

Serum Heparanase Level Is Decreased in Stable Coronary Artery Disease

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Significance of the Study

- Several recent reports have shown that heparanase plays an important role in stable to vulnerable plaque transformation.
- This study focused on the relationship between heparanase and stable plaque, rather than vulnerable plaque.
- This study showed that patients with stable coronary artery disease had lower levels of heparanase than patients with normal coronary arteries.

Keywords

Atherosclerosis · Diabetes mellitus · Heparanase

Abstract

Objective: Heparanase (HPA), mammalian endo- β -D-glucuronidase, separates heparan sulfate chains of proteoglycans and changes the structure of the extracellular matrix. We investigated whether serum levels of HPA differ in patients with stable coronary artery disease (SCAD) and subjects with normal coronary arteries. **Methods:** This study enrolled 92 patients with SCAD and 34 controls with normal coronary arteries. Levels of HPA were measured by a commercially available human HPA enzyme-linked immunosorbent assay kit. **Results:** Serum HPA levels were significantly lower in the SCAD group (137.5 [104.1–178.9] vs. 198.8 [178.2–244.9] pg/mL; $p < 0.001$). Serum HPA levels were significantly higher in subjects with diabetes mellitus (DM) compared to those

without DM ($p = 0.008$). Levels of HPA were lower in the SCAD group, both in the diabetic and nondiabetic subgroups, as compared to controls ($p < 0.001$ for both subgroups). Levels of HPA positively correlated with fasting blood glucose (FBG) ($r: 0.42$; $p < 0.001$). In multiple logistic regression analysis, serum HPA level (odds ratio [OR]: 0.975; 95% confidence interval [CI]: 0.966, 0.985; $p < 0.001$) and FBG (OR: 1.028; 95% CI: 1.010, 1.047; $p = 0.002$) were independently associated with SCAD. The receiver operating characteristic curve showed that HPA levels less than 160.6 pg/mL predicted SCAD with 65% sensitivity and 97% specificity (AUC: 0.80; 95% CI: 0.728, 0.878; $p < 0.001$). **Conclusion:** Diabetes and FBG levels were closely associated with serum levels of HPA. Low serum levels of HPA may predict SCAD in both diabetic and nondiabetic populations.

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Introduction

Atherosclerosis is a leading cause of death worldwide [1]. Atherosclerotic plaques are thickenings of the intima and are composed of inflammatory cells, lipids, proteoglycans, and vascular endothelial and smooth muscle cells. Since proteoglycans compose the extracellular matrix of the blood vessel wall, they are involved in the formation of atherosclerosis [2]. Proteoglycans, predominantly chains of chondroitin or dermatan sulfate, bind to serum low-density lipoproteins and cause lipid accumulation in the vessel wall. Therefore, dermatan sulfate and chondroitin sulfate proteoglycans are thought to be atherogenic [3]. However, the role of heparan sulfate (HS) proteoglycans in atherogenesis is controversial. HS proteoglycans are thought to be anti-atherogenic because they inhibit monocyte adhesion and growth of vascular smooth muscle cells. Deficiency of perlecan, the major HS proteoglycan in the subendothelial matrix, has been shown to reduce atherosclerosis by reducing lipoprotein involvement in mouse [4]. This study demonstrated that HS may have pro-atherogenic effects in mouse models with HS-deficient perlecan. These findings suggest that HS proteoglycans are atherogenic.

Heparanase (HPA), the only known mammalian endo- β -D-glucuronidase, is a multifunctional protein that has both enzymatic and nonenzymatic functions. It separates the HS chains of proteoglycans and changes the structure of the extracellular matrix. HPA is thought to be involved in various pathologies such as tumor angiogenesis, chronic inflammation, diabetic nephropathy, bone osteolysis, thrombosis, and atherosclerosis [5]. Previous experimental studies have demonstrated that HPA has a significant effect on the transition of stable plaque to a vulnerable state [6]. We investigated whether serum HPA levels differ in patients with stable coronary artery disease (SCAD) compared to subjects with normal coronary arteries.

