Evaluation of plasma catestatin levels in patient with coronary slow flow

Dursun Topal[1], Bedrettin Boyraz[2], Alkame Akgümüş[3], Tezcan Peker[4], Mustafa Yılmaz[5], Fahriye Vatansever Ağca[1], Selçuk Kanat[1], Ömür Aydın[6], Burhan Arslan[7]

[1]Department of Cardiology, University of Health Sciences, Bursa Yüksek İhtisas Training and Research Hospital, Bursa, Turkey
[2]Department of Cardiology, Bitlis Tatvan State Hospital, Bitlis, Turkey
[3]Department of Cardiology, Gemlik State Hospital, Bursa, Turkey
[4]Department of Cardiology, Bursa Doruk Hospital, Bursa, Turkey
[5]Department of Cardiology, Uludağ University School of Medicine, Bursa, Turkey
[6]Department of Cardiology, Kilis State Hospital, Kilis, Turkey
[7]Department of Cardiology, Mardin Kızıltepe State Hospital, Mardin, Turkey

DOI: 10.18621/eurj.372631

ABSTRACT

Objectives: Coronary slow flow (CSF) is characterized by delayed opasification of the coronary arteries in the absence of obstructive coronary disease. Catestatin has several cardiovasculer actions, in addition to diminihed sympato-adrenal flow. The study was to investigated associations between CSF and plasma catestatin levels.

Methods: This study included 45 CSF patients (37 males, mean age 48 ± 9.5 years) and 30 control individuals (24 males, mean age 48.4 ± 9 years). Coronary flow was quantified according to the TIMI (Thrombolysis in Myocardial Infarction) frame count method for coronary arteries. Serum catestatin levels taken from blood samples were measured by ELISA method. These parameters were compared between the groups.

Results: When compared with to the control group the serum catestatin levels was found higher in the CSF group. In addition to this, mean platelet volume was also significantly higher in patients with coronary slow flow.

Conclusions: Our study revealed that catestatin levels are increased in patients with CSF. Coronary slow flow that increased catecholaminergic sympathetic system activities seem to be among the reasons of endothelial dysfunction.

Keywords: Catestatin, coronary slow flow, coronary artery disease

Received: December 29, 2017; Accepted: March 30, 2018; Published Online: May 21, 2018

The coronary slow flow (CSF) phenomenon which was first described by Tambe *et al.* [1] in 1972 is characterized by delayed opacification of the distal vascularture in patients with normal coronary arteries. Endothelial dysfunction, occlusive disease of small coronary arteries, increase of vasomotor resistance have been suggested [2-5].

Endothelin-1 (ET-1) acts by reversing the effect of nitric oxide and it is known as the most potent vasoconstrictor agent. ET-1 has positive inotropic effects on cardiomyocytes and is mitogenic for smooth muscle cells. Increased ET-1 levels and increased

Address for correspondence: *Dursun Topal, MD., University of Health Sciences, Bursa Yüksek İhtisas Training and Research Hospital, Department of Cardiology,Bursa, Turkey, E-mail: dursuntopal@yahoo.com*

e-ISSN: 2149-3189

catecholaminergic activity are responsible for microvascular resistance [6, 7].

Catestatin is a 21 aminoacidic hydrofobic neuroendocrine peptide that is formed by dissociation of chromogranin-A [8]. It is co-stored in the secretory granule and co-released with catecholamine in adrenal chromaffin cells and adrenergic neurons. It is non-competitively inhibites nicotinic acetylcholine receptors and catecholamine secretion [9]. Besides, it was shown to have a direct negative inotrophic effect, catestatin shows an adverse effect towards ET-1's positive inotropic, lusinotropic and constrictor effect on coronary arteries. In addition to this; hypertrophic, mitogenetic and angiogenetic effects of ET-1 on vascular smooth muscle cells, are also inhibited by catestatin [10, 11]. Catestatin also distrupts contraction coupling over the calcium pathway by inhibiting phosphorization of Phospholamban by protein phosphokinase. In this way catestatin contributes to vasodilation in microvascular area [10].

