NÖRO PSİKİYATRİ ARŞİVİ
ARCHIVES OF NEUROPSYCHIATRY

RESEARCH ARTICLE

Arch Neuropsychiatry 2019;56:52–56
https://doi.org/10.29399/npa.22907

Plasma Neuropeptide-S Levels in Populations Diagnosed with Generalized Anxiety Disorder: A Controlled Study

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ABSTRACT

Introduction: Neuropeptide S (NPS) is a novel neuropeptide reported to be involved in fear-and stress-related conditions and their corresponding neuroendocrine processes. The aim of this study was to compare the plasma NPS levels in patients suffering from generalized anxiety disorder (GAD) and those of healthy controls.

Methods: A total of 40 subjects diagnosed with GAD and 40 healthy controls were recruited in the study. The Hamilton Anxiety Scale (HAM-A), Generalized Anxiety Disorder-7 (GAD-7), and Hamilton Depression Scale (HAM-D) were administered to all participants to determine the severity of participants' anxiety and concomitant depressive symptoms. The plasma NPS levels were measured from the fasting venous blood samples obtained from each participant.

Results: The median plasma NPS level was found to be significantly higher in the GAD group in comparison to the control group (28.8 pg/mL as against 19.1 pg/mL, p=0.01). A significant positive correlation was observed between the plasma NPS levels and HAM-A scores (rs=0.23, p=0.04) as well as the GAD-7 scores (rs=0.28, p=0.01). The p-value obtained from the correlation analysis between the plasma NPS levels and HAM-D scores was 0.052. A receiver operating characteristic (ROC) analysis revealed that the plasma NPS levels could enable the identification of GAD with 67.5% sensitivity and 62.5% specificity, when the cut-off value was determined as 25.06 pg/mL.

Conclusions: Our results support the view that plasma NPS levels, which has demonstrated anxiolytic effects on the central nervous system, is related to the severity of anxiety in GAD and could be considered as a candidate marker for the identification of GAD.

Keywords: GAD, generalized anxiety, neuropeptide-S, HPA, inflammation

Cite this article as: Baykan H, Baykan Ö, Durmaz O, Kara H, Hışmıoğulları AA, Karlıdere T. Plasma Neuropeptide-S Levels in Populations Diagnosed with Generalized Anxiety Disorder: A Controlled Study. Arch Neuropsychiatry 2019;56:52-56. https://doi.org/10.29399/npa.22907

INTRODUCTION

Generalized anxiety disorder (GAD) is a common psychiatric condition with a 4.3–5.9% rate of lifetime prevalence (1). This disorder is accompanied by excessive and irrational concern regarding future events that are beyond one's control and are unlikely to materialize. The prevalence of GAD in women is twice than that in men (1). Further, GAD is characterized by psychological symptoms such as feelings of restlessness and irritability as well as somatic components which include nausea, sleep disturbances, sweating, and hot flashes. Since the individual, familial, and global burden of GAD is associated with serious consequences, the available etiological findings are insufficient to explain the etiopathogenesis of this disorder (2). Apart from cognitive and affective theoretical models, studies have also investigated the neurobiological domains responsible for the mechanisms involved in the etiopathogenesis of GAD to demonstrate that the regions associated with emotional processes and involuntary fear responses, such as those of the the limbic system, are implicated in the neurobiology of GAD. Endocrinological and imaging studies reported hyperactivity and increased volumes in the amygdala as well as increased hypothalamic activity, related to the activation of the hypothalamic pituitary adrenal axis (HPA), as a result of chronic anxiety responses in patients with GAD (3–5). Increased HPA activation leads to the release of cortisols and catecholamines that result in the production of cytokines and increases inflammatory processes (4, 6). This data supports the notion that, in addition to cognitive and affective components, GAD includes a neuroendocrinological aspect as well, and the existence of somatic symptoms related to the hyperactivity of the adrenal system constitutes a prominent feature of this disorder.

