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Research Article

Serum Ghrelin in a Sample of Egyptian Epileptic Patients

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Abstract

Ghrelin is a multifunctional hormone constituted of 28 amino acids, which is the endogenous ligand of the synthetic growth hormone secretagogue receptor 1a (GHS-R) ligands. Ghrelin is expressed in different tissues including the central nervous system (CNS). In addition, circulating ghrelin can access different structures of CNS such as hippocampus and ventral tegmental area. Ghrelin has various neuroprotective properties, and it appears to be related to memory retention and formation. In Rolandic epilepsy, although many changes in the physiology of hormones in the neuroendocrine system of patients can occur, the causes of these changes have not been fully elucidated. There are also relations between seizure activity and stages of sleep, which has been shown to affect both endocrine function and sleep. The purpose of this study was to evaluate serum levels of ghrelin in a sample of Egyptian epileptic patients and to evaluate its possible correlations with clinical findings. A total of 60 patients (age: 6.5±1.9) currently receiving antiepileptic drug therapy for Rolandicepilepsy were studied. The control group consisted of 60 healthy volunteers (age: 6.6±2.1) matched for age and gender. In all participants, serum levels of ghrelin were measured using ELISA technique. In the Rolandic epilepsy patients, the mean serum ghrelin level was 175.8±49.1pg/ml, and this was significantly lower than the control group's level of 445.7±151.4pg/ml ($p < 0.001$). In conclusion, the significant lower ghrelin serum level in patients with Rolandic epilepsy may support the hypothesis of ghrelin's antiepilepticpotential and neuroprotective actions.

Keywords: Rolandic Epilepsy; Serum Ghrelin; Egyptian Patients

Introduction

Epilepsy is a group of neurological disorders characterized by recurrent epileptic seizures [1]. The cause of epilepsy in the vast majority of cases is unknown [2]. In some cases, epilepsy occurs as the result of direct traumatic brain injury, stroke, brain tumors, CNS infections, and birth defects. The end result is to trigger a process known as epileptogenesis [3]. Known genetic mutations are directly linked to a small proportion of cases [4].

Epileptic seizures are the result of abnormal excessive and synchronized neuronal activity in the cortex of the brain [5]. Childhood centro-temporal spikes, also known as Rolandic epilepsy and recently as self-limited epilepsy with centro-temporal spike, is a common disorder distinguished by both its peculiar history and the typical electroencephalography (EEG) signature. Most patients resolve spontaneously, and anti-seizure medication is seldom used. The cognitive outcome is good, yet the risk for learning and behavioral difficulties remains a challenge [6]. A set of children had significant impairments, related to comorbidities, such as attention-deficit–hyperactivity disorder or a history of a previous speech and language delay, as well as more sustained interictal epileptic activity, in addition to cognitive impact of anti-seizure medications [6].

Ghrelin, the "hunger hormone", is a polypeptide hormone synthesized by ghrelinergic cells in the gastrointestinal tract which functions as a neuropeptide in the central nervous system [7]. Ghrelin is a multifunctional hormone constituted of 28 amino acids, which is the endogenous ligand of the synthetic growth hormone secretagogue receptor 1a (GHS-R) ligands. The expression of Ghrelin in the central nervous system (CNS) including; the inter-nuclear space between the lateral hypothalamus, the arcuate nucleus (ARH), the ventromedial nucleus (VMN), the dorsomedial nucleus (DMN), the paraventricular nucleus (PVN) and the ependymal layer of the third ventricle.

Besides regulating appetite, ghrelin contributes to the regulation of energy consumption [8]. Ghrelin also activates the cholinergic–dopaminergic reward loop in projection to the ventral tegmental area and in the mesolimbic pathway. In vivo, Ghrelin injections increase the animal motive to seek food in the form of increased sniffing, foraging for food, and hoarding food [9].

Ghrelin levels are inversely correlated to weight, which suggests that ghrelin functions as an adiposity signal messenger between the body's energy stores and the brain [10]. In humans, ghrelin has been shown to be involved in sleep regulation. In a study, supplementation of healthy young males with ghrelin resulted in prolongation of slow-wave sleep as well as the non-rapid-eye-movement (NREM) sleep during the night. This property indirectly affects the pattern of seizure occurrence by alteration in stages of sleep. Epileptiform EEG discharges mostly are generated during NREM sleep and seizures generally occur in the NREM sleep [11].

Mostfocal seizures in adults are originating in tempro-limbic structures, namely, hippocampus and amygdala. Dysfunctional activity, due to epileptiformdischarge, has been shown to interrupt the closely related hypothalamo-pituitary axis [12].

