The Relationship Between Coronary Collateral Circulation and Serum Endocan Levels in Patients with Coronary Chronic Total Occlusions

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ABSTRACT

Introduction: Endocan, a dermatan sulfate proteoglycan, is synthesized and secreted by the vascular endothelium. It is involved in the pathogenesis of vascular diseases, organ-specific inflammations, and angiogenesis. The aim of the present study was to investigate the relationship between serum endocan level and development of coronary collateral circulation (CCC) in patients with stable angina pectoris.

Patients and Methods: Patients with stable angina with at least one chronic total coronary occlusion (CTO) (n= 44) and who had normal coronary artery (n= 29) were included in the study. Collateral degree was evaluated according to the Rentrop and Cohen's classification. Patients who had grade 0 or 1 collateral filling were included in the poorly developed collateral group (n= 19), and those with grade 2 or 3 collateral filling were included in the well-developed collateral group (n= 23).

Results: Endocan levels were significantly higher in patients in the well CCC group than in those in the poor CCC and control groups (p< 0.001). Endocan level is higher in patients with poorly developed CCC than in the control group. Endocan was only found as an independent predictor of well CCC in regression analysis (odds ratio 1.002, 95% confidence interval (CI) 1.001-1.005, p= 0.001). Receiver operating characteristic curve analysis detected an optimal cut-off value for endocan level of 1773.9 ng/L that predicted the presence of well development of CCC with a sensitivity of 84% and specificity of 63% (area under the receiver operating characteristic (ROC) curve: 0.749, 95% CI: 0.654-0.849, p< 0.001).

Conclusion: Elevated endocan level is associated with better CCC in patients with CTO. Endocan may play an important role in the development of CCC.

Key Words: Endocan; coronary collateral circulation; angiogenesis; coronary artery disease

Koroner Kronik Total Oklüzyonu Olan Hastalarda Serum Endokan Düzeyi ile Koroner Kollateral Dolaşım Arasındaki İlişki

ÖZET

Giriş: Bir dermatan sülfat proteoglikan olan endokan, vasküler endotelyum tarafından sentezlenir ve salgılanır ayrıca vasküler hastalıkların, organ-spesifik inflamasyon ve anjiyogenezin patogenezinde rol oynar. Çalışmamızda, stabil angina pektorisli hastalarda serum endokan düzeyiyle koroner kollateral dolaşım (KKD) gelişimi arasındaki ilişkiyi araştırmayı amaçladık.

Hastalar ve Yöntem: Çalışmamıza en az bir kronik total tıkanıklığı olan stabil angina hastaları (n= 44) ve normal koroner arteri olan kontrol grubu (n= 29) dahil edildi. Kollateral derecesi, Rentrop ve Cohen sınıflandırmasına göre değerlendirildi. Evre 0-1 kollateral doluşa sahip olan hastalar zayıf gelişmiş KKD grubuna (n= 19) , evre 2-3 kollateral doluşa sahip olan hastalar iyi gelişmiş KKD grubuna (n= 29) dahil edildi.

Bulgular: İyi gelişmiş KKD grubunda, zayıf gelişmiş KKD grubuna ve kontrol grubuna göre endokan düzeyleri anlamlı olarak yüksek bulundu (p< 0.001). Zayıf gelişmiş KKD olan hastalarda endokan düzeyi kontrol grubuna göre daha yüksektir. Endokan düzeyi, regresyon analizinde iyi gelişmiş KKD için tek bağımsız öngördürücü idi (OR 1.002, 95% güven aralığı (GA) 1.001-1.005; p= 0.001). İyi gelişmiş KKD öngörmede endokan düzeyi optimal kestirim değeri ≥ 1773, 9 ng/L olarak bulundu. Endokan düzeyi ≥ 1773.9 ng/L olması halinde iyi gelişmiş kollateral varlığını %84 duyarlılık ve %63 özgüllükle öngörmekteydi (AUC: 0.749, 95% GA: 0.654-0.849, p< 0.001).

Sonuç: Koroner kronik total oklüzyonu olan hastalarda KKD artmış endokan düzeyi ile ilişkilidir. KKD gelişiminde endokanın önemli rolü olabilir.

