Association between serum NUCB2/nesfatin-1 levels and erectile dysfunction

AKIF KOÇ[1], ABDULLAH GUL[1], ANIL ERKAN[2], MUHAMMET GUZELSOY[1], ALI RIZA TURKOGLU[1], SONER COBAN[1], RAHIME FEYZA KOLOGLU[3] and YASEMIN USTUNDAG[3]

[1]Department of Urology, Bursa Faculty of Medicine, Health Sciences University, Bursa Yuksek Ihtisas Training and Research Hospital, Bursa 16310, Turkey; [2]Department of Urology, Bursa Yuksek Ihtisas Training and Research Hospital, Bursa 16310, Turkey; [3]Department of Biochemistry, Bursa Yuksek Ihtisas Training and Research Hospital, Bursa 16310, Turkey

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Abstract. Erectile dysfunction (ED) is one of the most common complaints in the male sexual health field, with a multifactorial etiology yet to be fully elucidated. Nucleobindin 2 (NUCB2)/nesfatin-1, known for its regulatory role in food intake, can also regulate the vascular, neural and hormonal systems, all of which are of great importance in the etiology of ED. The present study included 43 men with ED and 40 healthy individuals without ED. The participants were assessed using the Turkish version of the International Index of Erectile Function (IIEF-5) to determine the presence and severity of ED. Serum NUCB2/nesfatin-1, total testosterone, fasting blood glucose, hemoglobin A1c, total cholesterol, low-density lipoprotein, high-density lipoprotein, very low-density lipoprotein, triglyceride and total prostate-specific antigen levels were all measured. The mean age of the participants was 46.77±9.87 years with an age range of 25-67 years. The mean ages of the ED and non-ED groups were 47.47±11.19 and 46.03±8.30 years, respectively. Patient age and serum biochemical parameters were found to be comparable between the two groups. The serum NUCB2/nesfatin-1 levels of the ED group were also revealed to be significantly lower compared with those of the non-ED group (P=0.019). There was a weak negative correlation between the serum NUCB2/nesfatin-1 level and the severity of ED according to the IIEF-5 score (r=-0.306; P=0.005). The receiver operating characteristic curve analysis of serum NUCB2/nesfatin-1 revealed a cut-off value of 1.25 ng/ml for distinguishing between the ED and non-ED groups (P=0.019). These findings suggest that reduced serum NUCB2/nesfatin-1 values may be implicated in the etiology of ED. Further studies are required to clarify the effect of NUCB2/nesfatin-1 on vascular physiology and erectile physiology or pathophysiology.

Correspondence to: Dr Akif Koç, Department of Urology, Bursa Faculty of Medicine, Health Sciences University, Bursa Yuksek Ihtisas Training and Research Hospital, Mimar Sinan District, 35 Emniyet Street, Bursa 16310, Turkey
E-mail: akifkoc@yahoo.com

Abbreviations: AUC, area under the curve; cGMP, cyclic guanosine monophosphate; DM, Diabetes mellitus; ED, erectile dysfunction; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein-cholesterol; HT, hypertension; IIEF-5, International Index of Erectile Function-5; NO, nitric oxide; NOS, nitric oxide synthase; NUCB2, nucleobindin 2; ROC, receiver operating characteristic; TC, total cholesterol; TG, triglyceride; tPSA, total prostate-specific antigen; TT, total testosterone; VLDL, very low-density lipoprotein-cholesterol

Key words: erectile dysfunction, human, lipoproteins, nucleobindin 2/nesfatin-1 protein, testosterone

Introduction

Erectile dysfunction (ED) has been defined as the inability to achieve and maintain a penile erection sufficiently to allow satisfactory sexual performance (1). According to previous studies, ~52% of men aged 40-70 years experience ED (2,3), with projections estimating a global prevalence of 322 million cases by 2025 (4). ED adversely affects psychosocial health and the quality of life (2). Penile erection is a complex physiological process that requires arterial dilatation, relaxation of the trabecular smooth muscle and activation of the corporeal veno-occlusive mechanism, necessitating an adequately functioning neural, vascular and endocrine environment (5). ED etiologically can be classified into the following three classes: Organic (such as neurogenic, hormonal, arterial, cavernosal, or drug-induced); psychogenic; and mixed-type ED (2,4). However, this classification should be used with caution, since the majority of patients with ED have a mixed etiology (2). ED is mostly of a mixed psychogenic and organic nature (4,6). An important cause of psychogenic ED is performance anxiety, that is, fear of inadequacy during sexual intercourse (7). Whilst developmental, cognitive, emotional and interpersonal factors that predispose men to sexual dysfunction have been previously identified in the etiology of psychogenic ED, the etiology is now considered to be primarily associated with a group of predisposing, triggering and maintaining factors (4,8). Specifically, psychogenic ED has been associated with performance anxiety and sexual confidence (8). However, depressive nature, loss of self-esteem, relationship concerns

