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The relationship of serotonin level with syndrome X and coronary artery disease

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ABSTRACT

Aims: Platelets secrete serotonin (5-hydroxytryptamine) which has several effects on the vascular wall and promotes thrombogenesis, mitogenesis, and proliferation of smooth muscle cells. We thought serotonin which relation with coronary artery disease (CAD) has been shown in various data might be important for etiology and diagnosis of cardiac syndrome X (CSX) which's physiopathology has not been elucidated yet. The aim of the study is to investigate relationship between serotonin level with CSX and stabile coronary artery disease.

Methods: Serum serotonin levels were measured by micro ELİSA procedure in three group of patients who underwent coronary angiography. First group consisted of 30 patients (17 male, 13 female, mean age 60.5±6.1) who has diagnosed CSX, second group consisted 22 patients (11 male, 11 female, mean age 60.5±6.1) who has diagnosed stable coronary artery disease and third group consisted 28 patients (10 male, 18 female, mean age 56.6±10) comprised the control group. All three groups of patients demographic, biochemical, hematological and echocardiographic data were recorded.

Results: The three groups were similar with respect to age, sex, body mass index and the frequencies of diabetes mellitus and smoking(p>.016). We detected average serum serotonin levels 49.7 ng/ml in syndrome X group, 41.8 ng/ml in CAD group and 44.8 ng/ml in control group. These values are not statistically significant between the groups monitored(p=.91).

Conclusion: The serotonin level of of CSX patients, stabile coronary artery disease patients and control group patients were measured in the serum which seperated from peripheral venous blood. Statistically significant difference in serum serotonin levels couldn't be detected between these three group.

Keywords: Serotonin, cardiac syndrome X, coronary artery disease

INTRODUCTION

Coronary artery disease (CAD) is defined as ischemia-induced pathological events that occur as a result of the myocardium not being fed due to occlusion in the coronary arteries. Many factors have a role in the etiology of CAD and atherosclerosis is the most blamed.

In a significant part of the patient group who went to the emergency room with chest pain and underwent coronary angiography, coronary angiography is found to be normal and vasospasm in the epicardial coronary arteries was not detected. Although there are many studies on the pathogenesis of cardiac syndrome X (CSX), the etiology of it has not been clearly revealed. In the formation of myocardial ischemia in patients with CSX, many hypotheses have been developed, such as mediation by α receptors,[1] coronary microvascular dysfunction, overestimation of cardiac pain,[2] increased vasoconstrictor response in smooth muscle cells due to the increase in membrane Na-H (sodium-hydrogen) exchanger channel activity, increased release of local vasoconstrictor autocoids such as angiotensin and endothelin, abnormal neural stimulation, and cardiac sympathetic dysfunction.[3]

Activated platelets release varying amounts of serotonin, which causes vasoconstriction and platelet aggregation.[4] Serotonin is a potent smooth muscle stimulant and vasoconstrictor. The release of vasodilator agents such as prostacyclin and prostaglandin E2 decreases with the release of local vasoconstrictor substances such as serotonin.[5] Serotonin level has been shown to be associated with CAD in various publications.[6]

Despite the fact that the role of serotonin in the physiopathogenesis of CAD has been the subject of various studies, the relationship between serotonin and CSX has

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not been studied yet. It has not yet been possible to clearly explain the cause of angina and the pathophysiology of angina in CSX patients. Alpha-receptor-mediated vasoconstriction in arterioles has been blamed in the mechanism of myocardial ischemia in CSX patients.[1] We thought that serotonin, which is known to be a vasoconstrictor agent, may be an important mediator in the etiology of CSX.

The aim of this study to investigate the relationship between serotonin level and CSX, which has not been examined in the literature before. Moreover, although studies have been conducted on it before, we were able to re-evaluate the relationship of serotonin level with CAD.

METHODS

Ethics

Upon obtaining the approval of Uludağ University Faculty of Medicine Clinical Researches Ethics Committee (Date: 26.05.2015, Decision No: 2015-11/27), the research was initiated. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patients

In this study, among the patients who went to Bursa Yüksek İhtisas Training and Research Hospital Cardiology Clinic and underwent elective coronary angiography for various reasons, 3 patient groups consisting of 30 patients with a diagnosis of syndrome X (group 1: Patients with angina chest pain, positive exercise tests suggestive of myocardial ischemia, and <50% stenosis lesions of the epicardial coronary arteries), 28 patients with normal coronary follow-up and non-cardiac evaluation (group 2: Patients with non-cardiac chest pain, negative stress tests that may indicate myocardial ischemia, and normal coronary angiography on coronary angiography), and 22 patients with stable coronary artery disease with obstructive coronary artery stenosis (group 3: A group of stable coronary artery patients with anginal complaints and a >70% stenosis lesion as a result of coronary angiography) were evaluated.

