

Evaluation of serum ghrelin, nesfatin-1, irisin, and vasoactive intestinal peptide levels in temporal lobe epilepsy patients with and without drug resistance: a cross-sectional study

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SUMMARY

OBJECTIVE: Epilepsy is a common disorder that affects the nervous systems of 1% of worldwide population. In epilepsy, one-third of patients are unresponsive to current drug therapies and develop drug-resistant epilepsy. Alterations in ghrelin, nesfatin-1, and irisin levels with epilepsy were reported in previous studies. Vasoactive intestinal peptide is among the most common neuropeptides in the hippocampus, which is the focus of the seizures in temporal lobe epilepsy. However, there is also lack of evidence of whether these four neuropeptide levels are altered with drug resistant temporal lobe epilepsy or not. The aim herein was the evaluation of the serum levels of nesfatin-1, ghrelin, irisin, and Vasoactive intestinal peptide in drug-resistant temporal lobe epilepsy patients and temporal lobe epilepsy (TLE) without drug resistance, and to compare them to healthy controls.

METHODS: This cross-sectional study group included 58 temporal lobe epilepsy patients (24 with drug resistant temporal lobe epilepsy and 34 with temporal lobe epilepsy who were not drug-resistant) and 28 healthy subjects. Nesfatin-1, ghrelin, irisin, and Vasoactive intestinal peptide serum levels were determined using enzyme-linked immunosorbent assay.

RESULTS: The serum ghrelin levels of patients with drug resistant temporal lobe epilepsy were seen to have significantly decreased when compared to those of the control group ($p < 0.05$). Serum nesfatin-1, vasoactive intestinal peptide, and irisin levels were seen to have decreased in the drug resistant temporal lobe epilepsy group when compared to those of the control and temporal lobe epilepsy groups; however, the difference was non-significant ($p > 0.05$).

CONCLUSIONS: The results herein suggested that ghrelin might contribute to the pathophysiology of drug resistant temporal lobe epilepsy. However, further studies are needed to confirm this hypothesis.

KEYWORDS: Ghrelin. Neuropeptides. Drug resistant epilepsy. Vasoactive intestinal peptide.

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INTRODUCTION

Epilepsy is a significantly prevalent neurological condition that affects about 50 million people worldwide¹. Approximately 25% of epileptic patients have drug resistance². Human temporal lobe epilepsy (TLE) is both the most prevalent seizure condition in adults³ and the most frequent reason for drug-resistant (pharmacoresistant) seizures⁴. Pharmacoresistant epilepsy is associated with poor quality of life, injuries, psychosocial problems, premature mortality⁵, and psychiatric problems⁶. Thus, finding new treatments is an urgent necessity⁷, and there is an unmet need for finding new antiepileptic drugs with novel targets and different mechanisms⁸. Furthermore, drug-resistant epilepsy is the cause of 80% of the expenditure of epilepsy⁴ and the mechanisms underlying pharmacoresistant epilepsy are not completely understood⁵. Therefore, major attention has been directed towards elucidating the mechanisms underlying drug resistance.

There are numerous hypotheses explaining the mechanisms related with refractory epilepsy, including methylation, impaired mitochondrial function, neural network, intrinsic severity, transporter, and target hypothesis⁹. The intrinsic severity hypothesis assumes that drug resistance is the result of high excitatory neurotransmission, which leads to elevated intensity and frequency of seizures⁹. A deterioration in the balance of inhibitory and excitatory systems in the brain leads to seizures, which have been defined as aberrant, extreme, and synchronous neural activity¹⁰. Neuropeptides are significant in the field of epilepsy due to their modifying roles¹¹ on inhibitory or excitatory neurotransmitters¹⁰. Therefore, neuropeptides draw attention as drug or biomarker candidates in the field of epilepsy research¹⁰.

