The relationship between coronary slow flow phenomenon and urotensin-II: A prospective and controlled study

Halit Zengin, Ali Rıza Erbay[1], Ali Okuyucu, Hasan Alaçam*, Serkan Yüksel, Murat Meriç, Korhan Soylu, Ömer Gedikli[2], Naci Murat**, Okan Gülel, Sabri Demircan[3], Filiz Akın[4], Özcan Yılmaz, Mahmut Şahin*

Department of Cardiology and *Biochemistry, Faculty of Medicine, **Department of Industry Engineering, Faculty of Engineering, Ondokuz Mayıs University; Samsun-*Turkey*

[1]Clinic of Cardiology, Bitlis State Hospital; Bitlis-*Turkey*

[2]Clinic of Cardiology, Artvin State Hospital; Artvin-*Turkey*

[3]Department of Cardiology, Faculty of Medicine, İstanbul Bilim University; İstanbul-*Turkey*

[4]Clinic of Cardiology, Kastamonu State Hospital; Kastamonu-*Turkey*

Abstract

Objective: The underlying mechanism of coronary slow flow (CSF) has not yet been clarified, although many studies have been conducted to understand its pathophysiology. In this study, we investigated the role of a very potent vasoconstrictor, urotensin-II (UII), in the pathophysiology of CSF. This prospective and controlled investigation aimed to evaluate the association between CSF and serum levels of UII.

Methods: Our study included 32 patients with slow flow in any coronary artery and 32 patients with normal coronary arteries. Coronary flow was calculated using the Thrombolysis in Myocardial Infarction (TIMI) frame count (TFC) method, and CSF was defined as TFC ≥39 for the left anterior descending artery, TFC ≥27 for the circumflex coronary artery, and TFC ≥24 for the right coronary artery. UII levels in blood samples obtained from both groups were measured by enzyme-linked immunosorbent assay (ELISA) method.

Results: UII levels were significantly higher in the CSF group than in the control group [122 pg/mL (71-831), 95 pg/mL (21-635), respectively; p<0.001]. High-density lipoprotein (HDL) levels were lower in the CSF group, and leukocyte counts were significantly higher. A positive correlation between UII and mean TFC (r=0.524, p=0.002) was found in the CSF group. The multivariate logistic regression analysis determined that UII, HDL, and cigarette smoking were independent indicators in predicting CSF (OR=1.010, 95% confidence interval 1.002-1014, p=0.019; OR=0.927, 95% confidence interval 0.869-0.988, p=0.019; OR=5.755, 95% confidence interval 1.272-26.041, p=0.021, respectively).

Conclusion: Serum UII levels were found to be significantly higher in the CSF group, suggesting that UII may be one of the underlying factors in the pathogenesis of CSF. *(Anatol J Cardiol 2015; 15: 475-9)*

Keywords: coronary slow flow, urotensin-II, TIMI frame count

Introduction

Coronary slow flow (CSF), defined as delayed circulation in the coronary arteries in the absence of an obstructive lesion, was first identified in 1972 by Tambe et al. (1). Vasomotor disorders, oxygen-hemoglobin dissociation, microvascular disease, metabolic disorders of myocardial cells, and endothelial dysfunction have all been implicated in its etiopathogenesis (2-4). Currently, circulatory disorder of the capillary system is mainly emphasized (5). Coronary angiography is the gold standard in the diagnosis of CSF, and coronary flow rate is quantitatively evaluated using the Thrombolysis in Myocardial Infarction (TIMI) frame count (TFC) method (6).

The endothelium performs several tasks, such as providing vascular homeostasis, adjusting vascular tone and permeability, and regulating inflammatory responses and angiogenesis. The normal function of the endothelium depends on the balance between endothelium-derived vasoconstrictor and vasodilator agents (7).

Urotensin-II (UII) is released from the endothelium as the most powerful vasoconstrictor peptide known. When compared with endothelin-1 (ET-1), it has 50-fold and 10-fold greater vasoconstrictor effects on arteries and veins, respectively (8). Plasma UII levels have been found to be high in the presence of hypertension, renal failure, congestive heart failure, diabetes, portal hypertension, and atherosclerosis (9-13). Although UII levels might be elevated in hypertension and coronary artery disease patients, there are no data available regarding whether serum UII levels are elevated in CSF patients (14). This prospective and controlled investigation aimed to evaluate the association between CSF and serum UII levels.