Patients over the age of 35 were included in the study, regardless of gender. The patient groups that were not included in the study due to exclusion criteria are listed in Table 1. Demographic characteristics, weight, height, concomitant diseases, habits, medications, blood tests, echo and ECG findings, exercise tests, coronary lesion status of the patients were examined in detail and recorded.

Effort Test

The patients were subjected to the maximal effort test according to the Bruce protocol. In cases where there is at least 1 mm horizontal and down sloping ST segment depression in at least 2 consecutive leads 80 ms after the J point, chest pain during the exertion test, and a decrease of 10 mmHg or more in systolic blood pressure compared to the initial blood pressure, the effort test was considered positive. Duke treadmill score (DTS) was calculated for each patient. A DTS score above +5 was considered low risk, between 4 and -10 was considered medium risk, and -11 and below was considered high risk for coronary artery disease.

Table 1. Exclusion criteria of the study

Left ventricular systolic dysfunction (ejection fraction	Chronic obstructive pulmonary disease
Presence of valvular heart disease or hypertensive heart disease	Liver or kidney dysfunction thyroid dysfunction
Patients with previous percutaneous intervention	Those who have had a cerebrovascular accident
Patients diagnosed with depression or using antidepressant agents	Current signs of infection
Patients with thrombocytopenia or thrombocytosis	Patients with acute pericarditis or myocarditis
Presence of ECG findings that complicate the interpretation of the effort test	Presence of permanent pacemaker, bundle branch block, severe atrio-ventricular conduction defects
Presence of diagnosis of malignancy	Hypertrophic or dilated cardiomyopathy
	>75 years old patient group

Echocardiography

All patients included in the study was subjected to transthoracic echocardiography by the same operator. Echocardiographic examination was performed using Vivid 7 Pro® (GE healthcare) echocardiography device and a 3.5 Mhz transducer. In parasternal long axis images, left ventricular systolic and end-diastolic widths, posterior wall and septum thicknesses, and left ventricular ejection fraction (IF) were measured with M-Mode. Apical four-chamber, apical five-chamber, parasternal long axis and parasternal short axis images were obtained by two-dimensional and color Doppler examination. Measurements were taken in apical four-chamber and apical five-chamber images using tissue Doppler, continuous wave, and pulsed wave Doppler.

Coronary Angiography

Selective coronary angiography were performed via the right femoral artery, using the Seldinger technique, using 6F Judkins catheters. Vasospastic angina was ruled out to rule out vasospastic angina by using a hyperventilation test (explained to breathe quickly and deeply for 5 minutes) during angiography.

Serum Serotonin Measurement

Venous blood samples were collected from all three patient groups between 9 am and noon during their outpatient follow-up. After the collected blood was centrifuged at 5000 rpm for 10 minutes, its serums were separated and stored in eppendorfs at -22 °C. When all the blood was ready, serotonin measurements were performed in one go in the biochemistry laboratory of our hospital. Serum

serotonin levels were measured using the commercial kit DAS-ELISA (double-antibody sandwich enzyme-linked immunosorbent assay) from Sunred Biotechnology.

In summary, 40 µl of serum was added to wells coated with human serotonin monoclonal antibody, then 10 µl of biotin-labeled ST-antibody and 50 µl of streptavidin-HRP were added to form an immune complex. The wells were washed 5 times with 350 µl of washing solution to remove unbound enzymes after incubation for 60 minutes at 37 °C. Then, 100 µl of chromogen solution A and B (1:1 ratio) was added to each well. This mixture was incubated for 10 minutes at 25 37 °C in an orbital mixer in the dark. Afterwards, 50 µl of stopper solution was added and the absorption levels at 450 nm were measured within 15 minutes. The amount of serotonin was calculated from the calibration curve and the results were expressed in nanograms/milliliter. The manufacturer reported intraday and interday coefficients of variation as <10% and <12%, respectively, and sensitivity as 0.38 ng/ml.

