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Percutaneous Coronary Intervention

Serum Endocan Levels Predict Drug-Eluting Stent Restenosis in Patients with Stable Angina Pectoris

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Background: Endothelial cell-specific molecule 1 (ESM-1 or endocan) is an immunoinflammatory marker strongly associated with inflammation, vascular endothelial dysfunction and atherosclerosis. We explored the relationship between serum endocan concentrations and coronary in-stent restenosis (ISR).

Methods: Fifty consecutive patients with ISR and 50 control subjects were included in this study. Clinical data and angiographic characteristics were collected. Serum endocan concentrations were measured using an enzyme-linked immunosorbent assay.

Results: All included patients were divided into four quartiles based on their concentrations of endocan: quartile 1 (0.62-1.31 ng/mL), quartile 2 (1.33-1.74 ng/mL), quartile 3 (1.75-2.77 ng/mL) and quartile 4 (2.78-4.24 ng/mL). The rates of ISR were 16%, 24%, 68%, and 92%, respectively. The patients in quartile 4 had significantly higher rates of ISR than the other groups ($p < 0.001$). Logistic regression analysis indicated that endocan concentration [odds ratio = 8.65, 95% confidence interval 3.56-20.94; $p < 0.001$] was an independent predictor of ISR. Receiver operating characteristic curve analysis was used to explore the relationship between endocan and ISR. Using a cutoff value of 1.625 ng/mL, endocan predicted ISR with a sensitivity of 86% and a specificity of 78%.

Conclusions: Our findings suggest that plasma endocan levels may be a novel biomarker of endothelial dysfunction in patients with ISR.

Key Words: Biomarker • Endocan • Endothelial cell-specific molecule 1 • Inflammation • In-stent restenosis

INTRODUCTION

In-stent restenosis (ISR) remains a vexing clinical problem, affecting a considerable portion of patients undergoing percutaneous coronary interventions, even in the drug-eluting stent (DES) era.[1] Therefore, it is important to find a reliable biomarker to predict coronary ISR in clinical practice. The development of coronary ISR is a complex pathophysiological process that includes a large number of inflammatory factors, and various cytokines playing important roles in inducing ISR via interrelated mechanisms.[2] The primary mechanisms include vascular inflammation, vascular remodeling induced by endothelial damage and excessive vascular smooth muscle cell proliferation and migration.[3] Novel therapeutic options to interfere with the pathophysiologic mechanisms responsible for ISR have recently been investigated.[4,5] There has also been increasing interest in the relationship between endothelial dysfunction and ISR. Endothelial cell-specific molecule 1 (ESM-1 or endocan) is a soluble dermatan sulfate proteoglycan mainly secreted by vascular endothelial cells,[6] and it is strongly

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associated with vascular endothelial dysfunction and atherosclerosis.[7-12] Endocan has also been reported to have prognostic significance in patients with hypertension,[7] chronic renal failures,[13] and acute myocardial infarction (AMI).[14] However, to the best of our knowledge, no clinical trial has been conducted to elucidate the relationship between endocan levels and coronary ISR. The aim of this study was to investigate the relationship between serum endocan levels and ISR after coronary stenting with DESs in patients with stable angina pectoris (SAP).

METHODS

Study population

Stable angina patients who had undergone coronary angiography between 01/01/2014 and 01/01/2016 were enrolled as the study population. Fifty consecutive patients (38 men, age: 57.4 ± 10.0 years) who displayed ISR on coronary angiography and who were free of exclusion criteria were enrolled as the ISR group. Fifty consecutive patients with a history of stent implantation who were free of ISR were enrolled as the non-ISR control group (35 men, age: 59.4 ± 9.7 years).

