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Endothelium biomarkers endocan and thrombomodulin levels in isolated coronary artery ectasia

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Abstract. – OBJECTIVE: Endothelial dysfunction may play an important role in the evolution of coronary artery ectasia (CAE). Endocan and thrombomodulin (TM) are two biomarkers released from the endothelium that are associated with dysfunction. We aimed to evaluate the levels of these markers in patients with isolated CAE.

PATIENTS AND METHODS: Thirty-two patients with isolated CAE and thirty-five sex- and age-matched control patients with normal coronary angiograms were enrolled. Serum endocan and TM concentrations were measured with an enzyme-linked immunosorbent assay kit.

RESULTS: The basal characteristics of the two groups were similar. Both endocan (1.19 ± 0.18 vs. 1.07 ± 0.15 ng/ml; $p = 0.006$) and TM (687.28 ± 150.85 *vs.* 571.27 ± 171.23 pg/ml; $p = 0.007$) were significantly increased in the CAE group compared to controls. However, no significant differences were detected in the concentration of these markers when we grouped the subjects according to the Markis classification.

CONCLUSIONS: We found higher endocan and TM levels in isolated CAE patients. However, these markers were not associated with CAE severity as assessed using the Markis classification. The results suggest that these markers play an important role in the development of isolated CAE.

Key Words:
Endocan, Thrombomodulin, Endothelial dysfunction, Isolated coronary artery ectasia.

Introduction

Coronary artery ectasia (CAE) is generally defined as abnormal diffusion or localized dilatation of a segment of a coronary artery that is 1.5 times larger than a normal coronary artery segment[1]. CAE had a reported incidence of between 1.2% and 7.4%[2-5]. A variety of etiologies, including congenital defects, inflammation, and atherosclerosis, are associated with the development of CAE, but atherosclerosis is the main cause in nearly half of all CAE cases[6,7]. Isolated CAE is defined as the absence of coronary artery stenosis and accounts for roughly 0.1-0.79% of all CAE cases[2]. In clinical settings, CAE is associated with adverse cardiovascular outcomes, including coronary spasm, thrombosis, distal embolization, dissection, and myocardial ischemia[8]. The exact pathophysiological mechanisms of CAE are not clearly understood; however, endothelial dysfunction, atherosclerosis, and vasculitis (inflammation) are all associated with CAE[9,10].

Endocan is a novel soluble molecule that is released from human endothelial cells and can be detected in blood[11]. It is thought to play an important role in vascular disorders caused by endothelial dysfunction[11-13].

Thrombomodulin (TM) is an integral membrane protein expressed on vascular endothelium that contributes to the development of endothelial dysfunction and atherosclerosis[14].

Endothelial dysfunction is considered to be the first step in the development of atherosclerosis[15]. Endothelial dysfunction and atherosclerosis are the two main factors that are thought to result in CAE[9,10]. Previous studies showed that TM and endocan are related to endothelial dysfunction[11-14]. Therefore, we investigated the relationship between these endothelial biomarkers and isolated CAE. To our knowledge, this is the first report to study the levels of TM in isolated CAE.

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Patients and Methods

Fifty-seven patients were prospectively diagnosed with CAE out of 3,296 total patients that underwent coronary angiography at Harran University Hospital and Mehmet Akif Inan Training and Research Hospital between June 2015 and January 2016. The exclusion criteria were as follows: acute coronary syndrome, coronary artery disease (CAD), significant valvular heart disease, heart failure (left ventricular ejection fraction < 40%), inflammatory diseases (acute or chronic), and hepatic and renal disorders. Twenty-five subjects were excluded based on these criteria. Finally, 32 patients were enrolled in the study and 35 patients with similar baseline characteristics and normal coronary angiograms were recruited as the control group. All patients underwent a detailed medical evaluation including clinical history, physical examination, routine blood analysis, lipid profile, electrocardiography, and echocardiography. Hypertension (HT) was defined as a systolic blood pressure (SBP) ≥ 140 mmHg and/or a diastolic blood pressure (DBP) ≥ 90 mmHg on repeated measurements, or being on an antihypertensive medication. Diabetes mellitus (DM) was considered to be present if fasting glucose was ≥ 126 mg/dl, or if the patient was taking an antidiabetic medication or adhering to an antidiabetic diet. Hypercholesterolemia was defined as total cholesterol > 200 mg/dl. The local Ethics Committee approved the study protocol and written informed consent was obtained from all participants.

