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Serum Orexin-A Levels in Childhood Generalized Epilepsy Syndromes

Gunce Basarir[1], Banu İşbilen Basok[2], Olgay Bildik[3], Nihal Olgac Dundar[4,5], Pinar Gencpinar[4,5]

1 University of Health Sciences, Haseki Training and Research Hospital, Department of Pediatric Neurology, Istanbul, Türkiye

2 University of Health Sciences, Behcet Uz Training and Research Hospital, Department of Medical Biochemistry, Izmir, Türkiye

3 University of Health Sciences, Diyarbakır Gazi Yaşargil Training and Research Hospital, Department of Pediatric Neurology, Diyarbakır, Türkiye

4 Izmir Katip Celebi University, Faculty of Medicine, Department of Pediatric Neurology, Izmir, Türkiye

5 Izmir Bayrakli City Hospital, Department of Pediatric Neurology, İzmir, Türkiye

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Abstract

Objectives: To evaluate the role of orexin-A in childhood generalized epilepsy by determining serum orexin-A levels in children with generalized epilepsy syndromes.

Methods: This cross-sectional, case-control study included 21 children diagnosed with generalized epilepsy syndromes and 21 age- and sex-matched healthy controls. Serum orexin-A levels were measured using the enzyme-linked immunosorbent assay method. All patients underwent detailed neurological examination, electroencephalography, and neuroimaging as part of routine clinical evaluation.

Results: No significant difference was observed in serum orexin-A levels between patients with generalized epilepsy syndromes and healthy control subjects (2745.3±2240.5 pg/mL and 2674.3±1794.5 pg/mL, respectively; p=0.697). The observed between-group effect size was negligible. In addition, serum orexin-A levels were not significantly associated with seizure frequency, seizure type, or electroencephalographic findings (all p>0.05).

Conclusions: Serum orexin-A levels were not significantly altered in children with generalized epilepsy syndromes and were not associated with seizure-related clinical features. Despite supportive preclinical evidence, these findings suggest that peripheral orexin-A measurements may have limited utility as a biomarker in pediatric generalized epilepsy, highlighting the need for larger, multimodal studies to clarify the role of orexin signaling in epilepsy.

Keywords: Childhood, generalized epilepsy, neuropeptides, orexin-A, seizure.

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Correspondence / Yazışma Adresi: *Pınar Gencpinar, Izmir Katip Celebi University, Department of Pediatric Neurology, 35620, Izmir, Türkiye e-mail: pinargencpinar@gmail.com*

Çocukluk Çağı Jeneralize Epilepsi Sendromlarında Serum Oreksin-A Düzeyleri

Öz

Amaç: Bu çalışmada, jeneralize epilepsi sendromu tanısı olan çocuklarda serum oreksin-A düzeyleri ölçülerek oreksin-A'nın çocukluk çağı jeneralize epilepsisinde rolünün değerlendirilmesi amaçlandı.

Yöntemler: Bu kesitsel, vaka-kontrol çalışmasına jeneralize epilepsi sendromu tanısı almış 21 çocuk ile yaş ve cinsiyet açısından eşleştirilmiş 21 sağlıklı kontrol grubu dahil edildi. Serum oreksin-A düzeyleri, enzim bağlı immünosorbent analiz yöntemiyle ölçüldü. Tüm hastalara rutin klinik değerlendirme kapsamında ayrıntılı nörolojik muayene, elektroensefalografi ve nörogörüntüleme yapıldı.

Bulgular: Jeneralize epilepsi sendromu olan hastalar ile sağlıklı kontrol olguları arasında serum oreksin-A düzeyleri açısından anlamlı fark saptanmadı (sırasıyla 2745,3±2240,5 pg/mL ve 2674,3±1794,5 pg/mL; p=0,697). Gruplar arasında gözlenen etki büyüklüğü ihmal edilebilir düzeydeydi. Ayrıca serum oreksin-A düzeyleri ile nöbet sıklığı, nöbet tipi veya elektroensefalografik bulgular arasında anlamlı ilişki bulunmadı (tümü için p>0,05).

