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Serum ghrelin levels are enhanced in patients with epilepsy

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Summary

Purpose: In patients with epilepsy, although many changes in the physiology of hormones in the neuroendocrine system can occur (especially in the sex hormones, for example), the causes of these changes have not been fully elucidated. There are also relations between seizure activity and stages of sleep. Ghrelin is the peptide hormone, which has been shown to affect both endocrine function and sleep. The purpose of this study was to evaluate serum levels of ghrelin in epilepsy patients.
Methods: A total of 35 patients currently receiving antiepileptic drug therapy (of these patients, 20 had primary generalized seizure and 15 had partial seizure) were studied. The control group consisted of 30 healthy volunteers matched for age and gender. In all participants, serum levels of ghrelin, cholesterol and triglycerides were measured and body mass index (BMI) was determined. Patients with endocrine, immune or any other chronic diseases were excluded.
Results: In the epilepsy patients, the mean serum ghrelin level was 158.81 $\pm$ 55.97 pg/ml, and this was significantly higher than the control group's level of 93.43 $\pm$ 21.33 pg/ml ($p < 0.001$). In terms of serum cholesterol, triglycerides and BMI, no significant differences were found between the epilepsy patients and the control group ($p > 0.05$).
Conclusions: The origin of higher serum ghrelin levels in epilepsy and their relation to seizures are not completely known. However, this elevation of serum ghrelin could contribute to the lengthening of NREM sleep in epilepsy patients, thereby playing a role in seizure occurrence. From another direction, high serum ghrelin levels could cause changes and/or dysfunction in hormone secretion and physiology via its effects on growth hormone, and thereby play a facilitating role in seizure occurrence.

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Introduction

Relations between epilepsy and the endocrine system have attracted the attention of researchers for many years.[1,2] It is known that the severity of epileptic seizures is related to temporary changes in secretions of circadian-modulated hormones.[3] For example, after severe primary or secondary generalized partial seizures, serum levels of prolactin, noradrenalin, vasopressin and oxytocin increase.[4] Changes in the balance of sex hormones have also been noted in patients with epilepsy.[5,6] In particular, when activity in the temporolimbic region is disrupted by recurrent or persistent interictal epileptiform discharges, a path can be opened for changes in the hypothalamo-pituitary regulation of gonadal secretion, possibly leading to dysfunction in the reproductive endocrine system.[7,8] A link between seizures and impairment of the hypothalamic pituitary adrenocortical system has also been shown.[9]

In patients with epilepsy, sleep disorders are common, but they are diverse and complex.[10,11] A connection between sleep and epilepsy has long been established,[12,13] and it has been shown that physiologic changes involved in the non-rapid-eye-movement (NREM) sleep are associated with increased susceptibility to seizures.[10,13] From this it is known that stages of sleep have an important role in seizure occurrence.[10,12]

Recently, the polypeptide hormone ghrelin has been recognized as an endogenous ligand to the growth hormone secretagogue receptor (GHSR), and has been isolated from stomach, hypothalamus and other tissues.[14,15] Although ghrelin is secreted mainly by stomach mucosa, it is widely expressed in other tissues, and in this way it may have both endocrine and paracrine effects.[15] Ghrelin has been reported to affect cardiac and gastrointestinal function, carbohydrate metabolism, adipose and reproductive tissue, cell proliferation and behavior as well as affecting sleep and pituitary hormone axis function.[14–16]

Given the multiple links between epilepsy, sleep and endocrine function on the one hand and between ghrelin, sleep and endocrine function on the other, the purpose of this study was to look for changes in serum ghrelin levels in patients with epilepsy. To our knowledge, this is the first clinical study to investigate this question.

Methods

The protocol of this study was approved by our institution's Ethics Committee. Each patient was informed about the study and signed a consent form before participating. A total of 35 patients currently being followed by and receiving treatment from the Department of Neurology at Firat University Medical Center were included in the study. Of these patients, 20 had primary generalized seizure and 15 had partial seizure, according to the International League Against Epilepsy (ILAE) classification.[17] In the primary generalized epilepsy group, there were 12 females and 8 males, with an overall mean age of 34.25 ± 10.37 years. The duration between diagnosing and initiation of therapy was 2.06 ± 0.27 years. Twenty patients in the primary generalized group had generalized tonic-clonic seizure; 16 were treated with valproic acid and 4 with phenytoin for seizure control. While before therapy the patients with generalized tonic-clonic seizures had an average of 9.08 ± 2.06 seizures per year, seizures had not occurred for approximately 5 months.