The inclusion criteria were as follows: (1) patients with single or multi-coronary lesions and who had received DESs. Most of the patients had a history of everolimus-eluting platinum chromium coronary stent implantation due to the predominant availability of this type of DES in state hospitals in our country. We only analyzed patients with everolimus-eluting platinum chromium coronary stents to overcome the possible confounding effect of different stent types on the progression of ISR; (2) patients who underwent repeat coronary angiography between 6 and 18 months following stent implantation as a result of stable angina symptoms and/or positive stress tests. The exclusion criteria were as follows: (1) angiographic confirmation of the existence of primary coronary lesions (except stented coronary segments) that were aggravated following stent implantation and the patients presented with acute coronary syndrome; (2) patients with a history of previous myocardial infarction, coronary artery bypass grafting, secondary hypertension, presence of severe anemia, valvular diseases, respiratory disease, left ventricular dysfunction (left ventricular ejection fraction < 50%) and hypertrophy; (3) patients suffering from related diseases that potentially affected their serum concentrations of endocan, such as malignant tumors, acute inflammation, autoimmune diseases, thyroid dysfunction, severe liver and kidney dysfunction or alcohol consumption. The study protocol conformed to the principles of the Declaration of Helsinki and was approved by the institutional ethics committee. Informed consent was obtained from each study participant.

Data collection

Basic clinical data including cardiovascular risk factors, angiography information, stent-related factors, and medication usage were collected and entered into a database. Venous blood samples were obtained on the morning of the repeat coronary angiographic procedure, following an overnight fast of at least 12 h. Blood samples were collected in plain tubes to measure serum endocan levels. Serum was separated from the blood after centrifugation for 10 minutes. The serum samples were stored at -80 °C until analysis. A sandwich enzyme-linked immunosorbent assay (ELISA) was used to determine the endocan levels. The ELISA kit (Boster Biological Technology, CA, USA) is commercially available and provides highly specific and sensitive results for detecting human endocan levels. The manufacturer reported an intraassay coefficient of variation between 5.5-7.9% and interassay coefficient of variation between 6.3-8.8%. Blood lipids, creatinine and high-sensitivity C-reactive protein (hs-CRP) values were also measured and recorded on admission. Coronary angiography was performed using the standard Judkins approach in all patients at 6 to 18 months following DES implantation, and was subsequently reviewed by two independent interventional cardiologists. ISR was defined as a luminal narrowing of 50% or more occurring in the segment with the stent, or within a 5-mm segment proximal or distal to the stent by quantitative coronary analysis evaluation.[15]

Statistical analysis

SPSS 20.0 software was used to analyze the data. Quantitative variables were presented as the means ± standard deviations, and categorical data as percentages. Differences between the groups were assessed

using either the Student's t test or Mann-Whitney U-test for continuous variables. The categorical data were analyzed using the chi-square test or Fisher's exact test where appropriate. Multiple logistic regression analysis was performed to identify the independent predictors of ISR. Variables showing marginal associations with ISR in univariate analysis were included in the regression analysis ($p < .20$). A receiver operating characteristic (ROC) curve was constructed for endocan to determine its accuracy in predicting the risk of ISR. p values < 0.05 were considered to be statistically significant.

RESULTS

Based on the inclusion and exclusion criteria, we included 50 consecutive patients with ISR and 50 patients without ISR as control subjects. There were no significant differences in baseline clinical characteristics and procedural variables between the two groups at repeat angiography; however, compared with the non-ISR group, the ISR group exhibited significantly higher concentrations of serum endocan, as well as longer stent lengths (Table 1). The patients were divided into the following

Table 1. Baseline clinical characteristic and procedural details of the study population