Statistical Analysis

SPSS 22.0 computer software was used for the statistical analysis of the cases. Numerical variables are shown as mean±standard deviation, and categorical variables as percentages. Shapiro-Wilk test and skewness-kurtosis coefficients were analyzed for normality analysis. Accordingly, the comparison of the groups was performed with the Kruskal-Wallis test for continuous variables and with Chi-square or Fisher's exact test for categorical variables. Since the number of groups compared was 3, the Bonferroni correction was performed and the p<.016 value was considered significant in the statistical measurements.

RESULTS

A total of 80 patients with similar demographics, 30 of them constituting the CSX group (group 1), 22 stable coronary artery patients with occlusive lesion on CAG (group 3), and 28 control group patients with negative exertion test (group 2), whose coronary arteries were normal and evaluated as non-cardiac, were included in the study.

There was no statistically significant difference between the groups in the evaluation performed in terms of gender, age and body mass index (BMI) of all 3 patient groups included in the study. When patients were examined in terms of coronary artery disease risk factors, the number of patients with hypertension was found to be 9 (30%) in the CSX patient group, 18 (81.8%) in the CAD group, and 8 (28.6%) in the control group. The number of hypertensive patients in the CAD group was statistically significantly higher (p<.001). Presence of diabetes, hyperlipidemia, smoking and CAD heredity, which are other CAD risk factors, are similar in all 3 groups (p>0.016).

Against the possibility of affecting the serotonin level, blood pressure and pulse/minute counts were measured just before taking the serotonin sample from the venous blood of the patients participating in the study, and it was understood that there was no statistical difference between the 3 groups in terms of the relevant results.

Demographic and clinical characteristics of all three patient groups are summarized in Table 2.

Table 2. Demographic and clinical characteristics of the groups

	Group 1 (n=30)	Group 2 (n=28)	Group 3 (n=22)	p
	(Syndrome X)	(Control)	(CAD)	
Age (years)	54.6±11.4	56.6±10	60.5±6.1	.08
Male gender, n (%)	17 (56.7)	10 (35.7)	11 (50)	.26
Female gender, n (%)	13 (43.3)	18 (64.3)	11 (50)	.26
BMI (kg/m^2)	28.6±3.6	29.9±3.88	30±3.4	.32
DM, n (%)	6 (20)	5 (17.9)	8 (36.4)	.25
HT, n (%)	9 (30)	8 (28.6)	18 (81.8)	<.001*
HL, n (%)	9 (30)	6 (21.4)	6 (27.3)	.75
Smoking, n (%)	8 (26.7)	14 (50)	5 (22.7)	.07
Heredity, n (%)	8 (26.7)	9 (32.1)	14 (63.6)	.017
SBP (mmHg)	122.58 ±12.72	120.88 ±13.1	128.18±11.70	.08
DBP (mmHg)	80.34±6.39	78.57±5.75	84.09±5.90	.018
MHR (Beats/min)	77±10	75±13	77±10	.31

*p<.016, CAD: Coronary artery disease, BMI: Body mass index, DM: Diabetes mellitus, HT: Hypertension, HL: Hyperlipidemia, SBP: Systolic blood pressure, DBP: Diastolic blood pressure MHR: Mean heart rate

Statistically similar features were observed when the left ventricular ejection fraction (LVEF), left ventricular ejection fraction (LVEF), left ventricular systolic and end-diastolic diameters (LVDSC, LVSS), left ventricular interventricular septum and posterior wall diameters were measured and analyzed echocardiographically in all 3 patient groups (Table 3).

As a result of the study, the hematological and biochemical data of the groups are summarized in Table 4. In terms of white blood cell count, hemoglobin and platelet counts, no significant difference was observed between the groups. Renal function tests, electrolytes, lipid parameters, brain natriuretic peptide (BNP) and Hs-CRP values obtained from the patients were found to be similar in all 3 groups, and the results are summarized in the Table 4.