In this study, we aimed to investigate the relation between CSF and plasma catestatin levels.

METHODS

The study included 45 patients (group I) with CSF (37 males/8 females, mean age 48 ± 9.5 years) and 30 patients (group II) with normal coronary arteries (24 males, mean age 48.4 ± 9 years). The study was approved by the Institutional Ethics Committee, and written consent was obtained from all patients. All patients were evaluated in terms of cardiovascular risk factors. Patients with acute or chronic renal failure, unregulated hypertension, occlusive coronary artery disease diagnosed in the past or recent angiography or who had been surgically or percutaneously treated for this diagnosis, patients with an open heart surgery history, patients with an active infection, patients below 18 years old, pregnants, patients have systolic dysfunction and patients with dysrhythmia have been excluded from the study.

Hyperlipidemia was defined as low-density cholesterol (LDL) was greater than 100 mg/dl or lipid-lowering medication was used. Blood pressure was measured of all patients. Hypertension was defined systolic blood pressure equal or greater than 140 mmHg and/or diastolic blood pressure equal or greater than 90 mmHg or under control by using antihypertansive drugs. Diabetes mellitus was defined as the fasting glucose level exceeding 126 mg/ dl on two seperate measurement or using of antidiabetic treatment.

All patients underwent coronary angiography due to stable angina pectoris in our clinic and preliminary diagnosis of ischemic heart disease and diagnosed as having normal coronary arteries. Coronary angiographies of the patients were evaluated and two groups were separated as having coronary slow flow according to the TIMI (Thrombolysis in Myocardial Infarction) frame countmethod and having normal coronary artery. Blood samples were obtained for basic hematological and biochemical parameters.

Plasma Catestastin Measurement

Samples taken for the measurement of catestatin levels were centrifuged for 10 minutes at 10.000 cycle than stored at -80°C until analysed. Obtained samples were measured by human catestatin Elisa kit and cathestatin levels were analysed.

Echocardiograhy

Echocardiography images were examined in the left lateral decubitus position. The measurements were examined after patients' admission to the hospital, with 3,5 MHz transduser and GE Vivid 7 Pro equipment, according to the guidelines of the American Society of Echocardiography [12]. Left ventricle end-diastolic diameters (LVEDd), left ventricle end-systolic diameters (LVESd) and left ventricle ejection fraction (LVEF) were obtained with modified Simpson method from apical 4 chamber views.

Documentation of Coronary Slow Flow

All the patients underwent selective coronary angiography in elective conditions with the standard technique. For imaging, lohexol (Omnipaque) was used as the contrast agent. TIMI frame count method first described by Gibson *et al*. [13] was used for documenting of coronary flow rates of all subjects and was determined for each major coronary artery in each patient and control subject.

Quantitative coronary angiographic measurements were examined with ACOM PC Lite version 2.0 (Siemens, München, Germany) programme. Flow

velocity was calculated by observing angiographic records taped at 15 square (15 fps) speed. For each coronary artery, elapsed time for the contrast to reach distal determinant points was defined as the frame count. As the starting point, the point where the contrast touches both sides of the coronary artery was taken. As the final point, mustage was taken for left anterior descending artery (LAD), first distal bifurcation of the longest branch was taken for circumflex artery (Cx) and the point where posterior descending artery (PD) branch gave its first sub branch, was taken for right coronary artery (RCA). TIMI frame count was normalized by comparing the length of LAD with RCA and Cx and corrected TIMI frame count (cTFC) was found. cTFC was obtained by dividing total sine frame count of LAD to 1.7. For LAD 36 ± 1, for Cx 22 ± 2 and for RCA 20 ± 3 average reference values were obtained. Since our angiography records were at 15 fps (frame speed per minute), obtained values were doubled and standardized according to the 30 fps velocity. Mean reference values above two standard deviation was accepted as CSF.

