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Research Article

Nesfatin-1 Levels in Cardiac Syndrome X Patients

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Abstract

Background & Aim: Nesfatin-1 is a novel anorectic neuropeptide with potent metabolic regulatory effects. Our aim is to investigate the association of nesfatin-1 levels and cardiac syndrome X (CSX).

Methods: The study group consist of 80 patients who underwent coronary angiography and were diagnosed as CSX according to the presence of exercise-induced typical angina pectoris and ischemia on myocardial perfusion scintigraphy with angiographically normal coronary arteries. For control subjects, we recruited 80 healthy gender- and age-matched individuals who were seen for health check-ups.

Results: The cases had significantly lower levels of Serum Nesfatin-1 than the subjects ($p < 0.001$). There is significant correlation between low Nesfatin-1 levels and the CSX presence (OR, 0.982; 95% CI; 0.969-0.995; p=0.005).

Conclusion: The study results of CSX indicated lower levels of serum Nesfatin-1 compared to the control group. Nesfatin-1 might function as a pathogenic factor for CSX through an inflammatory response as well as an impaired endothelium. We commend this labor to you and expect further research from you on the association of Nesfatin-1 and CSX.

Keywords: Inflammatory Response; Nesfatin-1 Protein; Impaired Endothelium; Cardiac Syndrome X

1. Introduction

Pain or pressure on chest is responsible for over 5 million individuals taken to emergency services in hospitals, annually [1]. Among these patients, cardiac syndrome X (CSX) cases are substantial in number. If chest pain is caused by angina or ischemia using cardiovascular strest test or myocardial perfusion scintigraphy (MPS) without

flow-limiting stenosis on coronary angiography [2]. CSX patients will have ischemic myocardium and impaired endothelium that might be also cause for angina [3].

Oh et al. (2006) revealed that Nesfatin-1 is hypothalamus secretion like an appetite checker [4]. It was initially evaluated as a satiety molecule involved in decreasing appetite and regulating metabolism. Maejima et al. [5] showed that acute and chronic effects of nesfatin-1 with melanocyte-stimulating hormones systematically cause anorexia. Subsequently it was found that nesfatin-1 influences growth and differentiation of the adipose tissue, inflammation, thermoregulation, pancreatic insulin secretion, glucose homeostasis in the liver, nutrient intake in the brain, sleep, fear, anxiety, stress, glucose homeo¬stasis; regulation of gastric emptying, gastric acid secretion, gastric motility, and reproductive functions [6].

Dai et al. [7] have shown that serum nesfatin-1 levels were lower in individuals with acute myocardial infarction (AMI) compared to the angina pectoris group and the control group, in a study involving 156 individuals. In addition, plasma nesfatin-1 levels were inversely correlated with high sensitivity C reactive protein (hs-CRP), neutrophil percentage, and Gensini score in the AMI group. They reported that low levels of nesfatin-1 may have an important role in the development of AMI [7]. It has been shown that nesfatin-1 has an effect on inflammation and coronary artery disease [7]. The aim of the present study is to assess the association of nesfatin-1 with cardiac syndrome X.

2. Materials and Methods

2.1 Patient Characteristics

The study group consist of 80 patients who underwent coronary angiography on an outpatient basis at the cardiology department of Sivas Numune Hospital between October 2015- April 2017 and were diagnosed as CSX according to the presence of exercise-induced typical angina pectoris and ischemia on myocardial perfusion scintigraphy with angiographically normal coronary arteries. For the control group, consecutive 80 individuals with no diagnosis on stress test were designated as gender- and age-matched. Exclusions were the cases with unstable pain on chest, previous MI, coronary vasosplasm and coronary atherosclerosis (inc. plaque and ectasia), valvular heart disease (except for mild), impaired kidney (SCr > 1.5 mg/dl in males and >1.4 mg/dl in females), and liver function (more than doubled the peak as lab ref.), systolic impairment in left ventriclesby echocardiography (EF <0.40) malignant tumors, haematological diseases, chronic or acute infection or inflammation, or steroid treatment.

For the diagnosis of hypertension should be systolic blood pressure of > 140 mm Hg or diastolic blood pressure of > 90 mm Hg or the current use of antihypertensive drugs. Diabetes mellitus was defined as the use of antidiabetic medication or a fasting blood glucose of ≥ 126 mg/dl. Smoking was described as the regular use of tobacco.