Anahtar Kelimeler: Endokan; koroner kollateral dolaşım; anjiyogenez; koroner arter hastalığı

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INTRODUCTION

The development of coronary collateral circulation (CCC) is an adaptation mechanism of the myocardium to protect itself from tissue damage due to chronic ischemia and infarction[1]. Well-developed collateral circulation was shown to decrease cardiovascular mortality and morbidity by reducing recurrent myocardial infarction, fatal arrhythmia, ventricular failure, and ventricular aneurysm formation[2]. Although diabetes mellitus, hypertension, smoking, exercise, chronic inflammation, oxidative stress, and duration and severity of myocardial ischemia are well known triggers of collateral development, angiogenesis significantly varies even among individuals with the same clinical status[3,4]. Vascular growth factors, such as vascular endothelial growth factor and transforming growth factor β, and immune system components, such as monocytes, macrophages, neutrophils, and lymphocytes, play an important role in the development of CCC. However, various mechanisms involved in the development of CCC are still not clear[5].

Endocan (endothelial-specific molecule 1) is a dermatan sulfate proteoglycan synthesized and secreted by activated vascular endothelium and plays a role in the pathogenesis of vascular diseases, organ-specific inflammations, tumor adhesion, migration, and angiogenesis[6-8]. A previous study demonstrated that endocan levels increased in patients with acute coronary syndromes. The authors asserted that increased levels of endocan are not associated with the severity of coronary artery disease as assessed by Gensini and SYNTAX scores[9]. Contradictory results exist about the relationship between serum endocan levels and development of collateral circulation in the literature[10,11].

In the present study, we aimed to clarify the controversial consequences of the relationship between endocan level and CCC. Additionally, we compared patients with well and poor CCC to the control group.

PATIENTS and METHODS

Study Population

A total of 73 patients who underwent elective coronary angiography with signs of ischemia (e.g., positive treadmill exercise test, ischemia in myocardial perfusion scintigraphy, patients with recently detected left ventricular wall motion abnormalities, or patients with stable angina pectoris) were enrolled to the study. Forty-four patients had chronic total coronary occlusion (CTO) in at least one major epicardial coronary artery, and 29 subjects had normal coronary anatomy. Patients with acute/chronic infective or inflammatory disease, connective tissue disease, chronic kidney disease (serum creatinine > 1.5 mg/dL), history of coronary artery bypass grafting, acute coronary syndrome within the last 1 year, severe valvular heart disease, hepatic and hemolytic disorders, active malignancy, and patients receiving immunosuppressive treatments were excluded from the study. Transthoracic echocardiography was performed in all patients, and left ventricular ejection fraction was calculated using the modified Simpson's method. Demographic, clinical, and laboratory parameters and angiographic results of patients were recorded. The local ethics committee approved the study protocol (2018/1368). Written consent was obtained from each patient.

Coronary Angiography and Evaluation of Coronary Collateral Circulation

Coronary angiography was performed using the standard Judkins technique. Coronary angiograms and the degree of coronary collateral development were evaluated by two experienced interventional cardiologists. The grades of coronary collateral filling were classified according to the Cohen-Rentrop method as follows: Grade 0, no filling of any collateral; Grade 1, filling of side branches by collateral vessels without viewing of the epicardial segment; Grade 2, local filling of the epicardial artery by collateral vessels; and Grade 3, complete filling of the major epicardial coronary artery by a collateral vessel[12]. If the patient had more than one vessel with collateral circulation, the classification of collateral grade was performed according to the best collateral filling. CTOs are described as the complete interruption of antegrade coronary blood flow on angiography with a duration of > 3 months[13]. SYNTAX score was calculated as previously described in the literature[14]. More than 50% of stenosis in at least one coronary artery was accepted as stenotic coronary artery disease, and the numbers of diseased vessels were recorded as one, two, or three vessel diseases. Patients with grades 0 and 1 were grouped as poor CCC (n= 19), whereas patients with grades 2 and 3 as good CCC (n= 25). Twenty-nine healthy subjects with normal coronary anatomy were included in the study as the control group.