Statistical Analysis

Statistical analyses were made with IBM SPSS (Statistical Package for the Social Sciences ver. 16.0, SPSS Inc, Chicago, Illinois, USA) statistical analysis package programme. Continuous variables were defined as mean ± standard deviation whereas categorical variables were defined as percentage (%). Among groups, parametric variables that show comparability to the normal range, were compared by Student t test while non-parametric variables that do not show comparability to the normal range, were compared by Mann-Withney U test. Correlation between continuous variables was determined by Pearson correlation coefficients. For statistical evaluations, $p < 0.05$ value was considered as significant for all the statistical analyses.

RESULTS

In both groups, male gender was observed significantly higher. In the CSF group, 8 (17.7%) of 45 patients whereas in the control group, 6 (20%) of 30 patients were female. No significant difference was found between these two groups ($p > 0.05$).Baseline clinical and demograhic parameters were similar. (Table 1).

When compared in terms of echocardiographic parameters, both groups were similar in left ventricular chamber diameters and systolic pulmoner arterial pressure parameters. Moderate or severe valvular insufficiencies or stenosis was not observed in all groups (Table 2).

Mean platelet volume (MPV) in the CSF group was found to be higher and statistically significant ($p = 0.01$) compared to the control group; in CSF group MPV was 8.04 ± 0.91 fL, and incontrol group MPV was 7.55 ± 0.53 fL. There was no significant difference between the groups, in terms of routine hematologic and biochemical parameters except MPV (Table 3).

Catestatin levels in CSF (2.1 ± 0.65 ng/ml) group

Table 1. Comparison of the clinical and demographic properties in coronary slow flow patients and controls

Variables	Group 1 (CSF) (n = 45)	Group 2 (CONTROL) (n = 30)	*p* value
Age (year)	48 ± 9.5	48.4 ± 9	0.841
Diabetes Mellitus (%)	6 (13.3%)	5 (16.6%)	0.910
Hypertension (%)	16 (35.5%)	10 (33.3%)	0.699
Dyslipidaemia (%)	4 (8.8%)	5 (16.6%)	0.721
(BMI) (kg/m^2)	30 ± 1.7	29.6 ± 1.9	0.271

Data are presented as mean ± standard deviation. CSF = coronary slow flow, BMI = body mass index

Table 2. Comparison of conventional echocardiographic parameters of coronary slow flow and control groups

Variables	Group 1 (CSF) (n = 45)	Group 2 (CONTROL) (n = 30)	*p* value
LVEF (mm)	62.5 ± 2.5	61.6 ± 2.3	0.120
IVST (mm)	10.2 ± 0.6	10 ± 0.6	0.260
LVEDd (mm)	48.3 ± 3.2	47.4 ± 1.9	0.170
LVESd (mm)	28.7 ± 1.5	28.31 ± 1.9	0.150
sPAP (mmHg)	22.3 ± 6.1	24.2 ± 3.5	0.250

Data are presented as mean ± standard deviation. LVEF = left ventricular ejection fraction, IVST = interventricular septum thickness, LVEDd = left ventricular end-diastolic diameter, LVESd = left ventricular end-systolic diameter, sPAP = systolic pulmonary artery pressure

was found to be statistically significantly higher compared to the control group (1.80 ± 0.41 ng/ml) (p = 0.016) (Table 3).

In the correlation analysis, we found a strong and positive linear relationship between TIMI frame count and serum catestatin levels (r = 0.417, p = 0.004). (Table 4).