The objectives of the present study are; to investigate the presence of changes in serum ghrelin in patients with Rolandic epilepsy and controls; and to determine the possible correlations with clinical

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parameters i.e. clinical epilepsy variables, IQ, and school performance based on school evaluation sheets.

Subjects and Methods

Type of Study

Case–control study.

Study Settings

National Research Centre, Department of Research on Children with Special needs, Dokki, Egypt.

Misr University for Science and Technology, 6th of October City, Egypt.

Study Population

Sampling method: Blood samples for serum separation.

Sample size: 60 cases and 60 controls.

1. **Cases group:** 60individuals aging from 4 to 12 years old diagnosed with epilepsy were recruited from National Research Centre, Research on Children with Special Needs department out-patients' clinic.

2. **Controls group:** 60 volunteering individuals from Misr University for Science and Technology Hospital matching age, and sex of cases group.

Study Procedures

The current study was done following the ethical procedures of the National Research Centre, Egypt. All cases were subjected to history taking and thorough clinical examination including general and neurological examination and the following:

1. Routine EEG.
2. MRI brain.
3. Serum level of Ghrelin.

Inclusion Criteria

1. 4-12 years old.
2. Rolandic epilepsy.
3. At least one attack in the last 6 months.

Exclusion Criteria

1. Obesity.
2. Abnormal MRI brain.
3. Developmental delay.
4. Dysmorphic features.
5. Known organic or metabolic disease.
6. IQ less than 70 by *Wechsler intelligence scale for children* (Arabic version),an individually administered intelligence test for children between ages of 4-16 years old developed by David Wechsler, it generates a full-scale IQ that represents a child's general intellectual ability, it also provides five primary index score:

- Verbal comprehension index.
- Visual spatial index.
- Fluid reasoning index.
- Working memory index.
- Processing speed index.

Biochemical Analysis

Blood samples were withdrawn from each participant in 5-ml vacutainer plain sterile tube, serum samples were isolated from each sample within 30minutes after collection at 3000 rpm centrifugation (Heraeus, Labofuge 400R, Germany). Serum samples were divided into aliquots and stored immediately at -80°C, until further processing. Serum ghrelin level was measured in each sample of the cases and controls using Enzyme-Linked-Immunosorbent-Assay (ELISA) method using human ghrelin ELISA kit (SUNLONG, Cat. no. SL1947Hu, China), according to the manufacturer's instructions.

Statistical Analysis

All data were recorded, entered and analyzed using the statistical package of social science (SPSS).

Results

A total of sixty healthy controls and sixty cases in the age range of 4 to 12 years diagnosed with Rolandic epilepsy. The mean age of the case group was 6.5±1.9 years; forty cases were males (66.7%) and twenty cases were females (33.3%). Eighteen of the cases have positive consanguinity, eighteen have similar condition in family and twenty-eight of them have history of delayed language development.

Table (1) shows the age mean of case group is 6.5±1.9 and age mean of the control group is 6.6±2.1, including 40 males (66.7%) and 20 females (33.3%), while the control group includes 38 males (63.3%) and 22 females (36.7%), with no statistically significant difference.

Table (2) mentions the clinical history of the studied cases

Table (3) shows that there is statistically significant difference between the case and the control groups regarding learning performance where 8 of the patients have good learning performance compared to 38 participants of the control group, 36 of the case group have fair learning performance compared to 16 participants of the control, and 16 of the case group have poor learning performance compared to 6 participants of the control group according to the official school evaluation sheets.

Table (4) shows that serum ghrelin was significantly lower in cases than in control.

Table (5) presents the correlation between serum ghrelin and frequency of attacks of the studied patients. There was significant negative correlation regarding serum ghrelin and the frequency of attacks in this study group.

Table 1: Age and sex of the studied groups.

Variables		Case (N=60)	Control (N=60)	P
Age (years)	Mean±SD	6.5±1.9	6.6±2.1	^0.746
	Range	4.0–12	4.0–12	
Gender (n, %)	Male	40 (66.7%)	38 (63.3%)	#0.787
	Female	20 (33.3%)	22 (36.7%)	

^Independent t-test.
#Chi square test

Table 2: Clinical history of the studied cases.

Variables		Mean±SD	Range
Age of onset (years)		2.9±0.8	2.0–4.0
Illness duration (years)		2.5±1.3	2.0–5.0
Frequency (attack/month)		2.6±1.6	1.0–7.0
		N	%
History of language affection		28	46.7
History of status epilepticus		2	3.3
EEG CTS	Unilateral	16	26.7
	Bilateral	44	73.3
Treatment	Levitracitam	28	46.7
	Oxacarbazepine	16	26.7
	Valproic acid	8	13.3
	Carbamazepine	8	13.3

Total = 60.
EEG: Electroencephalogram.
CTS: Centro-temporal spikes

Table 3: Learning performance of the studied groups.

