









Plasma Nesfatin-1 Levels and its Relationship with Anxiety Levels in Obesity Diagnosed Population: A Controlled Study

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Received: 17.02.2018

Accepted: 02.05.2018

ABSTRACT

Objective: Nesfatin-1 is a satiety neuropeptide involved in the regulation of metabolic pathways and food intake. Some studies have shown nesfatin-1 to be also associated with stress responses and stress-related behaviors. In the present study, we evaluated nesfatin-1 levels in obese individuals and investigated whether nesfatin-1 levels could be associated with anxiety levels in obese populations.

Methods: Fifty-eight obese (29 anxious and 29 non-anxious) patients and 25 healthy control subjects between 18 and 65 years old were enrolled in the study. Plasma nesfatin-1 levels were measured with a commercial enzyme-linked immunosorbent assay, and anxiety levels were measured using the Hospital Anxiety and Depression Scale.

Results: Plasma nesfatin-1 levels were significantly lower in both obese and anxious subjects and obese and non-anxious subjects than in the control group. No significant differences were found between the obese+anxious group and the obese+nonanxious group.

Conclusion: Our results suggest that plasma nesfatin-1 levels are decreased and not associated with anxiety levels in obese populations

Keywords: Nesfatin-1, Obesity, Anxiety

1. INTRODUCTION

Nesfatin-1 is a multifunctional peptide with pleiotropic functions secreting from different systems. The most established functions associated with nesfatin-1 have been reported as anorexigenic effect, feed regulation, and appetite (1). Studies on animals showed that nesfatin-1 is derived from nucleobindin-2 and is expressed in several regions of the central nervous system (CNS), including the limbic system, brainstem, and hypothalamus (2). Several studies showed that nesfatin-1-secreting neurons are distributed in the regions of the CNS associated with neurovegetative functions, such as food intake regulation (3). Some data revealed that nesfatin-1 is expressed in the regions other than the CNS in the human body, including adipose tissue, and has potential regulatory functions in association with some other metabolic hormones, such as ghrelin (4). There is also evidence supporting that nesfatin-1 is implicated in adipogenesis and obesity (5). Obesity and metabolic syndrome, as well as diabetes and cardiovascular diseases, have been associated with the dysfunction of nesfatin-1 and its associated pathways (6). Nesfatin-1 levels have been reported to be increased in obese individuals, whereas they have been found to be decreased in cases with anorexia nervosa (7). In line with this data, a positive correlation

between nesfatin-1 levels and body mass index (BMI) has been observed in some studies, whereas some conflicting data regarding a negative correlation between nesfatin-1 levels and BMI also exist (8). There is evidence of low concentrations of nesfatin-1 in populations with high BMI. A previous study showed a negative correlation between BMI and nesfatin-1 in non-obese populations (8). Nesfatin-1 has been reported to be expressed in several hypothalamic nuclei and regions associated with appetite and emotional status (9). Furthermore, central regulatory neuropeptides, such as neuropeptide Y, vasopressin, and oxytocin, that contribute to energy balance are expressed from neurons in the arcuate, paraventricular, and supraoptic hypothalamic nuclei similar to nesfatin-1. Although a specific nesfatin-1 receptor is still unknown, current data showed a proposed mechanism of action that involves nesfatin-1 interacting with a G protein-coupled receptor that results in an inhibitory hyperpolarization of neuropeptide Y/agouti-related peptide neurons that have an orexigenic effect in the arcuate nucleus (10). In addition, nesfatin-1 has been reported to stimulate oxytocin-mediated melanocortin that has an anorexigenic effect in the paraventricular nucleus (10, 11). Neurovegetative functions, including appetite and sleep, as well as some other hypothalamic

