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Inquiring about the link between urotensin-II and coronary collateral development in coronary artery patients with and without diabetes

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ABSTRACT

Aims: Coronary collateral circulation consists of vascular channels activated to maintain perfusion in major epicardial coronary arteries in severe stenosis or occlusion. Yet, coronary collateral development (CCD) in diabetic patients was previously proven to be poor. Urotesin-II (U-II) is famous for being the most potent vasoconstrictor agent, and plasma levels are known to elevate in diabetic patients and play an important role in diabetic complications. In this study, we inquired about the link between U-II levels and the development of coronary collaterals between diabetic and non-diabetic patients with coronary artery disease (CAD).

Methods: We recruited 31 diabetic and 30 non-diabetic patients with 95% or more coronary artery stenosis or occlusion and considered Rentrop's classification for grading collaterals. In this sense, while Rentrop grades 0-1 are regarded as poor CCD, Rentrop grades 2-3 correspond to well-developed collaterals. Moreover, we compared the patients' serum levels of U-II by the degree of CCD.

Results: The findings revealed that demographic characteristics did not significantly differ between the groups ($p > 0.05$). Although CCD seemed worse in diabetic patients than those without diabetes (DM), the finding was not statistically significant. However, the diabetic patients had significantly higher U-II levels than non-diabetic patients (388.1±314.2 vs. 229.8±216.9, $p=0.026$). Despite not being significant, U-II levels were higher in patients with poor CCD than those with well-developed collaterals in the non-diabetic group (370.6±298; 178.6±158.3, $p=0.2$). In the diabetic group, on the other hand, U-II levels were significantly higher in patients with poor CCD and significantly lower in patients with good CCD (582.7±316.4 and 180.4±121.6, respectively; $p < 0.0001$ for both).

Conclusion: Overall, our findings demonstrated a significant association between U-II levels and the development of coronary collateral circulation in patients with DM. We also determined that U-II levels were low in diabetic patients with good CCD, while those with poor CCD had higher levels of U-II.

Keywords: Diabetes, coronary collateral circulation, urotensin II

INTRODUCTION

Coronary artery disease (CAD) is the leading cause of mortality in many countries.[1] The human body hosts numerous collateral vessels connecting the major coronary arteries, and these coronary collaterals are potential channels in the human heart. In the case of a narrowing in the coronary arteries, these channels expand depending on the pressure gradient and provide an alternative flow path.[2]

A functional endothelium is essential in the development of collateral vascular networks that varies among individuals. Besides, a plethora of research demonstrated the impacts of diabetes mellitus (DM) on endothelial functions. For example, it was shown that increased glucose levels impair the structure and proliferation of endothelial cells and cause delays in various stages of the endothelial cell cycle.[3] Moreover, it was discovered that coronary collateral development (CCD) is poor in diabetic patients.[4]

Urotensin-II (U-II) is a peptide vasoactive substance similar to somatostatin that yields a more potent effect than endothelin-1, a noteworthy vasoconstrictor. It is known to be expressed in the brain, spinal cord, kidneys, and skeletal muscle and is often found in the myocardium, atrium, ventricles, and vascular endothelial/smooth muscle cells

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in the cardiovascular system. U-II was also discovered to contribute to endothelial cell permeability and to induce endothelial cell proliferation.[5] Moreover, plasma U-II levels were found to be high in renal failure, congestive heart failure, DM, hypertension (HT), and portal HT.[6]

In this study, we inquired about the relationship between U-II levels and CCD in diabetic and non-diabetic patients with CAD.

METHODS

The study was carried out with the permission of İnönü University Clinical Researches Ethics Committee (Date: 2011, Decision No: 128). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

We conducted this study with patients who underwent coronary angiography in the cardiology department between August 2011 and February 2012 and were found to have 95% stenosis or complete occlusion in their coronary arteries.

Exclusion Criteria

a. Pre-diagnosed rheumatic valve disease,
b. Moderate and severe stenosis/failure of other valves,
c. Undergoing percutaneous coronary intervention in the last 30 days,
d. Renal and hepatic failure,
e. Less than 95% stenosis in the relevant coronary artery,
f. Undergoing coronary artery bypass surgery,
g. Having an acute or chronic infectious disease.

Initially, we obtained anamnesis from all patients, performed their physical examinations, and noted down their risk factors for CAD (e.g., DM, HT, smoking, age, and sex) and antihypertensive, antidiabetic, and statin drugs they used. We next inquired about their history of myocardial infarction (MI), coronary bypass operation, and percutaneous coronary intervention and calculated their body mass indices (BMI). Then, we performed electrocardiograms and echocardiograms on the patients. While defining the presence of DM as a fasting blood glucose level ≥ 126 mg/dl, a spot blood glucose level ≥ 200 mg/dl, and/or the use of oral antidiabetic and/or insulin, we considered HT in the case of systolic blood pressure > 140 mmHg or diastolic blood pressure > 90 mmHg, or the use of blood pressure-lowering medication. In addition, while patients smoking for the past six months or more were defined as active smokers, those having smoked in the past were regarded as quitters. Finally, hyperlipidemia was accepted as positive in the case of measurement of total cholesterol > 200 mg/dl or low-density lipoprotein (LDL) cholesterol > 100 mg/dl or the use of lipid-lowering medication.