Routine Laboratory Tests and Measurements of Serum Endocan Levels

Blood samples for routine hematological and biochemical tests were collected before the procedure after 12 h of fasting. Serum levels of creatinine and urea and hematological values were determined using the standard methods. Patient's serum samples were separated by centrifugation at 1500g for 10 min and stored at -80°C until analysis. Serum endocan levels were calculated using an enzyme-linked immunosorbent assay (ELISA) kit with high sensitivity and specificity for detection of human endocan (Human Endothelial Cell-specific Molecule-1 ELISA Kit; Shanghai Yehua Biological Technology, Shanghai, PR China). All blood samples were routinely tested by ELISA in duplicate, and the results were averaged.

Statistical Analysis

Data collected during the research were analyzed using the SPSS 17.0 statistical package program (IBM Corp., Armonk, NY, USA). Descriptive statistics were expressed as mean ± standard deviation or median (interquartile range) for continuous variables and as number of cases (n) and percentages (%) for categorical variables. Kolmogorov-Smirnov test was used for normally distributed data. Pearson's or Spearman's correlation analysis was used to assess the relationships among parameters according to the normality of data. One-way ANOVA test was used to show

the differences between the groups in continuous numeric parameters with normal distribution and to compare endocan levels in accordance with the Rentrop-Cohen classification. Student's t-test was used to compare parametric continuous variables, and Mann-Whitney U test was used to compare nonparametric continuous variables. Logistic regression analysis was performed to identify the independent predictors of well CCC. Variables with a p-value of < 0.1 in univariate logistic regression analysis and variables considered to have clinical importance were included in a multivariate logistic regression model. The receiver operating characteristic (ROC) curve was used to show the sensitivity and specificity of endocan levels and optimal cut-off value for predicting well CCC. A p-value <0.05 was considered to be statistically significant.

RESULTS

A total of 73 subjects (25 well CCC, 19 poor CCC, and 29 healthy control) were included in the study. Clinical, laboratory, angiographic, and demographic characteristics of the groups are shown in Table 1. Baseline characteristics, laboratory parameters, and conventional risk factors for coronary artery disease were not different between all groups (p> 0.05). SYNTAX score, number of vessel disease, or localization of CTO was similar in patients in the poor and well CCC groups (p> 0.5). Endocan levels were significantly higher in patients in the well CCC group than in those in the poor CCC and control groups (Table 1 and Figure 1, p< 0.001). On the other hand, serum endocan levels were significantly lower in the control group than in patients in the well CCC and poor CCC groups (p< 0.001 and p= 0.04, respectively) (Figure 1). Furthermore, as per correlation analysis, the relationship between endocan and SYNTAX score (Spearman's rho = -0.137, p= 0.380) was found to be non-significant. Moreover, endocan (odds ratio 1.002, 95% confidence interval (CI) 1.001-1.005, p= 0.001) was only found as an independent predictor of well CCC in multivariate logistic regression analysis including age, syntax score, and presence of diabetes (Table 2). In ROC curve analysis, a cut-off value of 1773.9 ng/L identified well-developed collateral with a sensitivity of 84% and specificity of 63% (area under the ROC curve: 0.749, 95% CI: 0.654-0.849, p< 0.001) (Figure 2).

DISCUSSION

In the present study, we found a significant relationship between serum endocan levels and well CCC in patients with stable coronary artery disease. Although some studies compared endocan levels in patients with well and poor collaterals, to the best of our knowledge, this is the first study that included a normal coronary artery group[10,11]. Serum endocan levels were significantly higher in patients with CTO independent of the collateral's grade than in patients with normal coronaries. An increase in serum endocan level was an independent predictor of the development of good CCC.

Endocan is an endothelium-derived soluble dermatan sulfate proteoglycan secreted by inflamed endothelium that can bind various bioactive molecules related with intercellular signaling and adhesion, organ-specific inflammations, tumor progression, and angiogenesis. Thus, endocan regulates the differentiation, proliferation, adhesion, and migration of different cell types[15,16]. Endothelial dysfunction and vascular inflammation are considered as the main pathological processes in the initiation and progression of atherosclerosis[17]. In this context, Kose et al. demonstrated that serum endocan levels are significantly increased in patients with acute coronary syndromes[9]. Moreover, Ye et al. proposed that an increase in serum endocan level is an independent predictor of coronary slow flow[18]. A recent study demonstrated increased endocan levels in patients with coronary ectasia that is associated with endothelial dysfunction[19]. Another previous study showed that patients with cardiac syndrome X had higher serum endocan levels; therefore, endocan levels might be a good marker of microvascular disease[20]. The aforementioned emphasized the association between microvascular and macrovascular coronary artery disease and serum endocan levels.