Ghrelin is described as a new anticonvulsant¹², pleiotropic¹³, and orexigenic peptide, known to be expressed in the brain¹⁴. Alterations in ghrelin levels have been reported both in clinical¹⁵⁻¹⁷ and animal studies¹⁸. Irisin is defined as a myokine, which is produced in skeletal muscle with exercise¹⁹. In addition, FNDC5, the precursor of irisin, is present in the brain¹⁹. Significant alterations in serum levels of irisin²⁰ and FNDC5/irisin¹⁸ have been reported. Nesfatin-1 is a recently identified neuropeptide, produced in different areas of the brain²¹. Increased nesfatin-1 levels have been reported in clinical¹⁶ and animal studies¹⁸. Vasoactive intestinal peptide (VIP), a neuropeptide that contains 28 amino acids, is expressed in different areas of the brain²². VIP is among the neuropeptides in the hippocampus, which is the most common focus of seizures in TLE²². Although VIP can increase the electrical activity in various areas of the brain and may have a function in seizure pathology, VIP has not often been investigated in the field of epilepsy¹⁰.

In summary, in previous studies, significant alterations were reported in ghrelin¹⁵⁻¹⁸, irisin²⁰, FNDC5/irisin¹⁸, and

nesfatin-1^{16,18} levels. Despite this, there is also lack of evidence of whether these four neuropeptide levels are altered with drug resistant temporal lobe epilepsy (DRTLE) or not. Therefore, the aim herein was the investigation of possible alterations of these peptides in TLE patients with or without drug resistance.

METHODS

Study design

This cross-sectional study was conducted during the period comprehending November 2018 to March 2020. All of the study protocols received approval from the local Medical Ethics Committee of Van Yuzuncu Yil University. A written informed consent was given to the subjects before participating in the study. Of the 116 eligible subjects, nine were excluded due to refusal to give blood and nine, due to non-fasting status at the time of sampling. Moreover, 13 other subjects were excluded due to a body mass index (BMI) over 30.0 kg/m², one was excluded with a newly diagnosis of ankylosing spondylitis. Some subjects have two excluded criteria. Finally, 86 subjects remained for this study. The cross-sectional study was carried out on 58 TLE patients (24 with DRTLE and 34 with TLE that was not drug-resistant) (who attended epilepsy outpatient clinic at Van Yuzuncu Yil University, Neurology department) and 28 healthy subjects. All of the patients had a body mass index (BMI) of less than 30.0 kg/m². The control group included age- and BMI-matched subjects who did not have any chronic illness and a BMI of less than 30.0 kg/m². The exclusion criteria are BMI greater than 30.0 kg/m², chronic illness except epilepsy. "TLE is diagnosed by a history of characteristic partial seizure symptoms. The diagnosis is confirmed by the capture of a typical episode during an electroencephalogram (EEG) or video-EEG, with epileptiform activity over one or both temporal regions"²³. Despite significant advances involving both antiepileptic drugs and surgery in TLE treatment over recent decades, approximately one third of patients with this disease are only poorly controlled, or their seizures are resistant to drugs. "Drug resistant epilepsy may be defined as failure of adequate trials of two tolerated and appropriately chosen and used AED schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom"²⁴ by ILAE. This study has been reported in line with the STROBE criteria²⁵.

Ghrelin, nesfatin-1, irisin, and VIP assays

Blood was collected from the subjects at 08:00 and 12:00 h following one night of fasting. Centrifugation of the blood was performed for 5 min at 4,000 rpm. Storage of the serum was at -80°C until the testing. Serum ghrelin, nesfatin-1, irisin, and VIP

levels were measured using ELISA. Serum levels of the ghrelin (Cat No: YLA1024HU), VIP (Cat No: YLA0803HU), irisin (Cat No: YLA1361HU), and nesfatin-1 (Cat No: YLA0715HU) (all available commercially from YLbiont, Shanghai, China) were determined using ELISA kits.

Statistical analysis

In the study, ghrelin is considered for sample size calculation. From the previous studies²⁶, the standard deviation for ghrelin varies between 0.1 and 0.9. Thus, standard deviation was considered as 0.5. For the 95% of confidence coefficient and approximately 80% power value, Type I error is 0.05 (Z value is 1.96 for the 5% type I error), the effect size was defined by the researcher as 0.2. Based on this information, the necessary sample size was calculated by the equation " $n=Z^2 \times \sigma^2/d^2$ "

According to this equation, minimum sample size in each group was found as 24 [$n=(1.96^2 \times 0.5^2/0.2^2 @ 24)$].