	ISR group (n = 50)	Non-ISR group (n = 50)	p values
Cardiovascular risk factors			
Age (years)	57.4 ± 10.0	59.4 ± 9.7	0.33
Sex (male)	38 (76)	35 (70)	0.49
Hypertension	37 (74)	34 (68)	0.51
Diabetes	19 (38)	12 (24)	0.13
Family history of CAD	27 (54)	25 (50)	0.69
Current smoking	23 (46)	16 (32)	0.15
BMI (kg/m^2)	26.8 ± 5.5	26.4 ± 4.8	0.73
Laboratory data			
Total cholesterol (mg/dL)	197.4 ± 52.7	192.9 ± 47.9	0.65
Triglycerides (mg/dL)	168.4 ± 93.8	160.6 ± 76.8	0.64
HDL-C (mg/dL)	41.9 ± 8.4	44.3 ± 8.7	0.16
LDL-C(mg/dL)	116.0 ± 36.4	114.0 ± 39.2	0.79
HbA1c (%)	6.4 ± 0.9	6.1 ± 0.8	0.15
Creatinine (mg/dL)	0.88 ± 0.2	0.85 ± 0.2	0.54
Leukocytes ($10^9/L$)	7.5 ± 1.8	7.1 ± 1.7	0.28
hs-CRP (mg/L)	2.8 ± 1.9	2.2 ± 1.5	0.11
Endocan (ng/mL)	2.56 ± 0.88	1.43 ± 0.56	< 0.001
Medications			
ACE inhibitor or ARB	31 (62)	28 (56)	0.54
Beta-blocker	38 (76)	35 (70)	0.49
Statin	39 (78)	41 (82)	0.62
Antiplatelet	49 (98)	50 (100)	1
Angiographic characteristics			
Multivessel disease	30 (60)	26 (52)	0.42
Target vessel LAD	31 (62)	30 (60)	0.84
Target vessel LCX	14 (28)	15 (30)	0.83
Target vessel RCA	20 (40)	18 (36)	0.68
Multiple complex lesion	11 (22)	9 (18)	0.62
Procedural variables			
Minimal lumen diameter (mm)	0.31 ± 0.2	0.34 ± 0.2	0.45
Reference vessel diameter (mm)	2.95 ± 0.4	2.91 ± 0.3	0.57
Stent length (mm)	23.1 ± 6.3	20.7 ± 5.2	0.04
Stent diameter (mm)	2.82 ± 0.2	2.86 ± 0.2	0.36
Number of implanted stents (n)	1.86 ± 0.9	1.72 ± 0.9	0.45
Angiographic follow-up interval (m)	10.6 ± 3.5	9.9 ± 3.7	0.39

Data are expressed as mean ± SD, or number (%).
ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; CAD, coronary artery disease; HbA1c, glycosylated hemoglobin; HDL-C, high-density lipoprotein-cholesterol; hs-CRP, high-sensitivity C-reactive protein; ISR, in-stent restenosis; LAD, left anterior descending coronary; LCX, left circumflex coronary; LDL-C, low-density lipoprotein-cholesterol; RCA, right coronary artery; SD, standard deviation; multiple complex lesion, ≥ 2 complex lesions.

four quartiles based on their serum concentrations of endocan to evaluate the relationship between endocan quartiles and the presence of ISR: quartile 1 (0.62-1.31 ng/mL), quartile 2 (1.33-1.74 ng/mL), quartile 3 (1.75-2.77 ng/mL) and quartile 4 (2.78-4.24 ng/mL). There were 25 patients in each group, and the rates of ISR were 16%, 24%, 68%, and 92%, respectively. The patients in quartile 4 had significantly higher rates of ISR than the other groups ($p < 0.001$), and the rates of ISR increased progressively across the individual quartiles (Figure 1). Logistic regression analysis was used to assess the independent predictors of ISR. The factors that were correlated with ISR and the variables showing marginal associations in univariate analysis (diabetes, smoking, HDL-C, HbA1c and hs-CRP) were included in the regression analysis. The results showed that endocan level [OR 8.64, 95% confidence interval (CI) 3.56-20.94; $p < 0.001$] and stent length [OR 1.11, 95% CI 1.0-1.23; $p = 0.041$] were independent predictors of ISR in multivariate logistic regression analysis (Table 2). A ROC curve was used to explore the relationship between endocan and ISR, and the area under the curve for endocan was 0.859 (95% CI 0.684-0.865; $p < 0.001$). Using a cutoff value of 1.625 ng/mL, endocan predicted ISR with a sensitivity of 86% and a specificity of 78% (Figure 2). The patients with endocan values < 1.625 ng/mL had a significantly lower risk of restenosis compared with the rest of the study population.