functions, are related to and influenced by an individual's emotional state. As nesfatin-1 is a satiety neuropeptide involved in the regulation of metabolic pathways and food intake, some studies showed that nesfatin-1 is also associated with stress responses and stress-related behaviors (7, 12). A previous study conducted on rats showed that intracerebroventricular nesfatin-1 injections yield anxiety – and fear-related behaviors (9). Data on the association between nesfatin-1 and stress responses in human subjects are limited. However, in recent years, nesfatin-1 has been studied in subjects with psychiatric disorders, such as depression and anxiety in particular. Some studies found a relationship between nesfatin-1 levels and anxiety disorders, such as panic disorder, generalized anxiety disorder, and obsessive–compulsive disorder (13, 14, 15). In a previous study, nesfatin-1 levels were found to be relatively higher in patients diagnosed with panic disorder than in the control group (13). However, data regarding nesfatin-1 levels in patients diagnosed with generalized anxiety disorder are conflicting (15, 16), and studies examining nesfatin-1 levels in subjects with affective disorders are limited. One study showed nesfatin-1 levels to be significantly higher in subjects with major depression than in the control group, whereas another study reported decreased nesfatin-1 levels during manic episodes (17, 18). All of this data supports nesfatin-1 as a central neuropeptide participating in metabolic regulation, emotional state, and stress responses. To our knowledge, although nesfatin-1 levels have been studied in relation to obesity and some psychiatric disorders, such as depression and anxiety, data on the association between nesfatin-1 levels and anxiety in obese populations are limited. In the present study, we evaluated nesfatin-1 levels in obese individuals and investigated whether nesfatin-1 levels are associated with anxiety levels in obese populations.

2. METHODS

The study was performed in accordance with the Declaration of Helsinki. The local ethics committee of Balikesir University Faculty of Medicine approved the study (2014/04). Written informed consent was obtained from all the participants in the study. Fifty-eight obese patients and 25 healthy controls (non-obese and non-anxious) between 18 and 65 years old were enrolled in the present study. Patients were divided into two groups based on whether they had comorbid anxiety (29 obese and anxious and 29 obese and non-anxious). Patients were selected from those who applied to the psychiatry outpatient clinic and were diagnosed with anxiety disorder or from those who were directed to the psychiatry outpatient clinic due to their psychiatric complaints from the dietary outpatient clinic. All patients were diagnosed with obesity with BMIs measuring >30 kg/m². Diagnosis of obesity was made after BMI was measured by a dietitian. A psychiatric evaluation and diagnosis were made by a psychiatrist. Patients met the criteria for anxiety disorders, as confirmed by

the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, 4th Edition Axis I Disorders (SCID-I) (19). The control group was selected from individuals who had no psychiatric diagnoses, as confirmed by the SCID-I, as well as no obesity confirmed by BMI measurements <25 kg/m². The Hospital Anxiety and Depression Scale (HADS) was used to determine the severity of their anxiety (20). The HADS was developed to detect anxiety and depression levels in outpatient clinic settings. The Turkish version of the HADS was performed by Aydemir et al. with a cut-off value of 10 for anxiety to be used in the Turkish version (21). Patients with psychiatric or neurological diagnoses other than anxiety disorders, such as mental retardation, alcohol/substance use disorder, history of electroconvulsive therapy, history of psychiatric drug use in the last 3 months, a severe physical illness, such as diabetes or another endocrinopathy, cardiovascular diseases, malignancy, infectious diseases, and pregnancy, were excluded from the study. Patients were selected, instructed about the study, and handed written informed consent on their first visit. Venous blood samples were obtained between 07:00 and 08:00 AM in the morning of the second admission after an overnight fasting period. All blood samples were collected in standard ethylenediaminetetraacetic acid tubes (Becton, Dickinson, USA). After blood was withdrawn, plasma samples were centrifuged at 1300 \times g for 10 min. Plasma samples were placed into tubes, and 1% concentrated protease inhibitor cocktail (Sigma Aldrich, USA) was administered in all tubes. Samples were stored at -20 °C until further processing. Nesfatin-1 levels were measured using a commercial enzyme-linked immunosorbent assay kit (ELISA, RayBiotech, USA). All samples were processed in one batch without dilution. Intra-assay and inter-assay variabilities were $<10\%$ and $<15\%$, respectively, according to the manufacturer.