DISCUSSION

As a result of this study, catestatin levels in patients having CSF was found to be higher compared to the patients with a normal coronary angiography. In addition to this, in CSF patients with a higher TIMI frame count, catestatin levels were found to be higher

Table 3. Comparison of hematologic and biochemical parameters of coronary slow flow and control groups

Variables	Group 1 (CSF) (n = 45)	Group 2 (CONTROL) (n = 30)	*p* value
Leukocytes, /mm3	6,860 ± 1,161	6,423 ± 940	0.90
Hemoglobin, g/dL	14.2 ± 1.2	14 ± 1.2	0.470
Platelet, 10^3 /mm^3	249,620 ± 56,100	252,200 ± 36,210	0.820
Glucose, mg/dL	101 ± 23.8	96.3 ± 21	0.380
Urea, mg/dL	16.2 ± 5.1	17.3 ± 4.1	0.340
Creatinin, mg/dL	0.78 ± 0.1	0.84 ± 0.1	0110
Sodium, mg/dL	138.8 ± 2	141.9 ± 1.8	0.250
Potassium,mg/dL	4.1 ± 0.3	4 ± 0.3	0.120
AST, U/L	27.6 ± 7.8	28.6 ± 5.1	0.970
ALT, U/L	25.8 ± 8.2	25.9 ± 6.4	0.570
MPV, f/L	8.04 ± 0.9	7.55 ± 0.7	**0.01**
CRP, mg/dL	1.2 ± 0.6	1.2 ± 0.6	0.970
Catestatin, ng/ml	2.10 ± 0.6	1.80 ± 0.4	**0.016**

Data are presented as mean ± standard deviation. AST = aspartate transaminase, ALT = alanine transaminase, MPV = mean platelet volume, CRP = c-reactive protein

Table 4. Correlatioon between TIMI frame count and catestatin levels

		TIMI Frame Count	Serum Catestatin Levels
TIMI Frame Count	Pearson Correlation	1	0.417
	Sig. (2-tailed)		**0.004**
	N	45	45
Serum Catestatin Levels	Pearson Correlation	0.417	1
	Sig. (2-tailed)	**0.004**	
	N	45	45

**Correlation is significant at the 0.01 level (2-tailed). TIMI = Thrombolysis in Myocardial Infarction, N = number of the patients (Group 1)

than the patients having a lower TIMI frame count.

Endothelium normally secretes vasoconstrictor and vasodilator substances to regulate blood flow. In some situations this regulation is impaired and coronary blood flow is adversely affected.

While still carrying some question marks in its etiopathogenesis, CSF is a phenomenon characterized by late opacification of distal vasculartur of the epicardial coronary arteries without occlusive disease. Various mechanisms have been proposed for CSF phenomenon in terms of underlying pathology such as endothelial dysfunction, occlusive disease of small coronary arteries, increased vasomotor resistance [2-5].

Increased ET-1 concentrations in the small coronary arteries, increase coronary resistance by vasoconstrictive effect and cause myocardial ischemia in patients having angina pectoris and normal coronary angiography [13, 14].

Catestatin is a 21 aminoasitic peptide formed as a result of the hydrolysis of chromogranin A and is stored with catecholamines and released via exocytosis [15, 16]. Catestatin inhibites the catecholamine release, via non-competitive blockage of nicotinic receptors. It reverses ET-1's positive inotropic, lusinotropic and vasospastic effect on coronary arteries [9, 10].

Elevated serum catestatin levels have been shown in many studies in situations where catecholamine release is increased such as heart failure and myocardial infarction. In a study patients having heart failure, the group having NYHA (NewYork Heart Association) class 3-4 symptoms have significantly higher serum catestatin levels compared to the control group and group with NYHA class 1-2 symptoms [18, 19].

Besides, catestatin has cardioprotective effects on the cardiovascular system and these effects was shown in some studies. Catestatin's cardioprotective effect mainly occurs as a negative inotropic and antihypertansive effect via Ca++ metabolism on myocardium. In a study by Penna C *et al*. [19] in 2010, catestatin was found to improve post-infarction LV function and to decrease ischemia-reperfusion injury. It also has cardioprotective effect on endothelium. Catestatin blocks vasoconstructive effect of endothelin by inhibiting ET-1 receptor and causes vasodilation by two separate pathways; by stimulating histamin release via H1 receptor and by stimulating nitric oxide (NO) synthetase leading to NO formation.