Address for Correspondence: Dr. Halit Zengin, Ondokuz Mayıs Üniversitesi Tıp Fakültesi, Kardiyoloji Anabilim Dalı, Samsun-*Türkiye*
Phone: +90 362 312 19 19-2641 Fax: +90 362 457 71 46 E-mail: drhzengin@yahoo.com.tr

Accepted Date: 16.04.2014 **Available Online Date:** 28.04.2014

DOI:10.5152/akd.2014.5481

Methods

Patients with angiographically normal coronary arteries who underwent coronary angiography on suspicion of ischemic heart disease due to typical chest pain or ischemic findings on a treadmill exercise test or myocardial scintigraphy were included in this prospective study. All patients in both groups were selected from patients in whom elective coronary angiography was performed. None of the medications was discontinued before angiography.

The study group included 32 consecutive patients with CSF despite angiographically normal coronary arteries, and the control group included 32 consecutive patients with angiographically normal coronary arteries without CSF. Patients with a medical history of coronary artery disease, heart failure, uncontrolled hypertension, myocardial bridge, valvular heart disease, renal or hepatic dysfunction, acute coronary syndrome, spastic angina, or systemic disorders were excluded from the study.

Following coronary angiography, blood samples of the patients were tested for creatinine, glucose, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, hemoglobin, and leukocyte count. The clinical and demographic data of the patients were obtained from the hospital's database (Table 1).

The Ondokuz Mayıs University Medical Research Ethical Committee approved this study in 2013 (OMUKAEK2013/248).

Determination of Thrombolysis in Myocardial Infarction (TIMI) frame count (TFC)

Selective coronary angiography was performed using the standard Judkins technique at 15 frames/s in multiple angulated views. The left anterior descending coronary artery (LAD) and left circumflex coronary artery (CX) were visualized in a right anterior oblique projection with caudal angulations, and the right coronary artery (RCA) was visualized in a left anterior oblique projection with cranial angulations. During the coronary angiography, iopromide (Ultravist 370; Schering AG, Berlin, Germany) was used as the contrast agent in all patients. Other agents, such as nitrate, verapamil, and nicorandil, were not administrated.

TFC was calculated using the method of Gibson et al. (15). The first frame was defined as the frame in which the opaque material entered the coronary artery ostium, and the last frame was defined as the frame that was needed for imaging the distal landmark by the opaque material. The difference between the first and last frames was considered the TFC. Normal TFC values for the LAD, CX, and RCA were accepted as 36.2±2.6, 22.2±4.1, and 20.4±3.0 frames within 30 frames/s, respectively (at 30

Table 1. Demographic and clinical characteristics of the groups

	Slow coronary flow (n=32)			Normal coronary artery (n=32)			
	n	%	Mean±SD/Median (min-max)	n	%	Mean±SD/Median (min-max)	*P*
Clinical and hemodynamic parameters							
Age, years			57±10			54±9	0.25
Sex							
Male	17	53		18	56		0.81
Female	15	47		14	44		
Diabetes mellitus	7	22		10	34		0.27
Hypertension	18	56		18	62		0.64
Hyperlipidemia	16	50		8	28		0.07
Smoking	13	40		8	28		0.28
Systolic blood pressure (mm Hg)			130 (120-160)			137.5 (100-155)	0.706
Diastolic blood pressure (mm Hg)			80 (60-95)			80 (60-95)	0.21
Baseline medications							
Angiotensin-converting enzyme inhibitor	11	34.4		10	37		0.83
Angiotensin II receptor blocker	5	16		4	15		0.93
Beta-blocker	7	22		6	22		0.97
Calcium channel blocker	6	19		5	19		0.98
Aspirin	10	31.3		5	18.5		0.26
Statin	10	31.3		5	18.5		0.26
Thienopyridines	2	6.5		1	3.7		0.63

Mann-Whitney U test and chi-square test were used

frames/s) (15). Our images were acquired at 15 frames/s; therefore, all values were multiplied by 2.