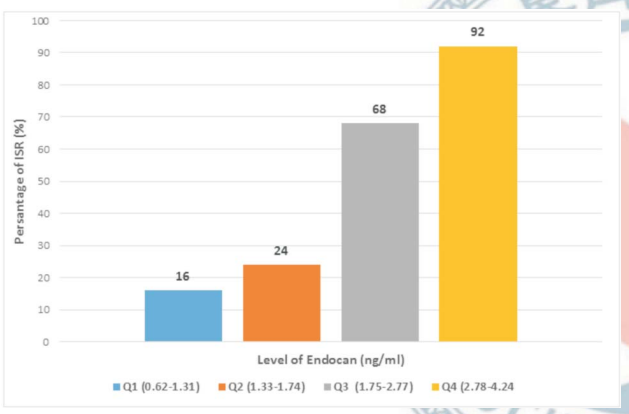

Figure 1. *ISR rate stratified by endocan quartiles. ISR, in-stent restenosis.*

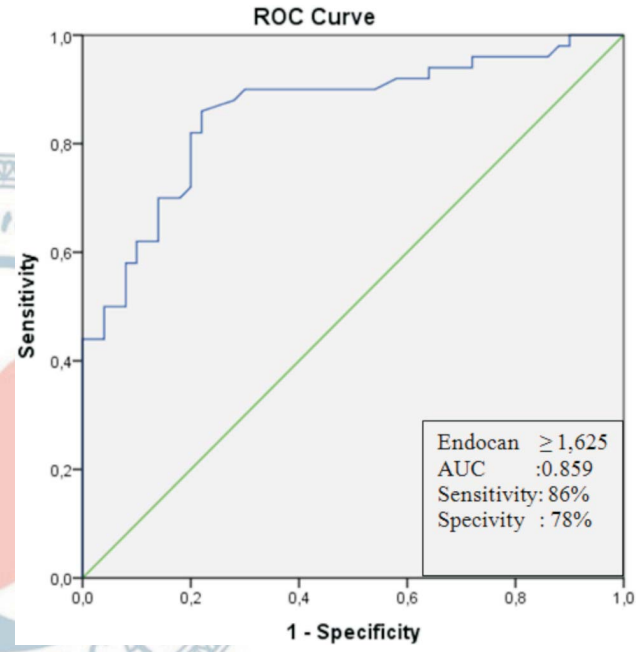

Figure 2. *The receiver-operating characteristics (ROC) curve analysis of endocan for predicting in-stent restenosis.*

Table 2. Univariate and multivariate analyses demonstrating the association between cardiovascular risk factors including serum endocan levels and the presence of ISR

Variable	β	Univariate			Multivariate		
		p	OR	(95% CI)	p	OR	(95% CI)
Diabetes	0.657	0.600	1.93	0.16-22.51			
Smoking	0.656	0.248	1.92	0.63-5.86			
HDL-C	-0.041	0.227	0.96	0.89-1.02			
HbA1c	0.094	0.877	0.91	0.27-3.01			
hs-CRP	0.145	0.451	1.15	0.79-1.68			
Endocan	2.157	< 0.001	8.64	3.56-20.94	< 0.001	7.15	3.29-15.53
Stent length	0.106	0.041	1.11	1.00-1.23	0.065	1.09	0.99-1.19

Multivariate logistic regression model including all the variables are shown in univariate analysis.
CI, confidence interval; HbA1c, glycosylated hemoglobin; HDL-C, high-density lipoprotein-cholesterol; hs-CRP, high-sensitivity C-reactive protein; OR, odds ratio.

DISCUSSION

To the best of our knowledge, this is the first study to investigate the relationship between serum endocan and coronary ISR. In this cross-sectional study, we found that higher serum endocan concentrations were associated with DES restenosis in patients with SAP. Beyond this, the rates of ISR increased progressively across the individual quartiles of endocan concentration, and this relationship persisted after adjusting for other well-known cardiovascular risk factors.