Materials and Methods

Study Population

This case-control study included 92 patients with SCAD who had atherosclerotic coronary stenosis of 70% or more on coronary angiography and a control group consisting of 34 subjects with normal coronary arteries confirmed by coronary angiography. The patient flow chart is shown in Figure 1. Hyperventilation test was performed on subjects who exhibited normal coronary anatomy to exclude vasospasm. Patients who had any of the following conditions were excluded from the study: acute coronary syndrome (including unstable angina pectoris), chronic total occlusion, a history

of coronary artery disease, structural heart disease, moderate to severe valvular heart disease, moderate to severe left ventricular hypertrophy, heart failure with reduced ejection fraction (<50%), stage 3–4 chronic kidney disease, chronic obstructive pulmonary disease, or abnormal thyroid/hepatic functions. Venous blood samples for serum HPA, fasting blood glucose (FBG), and cholesterol were taken, after fasting for 8 h, the day after coronary angiography. Patients who were administered heparin before their blood samples were taken for HPA were excluded from the study. All subjects were evaluated with transthoracic echocardiography to exclude structural and valvular heart disease before coronary angiography. Venous blood samples were also obtained before coronary angiography for renal function tests and hematologic parameters. Demographic characteristics, medications, and laboratory parameters of the study population were recorded. Hypertension was diagnosed depending on one of the following criteria: systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg, self-report of a physician's diagnosis, or history of any antihypertensive treatment on admission. Diabetes mellitus (DM) was diagnosed according to the criteria of the American Diabetes Association: an FBG level ≥ 126 mg/dL, a 2-hour post-load glucose level ≥ 200 mg/dL, self-report of a physician's diagnosis, or history of hypoglycemic medication taken at admission.

Coronary Angiography

Coronary angiography was performed via the femoral artery using the Judkins technique. An experienced interventional cardiologist, blinded to the study, took part in the evaluation of the angiograms. Unstable plaque, such as the presence of a thrombus, surface irregularity, hazy appearance, or ulceration on coronary angiography, was excluded from the study. No ad hoc coronary intervention (stent or balloon angioplasty) was performed in order not to induce vascular injury. Approximately 20–40 mL of a non-ionic contrast agent was used in all coronary angiographies.

Measurements of Serum HPA

Venous blood samples were collected using pyrogen-free tubes and centrifuged at 3,000 rpm for 10 min. Serum samples were stored at -70°C until the analysis. The analysis was performed with a commercially available Human HPA ELISA kit (Shanghai Sunred Biological Technology Co., Shanghai, China), as described [7]. The assay range was within 50 pg/mL–15 ng/mL. All serum samples were routinely analyzed twice, and the results averaged.

Statistical Analysis

Statistical analyses were performed using IBM Statistical Package for Social Science (SPSS) for Windows, Version 17.0. (IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov test was used to analyze the normality of the data. Descriptive statistics are reported as the mean \pm standard deviation for continuous variables with a normal distribution, median (25th–75th percentiles) values for continuous variables without a normal distribution, and frequency with percentages for the categorical variables. Categorical variables were compared using chi-square or Fisher's exact test, as appropriate. The relationships between the parameters were assessed using Pearson's or Spearman's correlation analysis, according to the normality of the data. The differences between the groups were tested using Mann-Whitney U or Student's *t* test, as appropriate. A receiver operating characteristic curve analysis and Youden's index were used to determine the optimal cutoff level for the serum