For continuous variables, descriptive statistics were presented as the mean and standard error of the mean (SEM), whereas the categorical variables were presented as counts and percentages. One-way ANOVA was used for comparison of the means of groups. The Duncan multiple comparison test was used for identification of the different means of the groups, followed by ANOVA. To determine linear relations among the variables, the Pearson correlation analysis was performed. Additionally, the chi square test was used for determining relations between categorical variables. Statistical significance was defined as $p<0.05$, and SPSS v.13 (Chicago, IL, USA) was used for the statistical computations.

RESULTS

Table 1 presents the demographic characteristics of subjects. There were no statistically significant differences in terms of age and BMI between the groups.

Serum ghrelin levels in the DRTLE group decreased significantly when compared to the control group ($p<0.05$).

Table 1. Demographic characteristics of subjects.

	Age (Mean±SEM)	BMI (Mean±SEM)
Control	30.92±1.54	25.16±0.64
TLE	26.44±1.22	23.55±0.55
DRTLE	31.75±2.62	24.01±0.52

TLE: Temporal lobe epilepsy patients without drug resistance; DRTLE: drug-resistant temporal lobe epilepsy patients; BMI: body mass index. Data are presented as Mean±SEM (standard error of mean).

The difference between the TLE and DRTLE groups in terms of ghrelin was non-significant (Figure 1A). Serum nesfatin-1 levels had increased in the TLE group, whereas they had decreased in the DRTLE group when compared to the control; however, both were non-significant (Figure 1B).

No statistically significant difference was observed between the TLE, DRTLE, and control groups with regards to the serum VIP levels (Figure 1C). Serum irisin levels had decreased in the TLE and DRTLE groups when compared to the control, and they had decreased in DRTLE compared to TLE group; however, both were non-significant (Figure 1D).

DISCUSSION

Increased¹⁵ or decreased^{16,17} ghrelin levels have been reported in studies on epilepsy patients. In a previous study, significantly decreased ghrelin levels in the brain and serum were found in acute PTZ-induced seizures; and PTZ kindling models, in rats¹⁸. It was suggested that ghrelin has antiepileptic²⁷ and neuroprotective²⁸ properties. In the present study, serum ghrelin levels had decreased in the TLE and DRTLE groups when compared to the control. This decrease was non-significant in TLE group, whereas it was significant in the DRTLE group. Aydin et al.¹⁷ suggested that the reason for the reduction of the ghrelin level may have been due to the high uptake of the neuropeptide by CNS for modulating epileptic discharges. Frago et al.²⁹ suggested that the anticonvulsant effects of ghrelin may be due to its actions on neuropeptide Y and gamma-aminobutyric acid (GABA). Therefore, in the present study, this decrease may be evaluated as a result of seizures; repetitive seizures may lead a decrease in the body's storage of ghrelin, which may have been responsible for the significant decrease in the serum levels of the DRTLE group.

Ghrelin has a role in a variety of neurophysiological process, including anti-inflammatory, neuroprotective, neurogenesis^{30,31}, anti-convulsant effects³⁰, learning and memory³¹, and can cross blood brain barrier³⁰. Ghrelin receptor GHSR1a is widely expressed in the body, involving prone areas in seizures, such as the hippocampus³⁰. The mechanism underlying its anti-convulsant properties remains unknown³⁰.