2.1. Statistical Analysis

Data were analyzed using the SPSS software version 15.0 (IBM Inc., Chicago, IL, USA). The ANOVA test was used to analyze the differences between mean values of the groups when normally distributed variables are homogenous. The Tukey test was used for post hoc comparisons. The chi-square test was used to compare categorical variables. The linear regression model was used to predict relationships between nesfatin-1 levels and independent variables. A P value <0.05 was considered as statistically significant.

3. RESULTS

Table 1 shows the sociodemographic characteristics, plasma nesfatin-1 values, plasma glucose levels, and BMI measurements. There were no significant differences between the three groups in terms of age ($p=0.057$), gender ($p=0.997$), tobacco use ($p=0.387$), and marital status ($p=0.825$). There were significant differences in

terms of BMI between the three groups ($p < 0.001$) (Table 1).

Table 1. Baseline characteristics of the study groups

	Obese	Obese	Non-obese	p
	+ anxious (n=29)	+ non-anxious (n=29)	+ non-anxious (n=25)	
Age (year) ^a	45.9±10.1	46.7±11.0	40.2±10.6	0,057
BMI (kg/m ²) ^a	37.2±5.4	38.3±5.5	22.4±2.1	<0.001 ^b
Glucose (mg/dL) ^a	101.7±11.5	104.0±9.1	92.7±9.4	<0.001 ^b
Nesfatin (ng/mL) ^a	28.4±7.1	28.7±6.9	34.6±10.9	0.014 ^b
Gender (female)	79.3%	79.3%	80.0%	0,997
Smoking (yes)	20.7%	10.3%	24.0%	0,387
Marriage (yes)	89.7%	86.2%	84.0%	0,825

^aMean±standard deviation, ^bStatistically significant, BMI: Body Mass Index.

In comparison, there were significant differences between the control group and both patient groups (obese+non-anxious and obese+anxious) ($p < 0.001$) (Table 2). Similarly, there were significant differences in terms of serum glucose levels between the three groups ($p < 0.001$) (Table 1). In comparison, there was a significant difference between the control group and both patient groups ($p = 0.004$ for obese+anxious group and $p < 0.001$ for obese+non-anxious group) (Table 2).

Table 2. Pairwise comparisons of the BMI, glucose, and nesfatin-1 levels between the study groups

	Obese	Obese	Non-obese
	+ anxious	+ non-anxious	+ non-anxious
BMI			
Obese+anxious	1	0,667	<0.001 ^b
Obese+non-anxious	0,667	1	<0.001 ^b
Non-obese+non-anxious	<0.001 ^b	<0.001 ^b	1
Glucose			
Obese+anxious	1	0,676	0.004 ^b
Obese+non-anxious	0,676	1	<0.001 ^b
Non-obese+non-anxious	0.004 ^b	<0.001 ^b	1
Nesfatin-1			
Obese+anxious	1	0,988	0.023 ^b
Obese+non-anxious	0,988	1	0.034 ^b
Non-obese+non-anxious	0.023 ^b	0.034 ^b	1

Table 3 shows the relationship between age, HADS scores, BMI measurements, and plasma nesfatin-1 levels. Plasma nesfatin-1 levels were found to be significantly different when compared in the three groups ($p = 0.014$) (Table 1). In post-hoc comparisons, plasma nesfatin-1 levels were significantly lower in obese and anxious subjects than in controls ($p = 0.023$), as well as in obese and non-anxious subjects than in controls ($p = 0.034$). There were no significant differences between the obese+anxious group and the obese+non-anxious group ($p = 0.988$).