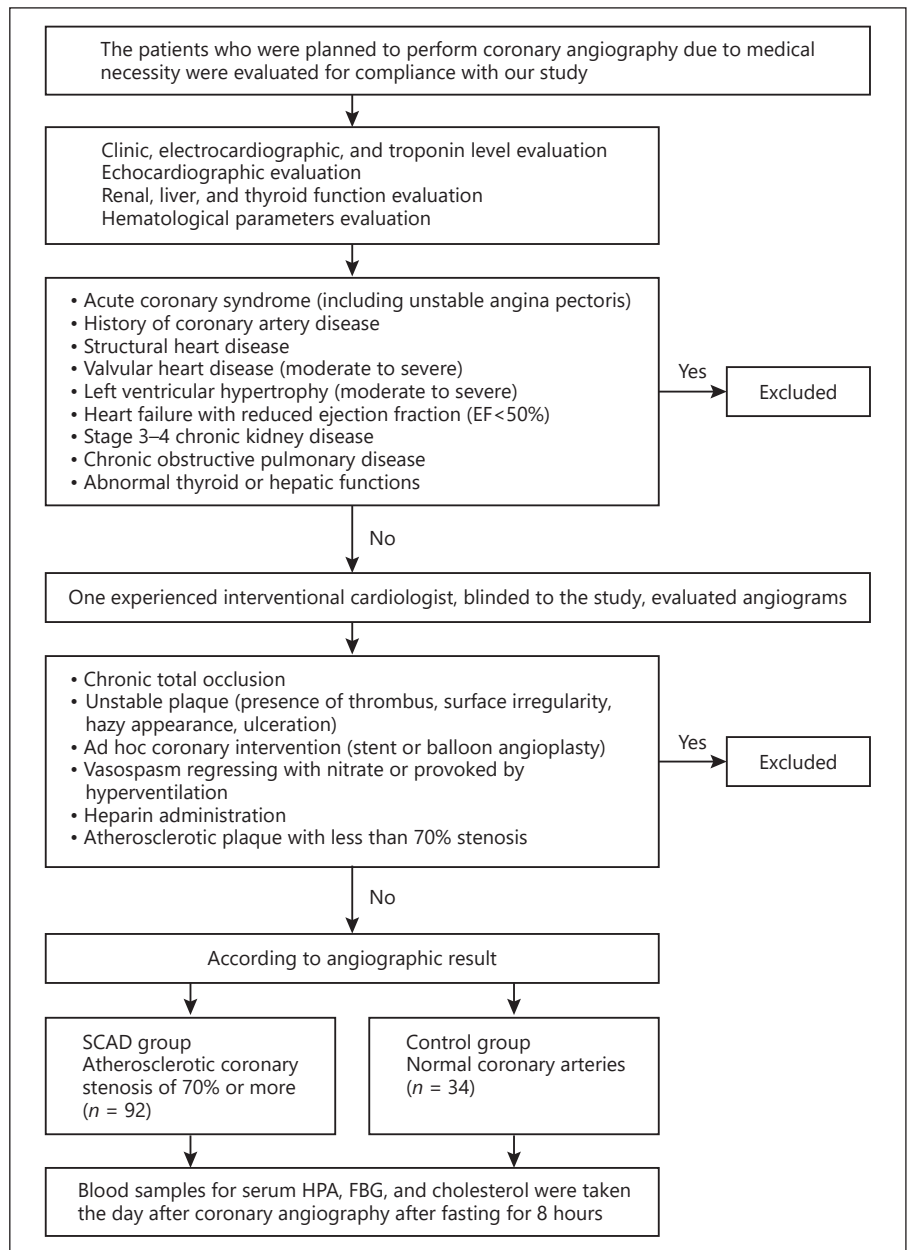


Fig. 1. Patient flow chart.

HPA value that best predicted SCAD. Multiple logistic regression analysis was used to identify the independent predictors of SCAD. p values less than 0.05 were considered statistically significant.

Results

The demographic, clinical, and laboratory characteristics of the study population are shown in Table 1. There was no difference between the control group and

the patient group with respect to age, gender, and body mass index. Cardiovascular risk factors including hypertension, DM, dyslipidemia, and smoking were similar between groups. Medications used at the time of angiography were similar between the groups as well. Serum levels of HPA were significantly lower in the SCAD group (137.5 [104.1–178.9] vs. 198.8 [178.2–244.9] pg/mL; $p < 0.001$) (Fig. 2). Serum levels of HPA positively correlated with FBG throughout the study population ($r: 0.42$; $p < 0.001$) (Fig. 3). Serum HPA levels were sig-