and psychosocial stresses may also be causes of psychogenic ED (9). By contrast, a number of factors have been reported to be responsible for the etiology of organic ED. There may be neurogenic causes namely multiple sclerosis, temporal lobe epilepsy, Parkinson's disease, stroke, Alzheimer's disease, spinal cord injury and cavernous nerve injury (radical pelvic surgeries, such as radical prostatectomy) (4,6). However, there may also be endocrinological causes, such as testosterone deficiency or hypogonadism and hyperprolactinemia (4,10). In addition, there may be vasculogenic causes, including atherosclerosis, hypertension (HT), hyperlipidemia, smoking, diabetes mellitus (DM) and pelvic irradiation (4,6). Although neurogenic, endocrinological and vasculogenic factors have been implicated in the development of ED, the complete etiological spectrum remains to be fully elucidated (4). Previous studies have proposed a potential link between NUCB2/nesfatin-1, a satiety regulator and ED (11,12).

NUCB2/nesfatin-1 is an adipocytokine that primarily regulates food intake. Increased levels of nesfatin-1 in the cerebroventricular system reduce food intake and the presence of an antibody that neutralizes nesfatin-1 in this system stimulates appetite (13). It has also been shown to function (e.g. modulator, activator) in numerous systems, such as the vascular, neural and hormonal systems (11,14,15), and is expressed in testis (16). In a study investigating the effect of NUCB2/nesfatin-1 on the vascular system, it was previously found that NUCB2/nesfatin-1 modulated peripheral arterial contractility and suppressed the vasodilator effect of nitric oxide (NO) (14), which brings to mind that the serum NUCB2/nesfatin-1 level may be high in patients with ED. By contrast, another previous study reported that NUCB2/nesfatin-1 could induce arterial vasodilation through significant changes in NO/cyclic guanosine monophosphate (cGMP) activity (17).

Given the physiological pathways shared by NUCB2/nesfatin-1 and penile erection, coupled with conflicting results in previous studies, the present study aimed to explore the relationship between NUCB2/nesfatin-1 and the presence and severity of ED.

Materials and methods

Patients. The present prospective cross-sectional study was conducted at the Department of Urology of the Health Sciences University Bursa Medical Faculty of Medicine (Bursa, Turkey). The protocol for the present study was approved by the Clinical Research Committee of Bursa Yuksek Ihtisas Training and Research Hospital (approval no. 2011-KAEK-25 2021/08-15), adhering to the provisions of the Declaration of Helsinki (18). Written informed consent was obtained from all participants.

Patient recruitment for the present study commenced in August 2021 and ended in April 2023. The present study was conducted at Bursa Yuksek Ihtisas Training and Research Hospital.

Following a comprehensive physical examination and medical history assessment, serum NUCB2/nesfatin-1, total testosterone (TT), fasting blood glucose (FBG), hemoglobin A1c (HbA1c), total cholesterol (TC), low-density lipoprotein-cholesterol (LDL), high-density lipoprotein-cholesterol (HDL), very low-density lipoprotein-cholesterol (VLDL), and triglyceride (TG) levels were measured in all cases. Participants aged >45 years underwent additional serum total prostate-specific antigen (tPSA) testing.

Venous blood samples were collected (~10 ml per patient) after 12 h overnight fasting (between 8 and 10 a.m. before any significant physical activity), placed in vacutainer plastic tubes (BD Biosciences) and processed according to clinical laboratory protocols within 30 min after venipuncture. FBG (cat. no. 3L82-22; Abbott Laboratories), HbA1c (cat. no. 030201002; Lifotronic Technology Co., Ltd), TC (cat. no. 04S9230; Abbott Laboratories), LDL (cat. no. 02R05-21; Archem Diognostic Ind. Ltd), HDL (cat. no. 02R06-21; Archem Diognostic Ind. Ltd), VLDL, TG (cat. no. 06T85-35; Archem Diognostic Ind. Ltd) and tPSA (cat. no. 7K70; Abbott Laboratories) levels were then measured using commercially available assay kits. Basal TT levels were determined using a commercially available ELISA kit (cat. no. 2P13-28; Abbott Laboratories) according to the manufacturer's protocols. Serum samples for NUCB2/nesfatin-1 were frozen and stored at -70˚C and measured using a standard Human Nesfatin-1 ELISA kit (cat. no. E3063Hu; Bioassay Technology Laboratory) based on the sandwich ELISA principle. The manufacturer's protocol was followed and no changes were made to the standard protocol.

Groups. The present study included 43 men with ED (ED group) and 40 healthy individuals (non-ED group). The participants were evaluated using the Turkish version of the five-item International Index of Erectile Function (IIEF-5) questionnaire (19). According to the IIEF-5 scores, ED severity was categorized as follows: i) 5-7, severe ED; ii) 8-11, moderate ED; iii) 12-16, mild-moderate ED; iv) 17-21, mild ED; and v) 22-25, normal sexual function.