Table 3. Echocardiographic data of groups				
	Group 1 (n=30)	Group 2 (n=28)	Group 3 (n=22)	
	(Syndrome X)	(Control)	(CAD)	p
SVEF (%)	59.66±4.21	58.25±3.40	60.54±3.48	.18
LVEDD (mm)	46.96±2.80	47.61±3.26	47.27±2.09	.63
LVESD (mm)	29.37±2.84	29.73±3.07	28.50±2.50	.54
IVS (mm)	9.96±2.02	9.73±2.70	10.45±0.80	.83
PW (mm)	9.51±1.78	8.96±2.97	10.13±0.46	.11

CAD: Coronary artery disease, LVEF: Left ventricular ejection fraction, LVEDD: Left ventricular end-diastolic diameter, LVESD: Left ventricular end-systolic diameter, IVS: Interventricular septum diameter, PW: posterior wall diameter

Table 4. Hematological and biochemical data of groups				
	Group 1 (n=30)	Group 2 (n=28)	Group 3 (n=22)	
	(Syndrome X)	(Control)	(CAD)	p
Wbc ($\times 10^3$)	7367±2110	7375±2210	7090±2090	.89
Hemoglobulin (gr/dl)	12.8±1.4	12.5±1.3	12.5±1.2	.80
Platelet	237670±70500	218600±57700	234040±77600	.86
Urea (mg/dl)	14.6±4.3	14.2±4.2	15.1±4.8	.85
S. crea (mg/dl)	0.69±0.21	0.66±0.16	0.71±0.17	.47
Sodium (mEq/L)	138±2	137±2	139±3	.02
Potassium (mEq/L)	4.3±0.4	4.1±0.2	4.4±0.4	.03
T. cholesterol (mg/dl)	184±33	188±40	168±46	.17
LDL-C (mg/dl)	112±29	115±35	101±37	.23
HDL-C (mg/dl)	47±14	44±9	42±16	.30
Triglyceride (mg/dl)	151±74	135 ±79	123±50	.45
BNP (pg/mg)	33.3±29.6	28.5 ±23.9	58.7±55.2	.04
Hs-CRP (mg/L)	6.45±11.9	6.87±8.54	7.38±5.81	.10

Wbc: White blood cell count, S. crea: Serum creatinin, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, T. cholesterol: Total cholesterol, Hs-CRP: High-sensitivity C-reactive protein, BNP: Brain natriuretic peptide, CAD: Coronary artery disease

Medications used by all three groups when recruited to the study are listed in Table 5. The number of patients using acetylsalicylic acid (ASA) was 7 (23.3%) in the syndrome X group, 22 (100%) in the CAD group, and 5 (17.9%) in the control group. There was a statistically significant excess of ASA use in the CAD group compared to the other groups (p<.001). The use of other drugs used by the patients and listed in the table was found to be at similar rates in all 3 groups.

We found that the mean serum serotonin levels were 49.7 ng/ml in the syndrome X group, 41.8 ng/ml in the CAD group and 44.8 ng/ml in the control group. These values

Table 5. Medications taken by the groups				
	Group 1 (n=30)	Group 2 (n=28)	Group 3 (n=22)	
	(Syndrome X)	(Control)	(CAD)	p
ASA, n (%)	7 (23.3)	5 (17.9)	22 (100)	<001*
Beta blocker n (%)	2 (6.7)	3 (10.7)	3 (13.6)	.70
ACEI n (%)	6 (20)	6 (21.4)	9 (40.9)	.18
CCB, n (%)	3 (10)	4 (14.3)	3 (13.6)	.87
Statin, n (%)	4 (13.3)	0	2 (9.1)	.14
Metformin, n (%)	5 (16.7)	5 (17.9)	5 (22.7)	.84
Sulfonylurea, n (%)	3 (10)	2 (7.1)	0	.32

*p<.016, CAD : Coronary artery disease, ASA : Acetylsalicylic acid, ACEI : Angiotensin converting enzyme inhibitor, CCB : Calcium channel blocker

were not statistically significant between the groups (p=.91). Statistical analysis of serotonin levels between groups is summarized in Table 6. In Figure (Central Illustration), serum serotonin levels between groups are given.

Table 6. Relationship between groups in terms of serum serotonin levels				
	Group 1 (n=30)	Group 2 (n=28)	Group 3 (n=22)	
	(Syndrome X)	(Control)	(CAD)	p
Serotonin (ng/ml)	49.7	44.8	41.8	.91