Both endothelial dysfunction and ISR are pathophysiological processes that involve the vascular wall and may be regarded as an abnormal vascular response or healing to injury. The status of the vascular wall, and especially the vascular endothelium, is the crucial component of the response to injury. The systemic and local milieu related to endothelial dysfunction favor cell proliferation, intimal hyperplasia, and vasoconstriction, all of which may contribute to the restenosis process.[16,17] Therefore, identifying markers of inflammation and surrogates of endothelial cell activation and/or dysfunction are of clinical relevance. Endothelial cell specific molecule-1, or endocan, is a soluble proteoglycan that is synthesized and secreted by activated vascular endothelium.[6] It can be detected in the circulation and it is an indicator of angiogenesis and endothelial cell activation[6]. Endocan can enhance the production of proinflammatory cytokines by endothelial cells, increase microvascular permeability and regulate leukocyte migration.[18,19] It has also been suggested to be a new endothelial mediator that can stimulate vascular smooth muscle cell proliferation and migration, and may thus contribute to neointima formation during atherogenesis.[19] Immunohistochemistry has demonstrated that endocan is highly expressed in these lesions.[20] In-stent restenosis and endothelial dysfunction are inter-related issues that probably stem from a common underlying pathophysiological mechanism: increased inflammation. Thus, our results of significantly higher concentrations of serum endocan in the ISR group may represent increased vascular inflammation. In this study, we excluded patients with inflammatory and infectious diseases. Furthermore, hs-CRP values, as an index of systemic inflammatory response, were similar between the groups, which is in disagreement with the hypothesis of increased vascular inflammation in patients with ISR.

Accumulating evidence suggests that endocan is a novel immunoinflammatory marker in atherosclerotic patients, and that it may also be a useful predictor of cardiovascular disease events.[9] Recently, there has been increasing interest in the relationship between endocan and coronary artery disease. It has been reported that endocan levels are independently associated with the presence and severity of coronary artery disease in hypertensive patients.[21] Kose et al. reported that serum endocan was over-expressed in patients with acute coronary syndrome.[8] Endocan has also been suggested to be a novel biomarker of endothelial dysfunction in patients with AMI, and it may be involved in the pathogenesis of AMI.[12] Furthermore, a high endocan level on admission to hospital has been reported to be an independent predictor of worse cardiovascular outcomes in patients with ST segment elevation myocardial infarction.[14]

Myointimal trauma induced by percutaneous coronary interventions (PCIs) affects the natural progression of atherosclerotic plaque and elicits a more aggressive local inflammatory response at the site of trauma.[22] Early elastic recoil, long-term vascular remodeling and neointimal hyperplasia are the predominant mechanisms for restenosis after PCI, and neointimal hyperplasia is the main mechanism of restenosis after coronary stent implantation.[22] In the present study, we investigated the relationship between serum endocan levels and ISR after coronary stenting with DESs in patients with SAP. We determined that serum endocan concentrations in the ISR group were significantly higher than those in the non-ISR group, and that higher concentrations of endocan may increase the risk of ISR. These results suggest that restenosis may be regarded as another aspect of endothelial dysfunction, considering the fact that coronary atherosclerosis can manifest as endothelial dysfunction at the very early stages of the disease and as ISR at the later stages. In the present study, there was no significant difference in hs-CRP value between the patients with and without restenosis. This finding is consistent with a recent study that failed to show increased ISR and late luminal loss among tertiles of baseline CRP in 1,650 consecutive patients undergoing successful DES implantation.[23] In contrast to findings of studies on bare metal stents, pre-procedural serum CRP levels do not appear to predict ISR in this setting.[24]

CONCLUSIONS

The single-center and cross-sectional design of this study and relatively small sample size may be regarded as the main limitations. In conclusion, this is the first study to demonstrate that increased serum endocan levels were an independent predictor of ISR in SAP patients with coronary DES implantation. Our findings suggest that serum endocan levels may be a novel biomarker of endothelial dysfunction in patients with ISR, and also that it may be involved in the pathogenesis of ISR. Our observations may provide a direction for future large-scale prospective studies which may more clearly define the possible clinical role of endocan in the prediction of ISR beyond the predefined risk factors such as stent length, stent size, stent type and diabetes.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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