All participants underwent a comprehensive clinical evaluation, including detailed medical and sexual history histories, to identify underlying medical conditions that may cause non-psychogenic ED, such as diabetes, cardiovascular disease or hormonal imbalances associated with organic diseases. Certain patients with ED had a medical condition that could cause non-psychological ED (the number of patients with HT, DM and coronary artery disease was 10, 11 and 5, respectively) (Table I), whilst others did not. Individuals with infectious diseases, liver problems, renal failure, a history of substance abuse or dependence, neoplasms or autoimmune disorders and psychological disorders were excluded. ED typically involves mixed (psychogenic and organic) etiologic factors (2,4). Therefore, it was not possible to separate patients with ED into purely organic or purely psychogenic subtypes of ED. Therefore, in the present study, the patient group was considered to consist mostly of the mixed type.

Statistical analysis. Data were processed and analyzed using the SPSS v.21.0 software (IBM Corp.). To ensure accuracy, the Kolmogorov Smirnov test was conducted for each group of continuous variables. Normally distributed measurement data were presented as the mean ± standard deviation and compared using the unpaired t-test. Non-normally distributed data were expressed as median (P25, P75) values and compared using the non-parametric Mann-Whitney U-test.

Table I. Comparison of the baseline characteristics between the groups.

Characteristics	ED group (n=43)	Non-ED group (n=40)	P-value
Age, years[a]	47.47±11.19	46.03±8.29	0.510
Smoking status			0.003
Smoker	21 (48.8)	7 (17.5)	
Non-smoker	22 (51.2)	33 (82.5)	
Hypertension			0.048
Present	10 (23.25)	3 (7.5)	
Absent	33 (76.75)	37 (92.5)	
Diabetes mellitus			0.028
Present	11 (25.58)	3 (7.5)	
Absent	32 (74.42)	37 (92.5)	
Coronary artery disease			0.435
Present	5 (11.6)	2 (5)	
Absent	38 (88.4)	38 (95)	
Nesfatin-1, ng/ml[b]	0.64 (0.54-1.23)	1.41 (0.63-4.1)	0.019
Fasting blood glucose, mg/dl[b]	96 (86-109)	86.5 (83.25-101)	0.111
Hemoglobin A1c, %[b]	5.69 (5.09-6.36)	5.39 (5.04-5.89)	0.121
Total testosterone, ng/dl[b]	435 (354-521)	413 (368-571)	0.668
Total cholesterol, mg/dl[a]	187.91±28.03	203.68±38.93	0.036
Very low-density lipoprotein-cholesterol, mg/dl[b]	27 (18.8-37.6)	29 (18.7-37.6)	0.888
Low-density lipoprotein-cholesterol, mg/dl[a]	106.68±31.43	126.27±38.55	0.013
Triglyceride, mg/dl[b]	107 (94-207)	146 (141-188)	0.685
High-density lipoprotein-cholesterol, mg/dl[a]	43.67±7.57	47.67±8.43	0.026
Total prostate-specific antigen, mg/dl[b]	0.79 (0.56-1.24)	0.81 (0.48-1.18)	0.757

[a]Mean ± standard deviation; [b]median (interquartile range). Values are expressed as n (%) unless otherwise indicated. ED, erectile dysfunction.

Categorical variables were presented as numbers and percentages and compared using the χ^2 and Fisher's exact tests. The correlation between the serum NUCB2/nesfatin-1 level and ED severity was assessed using Spearman's correlation coefficient. Logistic regression analysis was used to identify risk factors and predictors of ED. The diagnostic accuracy of serum NUCB2/nesfatin-1 for ED was evaluated using the area under the curve (AUC) values obtained from receiver operating characteristic (ROC) analysis. P<0.05 was considered to indicate a statistically significant difference. The minimum required sample size was calculated to be 40 participants for each group with an effect size of 0.82, a margin of error of 0.05 and a power of 0.95, using the two-tailed independent-samples t-test. This calculation was based on a previous study conducted by Ragab *et al* (12). Sample size estimation was undertaken using G*Power v. 3.1.9.4 (20).

Results

Baseline characteristics. The mean age of the participants was 46.77±9.87 (range, 25-67) years, with the ED and non-ED groups having mean ages of 47.47±11.19 and 46.03±8.30 yea rs, respectively. Age and serum TT, FBG, HbA1c, VLDL, TG and tPSA values were found to be statistically similar between the two groups (Table I). However, serum TC, LDL and HDL values were found to be significantly higher in the non-ED group compared with those in in the ED group (P<0.05). The mean serum NUCB2/nesfatin-1 level in the ED group was found to be significantly lower compared with that in the non-ED group (P<0.05; Table I). The mean IIEF-5 scores of the ED and non-ED groups were calculated to be 13.14±5.03 and 23.65±1.17, respectively. IIEF-5 scores were found to be significantly higher in the non-ED group compared with those in the ED group (P<0.001). The incidence of DM, HT and smoking were more prevalent in the ED group compared with those in the non-ED group (P<0.05; Table I).

