Plasma Nesfatin-1 Level May Be Associated with Disease Severity in Patients with Panic Disorder

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ÖZET:

Plazma nesfatin-1 düzeyi panik bozukluğu olan hastalarda hastalık şiddeti ile ilişkili olabilir

Amaç: Panik bozukluk (PB) hastaları ve sağlıklı kontrol grubunda son yıllarda keşfedilmiş peptid bir hormon olan nesfatin-1'in plazma düzeylerini karşılaştırmayı amaçladık.
Metod: Hasta grubundaki bireyler bir üniversite hastanesi psikiyatri polikliniğine başvuran ve PB tanısı konulan hastalar arasından seçildi. Yaş, cinsiyet, ağırlık ve Vücut Kitle İndeksi (VKİ) açısından benzer özellikleri olan sağlıklı bireyler ise çalışmanın kontrol grubunu oluşturdu. Her iki gruptaki tüm hastalara Panik Agorafobi Ölçeği (PAÖ) uygulandı. Plazma nesfatin-1 düzeylerini belirlemek için tüm katılımcılardan kan alındı ve analizler ELISA yöntemiyle incelendi.
Bulgular: PB hastalarının ortalama plazma nesfatin-1 düzeyi kontrol grubundaki bireylerinkinden daha yüksek bulundu (hasta grubunda 4.66 ± 2.39 ng/ml ve kontrol grubunda 2.13 ± 1.52 ng/ml). Panik bozukluğu olan hastalarda plasma nesfatin-1 düzeyleri ile PAÖ puanları arasında pozitif bir korelasyon olduğu tespit edildi.
Sonuç: Panik bozukluğu olan hastalarda anksiyete ve plazma nesfatin-1 düzeyleri arasında bir ilişki olabilir. Bu ilişkiyi araştıracak ileri çalışmalara ihtiyaç vardır.

Anahtar sözcükler: Panik bozukluk, nesfatin-1, anksiyete, açlık, tokluk, gıda alımı

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ABSTRACT:

Plasma nesfatin-1 level may be associated with disease severity in patients with panic disorder

Objective: We have compared the plasma levels of the recently discovered peptide hormone nesfatin-1 between patients with panic disorder (PD) and healthy control subjects.
Method: The subjects in the patient group were selected from the patients who presented to the psychiatry outpatient clinic of a university hospital and were diagnosed with PD. Healthy subjects similar to the patient group in terms of age, gender, weight, and BMI constituted the control group in this study. The Panic-Agoraphobia Scale (PAS) was applied to all subjects in both groups. Blood samples were collected to measure plasma nesfatin-1 level by using ELISA method.
Results: Mean plasma nesfatin-1 level (4.66 ± 2.39 ng/ml in patient group, 2.13 ± 1.52 ng/ml in control group) was higher in patients with PD than in the control group (p<0.001). A positive correlation between PAS scores and plasma nesfatin-1 levels was found in the patients with PD.
Conclusion: There may be a relationship between disease severity and plasma nesfatin-1 levels in panic disorder patients. Future studies investigating this relationship are needed.

Key words: Panic disorder, nesfatin-1, anxiety, starvation, satiety, food intake

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INTRODUCTION

Nesfatin-1 is a recently discovered hormone which is a peptide in structure. It contains 82 amino acids and is derived from NEFA/nucleobindin-2 (NUCB2) (1). This peptide has been found at high levels in the brain satiety center of the hypothalamus, paraventricular, supraoptic, arcuate and lateral nuclei (1,2). In addition, it was identified in the Edinger-Westphal nucleus, central amygdala nucleus, solitary tract nucleus, caudal raphe nucleus, locus coeruleus, periaqueductal gray matter, and dorsal motor neurons of the vagal nucleus (2). Authors have claimed that nesfatin-1 was responsible for the provision of appetite and metabolic regulation in hypothalamus (1,2). Oh et al. reported that nesfatin-1 given centrally reduced nutrient input, while intracerebroventricular (ICV) injection of antibodies against these peptides increased food intake (1).

It is well known that appetite and metabolic dysregulations are quite common in anxiety disorders. There are many neuroendocrine changes in the brains of patients with panic disorder (PD). The results of Sakai et al., which include slowing of glucose metabolism in the amygdala, hippocampus, and thalamus of patients with PD, have provided some biological evidence for those changes (3); however, the neurobiology of the relationship between PD, its severity, and appetite-metabolic status has not throughly been studied.

Food intake and the burning of calories are basically moderated by anorexigenic and orexigenic pathways.