Analysis of urotensin-II (UII)

Preparation of the samples

The bloods samples were collected into test tubes, and whole blood was allowed to clot at room temperature for 30 minutes. Then, the samples were centrifuged at 3000 x g for 10 minutes at 4°C. Following centrifugation, the serum was removed and transferred to a clean tube. All samples were stored at -80°C until analysis. The day before the study, all samples were dissolved at 2-8°C.

Measurement of serum human urotensin-II (UII) levels

UII levels were analyzed according to the manufacturer's instructions (Hangzhou Eastbiopharm Co. Ltd., Hangzhou, China). The principle of the test is a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). The plate was pre-coated with an antibody specific to UII, and the samples were added to the wells with a biotin-conjugated antibody specific to UII. Next, avidin-conjugated horseradish peroxidase was added to the wells and incubated. After chromogen solutions were added, the enzyme-substrate reaction was terminated by the addition of acid solution, and the color change was measured photometrically at 450 nm. The concentration of UII in the samples was determined by a standard curve, and the results were presented in pg/mL.

Statistical analysis

Based on studies carried out by two-sample t-test power analysis in order to calculate sample size, the testing power was 99.9% with 32 observation values from each group. Data were analyzed using IBM SPSS, version 21. The Kolmogorov-Smirnov test was used to analyze the normal distribution of the variables ($p>0.05$). Results are expressed as mean and standard deviation (SD) for normal distribution or median (min-max) for non-normal distribution. The independent t-test was used to compare continuous data of normal distribution, and the Mann-Whitney U test was used in cases of non-normal distribution. The chi-square test was used for categorical variables, and correlations between quantitative data were analyzed by Spearman's correlation test. Multivariate logistic regression analysis was used to find independent covariates for CSF. P values <0.05 were considered significant.

Results

Our study included 32 CSF and 32 normal coronary artery (NCA) patients. Both groups showed similar demographic and clinical characteristics (Table 1). In the CSF group, 13 patients had slow flow in all three vessels, two patients had slow flow in the LAD and CX, three patients had slow flow in the CX and RCA, three patients had slow flow in the LAD and RCA, five patients had slow flow in the LAD only, three patients had slow flow in the CX only, and three patients had slow flow in the RCA only.

Table 2. UII concentrations of groups

	Slow coronary flow (n=32) Median (min-max)	Normal coronary artery (n=32) Median (min-max)	*P*
Urotensin II, pg/mL	122 (71-831)	95 (21-635)	<0.001
UII - urotensin II; Mann-Whitney U-test was used			

Table 3. Biochemical and hematological parameters of the groups

	Slow coronary flow (n=32) Mean±SD/Median (min-max)	Normal coronary artery (n=32) Mean±SD/Median (min-max)	*P*
Fasting serum glucose (mg/dL)	99 (65-171)	101 (76-201)	0.17
Creatinine (mg/dL)	0.84 (0.5-1.3)	0.8 (0.5-1.9)	0.57
Total cholesterol (mg/dL)	192±30	190±33	0.76
LDL cholesterol (mg/dL)	123±26	108±30	0.056
HDL cholesterol (mg/dL)	40±10	48±15	0.03
Triglycerides (mg/dL)	133 (67-409)	147 (46-310)	0.54
Hemoglobin (g/dL)	13.7±1.3	13.4±1.5	0.42
Leukocytes ($10^3/mm^3$)	8.6±2.1	7.4±1.7	0.02
HDL - high-density lipoprotein; LDL - low-density lipoprotein. Independent t-test and Mann-Whitney U-test were used			

UII levels were found to be significantly higher in the CSF group compared with the NCA group [$p<0.001$; 122 (71-831) pg/mL and 95 (21-635) pg/mL, respectively] (Table 2). The UII levels in the CSF group were significantly higher in patients with three-vessel slow flow than in patients with one- or two-vessel slow flow (mean 388 pg/mL, 188 pg/mL, $p=0.004$).

HDL levels were significantly lower in the CSF group, and leukocyte counts were significantly higher (Table 3). A positive correlation was found between UII and mean TFC ($r=0.524$, $p=0.002$) in the CSF group. There was no significant correlation between urotensin-II levels and systolic blood pressure in these patients ($r=0.187$, $p=0.155$).