CAD : Coronary artery disease

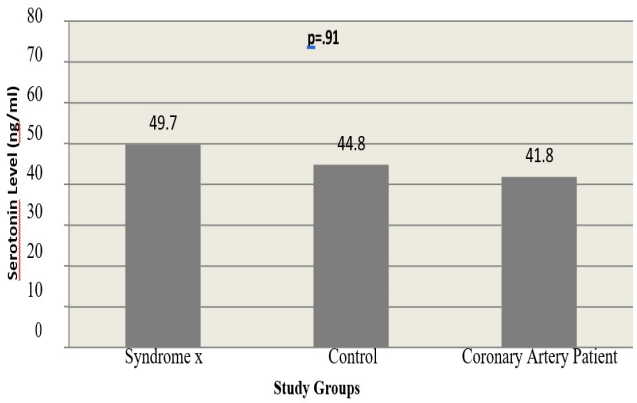

Figure (Central Illustration). Comparison of serum serotonin levels between groups

DISCUSSION

In this study, we aimed to review the relationship between coronary artery disease and serotonin levels and to examine the relationship between CSX disease, whose etiopathogenesis has not been clearly elucidated and whose relationship with serotonin has not been studied before, and serotonin.

More than 95% of serotonin in the human body is synthesized from enterochromaffin cells in the intestinal system, and the remaining part is synthesized from the raphe

nucleus and neuroendothelial cells in the brain.[7] Serotonin is not synthesized in platelets. These cells take serotonin from the plasma and store it. Experimental studies have suggested that platelets are associated with the development of atherosclerosis.[8-10] Some experimental studies in animals indicate that platelets are activated and aggregated in cases of coronary artery stenosis or endothelial damage.[4,11,12] Activated platelets release various amounts of serotonin, causing vasoconstriction[4,11,13] and cyclic flow reduction, leading to recurrent aggregation.[4] Moreover, serotonin stimulates the proliferation and migration of arterial smooth muscle cells through growth factor.[14,15] These effects are blocked by ketanserin, a specific serotonin receptor antagonist.[14,16] Serotonin has been shown to increase the proliferation of vascular endothelial cells.[17,18]

Various studies have indicated that platelet derivatives such as serotonin and thromboxane A2 have an important role in the physiopathogenesis of acute coronary syndrome.[19,20] In particular, increased serum serotonin concentrations were observed in blood collected from the coronary sinuses of patients with complex coronary lesions.[21] Similarly, blood serum obtained from the coronary sinus of patients with coronary artery disease has been shown to cause vasoconstriction in canine coronary arteries in vitro. This vasoconstrictor effect was abolished by serotonin receptor blockers.[22] In many previous studies, the vasoactive effects of serotonin were studied in animals with normal epicardial coronary arteries. Brum et al.[23] and Lamping et al.[24] reported that serotonin exerts a dose-dependent vasoconstrictor effect in damaged coronary artery endothelium of dogs. Chu and Cobb,[25] on the other hand, revealed that serotonin has a dose-dependent (consisting of vasodilation and vasoconstriction, respectively) biphasic effect in canine coronarys without endothelial damage. In addition, in vitro studies have shown that serotonin causes vasodilation in intact coronary arteries and vasoconstriction in damaged coronary endothelium.[26,27]

By binding to the 5-HT receptor on endothelial cells, serotonin stimulates the release of the relaxation factor, and by binding to the 5-HT2 receptors on the vascular smooth muscles, it stimulates their contraction.[28] If the vascular endothelium is normal, the relaxation factor is dominant and leads to vasodilation.[29] However, if the endothelium is damaged or dysfunctional, as in coronary artery disease, vasoconstriction via 5-HT2 receptors becomes dominant.[29-31] The platelet antiaggregation effect of sarpogrelate, which is a 5-HT2 receptor blocker, and its anti-anginal activity by increasing collateral circulation have been demonstrated in studies conducted.[32,33]

Serotonin levels of 96 patients with coronary artery disease and control group of 25 patients with normal coronary artery disease compared by Vikenes et al.[6] Serotonin levels were measured in the platelet-rich plasma of blood samples collected via the venous route, and the serotonin level was statistically significantly higher in the coronary artery disease group. In their study, Van den Berg et al.[21] investigated whether there was a difference between the serotonin levels of 39 patients with stable coronary artery disease with occlusive lesions and 13 patients with normal coronary artery control group. Blood samples were collected from 2 places, from the aorta and the coronary sinus of the patients, and their serotonin levels were studied. There was no difference between the two groups in terms of serotonin levels in blood samples collected from the aorta and coronary sinus. The difference between the coronary sinus serotonin concentration and the aortic serotonin concentration levels was found to be significantly higher in the coronary artery disease group. This data supports that serotonin is released into the coronary circulation in patients with occlusive coronary artery disease.