In the present study, significant reduction was found in serum ghrelin levels of the DRTLE group compared to the control. The interactivity between ghrelin-NPY/GABA in hypothalamic circuitry was reported^{12,31}. It was reported that the blockade of NPY receptors obstruct the anticonvulsant effects of ghrelin in rats' hippocampus³². Ghrelin supports the releasing of NPY presynaptically and, therefore, the releasing of GABA in the arcuate nucleus of hypothalamus¹². Chronic seizures lead a change in expression of NPY receptors resulting an increase in

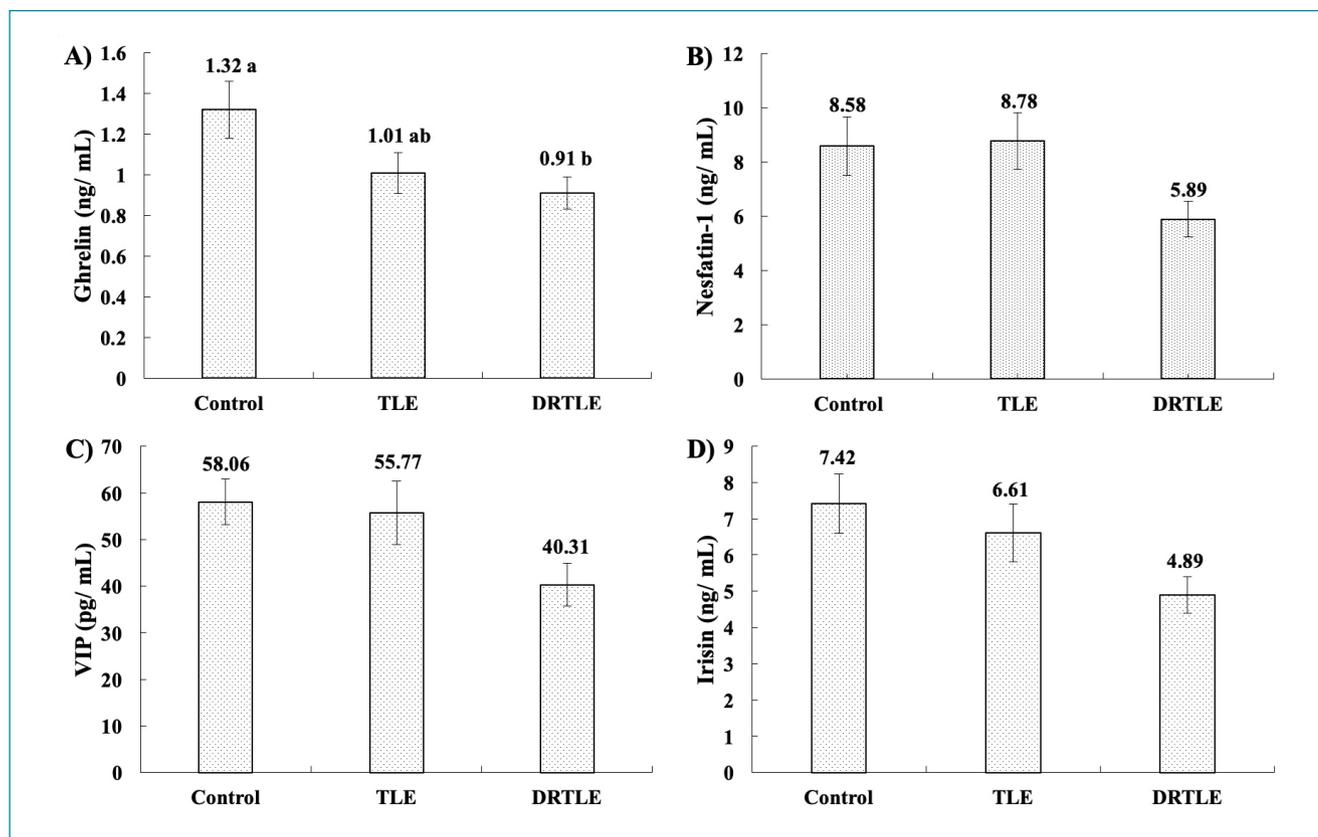


Figure 1. Serum neuropeptide levels of the control, TLE, and DRTLE groups.

Y2 and a decrease in Y1 receptors¹⁰. These changes in response to seizures may be a mechanism for dealing with hyper-excitability¹⁰. In the present study, the reduction of serum ghrelin levels might be due to the increased consumption of ghrelin to cope with chronic recurrent seizures which occurred in DRTLE.

