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Serum adropin as a predictive biomarker of erectile dysfunction in coronary artery disease patients

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Introduction Erectile dysfunction (ED) is associated with various comorbidities and an early diagnosis and treatment is necessary to avoid the development of these comorbidities. Unfortunately, there is no biochemical marker that can be used for early diagnosis of ED. Nitric oxide (NO) is released by nerve and endothelial cells in the corpora cavernosa of the penis and is believed to be the main vasoactive chemical mediator of penile erection. Adropin is a regulatory peptide which has effects on NO bioavailability and energy homeostasis. We hypothesized that adropin may contribute to the pathogenesis of ED because of the presence of both metabolic effects and the influence on NO bioavailability. To confirm this hypothesis, we investigated the relationship between ED and serum adropin and NO levels.

Material and methods Seventy-five ED patients were enrolled for this study and the patients were divided into two groups according to angiographic scoring. Serum NO and adropin levels were measured by the Griess reaction and ELISA method, respectively.

Results Serum adropin and NO levels were found to be lower in the group which has higher angiographic score and the difference in NO was statistically significant. Also, adropin has a significant correlation between IIEF scores in ED patients.

Conclusions This is the first study in the literature investigating the levels of adropin in ED patients having coronary artery disease. The adropin molecule shows a promising future in clarifying the etiopathogenesis of ED. More comprehensive and multicenter studies are needed to reveal the role of adropin in ED and the effects of treatment on this molecule.

Key Words: adropin ‹› ELISA ‹› erectile dysfunction ‹› nitric oxide

INTRODUCTION

Erectile dysfunction (ED) is defined as the inability to achieve or maintain an erection sufficient for satisfactory sexual performance. Vascular, neurogenic and muscular factors together have an important role in ED. This clinical entity primarily affects men older than 40 years of age [1]. ED is associated with aging and various comorbidities or conditions, including hypertension [2], hyperlipidemia [3], benign prostatic hyperplasia [4], cardiovascular disease (CVD) [5], psychological factors [6], diabetes mellitus (DM) [7]. Recently, ED has been shown to be associated with systemic and coronary endothelial dysfunction and with manifested coronary artery disease [1, 5].

Nitric oxide (NO) is produced from L-arginine by the enzyme NO synthase (NOS), in the presence of oxygen and cofactors [8]. Aside from exerting several critical anti-inflammatory, antithrombotic, and anti-atherosclerotic roles within blood vessels, NO dependent smooth muscle relaxation is also required for penile erection [9, 10]. NO is released by nerve and endothelial cells in the corpora cavernosa of the penis

and is believed to be the main vasoactive nonadrenergic, noncholinergic neurotransmitter and chemical mediator of penile erection [11].
Adropin is a newly discovered regulatory peptide encoded by the Enho gene (energy homeostasis associated). It was first found to be expressed in the liver and the brain of mice [12]. However, subsequent studies demonstrated adropin immunohistochemical reactivity in many tissues including those of the cerebellum, myocardium and endocardium [13]. Adropin expression was also shown in human coronary artery endothelium [14]. Adropin level in circulation is regulated by macronutrients in the diet [15]. The major biochemical effects of adropin are: reducing insulin resistance and dyslipidemia; regulation of nitric oxide bioavailability; metabolic adaptation to macronutrient and energy homeostasis [16].
We hypothesized that adropin may contribute to the pathogenesis of ED because of the presence of both metabolic effects and the influence on NO bioavailability. To confirm this hypothesis, we investigated the relationship between ED and serum adropin and NO levels.

MATERIAL AND METHODS

Patients

Seventy-five male patients with chest pain were enrolled from among the patients who had undergone coronary angiography at the Cardiology clinic of Turgut Özal University Hospital from September 2014 to December 2014. The inclusion criteria included all admitted patients between 37 to 82 years of age, with coronary artery disease confirmed by angiography. The exclusion criteria included other comorbidities associated with endothelial dysfunctions such as diabetes mellitus, renal or hepatic impairment, congestive heart failure, active inflammatory diseases, or a history of myocardial infarction in the past 6 months. ED patients were divided into 2 groups on the basis of angiographic Gensini scores: severe coronary artery disease (CAD) group (Gensini score >20) and mild CAD group (Gensini score ≤20).

Coronary angiography and Gensini scoring

Coronary angiography was performed using the Sones technique with filming of multiple views of each vessel by the same cardiologist. Significant CAD was considered present, when a stenosis of at least 50% of the lumen was found in a major coronary vessel. Moreover, the results of quantitative coronary angiography have also been expressed according to the Gensini scoring system [17]. Gensini score: stenosis <25% was recorded as 1 point, 25–49% was marked as 2 points, 50–74% was noted as 4 points, 75–90% was noted as 8 points, 91–99% was recorded as 16 points, 100% was recorded as 32 points. Factor: LM ×5, LCX opening ×3.5; left anterior descending artery, circumflex artery near, middle and far segments respectively ×2.5, 1.5, 1; right coronary ×1; D1 diagonal branch ×1; D2 diagonal branch ×0.5; left ventricular posterior branch ×1; obtuse marginal branch ×1, right posterior descending branch ×1, posterior collateral ×0.5. Gensini score = Σ (coronary stenosis × lesion factor). Gensini scoring systems were calculated by 2 cardiologists blind to the participants. Then, the mean of 2 measures was calculated. Patients with Gensini score 20 or higher were considered as serious coronary disease [18].