Table 1. Characteristics of stable coronary artery disease and control groups

	Control group (<i>n</i> = 34)	SCAD group (<i>n</i> = 92)	<i>p</i> value
Age, years	60.0±8.0	61.1±9.3	0.56
BMI, kg/m ²	27.8±2.8	27.4±3.3	0.47
Sex (male), <i>n</i> (%)	21 (61.8)	67 (73.6)	0.20
Dyslipidemia, <i>n</i> (%)	16 (47.1)	49 (47.5)	0.54
DM, <i>n</i> (%)	11 (32.4)	30 (32.6)	0.98
Hypertension, <i>n</i> (%)	15 (44.1)	46 (50)	0.56
Systolic BP, mm Hg	121.4±11.5	122.9±12.7	0.54
Diastolic BP, mm Hg	78.6±8.9	79.3±8.7	0.69
Smoking, <i>n</i> (%)	9 (26.5)	26 (28.3)	0.84
Current smoker, <i>n</i> (%)	6 (17.6)	19 (20.7)	0.71
Former smoker, <i>n</i> (%)	3 (8.8)	7 (7.6)	0.82
Pre-diagnosis drugs			
Beta-blocker, <i>n</i> (%)	4 (11.8)	10 (10.9)	0.89
ACE inh./ARB, <i>n</i> (%)	12 (35.3)	36 (39.1)	0.69
Ca antagonist, <i>n</i> (%)	7 (20.6)	16 (17.4)	0.68
Diuretic, <i>n</i> (%)	9 (26.5)	27 (29.3)	0.75
Statin, <i>n</i> (%)	13 (38.2)	43 (46.7)	0.39
OAD, <i>n</i> (%)	10 (29.4)	27 (29.3)	0.99
Laboratory parameters			
Urea, mg/dL	39.2±14.5	37.1±15.2	0.49
Creatinine, mg/dL	0.84±0.20	0.83±0.18	0.85
Hb, g/dL	13.4±1.4	13.8±1.8	0.25
Platelet, ×10 ³ /mm ³	235.8±87.4	230.2±59.7	0.74
Leukocyte, ×10 ³ /mm ³	8.52±2.84	7.89±3.28	0.34
FBG, mg/dL	109.8±37.8	114.2±44.7	0.62
Heparanase, pg/mL	198.8 (178.2–244.9)	137.5 (104.1–178.9)	<0.001

SCAD, stable coronary artery disease; BMI, body mass index; DM, diabetes mellitus; ACE inh., angiotensin-converting-enzyme inhibitor; ARB, angiotensin receptor blocker; OAD, oral antidiabetic; Hb, hemoglobin; FBG, fasting blood glucose; BP, blood pressure.

nificantly higher in subjects with DM compared those without DM ($p = 0.008$) (Fig. 2). HPA levels were lower in SCAD, both in the diabetic and nondiabetic subgroups, as compared to controls (DM [$n = 30$]; 143.9 [125.2–235.6] pg/mL vs. controls [$n = 11$]; 320.6 [227.5–397.4] pg/mL; $p < 0.001$ and non-DM [$n = 62$]; 129.8 [100.4–174.1] vs. controls [$n = 23$]; 195.4 [168.5–210.9] pg/mL; $p < 0.001$) (Fig. 4). In multiple logistic regression analysis, serum HPA level (odds ratio: 0.975; 95% confidence interval [CI]: 0.966, 0.985; $p < 0.001$) and FBG (odds ratio: 1.028; 95% CI: 1.010, 1.047; $p = 0.002$) were independently associated with SCAD (Table 2). The receiver operating characteristic curve showed that HPA levels less than 160.6 pg/mL predicted SCAD with 65% sensitivity and 97% specificity (AUC: 0.80; 95% CI: 0.728, 0.878; $p < 0.001$) (Fig. 5).

Discussion

This study showed that patients with SCAD have lower serum levels of HPA compared to patients with normal coronary arteries, both in diabetic and nondiabetic populations. Low serum levels of HPA may predict SCAD.