There are studies suggesting that endothelin and catecholamines are associated with slow coronary flow. Yazıcı *et al*. [20] investigated the relationship between plasma ET-1 levels and corrected TIMI frame counts in patients with CSF. They found that the corrected TIMI frame counts measured in LAD and Cx arteries was independent predictor of ET-1 level in patients with CSF. This result implies that the increase in ET-1 level may be responsible for increase in corrected TIMI frame count or CSF [20]. In another study, it was aimed to investigate the role of adrenergic activity in patients with CSF and its relationship to TIMI frame count on the pathogenesis of CSF. Correlation analysis established that both adrenalin and noradrenalin levels were correlated with TIMI frame counts of LAD and Cx arteries. Higher noradrenalin and adrenalin levels and correlation between TIMI frame count and ischemia in patients

with CSF suggest that increased adrenergic activity may be the manifestation of coronary slow flow [21].

In our study, catestatin levels were found to be significantly higher in the CSF patient group. In addition, in the CSF patient group, as the TIMI frame count increased, plasma catestatin levels increased as well. On the other hand, MPV was also significantly higher in patients with coronary slow flow according to literature [22]. Examining the pathogenesis of CSF, one of the possible causes is considered to be vasoconstruction at microvascular level. In this patient group, catestatin may be trying to reverse the vasoconstrictive effect of ET-1 and catecholamines and catestatin levels may be found increased in proportion to the severity of the disease. Besides, elevated catestatin levels may have a predictive value in understanding the severity of the disease in CSF. On the other hand, elevated catestatin levels can be considered as an indirect indicator of an increased catecholaminergic activity and increased coronary vasospastic presentation in this group. However, catestatin may be considered to be one of the factors affecting microvascular circulation. There is a need for more extensive studies.

CONCLUSION

Many reasons that might cause endothelium disfunction, are considered responsible for CSF pathophsiology. Increased catecholaminergic activity and sympathatic system activity are possible reasons of CSF vasospastic period. In our study, we found serum catestatin levels to be high in CSF patients. In addition to this, we also found that an increase in TIMI frame count is related with elevated catestatin levels in the CSF patient group. Plasma catestatin levels can be an indirect indicator of increased catecholaminergic activity and increased coronary vasospastic presentation. On the other hand, elevated serum catestatin levels in CSF may have a predictive value in understanding the severity of the disease.

Authorship Declaration

All authors listed meet the authorship criteria according to the latest guidelines of the International Committee of Medical Journal Editors, and all authors are in agreement with the manuscript.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