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Received: 20.09.2017, **Accepted:** 02.04.2018, **Available Online Date:** 20.04.2018

Neuropeptide S (NPS) is a novel neuropeptide, expressed mainly by the regions that modulate locomotor activity, wakefulness, appetite, and arousal in the central nervous system (7). Several studies have demonstrated that NPS and its G-protein coupled cell surface receptor, neuropeptide-S receptor (NPSR), are both expressed in brain regions such as the amygdala, hypothalamus, and thalamic regions that are considered pivotal in anxiety and stress-related responses (7). Some experimental animal studies also reported that the NPS/NPSR system is associated with the modulation of fear conditioning and HPA axis, with stress responses and anxiolytic effects (7–9). The central administration of the NPS has been demonstrated to be involved in the regulation of the HPA axis and increased plasma adrenocorticotropic hormone and corticosterone levels (10). Due to its unique pharmacological profile, including its anxiolytic and arousal-promoting effects, the NPS/NPSR system has been a point of interest in the treatment of mood-related disorders (11). In addition to NPS's in the central nervous system, in vivo studies conducted on animals revealed that the NPS/NPSR system is associated with the modulation of proinflammatory cytokines. Moreover, NPSR gene polymorphism was demonstrated to be associated with some systemic chronic gastrointestinal and respiratory diseases, since some animal studies have demonstrated that the NPS/NPSR system is distributed in the peripheral system, including the immune tissues, thyroid, mammary, and salivary glands (10, 12). While limited studies have been conducted on humans to investigate the role of NPS in psychiatric conditions, some reports support NPS/NPSR system's potential involvement in anxiety disorders, such as panic disorder (13, 14). However, as prior studies have only focused on the central NPS/NPSR system, studies including the peripheral measurements of the NPS, which presents a relatively practical and economical method, and investigations of NPS's permeability from the blood-brain barrier to determine the interactions between the peripheral and central NPS have not been conducted on human subjects. This would have been useful in determining the presence of NPS/NPSR system in anxiety-related conditions.

Therefore, the aim of this study was to investigate the plasma NPS levels in individuals diagnosed with GAD and determine whether there is a relationship between plasma NPS levels and the anxiety's severity in the condition. This study also aims to offer additional data regarding the systemic implications of the NPS/NPSR system in GAD.

METHODS

A total of 40 subjects diagnosed with GAD, according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR), and 40 healthy controls of similar age, gender, and body mass index (BMI) were recruited in the study. All the samples were aged between 18–65 and were recruited by a clinician from among the patients admitted in a psychiatry out-patient clinic subsequent to a detailed clinical assessment, which included the Structured Clinical Interview for DSM-IV Axis-I Disorders (SCID-I). Both the patient and control group displayed no signs of the presence of chronic, allergic or inflammatory diseases, pregnancy and substance abuse. They were not under any ongoing medication. The subjects suffering from co-morbid psychiatric conditions, including major depressive disorder, psychosis, bipolar disorder, mental retardation, substance use disorder, and organic mental disorder, in the patient group, were excluded from the study. All the participants had been off medication for at least eight weeks before the study. A written informed consent was obtained from each participant prior to their involvement in the study. This study was conducted in accordance with the Declaration of Helsinki. Further, the study protocol was approved by the Balıkesir University Clinical Research Ethics Committee (Date: 10.01.2014, Decision Number: 03/2014).

Socio-demographic variables, including age, sex, BMI were determined through the socio-demographic form, while anxiety and depression levels were assessed using the Hamilton Anxiety Scale (HAM-A) (15), Generalized Anxiety Disorder-7 (GAD-7) (16), and Hamilton Depression Scale (HAM-D) (17). The validity and reliability of the Turkish forms of HAM-A, GAD-7, and HAM-D were also reported (18, 19, 20). Further, fasting venous blood samples were obtained between 07:00–08:00 a.m. for the measurement of plasma NPS levels of each participant. Subsequent to the collection of the blood samples in the standard EDTA tubes (Becton, Dickinson, USA), plasma separation was conducted with a centrifuge at 1300 X g for 10 minutes. The plasma samples were placed in Eppendorf tubes, and then they were administered a 1% concentrated protease inhibitor cocktail (Sigma Aldrich product number: P8340) and stored at -20°C for further processing. Further, NPS was quantified using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Cloud-Clone Corp, USA). The intra- and inter-assay coefficients of variation (CV) were determined as <10% and <12%, respectively.

The SPSS Software version 15.0 (IBM Inc., Chicago, IL, USA) was employed for the statistical analyses. Normality was determined with the Kolmogorov-Smirnov or Shapiro-Wilk tests and visual tools. Furthermore, chi-square or Fisher exact tests were utilized to compare the categorical variables. An independent sample t-test or Mann-Whitney U test was employed for a comparison of the independent groups. Correlation analyses between the independent variables were measured with Pearson's or Spearman's correlation tests. Descriptive statistics for the data with the abnormal distribution was represented with median (minimum-maximum) values, while the mean ± standard deviation values were represented through the normally distributed variables. A receiver operating characteristic (ROC) analysis was applied to establish a cut-off value for the plasma NPS levels for the identification of GAD. The statistical significance level was considered as $p<0.05$.