Coronary Angiographic Evaluation

All patients underwent selective coronary angiography via the right or left femoral artery using a 6F diagnostic catheter using the Judkins technique. While preferring Philips Integris 5000 as the angiography device, we utilized Iopromide (Ultravist-370) or Iohexol (Omnipaque 350 mg/ml) as opaque material. Coronary arteries were visualized in the right and left oblique positions through cranial and caudal angulations. We took the measurements of all patients at the end-diastole, in the position where the coronary lesion was best seen and the lumen narrowed the most. Finally, we performed the angiographic grading of collaterals providing blood flow to the occluded coronary artery by Rentrop's classification.7

- **Rentrop Grade 0:** No visible filling of collaterals.
- **Rentrop Grade 1:** Very weak collateral flow without any epicardial filling of the target artery.
- **Rentrop Grade 2:** Partial epicardial filling of the target artery by opaque material through collaterals.
- **Rentrop Grade 3:** Complete epicardial filling of the target artery by opaque material through collaterals.

To measure U-II levels, we centrifuged the blood samples of the patients in 10 mL vacuum sterile K3-EDTA tubes at 5000 rpm for 10 minutes and then separated serums and plasmas. Plasma samples were collected into 1.5 mL Eppendorf tubes, stored in a deep freezer at -40 °C, and thawed on the research day. Then, we studied U-II kits (Uscn Life Science Inc. Wuhan, China, E90868Hu, 1120125000) by ELISA.

Statistical Analysis

While we present continuous variables as means and standard deviations, categorical variables are given as percentages. The pair-wise comparisons of the categorical variables were performed using Pearson's chi-square test or Fisher's exact test, and we compared continuous variables between the groups with independent samples t-test. While the relationship between U-II levels and CCD was sought using the Mann-Whitney U test and independent samples t-test in the non-diabetic and diabetic groups, respectively. All analyses were performed on SPSS 17.0 (SPSS Inc, Chicago, USA), and a p-value of <0.05 was considered statistically significant.

RESULTS

We recruited 31 diabetic patients with a mean age of 65.4±6.9 years and 30 non-diabetic patients with a mean age of 61.4±10.5 years. Of the patients, 46 (75.4%) were males, and 15 (24.6%) were females. The findings revealed no significant differences between the groups by sex, smoking, HT, MI, hyperlipidemia (including family history), and the use of beta-blockers,

angiotensin-converting-enzyme inhibitors (ACEI), statin, acetylsalicylic acid (ASA), and nitrate. In addition, no significant difference was observed between the two groups in echocardiographic measurements.(50.9±8.5 vs. 54.8±7.3; p=0.880) (**Table 1**).

Table 1. Clinical and demographic characteristics of groups

	Diabetes mellitus (+) (n=31)	Diabetes mellitus (-) (n=30)	p
Age, years,	65.4±6.9	61.4±10.5	0.873
Female Sex, n (%)	9 (29.0)	6 (20)	0.276
Hypertension, n (%)	16 (51.3)	13 (43.3)	0.912
Smoking, n (%)	12 (38.7)	15 (50)	0.597
History of myocardial infarction, n (%)	16 (51.6)	11 (36.7)	0.546
Systolic BP (mmHg)	133.8±17.4	131.5±21.9	0.347
Diastolic BP (mmHg)	82.8±10.4	80.6±12.2	0.076
CRP (mg/dl)	0.7±1.2	1.7±2.7	0.065
Serum creatinine (mg/dl)	0.91±0.5	0.88±0.4	0.865
Total cholesterol (mg/dl)	190±48	190±43	0.212
Low density lipoprotein cholesterol (mg/dl)	116±40	121±39	0.345
High density lipoprotein cholesterol (mg/dl)	35±8.5	37±9.6	0.923
Triglyceride (mg/dl)	190±165	159±106	0.413
Aspirin, n (%)	23 (74.2)	16 (53.3)	0.990
ACE-inhibitors/ARB, n (%)	10 (32.3)	6 (20.7)	0.665
Statins, n (%)	10 (32.3)	5 (16.7)	0.973
CCB, n (%)	11 (8.2)	12 (4.5)	0.298
Beta-Blocker, n (%)	13 (41.9)	15 (50)	0.869
Ejection Fraction, (%)	50.9±8.5	54.8±7.3	0.880