Atherosclerosis is the most common cause of death, despite an increase in interventional treatments. Different levels of lipids, fibroblasts, macrophages, smooth muscle cells, and extracellular matrix in intimal plaques lead to thrombosis in the arterial wall. The endothelium as well as the extracellular matrix, being continuously degraded and reorganized, play important roles in vascular remodeling. The role of the components of extracellular matrix, dermatan sulfate and chondroitin sulfate, in atherogenesis has been clarified [3]. Although the athero-

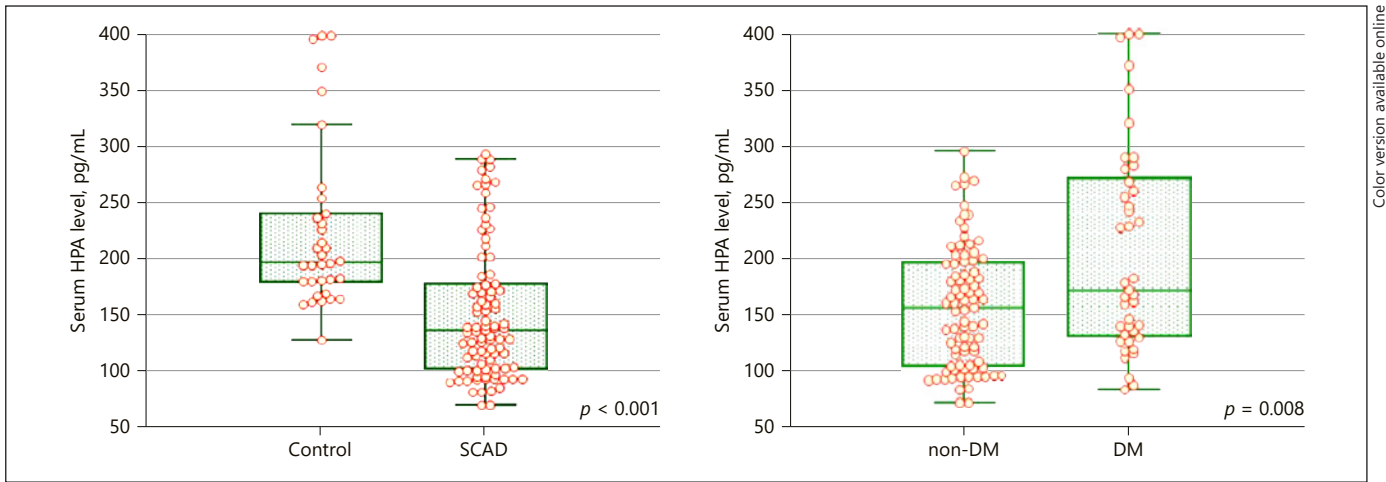


Fig. 2. Comparison of serum heparanase (HPA) levels between control and stable coronary artery disease (SCAD) patients and between patients with and without diabetes (DM vs. non-DM).

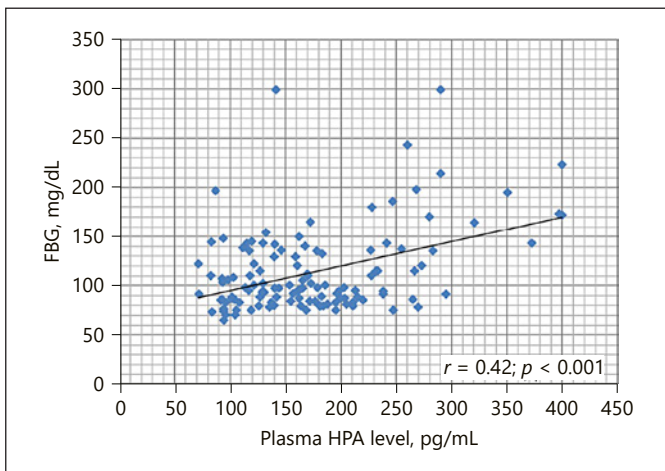


Fig. 3. Correlation between serum heparanase (HPA) level and fasting blood glucose (FBG).