RESULTS

The comparison of the demographic variables yielded the mean age of the patient group as 41.9±11.6, while it was 41.3±11.1 for the control group (t=-0.285, p=0.77). A total of 30% of the patient group and 32.5% of the control group was male (chi^2=0.058, p=0.80). Data regarding the age, BMI, plasma NPS levels, and clinical assessment measures has been represented in Table 1. No statistically significant difference between the groups in terms of their BMI (Z=-1.573, p=0.11) was found. The median plasma NPS level, determined as 28.8 (6.2–123.7), was significantly higher

Table 1. Comparison of age, NPS, BMI, and clinical measurements between patients of generalized anxiety disorder and healthy controls

	Patient Group	Control Group	Statistical values
Age (year) Mean ± SD	41.9±11.6	41.3±11.1	p=0.77, t=-0.285
NPS (pg/mL) Median (min-max)	28.8 (6.2–123.7)	19.1 (6.2–98.0)	p=0.01, z=-2.45
BMI ($kg/m^{2)}$ Median (min-max)	26.9 (18.8–41.6)	25.3 (17.5–33.1)	p=0.11, z=-1.573
HAM-A Median (min-max)	23.5 (9.0–33.0)	2.0 (0–15.0)	p <0.001, z=-7.697
HAM-D Median (min-max)	16.0 (10.0–25.0)	2.5 (0–8.0)	p <0.001, z=-7.715
GAD-7 Median (min-max)	13.0 (7.0–21.0)	2.0 (0–6.0)	p <0.001, z=-7.721

NPS: neuropeptide-S, BMI: body mass index, HAM-A: Hamilton Anxiety Scale, HAM-D: Hamilton Depression Scale, GAD-7: Generalized Anxiety Disorder 7, SD: standard deviation.

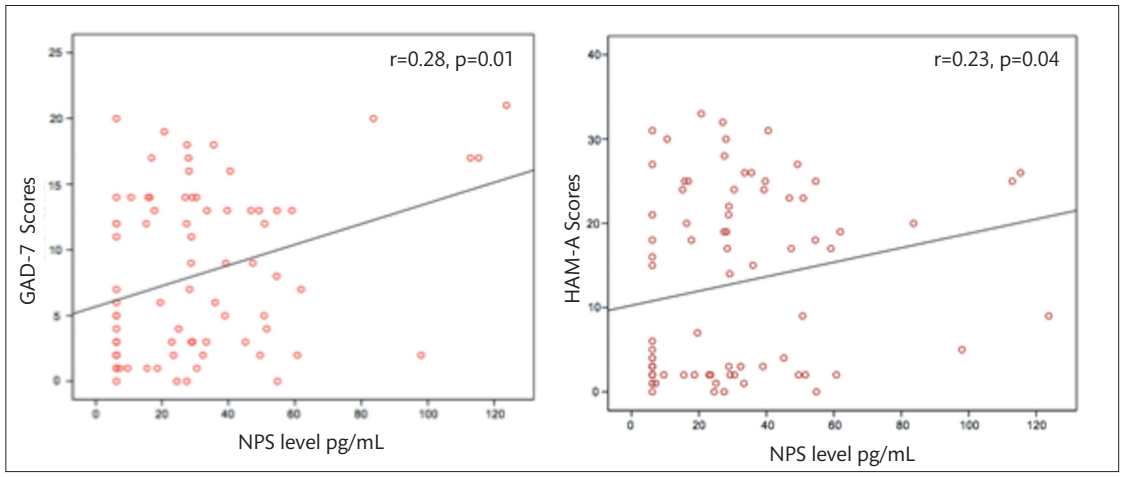

Figure 1. A correlation analysis between plasma NPS levels and anxiety scores; a significant positive correlation was observed between plasma NPS levels and both HAM-A and GAD-7 scores.