ACE; angiotensin converting enzyme, ARB; angiotensin receptor blocker, BP; blood pressure, CCB; calcium channel blocker;, CRP; C-reactive protein

In the diabetic group, while we detected poor CCD in 16 patients and good CCD in 15, they were discovered in 8 and 22 patients, respectively, in the non-diabetic group; however, the difference was not statistically significant (p=0.067). Moreover, the levels of CCD in the patients did not significantly differ by their CRP, uric acid, total, LDL, and high-density lipoprotein (HDL) cholesterol, and triglyceride levels, which was also the case between the diabetic and non-diabetic groups (**Table 2**). On the other hand, U-II levels were found to be significantly higher in the diabetic patients compared to non-diabetics (388.1±314.2 vs. 229.8±216.9; p=0.026; **Figure 1**). In the non-diabetic group, although we found the U-II levels of those with poor CCD (370.6±298) to be higher when compared to the patients with well-developed collaterals (178.6±158.3), the difference was not statistically significant (p=0.2). However, in the diabetic group, the U-II levels of the patients with poorly-developed collaterals (582.7±316.4) were found to be significantly higher than when compared to those with good CCD (180.4±121.6; p <0.0001; **Figure 2**).

Table 2. Demographic and clinical features according to collateral development

	Well-developed CCD (n=37)	Poor CCD (n=24)	p
Age, years,	62.1±9.8	65.5±7.2	0.512
Female Sex, n(%)	9 (24.3)	6 (25)	0.454
Hypertension, n(%)	17 (45.9)	12(50)	0.456
Smoking, n (%)	15 (40.5)	12(50)	0.675
Total cholesterol (mg/dl)	197±37	180±54	0.237
Low density lipoprotein cholesterol (mg/dl)	125±37	108±41	0.345
High density lipoprotein cholesterol (mg/dl)	36±8.8	36±9.4	0.923
Triglyceride (mg/dl)	175±152	175±120	0.413
Body mass index (kg/m²)	26.1±3.6	25.5±3.1	0.267

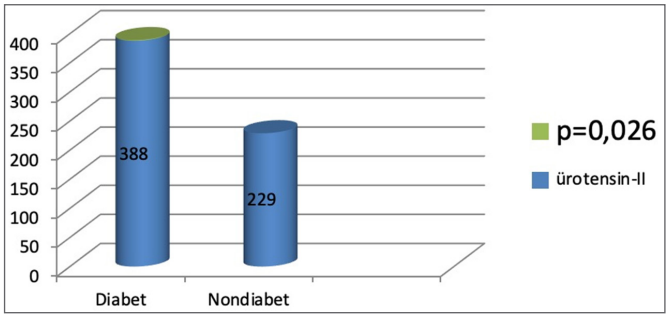

Figure 1. U-II levels were found to be significantly higher in the diabetic patients compared to non-diabetics.

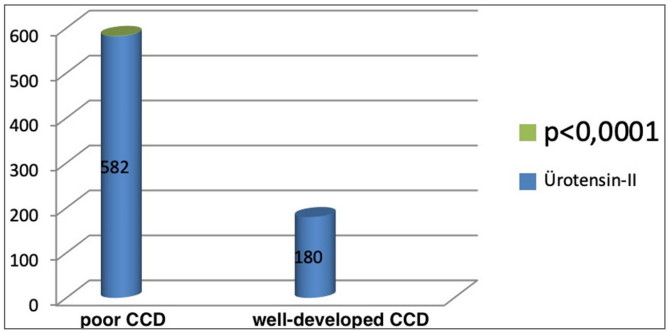

Figure 2. In the diabetic group, the U-II levels of patients with insufficient collaterals were found to be significantly higher than those with good CCD.

DISCUSSION

In this study, we investigated the relationship between U-II levels and CCD between diabetic and non-diabetic patients with CAD. While our findings showed a significant negative difference between U-II levels and CCD in diabetic patients, this difference was not statistically significant in the non-diabetic group.

CAD is the leading cause of mortality in many countries.1 Thus, the pre-detection and elimination of modifiable risk factors (e.g., DM, dyslipidemia, hypertension, obesity, and smoking) are known to reduce the risk of CAD significantly. On the other hand, severe stenosis or occlusion in the epicardial coronary arteries due to atherosclerotic or non-atherosclerotic causes results in loss of function or cell death in the myocardial

tissue supplied by the diseased coronary artery. In such situations, coronary collaterals become involved to be an alternative means of maintaining perfusion. In addition to preventing ischemia, coronary collaterals bear many beneficial effects, such as reduction of infarct area, prevention of left ventricular aneurysm development, improvement of left ventricular functions after infarction, reduction of coronary mortality, and prolongation of long-term survival. In a study by Williams et al., patients with well-developed collateral circulation had a higher ejection fraction, a lower left ventricular end-diastolic pressure, and a more limited wall motion disorder in the ischemic region.[8] The development of collateral vascular networks varies among individuals. Although the progression time and severity of coronary artery stenosis may be the most potent factors in this difference, it is also affected by DM, HT, hyperlipidemia, age, sex, drugs used, and endogenous mediators. A functional endothelium is essential in the process of CCD.