Association between NUCB2/nesfatin-1 and ED. A weak negative correlation between serum NUCB2/nesfatin-1 level and ED severity according to the IIEF-5 scores was found (r=-0.306; P=0.005; Fig. 1). Subsequent ROC curve analysis of the serum NUCB2/nesfatin-1 revealed a cut-off value of 1.25 ng/ml for distinguishing between the ED and non-ED groups (P<0.05), with an AUC value of 0.650 (95% CI, 0.53-0.77; Fig. 2). However, multivariate logistic regression analysis did not identify serum NUCB2/nesfatin-1 as a predictor for ED, whereas smoking and DM increased the probability of ED by 6.4 and 5.5 times, respectively (Table II).

In the subgroup analysis, when the without HT subgroups were compared, nesfatin-1 values were found to be significantly lower in the ED group (P<0.05). Similarly, when non-smokers were compared, nesfatin-1 values were found to be significantly

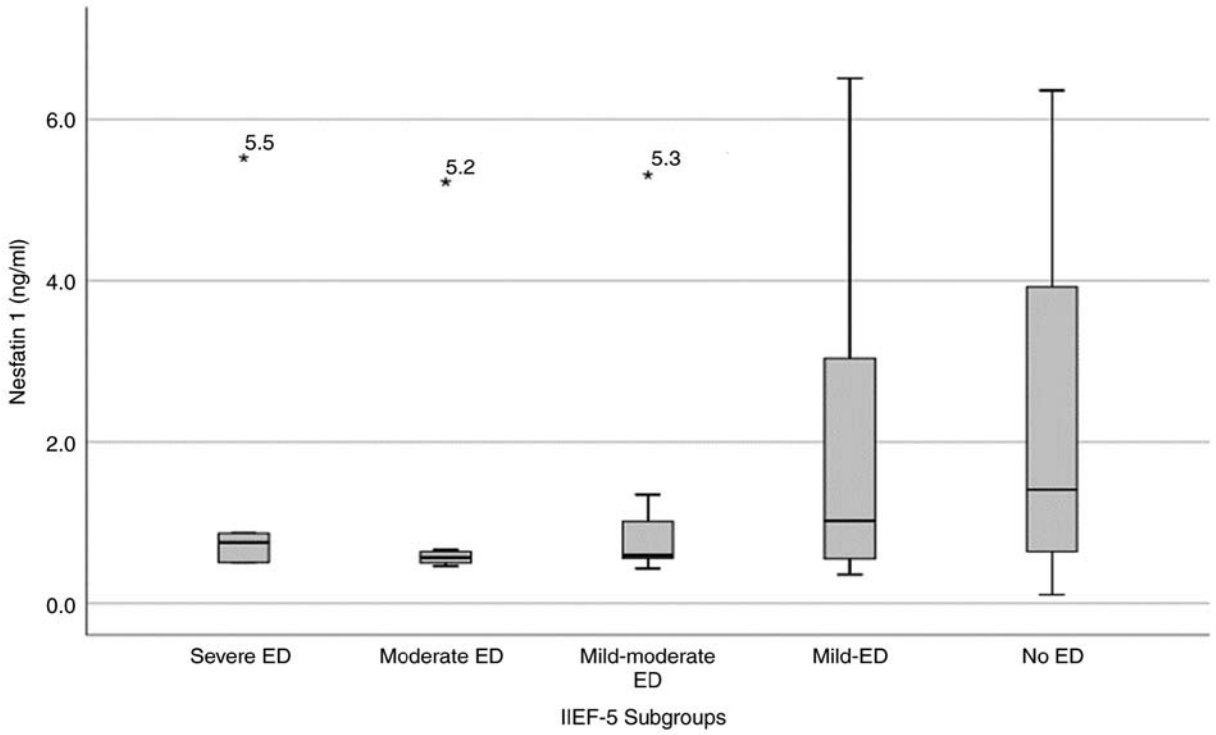

Figure 1. Correlation between the serum nesfatin-1 and ED severity. *Indicates outliers. ED, erectile dysfunction; IIEF-5, International Index of Erectile Function-5.