Two previous studies found a relationship between coronary collateral development and serum endocan level. Semet et al. showed the association of serum endocan levels with coronary collateral development[10]. The previous study concluded that levels of vascular cell adhesion molecule 1, a potential angiogenic molecule, were increased in patients with good collateral and positively correlated with serum endocan level. Moreover, serum endocan levels negatively correlated with high sensitivity C-reactive protein (hs-CRP). Gok et al. suggested that low endocan levels are independently associated with good CCC[11]. Furthermore, they have found that endocan levels are significantly and positively correlated with hs-CRP in contrast to the previous study. Our results support the hypothesis that serum levels of endocan are higher in patients with good CCC compatible with the study by Emet et al. In contrast to these two previous studies, the presence of the control group in our study makes it more powerful; however, the low number of participants is the most important limitation.

Previous studies demonstrated that elevated serum CRP levels were associated with impaired CCC[21]. If the endocan is a critical molecule that plays a role in the formation of new vessel, it is thought that serum CRP levels should be negatively correlated with serum endocan levels[7]. We think that the lack of CRP or any other inflammatory marker in our study does not constitute a significant limitation, since these markers do not provide additional information to comment on this topic. It appears to be more reasonable that serum endocan levels are higher in patients with good collateral development and negatively correlated with CRP levels, as Emet et al. and our study have determined[10].

The present study showed that a higher serum endocan level was associated with well CCC in patients with stable coronary artery disease who had at least one CTO. Serum endocan levels were an independent predictor for good collateral development. Moreover, serum endocan levels were lower in patients with normal coronary artery than in patients with poor and well CCC. Nevertheless, our study shows the possible effect of a novel

Table 1. Demographic characteristics, laboratory parameters and endocan levels of patients and control subjects

Variables	Control group (n= 29)	CTO with well CCC (n= 25)	CTO with poor CCC (n= 19)	p
Age, years (mean ± SD)	58.1 ± 10.5	63.0 ± 8.6	60.2 ± 9.5	0.183
Sex, male n (%)	19 (65.5)	18 (72)	15 (78.9)	0.6
Hypertension n (%)	17 (58.6)	11 (44)	12 (63.2)	0.39
Diabetes mellitus n (%)	17 (58.6)	16 (64)	13 (68.4)	0.783
Dyslipidemia n (%)	13 (44.8)	7 (28)	9 (47.4)	0.331
Smoking n (%)	13 (44.8)	10 (40)	9 (47.4)	0.879
Urea mg/dL	34.7 ± 9.7	33.8 ± 7.3	37.5 ± 9.7	0.365
Creatinine mg/dL	0.79 ± 0.21	0.79 ± 0.17	0.89 ± 0.21	0.154
Hemoglobin g/dL	13.7 ± 1.7	13.7 ± 1.8	14.2 ± 1.4	0.504
Platelet (1000/mm^3)	215 (196-256)	226 (191-255)	230 (207-281)	0.569
Leukocyte (1000/mm^3)	8.6 (7.7-11.1)	8.2 (7.1-9.2)	8.2 (6.7-9.7)	0.309
Medications n (%)				
Beta-blocker	7 (24.1)	22 (88)	17 (89.5)	< 0.001
ACE-I-ARB	16 (55.2)	16 (64)	13 (68.4)	0.625
Statin	13 (44.8)	13 (52)	9 (47.4)	0.869
Number of disease vessels n (%)				
One vessel disease		10 (40)	8 (42.1)	0.888
Two vessel disease		10 (40)	6 (31.6)	0.565
Three vessel disease		5 (20)	5 (26.3)	0.62
Position of chronic total occlusion n (%)				
LAD		6 (24)	6 (31.6)	0.576
Cx		6 (24)	3 (15.8)	0.504
RCA		13 (52)	10 (52.6)	0.967
Syntax score (mean ± SD)		12.1 ± 6.3	12.5 ± 5.5	0.802
Endocan levels (ng/L)	1408.1 (710.4-1553.6)	2194.1 (1849-3979.3)	1657.3 (928.8-2128.8)	< 0.001

CCC: Coronary collateral circulation, ACE-I: Angiotensin converting enzyme inhibitor, ARB: Angiotensin receptor blockers, Cx: Circumflex coronary artery, CTO: Chronic total occlusion, LAD: Left anterior descending coronary artery, RCA: Right coronary artery.