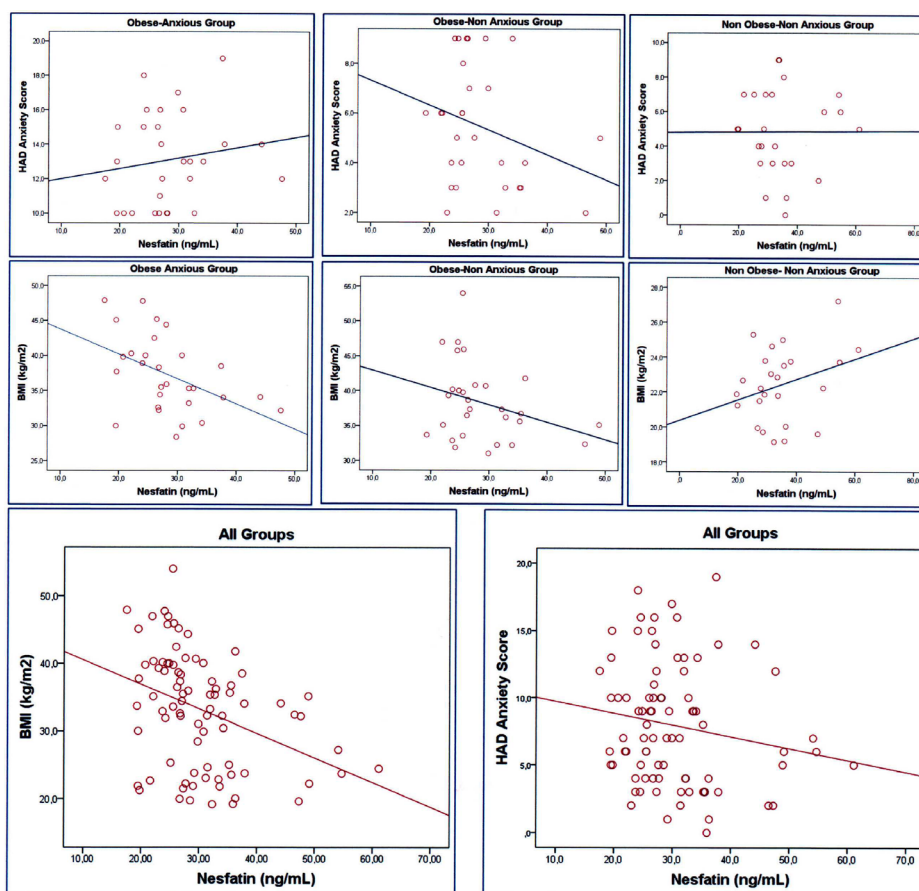
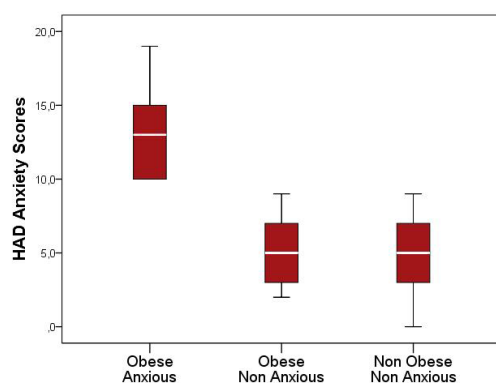


Fig 1. The specific anxiety scores visualized by histogram graphic. Distrubition of spesific anxiety scores in the groups were reflected.

Table 3. Correlations between age, HAD anxiety scores, BMI, and plasma nesfatin-1 levels

		Age	Nesfatin-1 Levels	BMI	HAD Anxiety Scores
Age	r	1	0,074	0,164	0,148
	P value		0,508	0,139	0,180
Nesfatin-1 Levels	r		1	-0,372	-0,168
	P value			0,001	0,129
BMI	r			1	0,359
	P value				0,001
HAD Anxiety Scores	r				1
	P value				

The multivariable linear regression model was used to identify the associations between age, HADS scores, BMI measurements, and plasma nesfatin-1 levels. The regression models were visualized by correlation graphics (Fig. 1). BMI has been identified as an independent variable in the prediction of plasma nesfatin-1 levels ($B=-0.384$, $p=0.001$), whereas age and HADS scores were not found to be associated with predicting nesfatin-1 levels ($B=-0.139$, $p=0.100$ for age and $B=0.113$, $p=0.592$ for HADS scores). Fig. 2 shows the specific anxiety scores as visualized by box plots.