Nesfatin-1, a neuropeptide, is expressed in many areas of the brain²¹. It induces satiety and is known for being a strong anorexigenic agent²¹. Its antiapoptotic and anti-inflammatory effects in the brain tissue of rats has been reported³³. In a previous study, serum levels of nesfatin-1 increased significantly in acute PTZ and PTZ-kindling in rats¹⁸. However, the serum nesfatin-1 levels of rats that received valproate treatment were ameliorated and non-significant when compared to the control¹⁸. Herein, serum nesfatin-1 levels had increased in the TLE group and decreased in the DRTLE group, but the differences were non-significant when compared to the control group. Antiepileptic drug treatment may ameliorate the increased nesfatin-1 levels of the serum. In a previous study, serum nesfatin-1 levels were reported to have increased, in newly diagnosed primary generalized epilepsy patients, approximately 160-fold higher than that of the

control; however, this increase was decreased via treatment with antiepileptic drugs, but remained approximately 10-fold higher than that of the control¹⁶. In the present study, this decrease may have been due to the long duration of antiepileptic drug treatment.

In the present study, the serum VIP level was also evaluated. VIP is defined as a neuroprotective³⁴ neuropeptide. In the present study, serum VIP levels in the TLE and DRTLE groups were similar to that of the control. These results were in accordance with previous studies, in which no significant changes were reported in the VIP levels in the hippocampus of TLE patients³⁵ and brain tissue of PTZ-kindled rats³⁶. Contrary to our results, increased serum and cerebrospinal fluid VIP levels were reported in children with seizure disorders³⁷. These contrary results may have been due to the age of the subject population.

Irisin is secreted from skeletal muscle with exercise³⁸. In recent years, its anti-inflammatory and antioxidative effects have drawn much attention from researchers³⁸. However, its role in the central nervous system is not well known. There are limited studies present on irisin in the field of epilepsy. In a previous

study, serum and brain FNDC5/irisin levels were significantly increased in PTZ-kindling, and acute PTZ-induced seizure groups in rats without antiepileptic drug treatment¹⁸. Herein, differences between the serum irisin levels of the control, TLE, and DRTLE groups were non-significant. In a previous study, it was found that chronic antiepileptic drug treatment (valproate) decreased the PTZ-induced increase in serum and brain irisin levels in PTZ-kindling in rats¹⁸. Significantly increased serum levels of irisin were reported in children with idiopathic epilepsy²⁰. These controversial results may have been associated with different factors; the present study conducted on adults and the subjects had therefore received longer antiepileptic drug therapy.

The strength of this study was that for the first time, to the best of our knowledge, the serum nesfatin-1, ghrelin, irisin, and VIP peptide levels in TLE and DRTLE patients were compared to healthy controls.

The limitations of our study are all patients being under antiepileptic drug therapy. Antiepileptic drug treatment and the age of epilepsy patients can influence the serum levels of ghrelin³¹. Second, the relation between these four peptide levels and the type of antiepileptic drug used did not investigate. In a previous study, it was reported that antiepileptic drug treatment could alter the serum levels of the ghrelin, FNDC5/irisin, and

nesfatin-1 compared to the group who were not under drug treatment in PTZ treated rats¹⁸. In further studies, these peptides should be tested in the same way, but should be done with a larger sample group with subgroups (age groups, type of antiepileptic drug treatment groups).

CONCLUSIONS

In conclusion, the results herein demonstrated decreased serum ghrelin levels in DRTLE patients when compared to the control. Therefore, the results herein suggested that ghrelin might contribute to the pathophysiology of DRTLE. However, future studies are necessary to confirm this hypothesis.

AUTHORS' CONTRIBUTIONS

OEE: Conceptualization, Formal Analysis, Writing – Original Draft. **AM:** Data Curation, Formal Analysis, Writing – Review & Editing. **AUK:** Conceptualization, Data Curation, Formal Analysis, Writing – Review & Editing. **MK:** Conceptualization, Formal Analysis, Writing – Review & Editing. **ZH:** Conceptualization, Formal Analysis, Writing – Review & Editing. **SK:** Conceptualization, Formal Analysis, Writing – Review & Editing.

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