Sonuç: Çalışmamızda, jeneralize epilepsi sendromu olan çocuklarda serum oreksin-A düzeylerinin sağlıklı kontrol grubuna göre değişmediği ve serum oreksin-A düzeylerinin nöbet ile ilişkili klinik bulgulardan etkilenmediği görüldü. Preklinik düzeyde destekleyici kanıtlar bulunmasına karşın, bu bulgular periferik oreksin-A ölçümlerinin pediatrik jeneralize epilepside biyobelirteç olarak sınırlı yararı olabileceğini düşündürmekte; epilepside oreksin sinyalizasyonunun rolünü açıklığa kavuşturmak için daha büyük örneklemli ve çok yönlü çalışmalara ihtiyaç olduğunu göstermektedir.

Anahtar kelimeler: Çocukluk çağı, jeneralize epilepsi, nöbet, nöropeptit, oreksin-A.

INTRODUCTION

Epileptogenesis is increasingly understood as the result of the contribution of several interacting mechanisms, including an imbalance between excitatory and inhibitory neurotransmitters, ion channel dysfunctions, abnormal neuronal network synchronization, neuroinflammatory processes, and alterations in neuromodulatory systems, which collectively transform normal brain circuits into hyperexcitable, seizure-prone networks[1]. Because epileptogenesis involves an evolving process characterized by functional, structural, molecular, and genetic/epigenetic alterations rather than a single discrete event, significant gaps remain in the understanding of epilepsy despite extensive preclinical and clinical research.

Orexins (hypocretins) are neuropeptides that play a role in regulating circadian rhythm, attention, modulation of emotions, arousal, appetite, sleep, and cognition[2]. Orexin signaling is mediated by two endogenous ligands, namely orexin-A and orexin-B. Orexin-A, a hypothalamic neuropeptide, has a key role in sleep–wake regulation and in the modulation of cortical and thalamocortical excitability[3]. Dysregulated orexinergic signaling may influence seizure threshold, particularly in sleep-dependent seizure patterns. Through its effects on glutamatergic and GABAergic neurotransmission, orexin-A can influence the stability of thalamocortical oscillations, which are central to the pathophysiology of generalized epilepsies[4]. In addition, the modulatory effects of orexin-A on autonomic, neuroendocrine, and inflammatory pathways suggest a potential link to multiple mechanisms involved in epileptogenesis, as orexin-A has been shown to exert anti-inflammatory and neuroprotective effects by modulating microglial activation and cytokine responses in the central nervous system[5]. Therefore, orexin-A has emerged as a plausible pathogenic modulator and a potential biomarker in generalized epilepsies. Despite supportive

results from animal studies, clinical evidence regarding the involvement of orexin-A in epilepsy remains limited and heterogeneous[4-5].

Considering the evidence that orexin-A is involved in neuroprotective and anti-inflammatory processes as well as in the modulation of cortical and thalamocortical network excitability, we hypothesized that alterations in serum orexin-A levels may be associated with the pathogenic mechanisms of generalized epilepsy syndromes. To address this question, we compared serum orexin-A concentrations between children with generalized epilepsy syndromes and healthy control subjects. In addition, we evaluated the associations between seizure semiology and frequency, electroencephalographic findings, and serum orexin-A levels.

METHODS

Study Design and Participants

An observational, cross-sectional case-control study was conducted, including 21 patients aged 3-18 years with a diagnosis of childhood generalized epilepsy who were followed in pediatric neurology outpatient clinics, and 21 sex- and age-matched healthy control subjects. Patients with a minimum follow-up duration of 6 months who met the diagnostic criteria for childhood generalized epilepsy syndromes, namely juvenile myoclonic epilepsy, childhood and juvenile absence epilepsy, and epilepsy with generalized tonic–clonic seizures alone, based on seizure semiology and electroencephalographic (EEG) characteristics, as defined by the latest International League Against Epilepsy (ILAE) guidelines[6,7], were included. Patients were excluded if they had structural and/or congenital anomalies of the central nervous system, a diagnosis of obesity or diabetes mellitus, chronic cardiovascular, respiratory, hepatic, or renal disease, or a history of chronic sleep disturbances. A standardized neurological examination, brain magnetic resonance imaging (MRI), and EEG recording using the international 10–20 system were performed in all patients. After a detailed explanation of the study procedures, written informed consent was obtained from the parents or legal guardians, with child assent obtained when applicable. This study was approved by the Institutional Ethics Committee (approval number: 299-2025, date: 10/12/2025), and conducted in accordance with the Declaration of Helsinki.