While DM is often shown to be among the significant risk factors for CAD, CAD is then the most apparent cause of morbidity and mortality in diabetic patients. Epidemiological research documented that at least 50% of deaths in diabetic patients can be ascribed to CAD.[9] It was also shown that increased glucose levels impair the structure and proliferation of endothelial cells and cause delays in various stages of the endothelial cell cycle.[10] Another factor contributing to the risk in diabetic patients is poor CCD. Endothelial dysfunction in diabetic patients stimulates negative remodeling in response to atherosclerosis. Then, the vasodilator response of endothelin to cytokines is impaired, and neovascularization and CCD remain insufficient in response to ischemia. It is also thought that Urotensin 2 may have an effect on coronary collateral by affecting angiogenesis through cell proliferation, migration and invasion, especially in diabetic patients. In many studies delving into collateral circulation, CCD was shown to be poor in diabetic patients. For example, Abaci et al. compared CCD in diabetic and non-diabetic patients and discovered lower collateral scores in the diabetic group.[11] Moreover, Islam et al.[12] compared 36 patients with DM and 50 patients without DM by CDD and found that the diabetic group demonstrated poorer CCD.4 A similar finding was reported in the study by Tatlı et al. where CCD was compared between the patients following acute MI. In our study, we found poorer CCD in the diabetic group compared to the non-diabetic group, but the difference was not statistically significant, which may be due to the small sample size in our study.

U-II is a peptide vasoactive substance with a more potent effect than endothelin-1, an important vasoconstrictor. Various studies previously showed that it bears vasoconstrictor, vasodilator, and neutral effects on vascular beds.[13] In general, while having a vasoconstrictor impact in coronary and radial arteries, it brings a vasodilator effect to pulmonary and abdominal arteries. U-II was previously shown to be expressed in the brain, spinal cord, kidneys, and skeletal muscle. It is often found in the myocardium, atrium, ventricles, and vascular endothelial/smooth muscle cells in the cardiovascular system. Plasma U-II levels correlate with congestive heart failure, essential hypertension, CAD, DM, and metabolic syndrome. The previous research consistently reported invcreased plasma levels of U-II in diabetic patients.[14] In fact, this increase occurs independently of plasma fasting glucose and Hemoglobin A1c. Totsune et al.[15] also showed increased U-II diabetic patients with normal renal functions compared to healthy individuals.

U-II expression is often found to be increased in atherosclerotic lesions in the coronary and carotid arteries and aorta.[16] Accordingly, it was suggested that elevated expression of U-II leads to vascular smooth muscle proliferation, accelerating the development of atherosclerotic plaque. In addition, it was shown that locally released U-II leads to coronary vasoconstriction and myocardial ischemia.[17] In a study, the researchers attained a positive correlation between carotid atherosclerosis and U-II levels in essential hypertensives compared to normotensives. The same study also reported positive relationships between plasma U-II levels and systolic blood pressure, carotid intima-media thickness, and plaque score.[18] In another study, patients with acute coronary syndrome had lower U-II levels than those with stable CAD and healthy controls.[19]

U-II boosts the expression of molecules, such as vascular cell adhesion protein-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and monocyte chemoattractive protein-1 (MCP-1), which are involved in the development and progression of diabetic atherosclerosis and contributes to vascular complications by promoting the expression of molecules (e.g., transforming growth factor-β (TGF-β)) that influence cell proliferation, differentiation, migration, and development.[20] Similarly, we concluded that U-II had an adverse effect on CCD in the diabetic group.

Limitations

The present study is not free of a few limitations. One of these may be related to our small sample size, and the other can be shown as the evaluation of CCD only by coronary angiography. Most collateral vessels are often 100 micrometers in diameter, but they must be above 100 micrometers to become angiographically visible. Collaterals with smaller diameters cannot be observed angiographically.

CONCLUSION

In a nutshell, we concluded a significant link between CCD and U-II levels in diabetic patients. Accordingly, while the diabetic patients with good CCD had low U-II levels, it was vice versa in those with poor CCD. U-II levels were found to be statistically significant in diabetic patients compared to the non-diabetic group. On the other hand, although non-diabetic patients with poor CCD had higher U-II levels than those with good CCD, the difference was not statistically significant. Our findings still need to be confirmed by prospective, longitudinal follow-up research with large sample sizes.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of İnönü University Clinical Researches Ethics Committee (Date: 2011, Decision No: 128).

Informed Consent: Written consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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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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