effects include nightmares, hypotension, and sleep disorders.[25]

Our study's sample size was too small to support the notion of melatonin as an actual antiepileptic agent. As another potential limitation, measurement of the 24-hour post-seizure melatonin level may not be enough elapsed time to assess the actual baseline levels of melatonin, although it seems as if that question has been addressed by other studies. The lower baseline levels of melatonin are more convincing for a possible antiepileptic effect, although the lower levels may also be due to a dysregulation of melatonin release, which is caused by the epilepsy itself, and not the other way around. Future studies with a larger number of study subjects that also determine the baseline levels of melatonin should be conducted.

Conclusion

Base melatonin levels in individuals with seizures were lower than those in healthy children. Moreover, we found that melatonin values increased during and immediately following seizure and then decreased to a lower level than that of healthy children by 24 hours after a seizure. The low plasma melatonin levels in patients with afebrile and febrile seizures, compared with healthy controls, coupled with a significant increase in hormone concentrations during seizures suggest that melatonin may act as an endogenous anticonvulsant. Further research is needed to test the role of melatonin in the pathophysiology and treatment of epilepsy.

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