Serum orexin-A measurement

In both the patient and control groups, blood samples were collected in the morning after overnight fasting. Venous blood samples were drawn into plain blood collection tubes (BD Vacutainer® SST II Advance Tube, 5 mL, 13x100 mm, USA). Within one hour of collection, samples were centrifuged at 1,500 g for 10 minutes to separate serum from cellular components. The obtained serum was aliquoted and kept at -80 °C until analysis. Serum orexin-A concentrations were measured using a commercially available quantitative ELISA kit (Elabscience Biotechnology Inc., TX, USA; Catalog No: E-EL-H1015, Lot No: IBC8AN48PN), in accordance with the manufacturer's protocol. The intra-assay and inter-assay coefficients of variation were <4.5% and <5.3%, respectively. The assay had a limit of detection of 37.5 pg/mL and a measurement range of 62.5–4000 pg/mL.

Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics, version 29. The choice of statistical tests was based on the distributional properties of the data and the type of variable analyzed. Normality of continuous variables was evaluated using the Shapiro–Wilk test together with skewness and kurtosis values. Continuous variables with normal distribution were summarized as mean ± standard deviation and compared between two independent groups using the independent samples t-test.

Variables that did not meet normality assumptions were analyzed using the Mann–Whitney U test. Categorical variables were reported as frequencies and percentages, and group comparisons were performed using the Pearson chi-square test. Serum orexin-A levels across seizure types were compared using the Kruskal–Wallis test. Correlations between serum orexin-A levels and ordinal clinical variables, including seizure frequency and the number of anti-seizure medications, were examined using Spearman's rank correlation coefficient. For the main patient–control comparison of serum orexin-A levels, the mean difference and its 95% confidence interval (CI), together with Hedges' g and its 95% CI, were calculated to describe the magnitude of the group difference. All analyses were two-sided, and statistical significance was set at $p < 0.05$.

RESULTS

No statistically significant difference was observed in serum orexin-A levels between patients with generalized epilepsy syndromes (n=21) and control subjects (n=21) (2745.33±2240.53 pg/mL vs 2674.30±1794.50 pg/mL, respectively; p=0.697). The mean difference was 71 pg/mL (95% CI, -1196.9 to 1338.9), and the standardized effect size was negligible (Hedges' g=0.03, 95% CI, -0.56 to 0.63). Demographic characteristics and serum orexin-A levels of patients and controls are presented in Table I. In addition, the distribution of serum orexin-A levels in both groups is illustrated in Figure 1.

Table I: Demographic characteristics and serum orexin-A levels of the study population

		Patient group (n=21)	Control group (n=21)	Total (n=42)	P value
Decimal age (year)	Mean±SD	11.6±3.6	11.3±4.5	11.5±4	0.78[a]
	Range	4.6-18.3	3.3-18	3.3-18.3	
Male/Female ratio	M (%)	11 (42.4)	9 (42.9)	20 (47.6)	0.54[b]
	F (%)	10 (47.6)	12 (57.1)	22 (52.4)	
Serum Orexin-A (pg/ml)	Mean±SD	2745.3±2240.5	2674.3±1794.5	2709.8±2005.2	0.697[c]
	Range	**373.3-10587.9**	**310.6-5036.4**	**310.6-10587.9**	

a Independent samples t-test, b Pearson chi-square test, c Mann–Whitney U test

F, female; M, male; n, number; pg/ml, picogram/milliliter; SD, standard deviation.