Despite coronary angiography without flow-limiting stenosis, CSX is characterized by typical anginal pain diagnosed after exclusion of non-cardiac pain. It is still a hopeless syndrome for both patients and physicians due to the lack of a standard diagnostic criteria, lack of consensus on pathophysiology, and ineffective treatment options. Various theories have been proposed to explain the pathophysiological abnormality, such as abnormal coronary flow reserve, insulin resistance, abnormal autonomic control, increased sodium hydrogen exchange activity, abnormal cardiac sensitivity, and microvascular spasm.[34] The most proven and accepted ideas are endothelial dysfunction and inflammation, and increased pain sensitivity.[35] Endothelial dysfunction disrupts the vasoconstriction/vasodilation balance, reduces the release of anti-inflammatory and antithrombogenic factors and decreases the availability of nitric oxide.[36]

Previously, the relationship between coronary artery disease and serotonin has been the subject of many studies, as mentioned above. In this study, we aimed to review the relationship between coronary artery disease and serotonin levels and to examine the relationship between CSX disease, whose etiopathogenesis has not been clearly elucidated and whose relationship with serotonin has not been studied before, and serotonin.

Previously, the relationship between coronary artery disease and serotonin has been the subject of many studies, as mentioned above. We could not detect any difference in serotonin levels between the coronary artery patients and the control group. As we mentioned above, the study conducted by Van den Berg et al.[21] on 39 patients is in line with the results of our study. Van den Berg et al.[21] found no significant difference in serotonin levels between the blood taken from the central aorta of both groups. Similarly,

serotonin levels of blood taken from the coronary sinuses were similar in both groups. The difference between aortic serotonin levels and coronary sinus serotonin levels was found to be significantly higher in patients with coronary artery disease.

In the study of Vikenes et al.[6], which we mentioned before, on 121 patients, unlike the results of our study, serotonin levels were found to be higher in coronary artery patients compared to the control group. In this study, as in our study, blood was obtained by peripheral venous route, but the serotonin level was studied from platelet-rich plasma, not from serum. Serotonin is known to be very rapidly metabolized and rapidly reabsorbed after release. Therefore, the relatively low levels of free circulating levels make it more meaningful to study serotonin levels from whole blood or platelet-rich plasma, not serum. Studying serotonin levels from serum is one of the limitations of our study. Measurement of serotonin levels in serum in our study may be one of the reasons underlying the inconsistency between the results of the study conducted by Vikenes et al.[6] and the study we conducted.

Studies that indicate that serotonin levels may play an important role in the etiology, diagnosis and treatment strategy of coronary artery disease in the aforementioned previous studies encouraged us to examine the relationship between CSX and serotonin, the physiopathogenesis of which has not yet been fully elucidated.

Endothelial dysfunction, one of the most frequently accused mechanisms in the etiology of cardiac syndrome X, may be causing serotonin's inability to vasodilate over 5-HT in the impaired endothelium, as in CAD, and develop ischemia as a result of the vasoconstriction effect on vascular smooth muscle (via 5-HT2). Based on this, we speculated that serotonin or 5-HT receptor blockers may have a role in the etiopathogenesis, diagnosis and treatment of CSX disease, which is known as a sub-branch of the stable coronary artery disease group and has a good prognosis but adversely affects the quality of life. We compared the serum serotonin levels of patients with CSX and the control group. No difference was observed between serum serotonin levels of both groups. As mentioned above, the measurement of serotonin level from serum, not whole blood or platelet-rich plasma, may have played a role in the emergence of this result. Moreover, the rapid metabolization and reabsorption of serotonin may explain the similarity of serotonin levels measured in peripheral blood between the two groups. Measurement of serotonin levels from the coronary sinuses or coronary arteries, as previously performed in animal studies, could change the course of the study. This can be considered among the important limitations of our study.

CONCLUSION

The relationship between CAD and serotonin has been examined in many previous studies. However, to the best of our knowledge, this study is the first to examine the relationship between cardiac syndrome X, which is predominantly caused by microvascular dysfunction, and serotonin, a vasoactive molecule. In this study, no significant difference was found between serotonin levels in coronary artery patients, syndrome X patients and the control group. It has not been possible to show the relationship of serotonin level with coronary artery disease and syndrome X.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Uludağ University Faculty of Medicine Clinical Researches Ethics Committee (Date: 26.05.2015, Decision No: 2015-11/27).

Informed Consent

All patients signed and free and informed consent form.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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