REFERENCES

[1] Tambe AA, Demany MA, Zimmerman HA, Mascarenhas E. Angina pectoris and slow flow velocity of dye in coronary arteries --a new angiographic finding. Am Heart J 1972;84:66-71.
[2] Lierde JV, Vrolix M, Sionis D, De Geest H, et al. Piessens J. Lack of evidence for small vessel disease in a patient with 'slow dye progression' in the coronary arteries. Cathet Cardiovasc Diagn 1991;23:117-20.
[3] Fung MM, Salem RM, Mehtani P, Thomas B, Lu CF, Perez B, et al. Direct vasoactive effects of the chromogranin A (CHGA) peptide catestatin in humans in vivo. Clin Exp Hypertens 2010;32: 278-87.
[4] Mahapatra NR. Catestatin is a novel endogenous peptide that regulates cardiac function and blood pressure. Cardiovasc Res 2008;80:330-8.
[5] Brunner F, Bras-Silva C, Cerdeira AS,Leite-Moreira AF. Cardiovascular endothelins: essential regulators of cardiovascular homeostasis. Pharmacol Ther 2006;111:508-31.
[6] Luscher TF, Tanner FC. Endothelial regulation of vascular tone and growth. Am J Hypertens 1993;6(7 Pt 2):283-93.
[7] Tamirisa P, Frishman WH, Kumar A. Endothelin and endothelin antagonism: roles in cardiovascular health and disease. Am Heart J 1995;130(3 Pt 1):601-10.
[8] Mahata SK, O'Connor DT, Mahata M, Yoo SH, Taupenot L, Wu H, et al. Novel autocrine feedback control of catecholamine release. A discrete chromogranin a fragment is a noncompetitive nicotinic cholinergic antagonist. J Clin Invest 1997;100:1623-33.
[9] Angelone T, Quintieri AM, Brar BK, Limchaiyawat PT, Tota B, Mahata SK, et al. The antihypertensive chromogranin A peptide catestatin acts as a novel endocrine/paracrine modulator of cardiac inotropism and lusitropism. Endocrinology 2008;149:4780-93.
[10] Liu L, Ding W, Li R, Ye X, Zhao J, Jiang J, et al. Plasma levels and diagnostic value of catestatin in patients with heart failure. Peptides 2013;46:20-5.
[11] Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, et al. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. J Am SocEchocardiogr 1989;2:358-67.
[12] Gibson CM, Cannon CP, Daley WL, Dodge JT Jr, Alexander B Jr, Marble SJ, et al. TIMI frame count: a quantitative method of assessing coronary artery flow. Circulation 1996;93:879-88.
[13] Kaski JC, Elliott PM, Salomone O, Dickinson K, Gordon D, Hann C, et al. Concentration of circulating plasma endothelin in patients with angına and normal coronary angiograms. Br Heart J 1995;74:620-4.
[14] Kaski JC, Cox ID, Crook JR, Salomone OA, Fredericks S, Hann C, et al. Differential plasma endothelin concentrations in subgroups of patients with angına and angiographically normal coronary arteries: Coronary artery Disease Research Group. Am Heart J 1998;136:412-7.
[15] Taupenot L, Harper KL, O'Connor DT. Mechanisms of disease: the chromogranin-secretogranin family. N Engl J Med 2003;348:1134-49.

[16] Helle KB, Corti A, Metz-Boutigue MH, Tota B. The endocrine role for chromogranin A: a prohormone for peptides with regulatory properties. Cell Mol Life Sci 2007;64:2863-86.
[17] Schiffrin EL, Deng LY, Larochelle P. Effects of a beta-blocker or a converting enzyme inhibitor on resistance arteries in essential hypertension. Hypertension 1994;23:83-91.
[18] Zhu D, Xie H, Wang X, Liang Y, Yu H, Gao W. Correlation of plasma catestatin level and the prognosis of patients with acute myocardial infarction. PLoS One 2015;10:e0122993.
[19] Penna C, Alloatti G, Gallo PM, Cerra MC, Levi R, Tullio F, et al. Catestatin improves post-ischemic left ventricular function and decreases ischemia/reperfusion injury in heart. Cell Mol Neurobiol 2010;30:1171-9.
[20] Yazıcı M, Balcı B, Demircan S, Yeşildağ O, Sağkan O, Şahin M, et al. [Relationship between plasma endothelin-1 levels and corrected TIMI frame count in patients with slow coronary artery flow]. Türk Kardiyoloji Dern Arş 2002;30:466-72. [Article in Turkish]
[21] Yazıcı M, Demircan S, Durna K, Şahin M. The role of adrenergic activity in slow coronary flow its relationship to TIMI frame count. Angiology 2007;58:393-400.
[22] Nurkalem Z, Alper AT, Orhan AL, Zencirci AE, Sari I, Erer B, et al. Mean platelet volume in patients with slow coronary flow and its relationship with clinical presentation. Turk Kardiyol Dern Ars 2008;36:363-7.