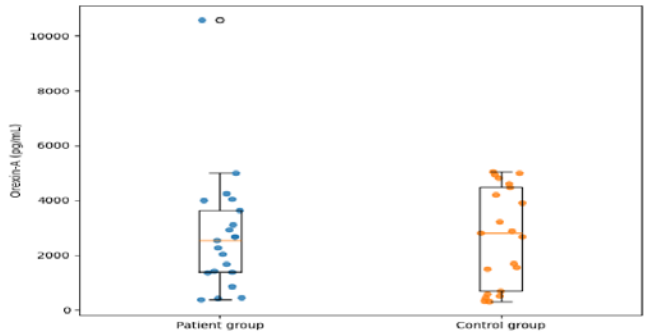

Figure 1. Box plot of serum orexin-A levels in the patient and control groups

Among the 21 patients with generalized epilepsy syndromes, 14 patients (66.7%) presented with absence seizures, three patients (14.3%) with myoclonic seizures, and four patients (19%) with generalized tonic-clonic seizures alone. Serum orexin-A levels did not differ significantly across seizure types (Kruskal-Wallis H=5.29, p=0.07). In the patient group (n = 21), seizure frequency ranged from seizure freedom to daily seizures: seven patients (33.3%) were seizure-free, seven (33.3%) experienced yearly seizures, three (14.3%) had weekly seizures, two (9.5%) had monthly seizures, and two (9.5%) had daily seizures. Serum orexin-A levels were not significantly associated with seizure frequency, as assessed by Spearman's rank correlation (ρ=-0.237, p=0.3). The most recent EEG findings were normal in 14 patients (66.7%) and showed generalized epileptiform abnormalities in seven patients (33.3%); patients with normal EEG findings and those with generalized epileptiform abnormalities showed comparable

serum orexin-A levels (Mann-Whitney U=35.0, p=0.31). At the time of serum sampling, anti-seizure treatment consisted of monotherapy in twelve patients (57.1%), two medications in seven patients (33.3%), and three medications in two patients (9.5%). The number of anti-seizure medications showed no significant correlation with serum orexin-A levels (Spearman's ρ=0.259, p=0.26).

DISCUSSION

Although our initial hypothesis anticipated an association between serum orexin-A levels and childhood generalized epilepsy syndromes, no significant differences or correlations were identified in the present study. Furthermore, the negligible effect size and wide confidence intervals crossing zero further support the absence of a clinically meaningful difference between groups. These findings contrast with a substantial body of preclinical evidence suggesting a key modulatory role for orexinergic signaling in neural networks involved in epilepsy. However, serum orexin-A levels may not reliably reflect central orexinergic activity within thalamocortical and limbic circuits, which are critically involved in generalized epilepsy. In addition, generalized epilepsy syndromes are heterogeneous, and orexin-related effects may be state-dependent, varying according to sleep–wake regulation, seizure burden, or network excitability. Compensatory mechanisms may further stabilize peripheral orexin-A levels despite potential alterations in central signaling. Importantly, our results underscore the complexity of translating experimental findings into clinical biomarkers.

The orexinergic system, particularly orexin-A, has been increasingly implicated in epilepsy-related mechanisms, primarily based on evidence from preclinical studies. Experimental animal models have demonstrated that orexin neurons modulate cortical and thalamocortical excitability through widespread projections to arousal-related brain regions, including the thalamus, cortex, locus coeruleus, raphe nuclei, and influence seizure threshold with orexin receptor antagonism by reducing seizure burden in rodent epilepsy models[8-10]. Pharmacological and genetic manipulation of orexin signaling in rodent models has been shown to alter seizure threshold, severity, and susceptibility to experimentally induced seizures[8,9,11]. Consistent with this notion, treatment with dual orexin receptor antagonists has been shown to reduce seizure incidence and severity in Kcna1-null mice, whereas SB-334867 (a selective orexin receptor type 1 antagonist) has been reported to elevate seizure threshold in mice[9,11]. Electrophysiological studies have demonstrated that orexin application causes membrane depolarization and enhanced excitability in specific neuronal populations, indicating that orexinergic signaling can directly increase neuronal responsiveness[12]. Furthermore, the neuroprotective and anti-inflammatory effects attributed to orexin-A suggest that its role in epileptogenesis may extend beyond direct modulation of neuronal firing[5].