for the patient group in comparison to the control group, with a median plasma NPS level of 19.1 (6.2–98.0) (Z=-2.45, p=0.01, Table 1). Median HAM-A, GAD-7, and HAM-D scores were also significantly higher in the patient group than that for the control group (p<0.001, Table 1). With respect to the severity classification of HAM-D scale (21), 23 subjects of the patient group (57.5%) had scores for mild depressive symptom (determined as 8–16 HAM-D scores) while 13 patients (32.5%) yielded moderate depressive symptom scores (determined as 17 to 23 of HAM-D scores). Only 4 patients (10%) gained scores indicative of severe depressive symptom scores (determined as >25 of HAM-D scores). Furthermore, no significant difference was observed between the groups of patients with mild, moderate, and severe depressive symptoms in terms of plasma NPS levels (p=0.607). In the correlation analysis, a significant positive correlation was observed between the plasma NPS levels and HAM-A scores as well as the GAD-7 scores in the patients (r_s=0.23 p=0.04, r_s=0.28 p=0.01, respectively) (Figure 1).

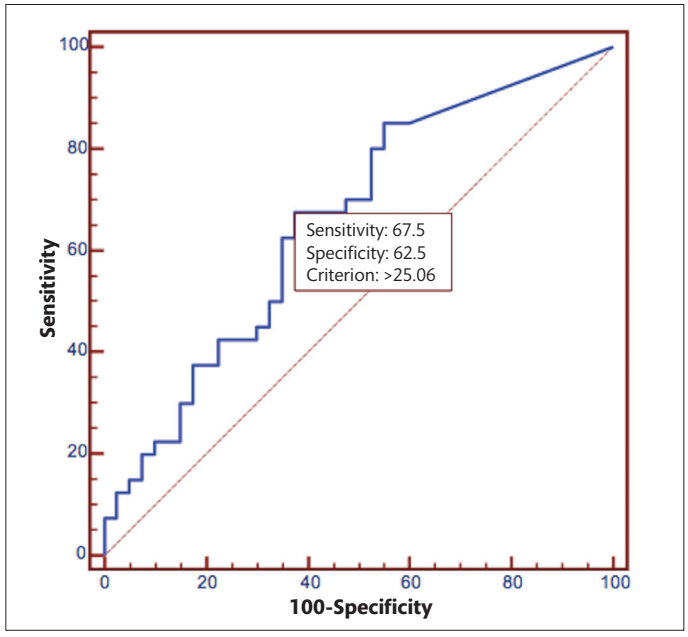

Figure 2. A ROC analysis to establish a cut-off value for plasma NPS levels in the identification of GAD. The ROC analysis revealed that plasma NPS levels could allow the identification of GAD with the cut-off value 25.06 pg/mL, with 67.5% sensitivity and 62.5% specificity. The remaining values were as indicated: Area Under the Ccurve (AUC)=0.658, 95%; confidence interval (CI)=0.543–0.760; P=0.009; ROC: A Receiver Operating Characteristic, GAD: Generalized Anxiety Disorder.

The p-value of the correlation analysis between plasma NPS levels and HAM-D scores was determined as 0.052.

A receiver operating characteristic (ROC) analysis revealed that the plasma NPS levels could enable the identification of GAD with a 67.5% sensitivity and 62.5% specificity (AUC=0.658, 95%; CI=0.543–0.760, p=0.009), when the cut-off value was 25.06 pg/mL (Figure 2).

DISCUSSION

In the present study, we investigated the relationship between plasma NPS levels and GAD. Our results revealed that the plasma NPS levels increased significantly in individuals diagnosed with GAD, compared to the healthy controls. There was also a positive correlation between the plasma NPS levels and severity of anxiety. As subjects with major depressive disorder were excluded, we found no significant correlation between concomitant depressive symptoms' severity and plasma NPS levels. Furthermore, the results from a rodent model of a depression study revealed that the central NPS administration did not lead to modifications in any depression-related behavior in the rats, but it was found to be specifically associated with anxiolytic effects (22). However, as there is no conclusive data regarding the relationship between NPS/NPSR system and depression, one should also consider the small sample size and the p-value of correlation between the depression scores and plasma NPS levels, which came extremely close to a statistically significant value, in the interpretation of our results. The correlation analysis between plasma NPS levels and concomitant depressive symptoms severity revealed a result that can be considered as a trend leaning toward significance. Thus, future studies involving subjects diagnosed with major depressive disorder may offer more robust data with regard to the relationship between NPS and depression.