Status	Case (N=60)	Control (N=60)	P
Good	8 (13.3%)	38 (63.3%)	<0.001*
Fair	36 (60.0%)	16 (26.7%)	
Poor	16 (26.7%)	6 (10.0%)	

#Chi square test.
*Significant

Table 4: Serum Ghrelin (pg/ml) in the studied groups.

Measures	Case (N=60)	Control (N=60)	P value
Mean±SD	175.8±49.1	445.7±151.4	<0.001*
Range	112.0–301.0	189.0–852.0	

#Chi square test
*Significant

Table 5: Correlation betweenserum ghrelin and frequency of attacks.

Parameter	r	P value
Frequency of attacks and serum ghrelin	-0.409	0.025*

#Pearson correlation
*Significant

Discussion

The hunger hormone (i.e., ghrelin) is a small peptide which consists of 28 amino acids. Acylated ghrelin and des-acyl ghrelin are the two circulating forms of ghrelin. Circulating ghrelin gains access to the CNS and reaches different structures as hippocampus and ventral tegmental area (VTA). Ghrelin has various neuroprotective properties andit seems to be associated with memory retention and formation. Suppression of microglia and astrocyte activation, as well as suppression of inflammatory mediators are involved in the aspects of ghrelin's neuroprotective effect. Ghrelin's anticonvulsant effect may involve neuropeptide Y (NPY) and gamma-aminobutyric acid (GABA) as NPY and GABA exert anti-epileptic effects in animal seizure models and ghrelin not only enhances NPY synthesis, but also increases GABA-ergic activity in the brain, Therefore, the stimulatory effect of ghrelin on NPY and GABA activities may contribute to the anti-epileptic properties of ghrelin.

The present study held on 60 epileptic patients and 60 healthy controls, their ages range from 4 to 12 years old, it was found that serum ghrelin level was significantly lower in cases than in controls, this corresponds to Dag's study that reported reduced levels of total ghrelin in serum and saliva of patients [13] and opposes Berilgen's study that found higher levels of total ghrelin in patients, both with focal and generalized epilepsy [14].

The results showed that serum ghrelin may be a diagnostic parameter to differentiate between patients and controls which can be used to differentiate between true fits and pseudo-fits later on.

It was found that serum ghrelin was significantly lower among cases with history of delayed language development and learning performance was significantly worse in cases than in controls, which means that the higher ghrelin level the better the learning performance including attention, concentration and memory which can be explained by enhancement of synaptic signaling by glutamate through the activation of ghrelin receptors in the hippocampus in which ghrelin promotes the synaptic accumulation of glutamate receptors and increases long-term potentiation, which is one form of synaptic plasticity that is thought to underlie learning and memory. The cognition-enhancing role of ghrelin may relate to theses molecular effects of ghrelin in the hippocampus.

This corresponds with Diano et al. [16] study in which theyfoundpromotion of long-term potentiation in the hippocampus, increasing in spine density of neurons in hippocampal CA1 region, and enhancing the performance in several types of hippocampal-dependent learning and memory tasks, upon ghrelin administration.

Moreover, there was a significant negative correlation between ghrelin and frequency of attacks in cases group, which means that ghrelin can reduce seizures' relapses and their severity. This corresponds toLuef study which found that the secretion of neuropeptide by both neurotransmitters signaling pathways and endocrine secretion were promoted by ghrelin. Neuropeptide exerts inhibitory effect on seizures [17], but Zeman et al. [18] found an increase in serum ghrelin level might contribute in prolongation of NREM sleep in those epileptic patients, thus may participate in seizure provocation.

It was also found that the highest level of serum ghrelin was with those patients treated with oxacarbazepine and levetiracetam (the difference wasn't significant though), which means that new anti-epileptic drugs could be considered as the safest antiepileptic drugs (AEDs) on cognition and learning performance and most protective against seizure occurrence in patients with Rolandic epilepsy comparing with those patients who received carbamazepine and valproic acid.

Serum ghrelin was lower among cases with bilateral EEG centrotemporal spikes, which mean that ghrelin has the ability to reduce seizure's severity that corresponds with the study of Obay which stated that previous treatment with ghrelin in rats delayed the time of onset of pentylenetetrazole which induced seizures [19].

Conclusion

In the current study the significant lower ghrelin's serum level in patients with Rolandic epilepsy than that of controls may support the

hypothesis of ghrelin's anti epileptic potential and neuroprotective actions.

Conflicting Interest

The authors declare that they have no conflict of interest

Consent to Participate

Signed consent taken from the parents of the children after explaining the aim of the study to complete the clinical diagnosis and blood sampling for biochemical analysis.

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