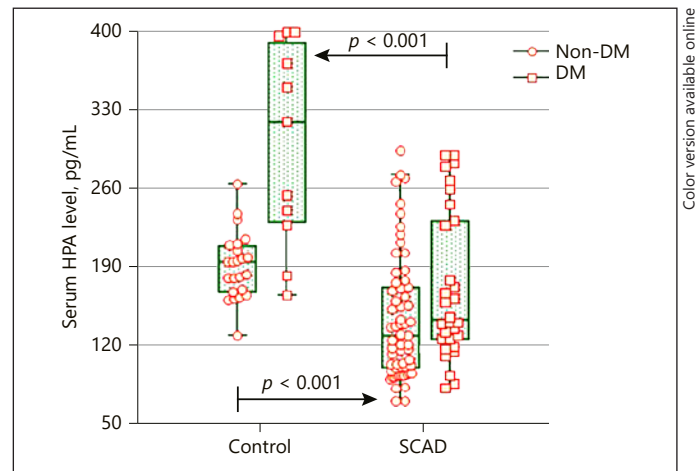


Fig. 4. Serum heparanase (HPA) levels of control and stable coronary artery disease (SCAD) patients in diabetic (DM) and nondiabetic (non-DM) subgroups.

genic and anti-atherogenic effects of HS have been demonstrated, the effect of HS on atherosclerosis remains to be clarified [4, 8].

HPA is the sole mammalian endo- β -glucuronidase that cleaves HS side chains from proteoglycans. The effect of HPA on atherosclerosis, diabetic nephropathy, and cancer metastasis has been studied in animal models [6, 9–11]. Baker et al. [6] demonstrated that the enzymatic cleavage of HS by HPA may influence the progression of atherosclerotic plaque in the coronary arteries of hyperlipidemic, diabetic swine. They showed that levels of HPA protein increased in thin cap fibroatheromas and plaques

with destructed internal elastic lamina compared to stable plaques [6]. Blich et al. [9] reported increased HPA staining in vulnerable plaques compared to stable plaques and controls in mice coronary specimens. These recent data have shown that HPA plays an important role in stable to vulnerable plaque transformation. In the future, HPA inhibitors may be considered for use in the prevention of vulnerable plaque. They reported a nine-fold increase in HPA levels in patients with acute myocardial infarction and a three-fold increase in patients with stable angina as compared to healthy subjects. This study compared healthy individuals with stable angina, 42% of whom were

Table 2. Logistic regression analysis for prediction of stable CAD

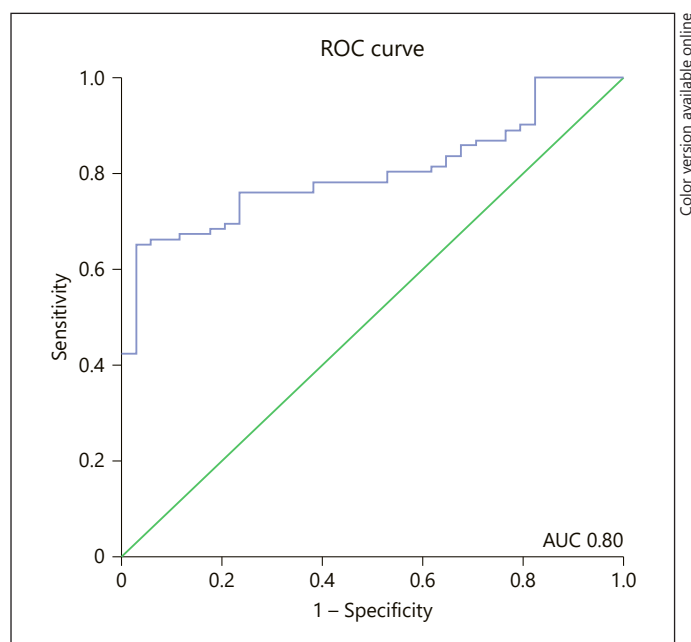
	p value	Exp(B)	95% CI for EXP(B)	
			lower	upper
Constant	0.079	49.754		
Age	0.6	0.984	0.927	1.046
Sex	0.096	0.417	0.149	1.169
FBG	0.002	1.028	1.010	1.047
Heparanase	<0.001	0.975	0.966	0.985

FBG, fasting blood glucose; CAD, coronary artery disease.

diabetic. A significant number of diabetic patients may explain the elevated HPA levels in patients with stable angina [9]. Baker et al. [12] induced vascular injury via endovascular stenting in wild mice overexpressing HPA. The time to thrombosis was significantly lower in the HPA-overexpressing mice. This study concluded that HPA is a powerful mediator of thrombosis in vascular injury [12].