In the partial epilepsy group, there were eight females and seven males, with an overall mean age of 27.43 ± 7.1 years. The mean time since diagnosis and initiation of therapy was 2.5 ± 0.40 years. While this group of patients had been experiencing an average of 10.64 ± 3.78 seizures per year before therapy, no seizures were observed in the last 4 months. In this group, 5 patients had simple partial seizure and were taking carbamazepine for seizure control, while the other 10 patients had complex partial seizure and were taking valproic acid (see Table 1).

Exclusion criteria were as follows: presence of any concurrent psychiatric disease; except for anti-epileptic drugs, regular drug use including oral contraceptives; other diseases (neurological, endocrinological, rheumatological, hematological, acute or chronic infectious and/or inflammatory), liver or kidney dysfunction, substance or alcohol abuse, or a body mass index (BMI) over 25. The control group consisted of 30 healthy volunteers matched for age and gender.

Each patient had a complete physical and neurological examination prior to being enrolled in the study. Electroencephalography (EEG) as well as computerized tomography (CT) or magnetic resonance imaging (MRI) of the brain was performed in all patients. Baseline laboratory investigations included white blood cell count, red blood cell count, creatinine, electrolytes, GOT, GPT, AP, γGT, partial thromboplastin time, sedimentation rate, C-reactive protein, serum iron, transferrin and glucose. Thyroid function tests were performed, and serum levels of cholesterol and triglycerides were measured. Serum lipid levels were specifically measured in this study due to their effect on the serum ghrelin levels. Pelvic ultrasono-

Table 1 Characteristics of epilepsy patients and healthy controls

	Primary generalized epilepsy (GTC) (n = 20)	Partial epilepsy (n = 15)		Controls (n = 30)
		Simple PE (n = 5)	Complex PE (n = 10)	
Age (years)	34.25 ± 10.37	29.5 ± 8.93	24.22 ± 5.21	28.34 ± 9.72
Gender				
Female	12	3	6	14
Male	8	2	4	16
Therapy				
CBZ	–	5	–	–
VPA	16	–	10	–
PHT	4	–	–	–

JME: juvenile myoclonic epilepsy; GTC: generalized tonic-clonic epilepsy; PE: partial epilepsy; CBZ: carbamazepine; VPA: valproic acid; PHT: phenytoin.

graphy was performed to exclude polycystic ovary disease in the female participants. Height and weight were measured in all patients and healthy control subjects.

Serum ghrelin levels were measured in fasting blood samples taken from participants' antecubital veins at 8 a.m. The blood samples were immediately centrifuged, and until the time of the ghrelin assay, sera were kept at −80 °C. Serum ghrelin concentrations were measured with a commercial radioimmunoassay (RIA) kit (Phoenix Pharmaceuticals Inc., Phoenix, AZ, USA) that employs I-125 labeled bioactive ghrelin as a tracer and a rabbit polyclonal antibody against full-length octanoylated human ghrelin. The assay detects both ghrelin and des-octanoyl-ghrelin. The sensitivity of the assay is 30 pg/ml, and the intra- and interassay coefficients of variation are <5% and <14%, respectively.

Data were evaluated statistically with SPSS 10.1 software. Between-group comparisons were made via one-way ANOVA, and the Tukey B and Scheffé tests were used as post hoc tests. Values obtained in this study are given as mean ± standard deviation. In statistical evaluations, $p < 0.05$ was accepted as significant.

Results

In the group of 35 epilepsy patients, serum ghrelin levels were significantly higher than in the healthy control group ($p < 0.001$, one-way ANOVA). When the group of 20 patients with primary generalized epilepsy and the group of 15 patients with partial epilepsy were each compared to the control group separately, their serum ghrelin levels were significantly higher ($p < 0.001$ in both groups, one-way ANOVA). When two groups of epilepsy patients were compared to each other, serum ghrelin levels in the partial epilepsy group were found to be significantly higher ($p < 0.001$, one-way ANOVA).