Neurotransmitters like neuropeptide Y (NPY), leptin, and norepinephrine are orexigenic, while some others such as melanocyte stimulating hormone (MSH), serotonin and corticotropin releasing factor (CRF) are anorexigenic (4). These neurotransmitters also play some roles in the regulation of anxiety states and stress. For example, NPY is an anxiolytic neurotransmitter,, whereas MSH, leptin, serotonin, and CRF lead to anxiety (5-7). Thus, they seem to be operating both in moderation of food intake and anxiety states. Some evidence supporting the idea that nesfatin-1 is related to both food intake and anxiety comes from the study published by Merali et al. in 2008 (8). They reported that the effects of central nesfatin-1 injection on stressors are related to behavior that reflects anxiety and/or fear in rats. Furthermore, ICV nesfatin injection raised the confusion strengthened by fear and they concluded that central nesfatin-1 injection led to anxiety and fear responses in rats.

Although a small number of studies regarding nesfatin-1 in animals are present in the literature, there is still a paucity of human studies on this topic. To the best of our knowledge, currently there is no human study examining nesfatin-1 in anxiety disorders. Given its possible role both in food intake and anxiety, we aimed to investigate whether its plasma levels are higher in patients with PD than in healthy control subjects.

METHOD

Thirty-three patients (19 female, 14 male), who applied to the psychiatry department of our university hospital and were diagnosed only with PD according to the DSM-IV criterion, were enrolled in the study as the patient group. Three of them (1 female and 2 males), who had been treated with anxiolytic drugs before their admission, were excluded from the study. A total of 30 drug naive patients (18 female and 12 male) with PD were enrolled into the study. Thirty healthy volunteers similar to the patient group in terms of weight, BMI, age, and gender (17 female and 13 male), were included in the control group. Total biochemical evaluation, hemogram, and electrocardiogram were carried out for all subjects. The co-occurrence of any severe physical illness, endocrinological pathology, infectious diseases, neoplasm, autoimmune disorder, gestation, obesity, abnormal lipid profile, history of alcohol and substance abuse or dependence or the presence of co-morbid depression or any other axis I disorder were assigned as exclusion criteria. All female subjects were examined for a possible effect of the menstrual cycle. Female subjects in the luteal phase were excluded. The Panic-Agoraphobia Scale (PAS) was applied to all subjects (9). Weight measurement and body mass index (BMI) calculations for all subjects were done meticulously using the same measurement instruments.

Blood samples for nesfatin-1 were drawn in the morning around 9 a.m. from a forearm vein at the end of an overnight fasting period of at least 8 hours. Tubes with a 2 milliliters capacity containing EDTA were used for collecting blood. The blood was carefully and immediately (in a few seconds) transferred from these tubes to centrifuge tubes which contained aprotinin (0.6 TIU/ml of blood) and immediately stored on ice.

Before the centrifuging, the centrifuge tubes were gently rocked several times to inhibit the activity of proteinases. After the centrifuge process at 1,600 x g for 15 minutes at 4°C plasma was obtained. The separated plasma was stored in a –80°C freezer until the time of assay. Plasma nesfatin-1 levels were measured using a commercial ELISA kit (Uscn Life Science, Wuhan, P.R.China). Some previous studies in the literature have used the ELISA method for measuring nesfatin-1 peptide levels (10-12). This study was approved by a local ethics committee (Gaziantep Ethics Committee of Clinical Investigations) and all participants provided written informed consent before enrollment in the study.

Statistics

The data were analyzed using the Statistical Package for Social Sciences version 12.0.1 (SPSS 2003). Significance level was accepted as $p<0.05$. The Student-t test was used to evaluate differences between groups in terms of age, gender, BMI, PAS, fasting glucose, and nesfatin-1 level. Normality of distribution of the data was verified by running a one-sample Kolmogorov-Simirnov test both for the study group and the control group. To compare categorical variables the chi-square test was used. The relationship between the nesfatin-1 levels and PAS scores in both groups was analyzed by using Spearman's rank correlations test.

RESULTS

There was no statistically significant difference in terms of demographic variables between the patient group and the healthy control group. The demographic and biochemical variables are shown in Table 1. In terms of BMI (23.12 ± 1.40 kg/m^2 in patients, 23.25 ± 1.37 kg/m^2 in control subjects) differences between the groups were not statistically significant (p=0.103). The mean PAS scores were statistically higher in patients with PD than in the subjects of the control group (p<0.001) (Table1).

Table 1: Mean values (or ratios) of study variables in panic disorder and control groups.

Variables	PD group (n= 30)	Control group (n= 30)	p
Age	28.24±5.42	26.63±4.35	0.654
Gender (female/male ratio)	18/12	17/13	0.496
BMI (kg/m^2)	23.12±1.40	23.25±1.51	0.103
PAS	22.89 ±4.26	2.67±2.46	<0.001
Glucose (mg/dl)	92.96±4.29	93.78±4.60	0.487
Nesfatin-1 (ng/ml)	4.66±2.39	2.23±1.43	<0.001

BMI: Body mass index, PAS: Panic-agoraphobia scale.