2.2 Myocardial perfusion scanning (MPS)

The standards set by Turkey Nuclear Association Working Group require one-day-and-exercise approach or stress test (dipyridamole Tc-99m MIBI protocol) for MPS, and in this study were duly complied. ‘No food or drink’ for the stress testing was stipulated for 4 or more hours. Calcium channel blocker or beta-blocker were no longer

medicated from before 48 hrs, provided that there were no contradictions, so that heartbeat or tension would not jump.Under the modified Bruce protocol, dose administration was made with 8-10 mCi before and 22 to 25 mCi after in stress/rest test. Target heart rate was computed with the formula of (220 - age) x 0.85. The effort test was ended on the maximum point that patient has faint, dyspnea, pain on chest, syncope, tachycardia or fibrillation, or ST-segment elevates or depresses by over 2 mm, AV block develops secondarily or tertiarily, or systolic pressure drops below the baseline by 10 mmHg or more, or jumps over 240 mmHg (or 120 mmHg diastolic) as seen on monitor. When the patient's heart rate reached at and above (.85 x peak), the intravenous administration of Tc-99m sestamibi (8-10 mCi as stress dose) was made, following dipyridamole (0.14 mg/kg/min x 4 min) if he or she was clinically debilitated for treadmill. And 30 minutes later, the patients were beginning to shoot, and until the late of the resting stage, about three hours later, Tc 99m sestamibi was administered again, yet at this time, with 22 to 25 mCi (nearly 3 x 8-10 mCi). Lastly, they underwent MPS 45-60 min after.

2.3 Coronary Angiography

During coronary angiography, Judkins method was applied as standard, in which femoral or radial catheter was used for coronary angiograms, and two angiography practitioners uninformed of the lab and test results examined them. As channel blockers, no adenosine, nitroglycerin or calcium were administered. Hyperventilation test was performed to detect arterial spasm and exclude the diagnosed patients. Coronary arteries were accepted as abnormal when any illuminated narrowing or irregularity was recognized.

2.4 Laboratory Measurements

Samples were taken from the peripheral veins of the patients having undergone fast during the previous night.Auto-analyzers were used to evaluate the hematological results; Abbott Cell-Dyn 3700 for the leukocyte counts,totally or differently, and Abbott Architect C16000 auto-analyzer for high-density lipoprotein (HDL) cholesterol, triglycerides, and fasting blood glucose levels on blood serum, totally and specifically(Abbott Lab, Abbott Park, IL, USA). The Friedewald equation was the formula to make computations of the concentrations of serum low-density lipoprotein (LDL) cholesterol.

The technique of immediate centrifugation and cold storage (-80°C) was applied for plasmas taken to measure nesfatin-1 levels for a length of time. On the analysis day, the measurements were realized through a commercial ELISA kit (Ranging: 31.2 to 2000 pg/mL, Sensitivity: < 10 pg/mL) (Enzyme-Linked ImmunoSorbent Assay, Boster Immunoleader, USA) in compliance with the guiding protocol, its appendix.

2.5 Statistical analysis

A software program was used to conduct any statistical techniques to analyze the results, SPSS for Windows v. 20.0 (SPSS, Chicago, IL, USA). The conventional descriptive statistics such as mean, standard deviation, rate, and frequency data were readily computed. Whether normal distribution was valid for persistent variables was found using the Kolmogorov-Smirnov test. Both Student's t-test and the Mann-Whitney U test were applied for parametric and non-parametric data, respectively. Inter-group comparison was performed for specific variables through Chi square test. Furthermore, Pearson correlation analysis was made along with logistic regression model established to

find out the parameters effects. With the calculation of standardized beta coefficient, statistical significance was identified as the area of p values < 0.05 at a confidence interval of 95% CI.

3. Results

Table 1 presents clinical and demographic characteristics of the population of study at baseline. The study and control groups were not statistically significantly different by the attributes of age, gender and body mass index, BMI and the conditions of hypertension, smoking, diabetes mellitus and dyslipidaemia, with family history.

Parameters	Control group (n = 80)	Patients with CSX (n = 80)	p
Age [years]	53.9 ± 9.5	54.8 ± 8.9	0.566
Body mass index [kg/m^2]	26.5 ± 3.3	26.3 ± 4.0	0.752
Female	32 (40.0%)	33 (42.0%)	0.853
Diabetes mellitus	12 (15.0%)	10 (12.5%)	0.503
Arterial hypertension	20 (25.0%)	18 (22.5%)	0.745
Dyslipidaemia	20 (25.0%)	18 (36.7%)	0.745
Family history	7 (8.75%)	10 (12.5%)	0.206
Smoking	12 (15.0%)	10 (12.5%)	0.745

Table 1: Baseline characteristics of the study groups (n = 160)

Data are given as mean ± standard deviation or number (percentage); CSX: Cardiac syndrome X

Table 2 shows laboratory results of the cases and other subjects. There is significant difference between Hs-CRP levels of the individuals in study and control groups (p=0.028). Nesfatin-1 levels measured on plasma samples from the patients were significantly lower than those from the control subjects (p < 0.001).