In terms of serum cholesterol, triglyceride levels and BMI values, no significant differences were found between the overall group of 35 epilepsy patients and the control group ($p > 0.05$, one-way ANOVA). When the primary generalized epilepsy group and the partial epilepsy group were compared separately to the control group in terms of serum cholesterol, triglyceride levels and BMI values, no significant differences were found ($p > 0.05$, one-way ANOVA). These findings in the epilepsy patients and healthy controls are summarized in Table 2.

Table 2 Ghrelin, cholesterol, triglycerides and BMI values in the epilepsy patient groups and control group

	Group of all epilepsy patients (n = 35)	Primary generalized epilepsy group (n = 20)	Partial epilepsy group (n = 15)	Control group (n = 30)	p-values
Ghrelin (pg/ml)	158.81 ± 55.97[a]	134.45 ± 39.29[a]	234.06 ± 36.47[a, b]	93.43 ± 21.33	<0.001
T cholesterol (mg/dL)	192.57 ± 42.69	195.90 ± 45.58	185.46 ± 38.89	187.98 ± 47.67	>0.05
Triglycerides (mg/dL)	164.51 ± 42.89	161.32 ± 32.85	169.06 ± 42.89	165.54 ± 58.93	>0.05
BMI	22.82 ± 3.75	24.25 ± 3.86	23.92 ± 2.68	22.75 ± 4.31	>0.05

Values are given as mean ± standard deviation.

[a] Comparison of patient group with control group via one-way ANOVA, $p < 0.001$.

[b] Partial epilepsy group compared to primary generalized group via one-way ANOVA, $p < 0.001$.

Discussion

To our knowledge, this is the first study to examine serum ghrelin levels in epilepsy patients. In the overall group of 35 epilepsy patients included in the study, serum ghrelin levels were found to be significantly higher than in the control group ($p < 0.001$, one-way ANOVA). Serum ghrelin levels in the 20 patients with primary generalized epilepsy and in the 15 patients with partial epilepsy were likewise found to be higher when these two patient groups were compared separately to the control group ($p < 0.001$, one-way ANOVA).

Due to complaints of hormonal imbalances seen in epilepsy patients, many studies have examined the relations between the endocrine system and epilepsy. It has been shown that epileptic seizures can change serum levels of some hormones, especially hypothalamic trophic hormones and pituitary hormones.[18,19] These changes can be observed immediately after epileptic seizures as acute and short-term changes in hormone levels. After generalized tonic-clonic or prolonged, severe secondary generalized partial seizures, serum levels of prolactin, vasopressin and oxytocin have been shown to rise.[3,4] Seizures can also lead to dysfunction in the hypothalamic—pituitary—gonadal axis, which is modulated by temporal and limbic cortex.[18] Regions in the limbic cortex such as the amygdala have especially dense reciprocal connections with the hypothalamus.[18] In the amygdala, the corticomedial nuclei stimulate the pulse frequency of gonadotropin releasing hormone (GnRH) secretion by the hypothalamus, while the basolateral nuclei inhibit the pulse frequency of GnRH.[18] Especially in female patients with temporolimbic epilepsy, it has been reported that reproductive endocrine dysfunction and disorders are not rare.[7] It has been suggested that temporal originated recurrent or persistent epileptiform discharges during interictal period, may promote the development of reproductive endocrine disorders by causing chronically changes in hypothalamopituitary regulation.[7] As a result, chronic changes in gonadal hormone secretion and menstrual cycle disorders may occur. It is in this way that reproductive endocrine disorders and reproductive dysfunction are seen in these female patients.[7,8] This situation is independent of the acute and short-term hormonal changes induced by seizures in epileptic patients, and is significant in terms of its being an example of epilepsy-induced chronic endocrine dysfunction.

Another link between epilepsy and endocrine function is that in patients receiving growth hormone (GH) replacement therapy, a reported side effect is epileptic seizures.[21] In a 10-year study that evaluated adverse events in GH replacement therapy, seizures were ranked seventh among side effects and were seen in 0.49% of patients.[22]

Ghrelin is a peptide hormone, and is an endogenous ligand of the growth hormone secretagogue receptor.[14—16] In humans, ghrelin has been isolated in stomach, hypothalamus and other tissues.[14—16,20] In addition to its effects on regulation of appetite and food intake, ghrelin has also been shown to play a role in regulating the release of GH, adrenocorticotropic hormone (ACTH) and prolactin.[15] In this regard, it may be proposed that high levels of ghrelin, by affecting GH and prolactin secretion and thereby disrupting hormonal equilibrium, may facilitate the occurrence of seizures. However, we do not know why serum ghrelin levels were elevated in the group of epilepsy patients in this study.