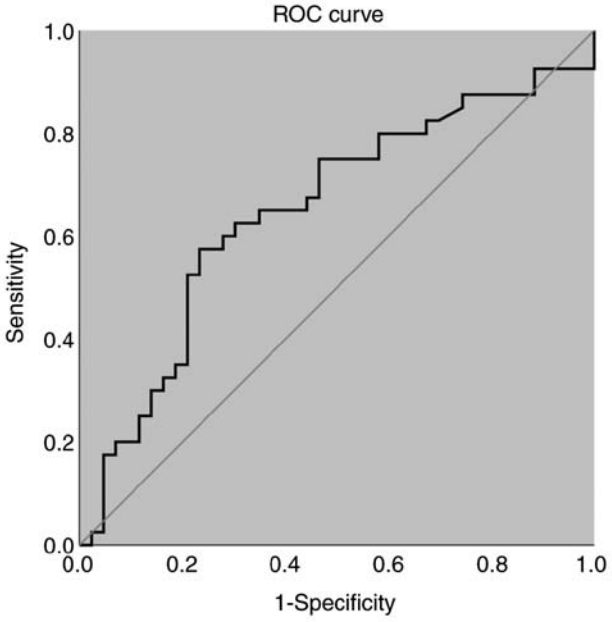

Figure 2. ROC analysis of serum nucleobindin 2/nesfatin-1 for the prediction of erectile dysfunction. Area under the curve, 0.650; cut-off value, 1.25 ng/ml (sensitivity, 0.575; specificity, 0.767). P=0.019. ROC, receiver operating characteristic.

lower in the ED group (P<0.05). However, no significance could be found in other subgroup analyses (Table III).

Discussion

The present study revealed that the serum NUCB2/nesfatin-1 level was reduced in patients with ED whilst being negatively correlated with ED severity. A cut-off value of 1.25 ng/ml for serum NUCB2/nesfatin-1 was established to differentiate ED cases. The presence of DM and smoking emerged as risk factors for ED, whilst serum NUCB2/nesfatin-1 was not a predictor of ED according to logistic regression analysis.

Nesfatin-1, initially identified by Oh *et al* (13) as a satiety molecule in the hypothalamus, is derived from NUCB2 (13). It is an 82-amino acid polypeptide derived from the post-translational processing of hypothalamic NUCB2, which consists of 396 amino acids (21,22). This peptide is mainly produced in the hypothalamus, particularly in the paraventricular nucleus, arcuate nucleus and nucleus of the solitary tract. However, it is also synthesized in peripheral tissues, such as the stomach, pancreas, adipose tissue and testes (23). Nesfatin-1 has a molecular weight of ~9.8 kDa and a half-life of 23.5 min (24,25). Whilst the exact degradation mechanism of nesfatin-1 remains to be fully understood, it is likely to be broken down by peptidases and proteases (25). Although it shows diurnal variations in its endogenous rhythm, there is no definitive evidence to support the idea that it follows a circadian pattern (26).

NUCB2/nesfatin-1 levels can be influenced by various factors, including nutrient status, hormonal regulation and energy homeostasis. Food intake significantly impacts its expression, with levels decreasing during fasting and increasing after feeding (27). A number of hormones, such as insulin, glucagon and gonadotropins, can also regulate its levels (28). Nesfatin-1 is involved in glucose and lipid metabolism, enhancing insulin secretion and action via stimulating insulin mRNA expression and/or by promoting Ca^{2+} influx through L-type channels (29). Its levels have been demonstrated to be elevated in obesity but reduced in other conditions, such as type 2 DM (30,31). Nesfatin-1 can also been

Table II. Logistic regression analysis of the data for evaluation of possible risk factors and predictors of erectile dysfunction in the present cohort.

	Univariate analysis		Multivariate analysis	
Variable	OR (95% CI)	P-value	OR (95% CI)	P-value
Smoking habit	4.5 (1.637-12.371)	0.004	6.4 (2.138-19.184)	0.001
Hypertension	3.7 (0.947-14.751)	0.060		
Diabetes mellitus	4.2 (1.087-16.542)	0.038	5.5 (1.272-24.275)	0.023
Nucleobindin 2/nesfatin-1	1.02 (0.999-1.047)	0.062		
Total cholesterol	1.01 (1.001-1.028)	0.041		
Low-density lipoprotein-cholesterol	1.01 (1.003-1.031)	0.018		
High-density lipoprotein-cholesterol	1.06 (1.006-1.128)	0.030		

OR, odds ratio.

Table III. Comparison of the nesfatin-1 levels between the subgroups based on with/without comorbidity.

Patients	N	ED group	N	Non-ED group	P-value
Without DM	32	6.24 (5.16-24.15)	37	14.26 (6.45-39.22)	0.660
With DM	11	7.42 (5.53-8.17)	3	13.93 (4.87-13.93)	0.312
Without HT	33	6.21 (5.31-14.66)	37	14.26 (6.45-42.87)	0.033
With HT	10	7.07 (5.41-12.58)	3	12.71 (4.87-12.71)	0.612
Without smoking	22	6.48 (5.49-11.00)	33	14.26 (6.45-48.06)	0.034
With smoking	21	6.44 (5.19-15.02)	7	12.71 (5.45-30.44)	0.507

Values presented as the median (interquartile range). DM, diabetes mellitus; HT, hypertension; ED; erectile dysfunction.

shown to modulate multiple signaling pathways (e.g. AKT kinase/AMP-dependent protein kinase/mammalian target of rapamycin pathway), including the mTOR/STAT3 signaling pathway, which is instrumental in glucose homeostasis and hepatic insulin sensitivity, although no specific receptor has been identified (32). In addition, this peptide serves various roles in growth (e.g. intrauterine and postnatal), reproductive function, stress response and cancer progression (28,33-36).