The independent variables that were used for the multivariate logistic regression analysis were: sex, diabetes mellitus, hypertension, hyperlipidemia, smoking, total cholesterol, low-density lipoprotein, high-density lipoprotein, urotensin, and systolic blood pressure. The multivariate logistic regression analysis suggested that UII, HDL, and cigarette smoking were independent indicators in predicting CSF (OR=1.010, 95% confidence interval 1.002-1014, $p=0.019$; OR=0.927, 95% confidence interval 0.869-0.988, $p=0.019$; OR=5.755, 95% confidence interval 1.272-26.041, $p=0.021$, respectively).

Discussion

This investigation showed a significant positive correlation between UII and CSF. Previous studies have suggested an impaired balance between vasoconstrictor and vasodilator factors in the etiology of CSF. High levels of ET-1 may slow down circulation by increasing coronary resistance (16). Çelebi et al. (17) encountered higher levels of plasma ET-1 in CSF patients than in normal coronary artery diseases. ET-1 that is released from the endothelium due to ischemia/reperfusion injury may cause intensive and continuous microvascular constriction. It has been stated that ET-1 levels may predict no-reflow after primary percutaneous coronary intervention (PCI) in patients with acute ST elevation (18). UII has a vasoconstrictive effect that is 50 times greater than ET-1; however, whether UII plays a role in the pathophysiology of CSF has not yet been investigated. To the best of our knowledge, this investigation is the first to reveal the association between UII and CSF.

The heart is one of the major sources of UII (19). UII causes vasoconstriction in endothelium-denuded coronary arteries in various species, including rats, cynomolgus monkeys, dogs, and humans (20, 21). UII may play an etiological role in the development of essential hypertension, due to its potent vasoconstrictive characteristics (22). UII is also associated with left ventricular hypertrophy, fibrosis, and myocardial infarction (23). The expression of UII is upregulated in the endothelial, myointimal, and medial smooth muscle cells of atherosclerotic human coronary arteries (24). Ban et al. (25) demonstrated a positive correlation between UII levels and systolic blood pressure and carotid intima-media thickness. Our findings suggest that UII may also lead to CSF through a similar mechanism with ET-1.

Chai et al. (26) encountered higher levels of UII and endothelin in coronary artery disease (CAD) patients than in a healthy control group. In addition, UII levels after PCI were found to be higher than pre-procedure levels in CAD. The expression of UII was found to be significantly higher after balloon angioplasty in rats. In addition, intima thickness was found to be lower in patients treated with a selective UII receptor antagonist following angioplasty than in a control group (27). The increased level of UII following balloon angioplasty might be due to impaired coronary circulation due to microembolization. In our investigation, we also found that occlusion of small vessels might cause CSF due to high levels of UII, through a similar mechanism in which increased microvascular resistance is considered responsible for CSF.

Hawkins et al. (28) reported that CSF patients were more obese and had lower HDL levels than a control group. They also stated that male sex and BMI were independent indicators in predicting CSF (28). In another investigation, TFC was found to be higher in cigarette smokers than in non-smokers (17). In our investigation, HDL levels were significantly lower in the CSF group. In addition, low HDL levels and cigarette smoking were determined to be independent indicators in predicting CSF. Both low HDL level and cigarette smoking are risk factors for CAD. These findings suggest that CSF may be a diffuse microvascular atherosclerotic disease.

Higher plasma/serum CRP, IL-6, and uric acid concentrations have been found in CSF patients than in patients with normal coronary diseases (29-31). Similarly, Xai et al. (32) found higher levels of postprandial blood glucose, platelet count, and hs-CRP in their CSF group compared with their control group. They determined that hs-CRP is an independent predictor of CSF and that inflammation may be one of the essential factors of CSF. UTII may also play a role in inflammation (22). Similarly, in our investigation, leukocyte count was higher in the CSF group than in the control group.

Study limitations

Medical conditions that may affect UII, like hyperlipidemia, metabolic syndrome, and diabetes, were not excluded from the study, but their distributions were similar in both groups. We conducted our investigation on a small group; therefore, the findings need to be supported by larger randomized, control studies.