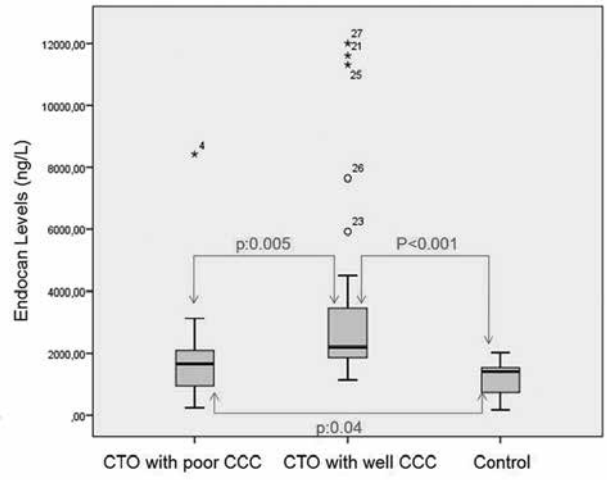

Figure 1. Serum endocan concentration in control subjects and in patients with well and poor coronary collateral circulation.

marker in neovascularization that should be investigated with further large studies.

Study Limitations

Our study has several limitations. First, a small sample size is the major limitation of our study. Second, the degree of coronary collateral development was assessed visually during routine angiography in a semi-quantitative way using the Rentrop score. Intravascular hemodynamic assessment (coronary flow index) might change our results. Peripheral blood sample was used to detect serum endocan levels. Coronary artery or coronary sinus sampling may be more accurate. Finally, the lack of inflammation markers (e.g., hs-CRP, interleukins, and fibrinogen) may also be considered as a limitation.

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Table 2. Multivariate logistic regression

	p	Exp (B)	95% CI for EXP (B) lower	Upper
Age	0.123	1.070	0.982	1.166
Syntax score	0.958	1.003	0.891	1.130
Diabetes mellitus	0.234	2.620	0.536	6.789
Endocan	0.001	1.002	1.001	1.005

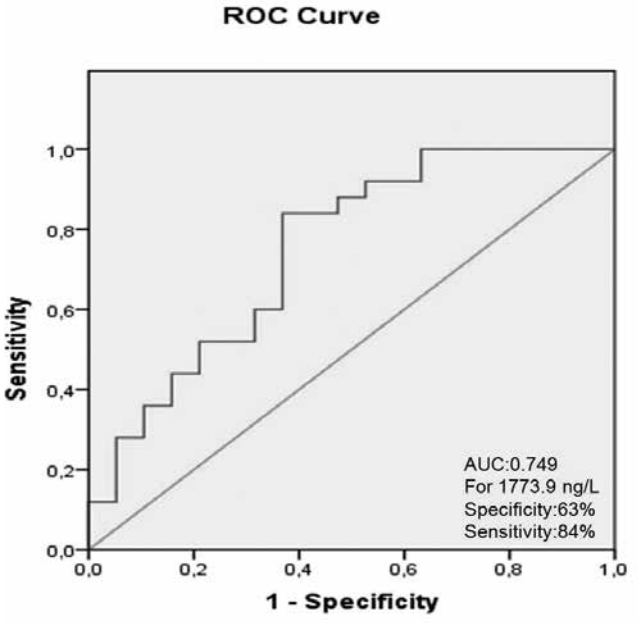

Figure 2. Receiver operating characteristic curve analysis for endocan in coronary collateral circulation.

CONFLICT of INTEREST

The authors reported no conflict of interest related to this article.

AUTHORSHIP CONTRIBUTIONS

Concept/Design: AG, SÖ

Analysis/Interpretation: AG, SÖ, AY

Data Acquisition: AG, SÖ, SE

Writting: YA, AG

Critical Revision: SÖ, AG, CK

Final Approval: All of authors.

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