The question of whether diabetes, HT and smoking, which are among the main etiological factors of ED, can mediate effects on serum nesfatin-1 levels remain unknown. As previously found by Zhai *et al* (37) in a review and meta-analysis, studies evaluating the relationship between circulating nesfatin-1 and diabetes have yielded conflicting results (37). Whilst a number of studies have demonstrated high levels of nesfatin-1 in patients with type 2 diabetes (38,39), others have reported lower levels of nesfatin-1 in such patients (40-43). In a previous study involving experimental HT models, no significant difference was found between nesfatin-1 levels in serum, urine and renal tissue samples of control and HT models (Angiotensin II-induced model) (44). However, in another previous study, where patients with essential HT were compared with the control group, serum nesfatin-1 levels in the patient group were found to be significantly higher (45). To the best of our knowledge, in the current literature, no study on the relationship between smoking and serum nesfatin-1 levels could be found. Therefore, it was not possible to draw a conclusion on the effect of diabetes, HT and smoking on serum nesfatin-1 levels according to the present study.

Numerous studies have previously explored the potential role of nesfatin-1 in female physiology, which included animal models and clinical studies. Human studies have mostly focused on polycystic ovary syndrome and gestational DM. Controversial findings have been made regarding nesfatin-1 levels in women with polycystic ovary syndrome, with studies suggesting higher levels (46,47) and others suggesting lower levels (48,49). In a previous meta-analysis, nesfatin-1 concentrations in patients with gestational diabetes were also conflicting. In total, three studies reported lower circulating levels of nesfatin-1 compared with those of healthy controls, whilst four studies observed higher levels of nesfatin-1 compared with those in healthy controls (50). In another study on premature telarche and serum nesfatin-1 levels in young female individuals (aged 4-8 years), serum nesfatin-1 levels were found to be significantly higher in patients with premature telarche compared with those in the healthy control group (51). In a previous animal study, inhibition of the hypothalamic expression of nesfatin-1 was found to delay puberty in female rats. Intracerebroventricular injection of nesfatin-1 in adolescent female rats, especially during fasting, was also observed to increase serum luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels (52). In another animal

study, nesfatin-1 levels fluctuated during pregnancy in normal pregnant mice (nesfatin-1 serum levels increased significantly on day 14.5 and then decreased rapidly on day 19.5). Evidence has also been provided that activation of Th17 cells, which may be an important regulator of pregnancy maintenance, may be regulated by nesfatin-1/NUCB2. Therefore, it was determined that nesfatin-1 may be an important molecule for pregnancy and fertility (53). In a study by Kim *et al* (54), NUCB2 mRNA and nesfatin-1 protein expression were detected in the ovaries and uterus of mice. NUCB2/nesfatin-1 expression in both organs responded to various hormonal manipulations (such as pregnant mare serum gonadotropin administration, ovariectomy, 17β-estradiol injection) in the direction of increase (administration of pregnant mare serum gonadotropin, 17β-estradiol injection) or decrease (ovariectomy). It was thereby concluded that NUCB2/nesfatin-1 expression in the ovary and uterus of mice can be regulated through the hypothalamus-pituitary-ovarian axis, where NUCB2/nesfatin-1 is a local regulator of ovarian steroidogenesis and uterine function (54). Although research on the levels and physiologic roles of NUCB2/nesfatin-1 in women is ongoing, available evidence suggests that it may influence various aspects of female physiology, including reproductive functions and stress responses (55).