Coronary angiography was performed routinely using Judkin's technique with six French catheters inserted through femoral or radial arteries without the use of nitroglycerin. Angiograms were evaluated by two cardiologists in a blinded manner. Isolated CAE was diagnosed if the diameter of the localized or diffuse dilated coronary artery was 1.5 times larger than that of the adjacent normal vessel and there was no significant stenotic lesion. According to the Markis classification, the degree of CAE was categorized as follows: type 1, diffuse ectasia in two or three vessels; type 2, diffuse ectasia in one vessel and localized disease in another vessel; type 3, diffuse ectasia in a single vessel; and type 4, segmental localized ectasia[16].

Complete hematological count, glucose level, lipid profile, liver enzyme level, and creatinine concentration were analyzed in peripheral venous blood samples obtained after 12 hours of fasting on the day of coronary angiography. The neutrophil-to-lymphocyte ratio (NLR) was obtained by dividing the neutrophil count by the lymphocyte count. Serum was obtained by centrifugation at 3,000 rpm for 15 minutes and stored at -80ºC for analysis of TM and endocan. Both serum endocan and TM levels were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) kit with high sensitivity and specificity for detecting human endocan (Cusabio Bioscience Inc, Wuhan, China). The minimum detectable concentrations of endocan and TM were 0.039 ng/ml and 7.8 pg/ml, respectively. The intra-and inter-assay coefficients of variation were less than 8% and 10%, respectively, for both biomarkers.

Statistical Analysis

SPSS for Windows software (ver. 22.0; IBM SPSS, Statistics for Windows, Armonk, NY, USA) was used for all statistical analyses. A Shapiro-Wilks test was used to evaluate the normality of the distributions of continuous variables. To assess normally distributed variables, an independent sample t-test was used, and for non-normally distributed variables, a Mann-Whitney U test was used. Means and standard deviation were calculated for continuous variables, and categorical variables are shown as percentages. Comparisons between all types of CAE were performed using one-way ANOVA and Tukey's post hoc test was applied. Univariate and multivariate logistic regression analyses were performed to determine if the relationships between the biomarkers and isolated CAE were independent. Variables significant at $p<0.1$ in the univariate analyses were included in the multivariate logistic regression analysis. Odds ratios (ORs) and 95% confidence intervals (CIs) were also calculated in the multivariate logistic regression model. Receiver operating characteristics (ROC) curve analysis was performed to determine the sensitivity and specificity for each biomarker, and to determine the cut-off values for discriminating subjects with isolated CAE. In all of the analyses, $p<0.05$ was considered statistically significant.

Results

The study included 32 subjects in the CAE group and 35 in the control group. The baseline characteristics of the study population are shown in Table I. There were no significant differences in baseline clinical characteristics, including age,

Table I. Clinical and laboratory characteristics of the study population.

	Isolated CAE (no. = 32)	Control (no. = 35)	*p*
Age, years	54 ± 8	53 ± 5	0.813
Female gender, n (%)	13 (40)	12 (34)	0.614
Hypertension, n (%)	14 (43)	13 (37)	0.451
Smoking, n (%)	15 (46)	14 (40)	0.419
BMI, kg/m^2	28.1 ± 2.7	27.9 ± 1.7	0.214
Creatinine, mg/dl	0.80 ± 0.12	0.78 ± 0.09	0.372
Fasting glucose, mg/dl	98.1 ± 8.9	95.4 ± 7.8	0.311
Total cholesterol, mg/dl	199.8 ± 42.4	196.7 ± 28.4	0.772
LDL, mg/dl	114.2 ± 31.4	102.7 ± 24.5	0.239
HDL, mg/dl	39.5 ± 8.4	38.8 ± 4.7	0.921
Triglyceride,mg/dl	234 ± 09.5	209.7 ± 89.4	0.542
Hemoglobin, g/dl	15.4 ± 1.7	15.2 ± 1.1	0.704
NLR	1.88 ± 0.56	1.82 ± 0.41	0.592
hsCRP, mg/dl	0.51 ± 0.14	0.49 ± 0.08	0.763
Endocan, ng/ml	1.19 ± 0.18	1.07 ± 0.15	0.006
TM, pg/ml	687.28 ± 150.85	571.27 ± 171.23	0.007