Parameters	Control group (n = 80)	Patients with CSX (n = 80)	p
Glucose [mg/dL]	118.4 ± 44.1	124.1 ± 59.7	0.490
Creatinine [mg/dL]	0.88 ± 0.2	1.00 ± 0.4	0.266
Uric acid [mg/dL]	6.8 ± 2.1	6.2 ± 1.7	0.580
WBC count [10³/mm³]	9.8 ± 2.4	10.3 ± 2.6	0.269
Hemoglobin [g/dL]	13.4 ± 1.7	13.7 ± 1.5	0.255
Platelet count [10³/mm³]	242.4 ± 62.4	238.2 ±56.8	0.671
Total cholesterol [mg/dL]	172.0 ± 79.6	180.1 ± 77.4	0.615
Triglyceride [mg/dL]	124.0 (80.0-190.0)	123.5 (78.25-161.25)	0.683
LDL-cholesterol [mg/dL]	113.1 ± 57.3	116.0 ± 58.7	0.790
HDL-cholesterol [mg/dL]	41.0 (33.5-48.0)	43.5 (35.0-49.0)	0.820
Hs-CRP [mg/L]	3.0 (1.1-4.7)	4.8 (2.6- 6.6)	0.028
Nesfatin-1 [pg/mL]	121.4 ± 31.2	104.5 ± 30.8	< 0.001

LVEF [%]	60.0 ± 4.9	58.2 ± 5.1	0.442

Table 2: Comparisons of laboratory findings and nesfatin-1 levels

Data are given as mean ± standard deviation, number (percentage) or median (interquartile range); HDL - high-density lipoprotein; Hs-CRP-high-sensitivity C-reactive protein; LDL - low-density lipoprotein; LVEF - left ventricular ejection fraction; CSX - cardiac synrome X; WBC- white blood cells.

Multivariate logistic regression analysis was conducted in order to revealpotential perplexing pathogenic causes of CSX disease, and nesfatin-1 levels were low in significant and independent association with CSX (OR: 0.982; 0.969-0.995 at 95% CI, p=0.005) (see Table 3).

Univariable OR (95% Cl)	**p**		**Multivariable OR (95% Cl)**	**p**
Hs-CRP	1.127 (1.008-1.261)	0.036	1.099 (0.982-1.230)	0.100
Nesfatin-1	0.980 (0.967-0.992)	0.002	0.982 (0.969-0.995)	0.005

Table 3: Multivariate logistic regression analysis predicting cardiac syndrome X

CI - confidence interval; Hs-CRP - high-sensitivity C-reactive protein; OR - odds ratio

4. Discussion

It was revealed that nesfatin-1 levels were significantly lower in patients with CSX than patients in control group, in the present study.

Out of the patients who have typical complains and test results of angina pectoris, 10% to 30% are categorized as “normal” or “near normal” in terms of their epicardial arteries recognized under on coronary angiography. Among those cases, the individuals with pain or pressure on chest, which is mostly provoked by exercises, have narrowing or irregularities on myocards and/or ST segment depression, ischemia-like discomfort on chest, induced by any factor or not, and they are evaluated as CSX in studies. Nevertheless, a group of patients due to hypertrophy in left ventricles, spasm in coronary arteries, and cardiomyopathy must have been excluded when arrived at the evaluation stage.

All these exlusions mean much more labor and money for our health care sector to discover the real factors of chest pain, spent on diagnosis tests and laboratory works which are substantially expensive. Moreover, CSX diagnosis vitally reduces the quality of life even though there is no additional risk of death.

This diagnostic case includes heterogeneity among any could-be pathogenetic factors, even derived from non-cardiac effects. The most notorious one is impaired endothelium in the collateral coronary circulation [8, 9].

In natural metabolism, myocards are demanding and simultaneously coronary veins are supplying blood flowing in there. Its increasing quantity when dilated, as "coronary blood flow reserve", primarily indicates the vasodilator capacity in microcirculation. If the artery has more resistance and/or less reserve, the spread between the demand and supply may not be closed, leading to myocardial ischemia, contributing factor to the pathogenesis of CSX disease, *called 'microvascular angina'.* The real cause or maybe causes for such coronary irregularities have not yet been totally discovered.