However, whether nesfatin-1 serves a role in the physiology of erection and/or ejaculation remains controversial. A recent study by Chen *et al* (56) provided information on erection physiology and ED pathophysiology. Using mouse models, a type 2 DM-like model was created and found that nesfatin-1 treatment had an ameliorating effect on ED, a complication of DM. This previous study also found nesfatin-1 can improve both glucose metabolism disorders and diabetic ED in ED mice with type 2 DM, which may be mediated by nesfatin-1 promoting the conversion of corpus cavernosus smooth muscle cells to a contractile phenotype, through the PI3K/AKT/mTOR signaling pathway. Intracavernosal pressures of the diabetic ED group was found to be improved with nesfatin-1 treatment. The smooth muscle/collagen fiber ratio in the cavernosal tissue, which had decreased with the development of diabetes, increased by nesfatin-1 treatment and the phenotype structures of muscle cells in this tissue, which were impaired with diabetes, were improved by nesfatin-1 treatment (56). Although not directly associated with erection and ejaculation physiology, there have been studies investigating the role of nesfatin-1 in various reproductive functions that may potentially affect erection and ejaculation physiology. Gao *et al* (16) found that nesfatin-1 is involved in the regulation of the hypothalamo-pituitary-gonadal axis. Specifically, it was shown that nesfatin-1 can affect the expression of the reproductive hormones gonadotropin-releasing hormone, LH, FSH and testosterone, which are critical for the maintenance of reproductive functions and erectile function (16). In another study, Ranjan *et al* (57) reported that nesfatin-1 is expressed in various reproductive tissues, including the testis, where it serves a role in spermatogenesis and steroidogenesis. It was specifically found in Leydig cells and has been shown to facilitate testosterone production and maturation of testicular functions (57). Nesfatin-1 has also been found to inhibit acrosome reaction in sperm within the epididymis, which is a crucial step for fertilization. This suggests that nesfatin-1 may have a regulatory role in sperm maturation and function before ejaculation (58).

In the context of erection physiology, the NO/cGMP signaling pathway serves a critical role in the regulation of erectile function (59). NO is synthesized from L-arginine by NO synthase (NOS) in endothelial cells. NO is then released from the endothelium and the cavernous nerve with sexual stimulation, subsequently activating the guanylate cyclase, which converts GTP to cGMP. This conversion reduces intracellular calcium levels, leading to the relaxation of penile smooth muscles and increased blood flow, thereby facilitating erection (4,60). Disruptions in any of these processes can potentially result in ED (61).

Yamawaki *et al* (14) previously examined the possible effect of nesfatin-1 on increasing blood pressure through the modulation of endothelial function in rats. It was demonstrated that the administration of nesfatin-1 to isolated mesenteric arteries inhibited relaxation induced by sodium nitroprusside (a NO donor) through the impairment of cGMP production. Furthermore, intravenous administration of nestin-1 significantly increased blood pressure and resisted SNP-induced blood pressure decreases (14). Based on these aforementioned results, it was therefore hypothesized to find a higher serum NUCB2/nesfatin-1 level in patients with ED compared with that in patients without ED. By contrast, serum nesfatin-1 of patients in the ED group was found to be significantly lower compared with that in patients in the non-ED group, corroborating the findings reported by Ragab *et al* (12) and Sun *et al* (11).

In a previous study investigating the effects of nesfatin-1 on the rat thoracic aorta, Barutcigil and Tasatargil (17) reported that NUCB2/nesfatin-1 did not affect the aortic tonus but induced vasodilation in phenylephrine-constricted rat thoracic aorta. This vasodilator effect was significantly abolished by the mechanical removal of the endothelium and completely inhibited by both molecules by the addition of N-nitro-L-arginine methyl ester (an inhibitor of NOS) and H-(1,2,4)oxadiazolo(4,3-a)quinoxalin-1-one (a soluble guanylate cyclase inhibitor) to tissue cultures. Therefore, it was concluded that nesfatin-1 could induce arterial vasodilation through endothelium-dependent mechanisms, which is closely associated with the presence of endothelial cells and the production of NO and cGMP in the rat thoracic aorta (17). In addition, previous human studies have reported normal blood nesfatin-1 concentrations of <10 ng/ml (39,42). The discrepancies between the results of the aforementioned studies may be due to the different concentrations of nesfatin-1 administered. Yamawaki *et al* (14) used a nesfatin-1 concentration of 100 ng/ml, higher compared with the 10 ng/ml administered by Barutcigil and Tasatargil (17). The use of a higher concentration of nesfatin-1 may have affected Ca^{2+} hemodynamics or the NO/cGMP pathway. The concentration of nesfatin-1 administered in the study by Yamawaki *et al* (14) exceeded that of normal human blood levels, underscoring the need for further studies.

Mori *et al* (62) previously found that nesfatin-1 can increase NO production in a dose-dependent manner in human umbilical vein endothelial cells (62). Another study observed an increase in cGMP levels in rat heart cells exposed to nesfatin-1 (63). However, whether decreased serum nesfatin-1