BMI: body mass index, CAE: coronary artery ectasia, HDL: high-density lipoprotein, hsCRP: high sensitive Creactive protein, LDL: low-density lipoprotein, NLR: neutrophil-to-lymphocyte ratio, TM: thrombomodulin. Bolded data indicate significance.

gender, smoking status, and body mass index (BMI), between the CAE and control groups. Also, the laboratory findings, including fasting glucose, serum creatinine, serum hemoglobin, high-sensitivity C-reactive protein (hsCRP), and serum lipid panels, were similar between the groups (Table I).

Ectasia involved the left anterior descending artery in 15 cases (46%), the left circumflex artery in 13 cases (40%), and the right coronary artery in 21 (65%) cases. According to the Markis classification, there were six (19%) type 1, seven (22%) type 2, eight (25%) type 3, and 11 (34%) type 4 subjects.

Both the TM (687.28 ± 150.85 *vs* 571.27 ± 171.23; $p = 0.007$) and endocan levels (1.19 ± 0.18 *vs* 1.07 ± 0.15; $p = 0.006$) were significantly higher in the isolated CAE group compared to the control group. We found no significant difference in the levels of these markers between groups when we grouped the subjects according to the Markis classification. In the post-hoc analysis, we found no significant difference in the TM or endocan concentration between groups (Figure 1). There were also no statistical differences in the hsCRP level or and NLR between the CAE and control groups.

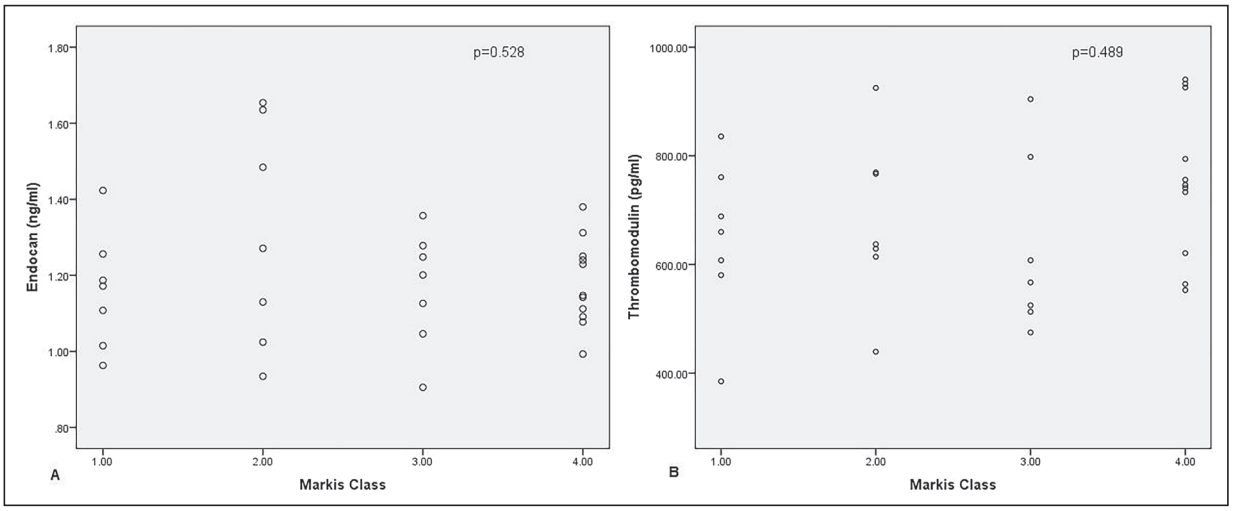

Figure 1. Scatter plots for levels of endocan ***(A)*** and thrombomodulin (TM) ***(B)*** according to the Markis classification in the isolated coronary artery ectasia (CAE) group.