**Fig 2.** The regressions models visualized by correlation graphics

4. DISCUSSION

In our study, we have found a negative relationship between plasma nesfatin-1 levels and BMI. In line with current data, our results support that nesfatin-1 levels may be involved in the development of metabolic abnormalities and obesity. Although studies regarding nesfatin-1 levels in obesity are confounding, our results are consistent with data reporting a negative correlation between nesfatin levels and BMI (8, 22, 23). Animal studies showed that central administration of nesfatin-1 has been associated with decreased food intake, as well as increased anxiety – and fear-enhancing effects (9). Anxiety-related functions of nesfatin-1 levels have been described not only by directional effects but also by interrelational pathways in the CNS. Similar to nesfatin-1, the corticotropin-releasing hormone (CRH) is one of the peptides with anorexigenic and anxiogenic effects that is abundantly found in the hypothalamic region. Recent studies found that nesfatin-1 and CRH act in a collaborative and serial manner, as CRH expression is influenced by the hypothalamic nesfatin-1 neurons (9). Activation of the melanocortin system, which

has been shown to regulate food intake and anxiety-related behaviors, has also been assumed to be involved in the anxiogenic effects of nesfatin-1 (24). Nesfatin-1 has also been associated with the inhibition of neuropeptide Y, which has anxiolytic effects (25). These experimental studies support the association between anxiety and nesfatin-1. Moreover, in a human study, plasma nesfatin-1 levels have been positively correlated to levels of anxiety in obese populations (26). However, there is some evidence to support that low nesfatin-1 levels are associated with anxiety disorders (15), whereas some studies report no relationship (16). Additionally, a study conducted on obese subjects found a sex-specific, positive correlation between plasma nesfatin-1 levels and anxiety in favor of the female population (27). In a recent study, a sex-specific inverse correlation was observed between NUCB2/nesfatin and anxiety levels, whereas there was no significant change in NUCB2/nesfatin following improvement of anxiety (28). However, our results showed no association between anxiety and plasma nesfatin levels in obese populations. Although our samples were predominantly women, no relationship was observed between anxiety and plasma nesfatin-1 levels. The association between nesfatin-1 levels and anxiety is a complicated issue that needs to take several factors into consideration. For instance, the peripheral measurement of a central acting peptide may not be sufficient to determinate its central role. As animal and clinical studies indicated that central nesfatin-1 is involved in the regulation of emotional and stress responses, results of the studies that measured peripheral nesfatin-1 levels in psychiatric conditions are confounding (9, 15, 16, 29). In one study, the authors suggested that nutritional status can be associated with different expressions of nesfatin-1 levels in psychiatric populations (15). Although nesfatin-1 has been shown to have the capacity to cross the blood–brain barrier, the effect of peripheral nesfatin-1 on the CNS or interaction between central and peripheral nesfatin-1 has not been well established. However, as studies focused on peripheral measurements of other regulatory neuropeptides, such as neuropeptide Y and oxytocin, which are involved in central processes and have been related to mood disorders and stress responses (30, 31), future studies are required to clarify the association between peripheral nesfatin-1 levels and anxiety disorders, as well as stress-related responses. There are some limitations to our study. First, the sample size was small. Second, individual factors, such as feeding habits and sedentary lifestyles, have not been taken into consideration during the study. Only performing peripheral measurements of nesfatin-1 levels is also a limitation of the study.

5. CONCLUSION

In conclusion, our results suggest that peripheral nesfatin-1 levels are not associated with anxiety levels in obese populations. Further studies with a larger sample size are warranted to determine whether there is an association between anxiety and peripheral nesfatin-1 levels.

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How to cite this article: Baykan H., Baykan O., Durmaz O., Gulsah O. E., Kara H., Gorgulu S. A., Yaman A., Karlidere T. Plasma Nesfatin-1 Levels and its Relationship with Anxiety Levels in Obesity Diagnosed Population: A Controlled Study. *Clin Exp Health Sci* 2019; 9: 107-111. DOI:10.33808/clinexphealthsci.566075