levels can lead to reduced NO and/or cGMP levels in endothelial and/or smooth muscle cells remain unknown. This necessitates further cellular and animal or human studies. To confirm the present findings and elucidate the role of nesfatin-1 in erectile dysfunction, culturing human endothelial and smooth muscle cells from penile tissues should be considered to investigate the effects of varying nesfatin-1 levels on NO and cGMP production. By manipulating nesfatin-1 concentrations in the culture media, changes in NO and cGMP levels using appropriate biochemical assays can be directly measured. Various techniques, such as reverse transcription-quantitative PCR and Western blotting, can be used to assess the expression levels of genes and proteins involved in NO synthesis and cGMP signaling pathways in response to altered nesfatin-1 levels. Developing animal models, such as nesfatin-1 knockout mice or rats, to study the impact of nesfatin-1 deficiency on erectile function should also be considered. Erectile responses in such models can be evaluated through intracavernosal pressure measurements following electrical stimulation of the cavernous nerve. In addition, future studies can administer nesfatin-1 to animal models with induced erectile dysfunction to observe potential therapeutic effects. This may be used to determine if nesfatin-1 supplementation can restore normal erectile function and normalize NO/cGMP levels. If future studies can show that low serum nesfatin-1 levels can cause a decrease in the amount or activity of molecules important for erection physiology, such as NO and cGMP, especially in penile tissue, it will facilitate the understanding into the low serum nesfatin-1 levels observed in patients with ED, as reported in the present study and previous studies by Ragab *et al* (12) and Sun *et al* (11). Although definitive conclusions regarding the physiological effects of nesfatin-1 cannot be drawn from the current literature, nesfatin-1 appears to be an important molecule in the physiology of erection and the pathophysiology of ED.

To determine whether nesfatin-1 can inhibit the vasodilator effect of NO in the intracavernosal region during sexual stimulation in patients with ED, a more challenging but effective method would involve measuring and comparing intracavernosal serum nesfatin-1 levels in patients with ED and healthy men during sexual stimulation. This hypothesis warrants further investigation.

Recent studies have explored the relationship between nesfatin-1 and testosterone. Seon *et al* (64) demonstrated that nesfatin-1 was regulated by testosterone, revealing a reduction in NUCB2 mRNA expression in the pituitary glands of mice after castration, which was reversed with testosterone replacement (64). Another previous study examined the cellular distribution and regulatory patterns of NUCB2/nesfatin-1 in mammalian testes and revealed the specific expression of NUCB2/nesfatin-1 in Leydig cells (28). Sun *et al* (11) previously reported a significantly lower serum testosterone level in the ED group compared with that in the control group, consistent with the lower nesfatin-1 levels of the former (11). However, the present study did not reveal a significant difference between the serum testosterone levels of the two groups.

To establish the association of a factor with disease, it would be ideal if the characteristics of the study groups were as similar as possible, except for the presence of the factor and the disease. Unfortunately, in the real world, achieving study groups with identical characteristics is nearly impossible for several reasons. Participants have inherent individual differences in genetics, lifestyle and environmental exposures that are difficult to control completely. In addition, ethical and logistical constraints limit the ability to manipulate or control certain factors in human studies. Random variations and unforeseen confounding factors can also introduce differences that are not accounted for at the outset. These aforementioned challenges render it difficult to create perfectly matched study groups solely differing by the factor and the disease in question.

A number of factors, such as age, diabetes and fat mass, can also influence NUCB2/nesfatin-1 levels. It may be beneficial to stratify subjects by age and then categorize them according to their physical condition, such as by comparing patients aged 40-50 years with diabetes and EDs with those without EDs, which may provide more precise findings. With 43 subjects with ED and 40 without ED included in the present study, the sample size may not be sufficient. This is a limitation of the present study and may be an aim of future studies.

As one of the results of this study, a weak negative correlation was detected between serum NUCB2/nesfatin-1 level and ED severity. This slight correlation suggests that it is uncertain. This can be considered as a potential limitation of the present study and may serve the purpose of future studies.

The present study has certain other limitations. The sample size was relatively small. In addition, the quantity of components in the NO/cGMP pathway was not evaluated or quantified. Serum NUCB2/nesfatin-1 levels were also not measured during sexual activity or upon stimulation.

The idea of investigating the development of ED and the change in nesfatin-1 serum levels over time appears prudent. It would undoubtedly be valuable to determine a correlation between the serum nesfatin-1 levels of a group studied over time and ED development and/or ED severity in the same investigated group to show the ED/nesfatin-1 relationship.

In conclusion, the present study showed that serum NUCB2/nesfatin-1 levels of patients with ED were significantly lower compared with those of healthy individuals. There was also a weak negative correlation between the serum NUCB2/nesfatin-1 level and ED severity. Although this finding does not definitively establish low levels of nesfatin-1 as a factor involved in the etiology of ED, it suggests a potential association. Further studies are needed to resolve the conflicting results regarding the effect of NUCB2/nesfatin-1 on vascular physiology and to elucidate the effects of this molecule on erectile physiology and/or pathophysiology.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

AK took part in protocol/project development, data collection and management as well as manuscript writing/editing. AG and AE took part in data analysis and manuscript writing/editing. MG, ART and SC participated in data collection or management. RFK and YU took part in the biochemical analysis of serum samples and data collection. AK and AG confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The present study was approved by the Clinical Research Committee of the Health Sciences University Bursa High Specialty Training and Research Hospital (approval no. 2011-KAEK-25 2021/08-15). Written informed consent was obtained from all participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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