Multivariate logistic regression analysis revealed that both endocan and TM levels were independently associated with isolated CAE (for endocan, OR = 1.214, 95% CI: 1.034-1.96, $p < 0.05$; for TM, OR = 1.047, 95% CI: 1.193-1.331, $p < 0.05$). ROC curve analysis was performed to determine the discriminatory capacity of endocan and TM levels. The area under the curve (AUC) value was 0.709 for endocan (95% CI: 0.580-0.838, $p < 0.001$) with a cut-off value of 0.125, and the sensitivity and specificity were 65.6% and 69.0%, respectively (Figure 2). The AUC value was 0.698 for TM (95% CI: 0.566-0.830, $p < 0. 001$) with a cut-off value of 624.82, and the sensitivity and specificity were 62.5% and 62.1%, respectively (Figure 3).

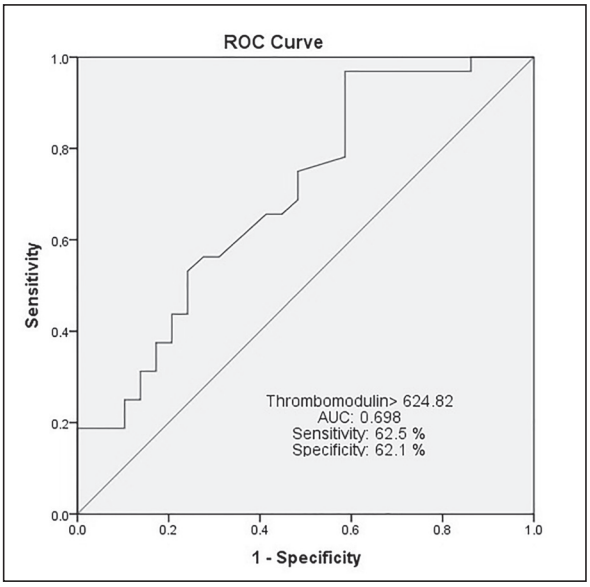

Figure 3. ROC curve for TM level to detect isolated CAE.

Discussion

In this report, we analyzed endocan and TM concentrations in the blood and found that subjects with isolated CAE had significantly higher levels of these biomarkers compared to the control group, who had angiographically normal coronary arteries. However, concentrations of these biomarkers were not associated with the extent of CAE.

CAE is a clinical entity defined as inappropriate dilatation of the coronary arteries. The precise mechanism underlying the evolution of CAE is not fully understood. However, several hypotheses have been proposed to explain the pathophysiology of this phenomenon[3]. Atherosclerosis is the most widely accepted hypothesis, since atherosclerosis and CAE have similar risk factors and histopathological features. The main findings in the ectatic segments were lipid deposition and disruption of the vascular media layer. In addition, approximately 50% of CAE cases had CAD[1,17]. Endothelial dysfunction is accepted as the initial step towards atherosclerosis[15]. Chronic overstimulation of the endothelium resulting in nitric oxide (NO) exposure represents one of the theories posited to explain the etiopathogenesis of CAE. Inappropriate production of NO is thought to cause the destruction of the coronary artery media layer, which in turn causes abnormal dilatation leading to CAE[3,6]. CAE is more commonly observed in patients who used herbicide sprays that promote NO overstimulation[18]. Increased concentrations of adhesion molecules, such as E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular adhesion molecule-1 (VCAM-1) in CAE patients are also considered proof of endothelial dysfunction[19].