Despite that defect, the appearance of CSX is often attributed to they way of its reflection like chest pain and behind this, myocardial ischemia and endothelial impairment as well [3]. In patophysiology, CSX appears with the mechanisms in which metabolism is insulin-resistant, auto-control irregular, and microcirculation spasmodic. The most reasonable evidence is impairment on endothelium that causes to diminish the quantity of nitric oxide and hence the quality of any agents against inflammation and coagulation and derange the balance of constriction and dilation in vascular arteries [10]. Among these patients, the amount of nitric oxide is reduced up to basal levels while endothelin-1 levels are increasing [11].

The impaired endothelium could be mainly the best precursor for higherlikelihood of adverse consequences [12]. In literature the studies on the relationship of CSX and inflammatory response come up with innovative approachs for treatment of such a puzzling condition. For instance, for CSX patients early detection of symptoms like severe and repetitive angina pectoris could be possible by means of monitoring construction of arteries' wall and their function through inflammatory markers as well as noninvasive measures.This facility can also be beacon of hope for those patients having poor endothelium with no better chance of prognosis, even with aggressive operations.Furthermore, intravascular ultrasonograpy has developed so that CSX patients can be provided more specific diagnosis like with plaques in atheroma and thicknesses in intima [13].

There is in addition association between impairment in endothelium and more CRP markers for chronic vasoinflammatory conditions [14]. The relationship of CSX patients' virtually incessant pain on chest and higher CRP levels have been proven as well as with greater number of ischemic episodes identified through 24-hour Holter monitoring [15]. CRP, a biomarker of inflammation, is naturally rising with inflammatory responses in the cases of pain, injury, infection, etc. [16], and so the angina presence increased as a result of ischemic events, per se, must be increasing factor for CRP. Nonetheless, regardless of chest pain, in fact, CRP is not only a direct response to pain but also CRP levels have pathogenic impact on CSX separately, on the ground that CRP is related to the ischemia-oriented episodes. As related to CSX, the connection of CRP levels and carotid artery stiffness has in the meantime been discovered [17]; among CSX patients CRP levels were significantly higher than normal as well as a stiff carotid artery. Additionally, there may exist a causality between inflammation and impairment of endothelium along with their would-be common factor, the present plaque in subclinical atherosclerosis.

Some studies in the last decade have demonstrated that pathological functions of adipose tissue can be associated with increased cardiovascular disease risk, not only due to the effect of the hypothalamus nucleus [4] on the regulation of cardiovascular function but also by activating the autocrine/paracrine/endocrine pathway of chemical

mediators released from adipose tissue like nesfatin-1 [18]. A major hormone giving sense of satiety is Nesfatin-1 [4]. In a number of trials conducted over the rats, Nesfatin-1 was intracerebroventricularly administered or intraperitoneally applied, and then dietary intake was reduced [4]. This type of peptide is related to hepatic (*esp.* diabetes mellitus) [19], polycystic (*in particular*, ovarian syndrome (PCOS) [20], psychiatric (21) or neurogenic [22] dysfunctions, as reported in recent times.

Bonnet et al. [23] have revealed an association between inflammation of the brainstem and hypothalamus and activation of neuron expressing nesfatin-1. One of the most important pathophysiological mechanisms of CSX is inflammation, and recent studies have shown anti-effects of that peptide against inflammation and oxidase [24]. It is reported that Nesfatin-1 that has been intravenously injected can inhibit producing nitric oxide and then induce constriction of vascular vessels, leading to high tension [25]. Ayada et al. [26] have demonstrated in their study particularly relating to rats being under restraint stress chronically that when nesfatin-1 is regularly infused into peripheral vein, this can inhibit the endothelial synthesis of nitric oxide. Dai et al. [7] have reported that plasmic concentrations of nesfatin-1 were substantially decreased among patients with AMI. Moreover, these concentrations have negative association with CRP and neutrophil percentage, which are important predictors of CSX development, in AMI patients [7].

Osaki et al. [27] showed that 6-hydroxydopamine, which makes chemical sympathectomy, might rise the presence of nesfatin/nucleobind-2 in thickened subcutaneous tissues. Thus, it could possibly be inferred that the expression of nesfatin-1 could be suppressed by sympathetic activity could suppress nesfatin-1 expression. Increased activity relating to sympathetic nervous system is also closely associated with endothelial dysfunction [28].

In this study, we have consensus with the finding of Rencio Mayoral et. al. [29] and Rinkevich et. al. [30] that myocardial microcirculation system could be impaired by subclinical inflammatory responses though epicardial blood flow is normal in coronary arteries.

We conclude that among CSX patients plasmatic concentration of nesfatin-1 is lower than normal, which could have pathogenic impact on CSX phenomenon with a number of factors including inflammatory responses and impaired endothelium. We consider that the relationship between CSX and nesfatin-1 should be challenged with further researches.

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