Evaluation of erectile function

The erectile function was evaluated using the 5-item version of the International Index of Erectile Function (IIEF-5) questionnaire [18] by one examiner. ED was considered present, when IIEF-5 score was ≤21 [19].

Blood sampling

Venous blood samples were collected before coronary angiography after the patients were fasting overnight. Serum fraction was obtained by centrifugation (2000×g, 10 min, and 4°C) after storing the whole blood at room temperature (approximately 15 min). Serum samples were stored at -80°C until analysis.

Biochemical analysis

Fasting glucose was measured using the glucose oxidation method, and total cholesterol (TC), triglyceride (TG), and low-density lipoprotein cholesterol (LDL-C) were determined by enzyme colorimetric assay using a Roche autoanalyzer (Cobas e601, Roche, Japan). High-density lipoprotein cholesterol (HDL-C) was measured using a precipitation-based method. The serum total testosterone level was assayed using an electrochemilluminescence method (Cobas e601, Roche, Japan). Baseline demographic and laboratory data characteristics of the groups are shown in Table 1.

Measurement of serum nitric oxide level

NO levels in serum were determined spectrophotometrically, based on the reduction of NO3− to NO2 − by VaCI3. Nitric oxide level was measured by the Griess reaction. Sodium nitrite and nitrate solutions

Table 1. *Baseline demographic and laboratory data characteristics of the groups*

	Mild CAD (n = 38)	Severe CAD (n = 37)	Minimum	Maximum
Age (years)	59	62	36	83
BMI	28	29	19	41
IIEF	19	16	5	25
TC (mg/dl)	185	187	109	276
HDL (mg/dl)	42	40	22	64
LDL (mg/dl)	111	110	11	193
VLDL (mg/dl)	31	32	10	77
TG (mg/dl)	166	161	32	598
Testosterone (ng/dl)	403	329	42	808

CAD – coronary artery disease; BMI – body mass index; IIEF – International Index of Erectile Function; TC – total cholesterol; HDL – high-density lipoproteins; LDL – low-density lipoproteins; VLDL – very low-density lipoproteins; TG – triglycerides

Table 2. *Correlation between adropin, cardiac risk factors, IIEF and Gensini scores*

	Adropin	IIEF score	Gensini score
Age (years)	0.000 r = 0.396[2]	0.000 r =-0.494[1]	0.259[1]
BMI	0.126[2]	0.603[1]	0.063[1]
Testosterone (ng/dl)	0.002 r = 0.372	0.446[1]	0.064[1]
TC (mg/dl)	0.136[2]	0.292[1]	0.583[1]
HDL (mg/dl)	0.027 r =-0.251[2]	0.723[1]	0.022 r =-0.260[1]
LDL (mg/dl)	0.342[2]	0.283[1]	0.469[1]
VLDL (mg/dl)	0.027 r = 0.250[2]	0.908[1]	0.932[2]
TG (mg/dl)	0.024 r = 0.256[2]	0.727[1]	0.960[2]
Adropin	–	0.001 r =-0.372[2]	0.978[2]

[1]Pearson correlation test; [2]Spearman correlation test
BMI – body mass index; IIEF – International Index of Erectile Function; TC – total cholesterol; HDL – high-density lipoproteins; LDL – low-density lipoproteins; VLDL – very low-density lipoproteins; TG – triglycerides

Table 3. *Serum adropin and NO levels of the groups*

Variables	Mild CAD (n = 38)	Severe CAD (n = 37)	p	r
Adropin (pg/ml)	17.92 ±16.9	18.7 ±15.86	0.66	-49
NO (µmol/l)	542.47 ±374.08	473.15 ±235.02	0.04*	-42

Results are expressed as mean ±SD
*statistically significant
CAD – coronary artery diease; NO – nitric oxide

(1, 10, 50, 100 μM) were used as standards. Serum samples were deproteinized prior to assay. Samples were added to 96% cold ethanol (1/2 v/v) and then vortexed for 5 min. After incubation for 30 min at +4°C, the mixture was centrifuged at 14 000 rpm for 5 min and the supernatants were used for the Griess reaction [20].

Measurement of serum adropin level

The serum concentrations of adropin were determined by commercially available ELISA kits (Human Adropin ELISA Kits; Eastbiopharm Co. Ltd; Hangzhou, China) according to the manufacturer's instructions. The intra- and inter-assay coefficient of variation of kit was <8% and <10%, respectively. Assay range for adropin: 1.56–100 pg/ml.