Endocan is a soluble proteoglycan released from the endothelium that is thought to play an important role in vascular endothelial disorders. Increased endocan concentrations have been reported in some disorders, including kidney disease, atherosclerosis, tumor progression, and inflammatory conditions. The results of above investigations suggest that endocan is a potential

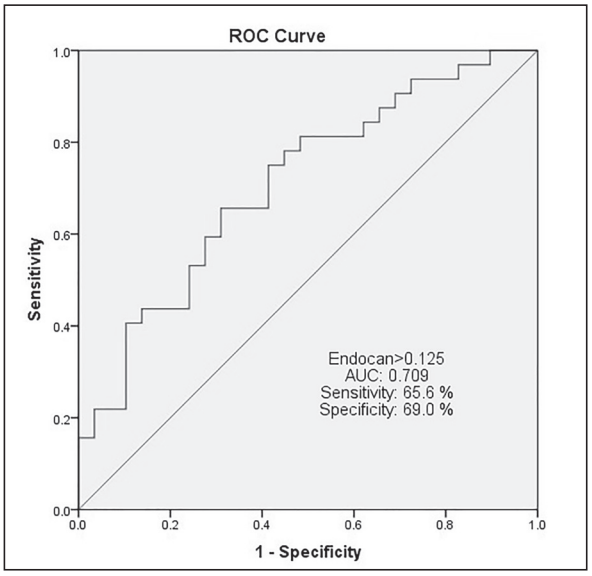

Figure 2. Receiver operating characteristic (ROC) curve for endocan level to detect isolated CAE. AUC, area under the curve.

indicator of vascular endothelial dysfunction[11]. Some scholars demonstrated elevated endocan levels in sepsis[20]. In addition, higher endocan concentrations have been detected in systemic vasculitic diseases, such as Behçet's disease and systemic sclerosis[21,22]. Endocan levels are strongly associated with carotid intima media thickness (cIMT) and flow-mediated dilatation (FMD)[11,23]. FMD is the standard non-invasive test for evaluating endothelial function. Turan et al[24] studied endocan levels in isolated CAE patients and showed a significant association between endocan level and presence of CAE. In our paper, we found higher endocan concentrations in the isolated CAE group compared to the control group. However, there was no correlation between endocan level and extent of isolated CAE. In contrast, Turan et al[24] found a significant correlation between these parameters.

TM is a transmembrane glycoprotein expressed on the vascular endothelial surface. It plays a regulatory role in endothelial thromboresistance and has anticoagulant, antifibrinolytic, and anti-inflammatory properties[14]. Previous studies have shown that in cases of endothelial injury and dysfunction, soluble TM concentrations are increased in the circulation[14,25]. Higher TM levels have also been detected in vascular disorders, such as atherosclerosis, CAD, cardio-embolic stroke, sepsis, and acute respiratory distress syndrome[14,26-28]. We detected higher circulating TM concentrations in the CAE group compared to the control group. However, the severity of CAE did not correlate with the TM concentration in our study.

In our work, the NLR, a simple marker of systemic inflammation, and hsCRP level were similar between the CAE and control groups. The results of previous reports were similar to those of our study[24,29,30]. However, endocan and TM levels were elevated in the CAE group compared to the controls in this study. These findings suggest that the mechanisms underlying the development of CAE may involve endothelial dysfunction, as well as a systemic inflammatory response.

The main limitation of this report was the relatively small sample size, which limited our ability to detect significant associations between the levels of analyzed biomarkers and CAE severity. The other limitation was that the diagnosis of isolated CAE was established without using intravascular ultrasound, which can be useful for detecting atherosclerotic plaques not visible on coronary angiography.

Conclusions

We demonstrated that plasma endocan and TM concentrations were elevated in patients with isolated CAE, but that the levels of these biomarkers did not reflect the degree of isolated CAE. To the best of our knowledge, this was the first study to demonstrate a relationship between TM and isolated CAE. Endothelial dysfunction may be involved in the pathogenesis of isolated CAE. Studies with larger samples are needed to understand the role of endothelial molecules in the etiopathogenesis of CAE.

Note

The findings of this study were presented as an abstract at the 13th Complex Cardiovascular Catheter Therapeutics (C^3) Conference in Orlando (FL, USA) June 2017.

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Authors' Declaration

All authors contributed to the conception and design of the study; acquisition or analysis and interpretation of the data; drafting of the article; and critical revision pertaining to intellectual content. All authors gave their final approval regarding the version of the manuscript to be published.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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