Although the rodent and experimental studies that investigated the role of NPS/NPSR in the central nervous system reported that NPS is associated with robust anxiolytic effects, it has also been implicated in a few other prominent neuro-chemical processes, including the activation of HPA axis and modulation of pro-inflammatory cytokines, the neuroendocrinological systems related to anxiety-related disorders (12, 23, 24). Additionally, a recent study discovered a naturally-occurring gene polymorphism of NPSR that is involved in distorted sympathetic arousals and anxiety-related emotional processes in people afflicted with panic disorders (PD) (14) In GAD, somatic symptoms associated with adrenergic hyperactivity, such as palpitations, nausea, sleep disturbances, sweating, are the most commonly encountered symptoms which are similar to those experienced in PD. In this regard, we hypothesized that

NPS, which is a neuropeptide associated with neuro-vegetative and neuroendocrinological pathways, could also be associated with GAD, an anxiety disorder that presents somatic symptoms. Our results, which showed increased levels of NPS and a positive correlation between anxiety levels and plasma NPS levels in GAD, imply that in comparison to the central measures in anxiety-related conditions, peripheral NPS levels may contribute to the creation of a defense system in people suffering from anxiety conditions and may lead to an activated reactive process in response to anxiety. A limitation of our study was that we could not evaluate the plasma cortisol levels and inflammatory markers to examine the HPA axis and inflammation processes involved in GAD in our study, due to the blood sample collection method and insufficient funding. Thus, we cannot exclude the possibility that the measured plasma NPS levels may have been modulated by, and in association with, the HPA axis and the inflammatory processes, which have been demonstrated to be related with both GAD and NPS/NPSR systems. Moreover, although we observed increased levels of NPS in a clinical anxiety condition, without any other measure regarding the NPS/NPSR system, this result is not sufficient to reflect the role of the NPS/NPSR system in GAD.

In the present study, with a cut-off value determined at 25.06 pg/mL with 67.5% sensitivity and 62.5% specificity, the plasma NPS levels were found to be significantly associated and positively correlated with the anxiety levels observed in GAD. Thus, in this regard, we suggest that our results may prompt future studies in the investigation of plasma NPS levels as a candidate marker in the identification of GAD.

There are some other limitations that should be considered in the interpretation of the results of our results. The assessment of the NPS/NPSR system was conducted through the measurement of only the peripheral plasma NPS levels, which comprises a limited method to arrive at a precise interpretation. As mentioned earlier, previous reports regarding the NPS system have mainly documented the findings obtained with regard to the central nervous system as well as components other than NPS levels, such as NPSR levels and genetic receptor variations, while our results included only the plasma NPS levels, which is a peripheral measure with regard to the NPS/NPSR system. Additionally, the assessment of biochemical markers associated with both the NPS and GAD, such as inflammatory markers and cortisol levels, could have enhanced the findings of our study. Furthermore, it is also possible that NPS receptor's insensitivity or dysfunction, which were not investigated in our study, might have affected the plasma levels of NPS in GAD patients. Although no significant correlation was found between the plasma NPS levels and depression measures, in considering the limited sample size and p-value 0.052, we cannot completely exclude the impact of concomitant depressive symptoms, which are extremely common among GAD patients, on the plasma NPS levels.

In conclusion, to the best of our knowledge, this is the first study to investigate the relationship between NPS levels and an anxiety disorder, GAD, in human subjects. The present study yielded a significant relationship between anxiety and plasma NPS levels in patients with GAD, as a significant contribution to the field of research regarding the involvement of the central NPS/NPSR system in anxiety-related conditions. Future studies with a larger sample size, more comprehensive methodology to investigate the NPS/NPSR system, in a well-determined population are warranted to highlight the neurobiological aspect involved in GAD and for the enhancement of future therapeutic approaches.

Ethics Committee Approval: This study was conducted in accordance with the Declaration of Helsinki. Further, the study protocol was approved by the Balıkesir University Clinical Research Ethics Committee (Date: 10.01.2014, Decision Number: 03/2014).

Informed Consent: A written informed consent was obtained from each participant prior to their involvement in the study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - HB, ÖB; Design- ÖB, AAH; Supervision- HB, HK, AAH; Resource– ÖK, HB, HK; Materials- HB, ÖB; Data Collection and/or Processing - HK, AAH; Analysis and/or Interpretation - OD, TK; Literature Search- OD, HB; Writing Manuscript - OD, TK; Critical Review - TK.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This study was funded by Balıkesir University Coordinatorship of Scientific Research Projects (2014/71).

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