Ghrelin has been shown to be a factor in sleep regulation in humans.[14] In a study in which healthy young males were given ghrelin, the time spent in slow-wave sleep increased overall during the night and non-rapid-eye-movement (REM) sleep was increased during the night.[14] Stages of sleep are also related to patterns of seizure occurrence.[10,12,13] Epileptiform EEG discharges probably multiply during NREM sleep, given that seizures generally emerge in the NREM sleep. NREM is characterized by a background of synchronized cellular discharge patterns and decreased tone, which are also a sign of decreased wakefulness. In contrast, REM sleep resists the emergence of epileptiform EEG potentials.[12] In this study, in both the patient and control groups, overnight fasting serum ghrelin levels were measured at 8 a.m. It is known that ghrelin secretion in healthy, non-obese individuals shows a diurnal course and that ghrelin levels increase at night.[23] Furthermore, in patients with Prader-Willi syndrome which typically have elevated ghrelin levels, serial measurements in both the patient and control groups showed that ghrelin level elevation was consistent with a diurnal pattern and that in every measurement ghrelin levels were higher than in the control group.[24] Thus, although a single measurement is made, always at 8 a.m., our results clearly indicate that serum levels of ghrelin in the group of epilepsy patients were significantly higher than the control group.

Thus, given ghrelin's capacity to prolong NREM sleep—the stage in which seizures tend to occur—the high serum levels of ghrelin in our epilepsy patients might be interpreted as a contributing factor in the occurrence of seizures.

In the patients with partial epilepsy, serum ghrelin levels were significantly higher than in the patients with primary generalized epilepsy ($p < 0.001$, one-way ANOVA). The difference in

ghrelin levels between the two epilepsy groups is an interesting finding, and might be due to the epileptic discharges having different origins and routes of spread. The difference in ghrelin levels found between the two groups of epilepsy patients might be explained in terms of epileptiform discharges. It is known that in adults, most localized seizures originate in temporolimbic structures that include hippocampus and amygdala.[25,26] Dysfunction in the temporolimbic region due to epileptiform activity has been shown to disrupt hypothalamopituitary regulation.[6–8,18] This suggests a possibility that the higher serum ghrelin levels we observed in patients with partial epilepsy are mediated by the effects of epileptiform discharges on the regulation of ghrelin by the hypothalamus.

The use of antiepileptic drugs by the patients in this study raises the question of whether those drugs might affect serum ghrelin levels. We have not encountered any information regarding this question. Neuroleptic agents, which affect the central nervous system, have been reported to increase serum ghrelin levels.[27] However, the mechanisms of action of anticonvulsant agents are different from those of neuroleptic drugs.

However, the capacity of epilepsy to impair regulation in the hypothalamopituitary axis independent of medication has been reported.[9] Among the patients with epilepsy, 10 in the partial epilepsy group and 16 in the generalized epilepsy group were using the anticonvulsant agent valproic acid.

A majority of patients in each group were receiving valproic acid therapy, and the finding that significantly different levels of elevated serum ghrelin between the groups is a phenomenon possible independent from drug effects. It is therefore possible that the high serum levels of ghrelin observed in our epilepsy patients were independent of drug effects.

However, it is not known whether serum ghrelin levels differ in epilepsy patients in terms of whether seizures are controlled by drug therapy. In the epilepsy patients, serum ghrelin levels were measured in the interictal period. Also, the patients' seizures were under control with anticonvulsant drugs, and none of the patients had experienced a seizure for approximately the last 4 months. Whether epileptic seizures affect serum ghrelin levels acutely is also unknown. We feel that further studies can shed light on these questions.

In summary, given ghrelin's capacity to affect neuroendocrine physiology and the stages of sleep, both of which are linked to epilepsy, it may be suggested that high serum levels of ghrelin found in epilepsy patients indicate a predisposition toward seizure activity.

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