Conclusion

The higher levels of UII in the CSF group suggest that UII may be one of the underlying factors in the pathogenesis of CSF.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

Authorship contributions: Concept - H.Z., A.R.E., F.A.; Design - A.R.E., O.G.; Supervision - M.M., K.S.; Resource - Ö.G., O.G., S.D.; Materials - H.A., A.O.; Data collection &/or processing - H.A., A.O., F.A.; Analysis &/or interpretation - N.M., M.M., K.S.; Literature search - S.Y., M.Ş.; Writing - H.Z., S.Y.; Critical review - N.M., O.G., S.D.; Other - Ö.Y., M.Ş.

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. [CrossRef]
2. Wilson RF, White CW. Intracoronary papaverine: an ideal coronary vasodilator for studies of the coronary circulation in conscious humans. Circulation 1986; 73: 444-51. [CrossRef]
3. Vrints C, Herman AG. Role of the endothelium in the regulation of coronary artery tone. Acta Cardiol 1991; 46: 399-418.
4. Mosseri M, Yarom R, Gotsman MS, Hasin Y. Histologic evidence for small-vessel coronary artery disease in patients with angina pectoris and patent large coronary arteries. Circulation 1986; 74: 964-7. [CrossRef]
5. Kaski JC, Tousoulis D, Galassi AR, McFadden E, Pereira WI, Crea F, et al. Epicardial coronary artery tone and reactivity in patients with normal coronary arteriograms and reduced coronary flow reserve (Syndrome X). J Am Coll Cardiol 1991; 18: 50-4. [CrossRef]
6. Sudhir K, Mullen WL, Hausmann D, Fitzgerald PJ, Chou TM, Yock PG, et al. Contribution of endothelium-derived nitric oxide to coro-