Recent preclinical evidence further supports the involvement of orexinergic signaling in generalized epileptic networks. A study using genetic absence epilepsy rats demonstrated that activation of orexin type-2 receptors via the selective agonist "YNT-185" significantly suppressed spike-and-wave discharges, reduced cumulative seizure duration, and modulated sleep architecture, suggesting that orexinergic modulation may influence absence seizure expression through sleep-wake regulatory mechanisms[13]. Moreover, a comprehensive analysis of thalamic and somatosensory cortical orexin receptor type 1 (OX1R) protein expression in Wistar Albino Glaxo rats from Rijswijk (WAG/Rij rats - a genetic model of absence epilepsy) revealed reduced orexin receptor expression in

symptomatic animals compared with controls, indicating an altered orexinergic phenotype that may contribute to absence epileptogenesis and network dysfunction in generalized epilepsy models[14].

Despite these supportive findings from animal studies, clinical data addressing the role of orexin-A in human epilepsy remain limited, heterogeneous, and conflicting. Available clinical investigations have primarily focused on small cohorts and have reported variable results[15-17]. In a recent pediatric study, plasma orexin-A levels did not differ significantly among children with recent seizures, children with epilepsy in remission, and healthy controls, and no associations were found with seizure type or EEG/MRI findings, suggesting a lack of clear peripheral orexin changes in childhood epilepsy[15]. In contrast, an earlier adult study reported lower basal orexin levels in the drug-resistant focal epilepsy group than in the control group, and orexin levels tended to increase postictally, particularly following nocturnal seizures, indicating a complex and potentially state-dependent relationship between orexin and seizure activity[16]. Another clinical investigation found lower serum orexin-A concentrations in patients with epilepsy than in parasomnia controls, with postictal increases observed after seizures during polysomnographic monitoring, a pattern which was interpreted as possibly reflecting blood-brain barrier changes or compensatory peptide synthesis during ictal events[17]. Notably, previous clinical studies have largely included heterogeneous epilepsy populations or focal epilepsies, whereas data specifically addressing orexin-A levels in pediatric generalized epilepsy syndromes remain scarce. Collectively, these studies indicate that although orexin alterations may occur in epilepsy, the direction and context of such changes vary according to patient population, seizure timing, and methodological differences, highlighting the need for larger, longitudinal clinical investigations to better define the contribution of orexin signaling to human epilepsy pathophysiology.

The present findings should be interpreted in light of several limitations, particularly the relatively small sample size. Second, serum orexin-A levels may not accurately reflect central orexinergic activity within epileptogenic networks. Third, although blood samples were obtained in the morning under fasting conditions, orexin-A is influenced by circadian and sleep-wake related mechanisms, and these factors may still have affected peripheral measurements. Fourth, the interval between the last seizure and blood sampling was not standardized, and peri-ictal timing may have influenced serum orexin-A levels. Fifth, the inclusion of different generalized epilepsy syndromes may have introduced clinical and biological heterogeneity. Finally, anti-seizure medication exposure may also have affected serum orexin-A levels. Accordingly, these factors should be considered when interpreting the present findings.

CONCLUSION

Serum orexin-A concentrations were not significantly altered in children with generalized epilepsy syndromes and were not associated with seizure-related clinical or EEG features. In addition, the negligible effect size observed in the present study further supports the limited discriminatory utility of peripheral serum orexin-A levels between patients and controls in this clinical setting. Despite robust preclinical evidence implicating the orexinergic system in epileptogenesis, our findings suggest that peripheral orexin-A measurements may have limited utility as a clinical biomarker in pediatric generalized epilepsy. To our knowledge, this is the first clinical study to specifically evaluate serum orexin-A levels in a pediatric population with generalized epilepsy syndromes. Importantly, our results underscore the complexity of translating experimental findings into clinical biomarkers and highlight the need for larger, multimodal studies incorporating central measurements and sleep-related parameters to elucidate the role of orexin signaling in epileptogenesis.

Author Contributions: Concept – BIB, NOD, PG; Design – BIB, NOD, PG; Data Collection or Processing –

GB, OB; Analysis or Interpretation – GB, BIB, PG; Literature Search – GB, OB, BIB; Writing – GB. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Ethics Committee Approval: This study was approved by the Institutional Ethics Committee (approval number: 299-2025, date: 10/12/2025), and was conducted in accordance with the Declaration of Helsinki.

Conflict of Interest: The author(s) declare that there is no financial conflict of interest related to this article.

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