In terms of fasting plasma glucose level (92.96 ± 4.29 mg/dl in patients and 93.78 ± 4.60 mg/dl in control subjects), there was no statistically significant difference between groups (p<0.487). The average nesfatin-1 level in patients with panic disorder was 4.66 ± 2.39 ng/ml, and 2.23 ± 1.43 ng/ml in the control group. The difference in mean plasma nesfatin-1 level between groups was statistically significant (p<0.001). Post-hoc power analysis yielded a power of 0.89 for the nesfatin-1 level. There was a positive correlation between plasma nesfatin-1 levels and PAS scores in the patient group (r=0.467, p=0.011) but not in the control group. The mean plasma nesfatin-1 levels were 4.54±2.27 ng/ml in male and 4.78±2.50 ng/ml in female patients and 2.17±1.20 ng/ml in male and 2.29±1.66 ng/ml in female control subjects. There was no statistically significant gender difference in terms of mean plasma nesfatin-1 level within either group.

DISCUSSION

Nesfatin-1, a newly discovered satiety peptide, has been reported to reduce food intake when injected centrally (8). Previously studied similar peptides related to the hunger-satiety system are known to affect physiological processes in addition to their regulatory effects on eating behavior. It is also clear that irregularities in physiological processes may activate some stress-related systems (13-16). On the other hand, stress by itself, not only changes food intake but also cause stress responses, including secretion of glucocorticoids (17-20). At the same time, starvation has been claimed to increase the sensitivity to stress in the hypothalamo-pituitary-adrenal axis (14,21,22). All these findings support the literature reports that peptide hormones, such as CRF, leptin, ghrelin, orexin, neuropeptide Y, and cholecystokinin, not only centrally keep food intake under control, but also modulate stress responses (14,15,23-27).

Previous data obtained from animal studies point out a potential role of nesfatin-1 in the processes related to the anxiety response to stress situations similar to that of above mentioned peptide hormones. The positive correlation we found between plasma nesfatin-1 levels and scores obtained from the PAS also supports this suggestion about nesfatin-1 hormone.

Panic disorder is accepted as one of the major anxiety disorders shown to be controlled by some genetic factors. (28). In our study, subjects with panic disorder had higher levels of mean plasma nesfatin-1 hormone than healthy controls (p<0.001). Additionally, a positive correlation between panic disorder severity and plasma nesfatin-1 level was found in the patient group. Our findings are supported by the study published by Merali et al. in 2008 (8). They reported that central nesfatin-1 injection led to anxiety and fear responses in rats. Similarly the, anxious group represented by patients with PD in our study, had higher mean plasma level of nesfatin-1 peptide than the control group. It is perhaps important to note that the permeability of the blood brain barrier to circulating nesfatin-1 peptide has been previously shown in the

literature (29,30).

As previously demonstrated by Price et al., nesfatin-1 inhibits Neuropeptide Y (NPY) neurons in the arcuate nucleus (31). In addition, NPY also plays a role in the regulation of stress and anxiety as shown by some recent studies. Additionally, some recent animal studies demonstrated that NPY has anxiolytic and anti-depressant effects (7). All these data support the idea that nesfatin-1 is a peptide hormone which may be related to anxiety states. Another study published by Goebel et al. showed that the majority of stress activated neurons of the raphe nucleus and locus coeruleus were immunolabeled with nesfatin-1 (32). They concluded that nesfatin-1 neurons were part of the hypothalamic and hindbrain neuronal cell groups and were activated by stress. Their findings also additionally support a possible interaction of nesfatin-1 and anxiety states.

In a study done by Oh et al., ICV administration of α-MSH (a melanocortin agonist) was reported to lead to gene encoding (NUCB2) in the paraventricular nucleus (1). Thereupon, the authors claimed that nesfatin-1 could lead to anxiety by activating the melanocortin system. In previous studies, the anxiogenic effects of the melanocortin system was thought to be due to increasing ACTH (26) and a negative influence on the GABAergic system (6). In addition to the suggestion that nesfatin-1 could lead to anxiety, Oh et al. also showed a decrease in nesfatin-1-induced satiety when SHU9119 (melanocortin receptor antagonist) was administered ICV.

Small sample size, the cross sectional design of the study, and having blood samples collected only once from PD patients make the interpretation and generalization of the findings of our study difficult. Additionally, the absence of data about the duration of panic disorder in PD patients is another limitation of the study. On the other hand, if some other parameters like ghrelin and leptin were measured besides nesfatin-1, the complex relationship among them would be more understandable. It may be worthwhile to mention that co-morbid depression in the study group would be a confounder if it were not part of the exclusion criteria.

This is the first study showing high levels of plasma nesfatin-1 in patients with PD (an anxiety disorder) and discussing its potential role in anxiety states in addition to its previously known effects on appetite and food intake. Findings of this study are consistent with the results of previous animal studies that showed anxiogenic effects of nesfatin-1 hormone. Future studies are needed to clearly understand the complex relationship between food intake and anxiety states and how the newly discovered statiety hormone nesfatin-1 fits into this picture.

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