nary arterial distensibility: an in vivo two-dimensional intravascular ultrasound study. Am Heart J 1995; 129: 726-32. [CrossRef]
7. Kharbanda RK, Deanfield JE. Functions of the healthy endothelium. Coron Artery Dis 2001; 12: 485-91. [CrossRef]
8. Maguire JJ, Kuc RE, Davenport AP. Orphan-receptor ligand human urotensin II: receptor localization in human tissues and comparison of vasoconstrictor responses with endothelin-1. Br J Pharmacol 2000; 131: 441-6. [CrossRef]
9. Ong KL, Lam KS, Cheung BM. Urotensin II: its function in health and its role in disease. Cardiovasc Drugs Ther 2005; 19: 65-75. [CrossRef]
10. Lim M, Honisett S, Sparkes CD, Komesaroff P, Kompa A, Krum H. Differential effect of urotensin II on vascular tone in normal subjects and patients with chronic heart failure. Circulation 2004; 109: 1212-4. [CrossRef]
11. Erbay AR, Meriç M, Alacam H, Zengin H, Akın F, Okuyucu A, et al. Serum urotensin II Levels in patients with non-dipper hypertension. Clin Exp Hypertens 2013; 35: 506-11. [CrossRef]
12. Nar G, Soylu K, Akçay M, Gülel O, Yüksel S, Meriç M, et al. Evaluation of the relationship between arterial blood pressure, aortic stiffness and serum Endothelin-1 levels in patients with essential hypertension. Clin Exp Hypertens 2013; 35: 589-94. [CrossRef]
13. Bousette N, Patel L, Douglas SA, Ohlstein EH, Giaid A. Increased expression of urotensin II and its cognate receptor GPR14 in atherosclerotic lesions of the human aorta. Atherosclerosis 2004; 176: 117-23. [CrossRef]
14. Heringlake M, Kox T, Uzun O, Will B, Bahlmann L, Klaus S, et al. The relationship between urotensin II plasma immunoreactivity and left ventricular filling pressures in coronary artery disease. Regul Pept 2004; 121: 129-36. [CrossRef]
15. 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. [CrossRef]
16. Kaski JC, Cox ID, Crook JR, Salomone OA, Fredericks S, Hann C, et al. Differential plasma endothelin levels in subgroups of patients with angina and angiographically normal coronary arteries. Coronary Artery Disease Research Group. Am Heart J 1998; 136: 412-7. [CrossRef]
17. Çelebi H, Çatakoğlu AB, Kurtoğlu H, Şener M, Hanavdeloğulları R, Demiroğlu C, et al. The relation between coronary flow rate, plasma endothelin-1 concentrations, and clinical characteristics in patients with normal coronary arteries. Cardiovasc Revasc Med 2008; 9: 144-8. [CrossRef]
18. Niccoli G, Lanza GA, Shaw S, Romagnoli E, Gioia D, Burzotta F, et al. Endothelin-1 and acute myocardial infarction: a no-reflow mediator after successful percutaneous myocardial revascularization. Eur Heart J 2006; 27: 1793-8. [CrossRef]
19. Charles CJ, Rademaker MT, Richards AM, Yandle TG. Urotensin II: evidence for cardiac, hepatic and renal production. Peptides 2005; 26: 2211-4. [CrossRef]
20. Douglas SA, Sulpizio AC, Piercy V, Sarau HM, Ames RS, Aiyar NV, et al. Differential vasoconstrictor activity of human urotensin-II in vascular tissue isolated from the rat, mouse, dog, pig, marmoset and cynomolgus monkey. Br J Pharmacol 2000; 131: 1262-74. [CrossRef]
21. Bottrill FE, Douglas SA, Hiley CR, White R. Human urotensin-II is an endothelium-dependent vasodilator small arteries. Br J Pharmacol 2000; 130: 1865-70. [CrossRef]
22. Pakala R. Role of urotensin II in atherosclerotic cardiovascular diseases. Cardiovasc Revasc Med 2008; 9: 166-78. [CrossRef]
23. Hunt SA, Baker DW, Chin MH, Cinquegrani MP, Feldman AM, Francis GS, et al. ACC/AHA Guidelines for the evaluation and management of chronic heart failure in the adult:executive summary. Circulation 2001; 104: 2996-3007. [CrossRef]
24. Hassan GS, Douglas SA, Ohlstein EH, Giaid A. Expression of urotensin-II in human coronary atherosclerosis. Peptides 2005; 26: 2464-72. [CrossRef]
25. Ban Y, Watanabe T, Suguro T, Matsuyama TA, Iso Y, Sakai T, et al. Increased plasma urotensin-II and carotid atherosclerosis are associated with vascular dementia. J Atheroscler Thromb 2009; 16: 179-87. [CrossRef]
26. Chai SB, Li XM, Pang YZ, Qi YF, Tang CS. Increased plasma levels of endothelin-1 and urotensin-II in patients with coronary heart disease. Heart Vessels 2010; 25: 138-43. [CrossRef]
27. Rakowski E, Hassan GS, Dhanak D, Ohlstein EH, Douglas SA, Giaid A. A role for urotensin II in restenosis following balloon angioplasty: use of a selective UT receptor blocker. J Moll Cell Cardiol 2005; 39: 785-91. [CrossRef]
28. Hawkins BM, Stavrakis S, Rousan TA, Abu-Fadel M, Schechter E. Coronary slow flow-prevalence and clinical correlations. Circ J 2012; 76: 936-42. [CrossRef]
29. Kalay N, Aytekin M, Kaya MG, Özbek K, Karayakalı M, Söğüt E, et al. The relationship between inflammation and slow coronary flow: increased red cell distribution width and serum uric acid levels. Turk Kardiyol Dern Ars 2011; 39: 463-8. [CrossRef]
30. Li JJ, Qin XW, Li ZC, Zeng HS, Gao Z, Xu B, et al. Increased plasma C-reactive protein and interleukin-6 concentrations in patients with slow coronary flow. Clin Chim Acta 2007; 385: 43-7. [CrossRef]
31. Madak N, Nazlı Y, Mergen H, Aysel S, Kandaz M, Yanık E, et al. Acute phase reactants in patients with coronary slow flow phenomenon. Anadolu Kardiyol Derg 2010; 10: 416-20. [CrossRef]
32. Xia S, Deng SB, Wang Y, Xiao J, Du JL, Zhang Y, et al. Clinical analysis of the risk factors of slow coronary flow. Heart Vessels 2011; 26: 480-6. [CrossRef]