Statistical analysis

All data were expressed as mean ±standard errors of the mean (SEM). Statistical analyses were performed using a software program (SPSS 16.0 for Windows, Chicago, IL, USA). The Kruskal Wallis test was used to analyze the significance of the differences among groups. Post Hoc analysis was done with the Mann Whitney U test. Continuous variables with normal distribution were evaluated by Pearson correlation analysis and continuous variables with abnormal distribution were evaluated by Spearman correlation analysis. For tests of significance a p value of less than 0.05 was considered to be significant.

RESULTS

In this study, there were a negative significant correlation between serum adropin levels and IIEF scores (Table 2).
Also, there was a significant correlation between age and body mass index (BMI) with Gensini scores and a negative correlation with IIEF, as expected (Table 2). Serum adropin and NO levels were found to be lower in the severe CAD group than in the mild CAD group and the difference in NO was statistically

significant while the difference in adropin was not (Table 3).
In correlation analysis, serum adropin levels were found to be negatively correlated with Gensini scores.

DISCUSSION

To our knowledge, this was the first study to determine the association between adropin levels and ED. We found that the mean adropin level was significantly lower in patients with ED.
Erection is a vascular phenomenon under a psychological control in a hormonal environment. ED is defined as the inability to obtain and to maintain sufficient erection for satisfactory intercourse. Incidence of ED dramatically increases in men suffering from DM, hypercholesterolemia, and cardiovascular diseases [21]. Organic ED results mainly from vascular problems due to atherosclerosis. Because penile arteries have the smallest diameter in the vascular network, ED could be a first symptom of a more generalized vascular pathology [22]. The vascular endothelium is an important factor in the durability of vascular homeostasis. Loss of the functional integrity of the endothelium and subsequent endothelial dysfunction, the first step of atherosclerosis, causes a significant reduction of blood flow to tissue and negatively impacts the erectile function. Endothelial cell homeostasis is maintained in part through the synthesis of NO [14]. NO has a key role in penile erection and impairment of NO bioactivity is propounded to be the most important pathological mechanism in ED [23].
Adropin participates in NO bioavailability and affects inducible NOS expression [24]. Adropin-treated endothelial cells exhibit greater proliferation, migration and capillary-like tube formation and less permeability and tumor necrosis factor-α-induced apoptosis [14]. Topuz et al. evaluated endothelial dysfunction and flow-mediated dilatation in type II diabetes mellitus patients. They found a positive correlation between plasma adropin levels and flow-mediated dilatation values and the authors suggested that adropin levels could be used in quantifying endothelial dysfunction [25].
Plasma adropin levels are found to be lower in pediatric obstructive sleep apnea (OSA) patients, especially when associated with endothelial dysfunction [26]. After adenotonsillectomy, adropin amounts return to within normal values [26].
In a study conducted on cardiac syndrome X (CSX) patients, serum adropin levels were found to be significantly lower than healthy subjects and therefore it was assumed that lower serum adropin level is an independent risk factor for CSX [27] Wu et al. found that low serum adropin levels were associated with coronary atherosclerosis in type II diabetic and non-diabetic patients and the authors asserted that lower adropin levels might be a novel predictor of coronary atherosclerosis [28].
In the literature, low serum adropin levels have been reported in many diseases, which have endothelial dysfunction in its etiology, such as OSA, CSX and atherosclerosis. But the role of adropin has not been investigated up to now. Due to the presence of endothelial dysfunction in its etiology we hypothesized, that in ED patients, serum adropin levels should be correlated with the severity of the disease. Such as, endocan (serum endothelial cell specific molecule-1) is a serum marker in some endothelial-related disorders. Karabakan et al. reported the significant correlation between ED and serum endocan level, that can assist in the evaluation of endothelial pathologies in the etiology of ED [29]. In our study, we showed a negative significant correlation between plasma adropin levels and the IIEF scores. Although our results showed higher adropin levels in the severe CAD group, the difference between the groups was not statistically significant.

Probable causes:

a. The pharmacokinetics of adropin in circulation is virtually unknown. So, a single measurement may not be enough to evaluate adropin levels.
b. The genes encoding adropin are affected by the nutrient content of the diet and we could not standardize the diet of individuals in our study population, so this factor may have influenced our results.
c. Adropin is a small protein consisting of 43 amino acid residues [30], some might have been degraded before analysis because the samples were not placed into tubes containing protease inhibitors.
d. The most important limitation of our study is the small number of patients. Also, we have not measured other risk factors for ED parameters such as levels of oxidized-LDL, homocysteine, hs-CRP or IL-6.

CONCLUSIONS

This is the first study in the literature investigating the levels of adropin in ED patients having coronary artery disease confirmed by angiography. More comprehensive and multicenter studies are needed to reveal the role of adropin in ED pathogenesis and the effects of treatment on this molecule.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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