HPA levels seem to be elevated in cases where the internal elastic lamina is damaged, such as cases involving vulnerable plaque, vascular injury, and acute myocardial infarction in contrast to stable plaque. Previous studies have demonstrated that plasma HPA is significantly associated with FBG levels, but not with HbA_{1c} in diabetic patients [13, 14]. A study in mice showed that HPA inhibitors reduced blood glucose [15]. An in vitro study found that hyperglycemia induces rapid release of HPA from endothelial cells [16]. We showed a positive correlation between FBG and serum levels of HPA. On the other hand, serum HPA level was higher in the diabetic group; this finding is compatible with previous studies. On the contrary, the proportion of diabetic patients and FBG was similar in the control group and the patient group in our study.

Perlecan is the major HS proteoglycan in the subendothelial matrix. A previous study demonstrated that perlecan is expressed only in the subendothelial matrix in a normal aorta. Perlecan expression is markedly increased in the fibrous cap as well as in the core plaque in atherosclerosis of the aorta. The same study showed that perlecan deficiency is associated with a reduced atherosclerotic process [4]. Since HPA separates the HS chains of proteoglycans, increased HPA levels may decrease perlecan levels in the subendothelial matrix. As a result, a decrease in perlecan levels will reduce atherosclerosis. Decreased HPA levels in patients with SCAD compared to patients with normal coronary arteries might be a result of this HPA-perlecan relationship.

**Fig. 5.** Receiver operating characteristic (ROC) curve analysis of serum heparanase levels for the prediction of stable coronary artery disease.

Apart from the stable plaques, elevated HPA levels in acute inflammation may be triggered by internal elastic lamina damage (acute coronary syndrome, vulnerable plaque, and vascular injury). HPA levels increase along with other inflammatory markers during vascular injury [17]. A recent study revealed that HPA might have distinct dual roles, depending on inflammatory status. Osteolysis mediated by osteoclasts predominates, and further destabilizes, stable plaques with the activation of macrophages. In the absence of inflammation, HPA is associated with osteogenic differentiation of mesenchymal cells resulting in stable plaques [18, 19]. This dual function of HPA, which is affected by the presence of inflammation, might yield different serum levels of HPA at various stages of plaque progression.

Smoking has been shown to play a role in the stages of atherosclerotic processing such as the oxidation of low-density lipoproteins, pro-inflammatory effects, vascular remodeling, and plaque destabilization [20]. With smoking cessation, the occurrence of coronary artery disease decreases by 50% in the first year, while the risk of stroke and acute coronary syndrome reaches the risk level of nonsmokers within 5–15 years [21]. Therefore, the rates of current and former smokers were kept similar in our study, and the effect of smoking on the results of our study was eliminated. The acceleration of atherosclerosis

in diabetes may be explained by hyperglycemia, the accelerated formation of advanced glycation end products, increased oxidative stress, hypertriglyceridemia, a high low-density lipoprotein/high-density lipoprotein ratio, hyperinsulinemia, and genetic variables. The beneficial effect of tight glycemic control has not been shown in studies; instead, increased mortality of patients with tight glycemic control has been observed [22, 23]. Dual effects of hyperglycemia on endothelial cells and cardiomyocytes to enhance coronary lipoprotein lipase activity is a possible explanation for how DM increases atherosclerosis [24]. The beneficial effects of lowering lipids on atherosclerosis are clear in type 2 diabetes [25]. Thus, it is likely that lipids play a more important role than glucose in atherosclerosis associated with type 2 diabetes. It may be said that the other mechanisms that trigger atherosclerosis in diabetics dominate the HPA effect. Since atherosclerosis is a complex process that is linked to multiple factors, it is not possible to simply explain the whole process with a single enzyme. Nevertheless, our study pointed out the possible effect of a novel enzyme in atherogenesis, which should be investigated in further studies.

The primary limitation was that our study was an observational study with a relatively small number of patients. Second, plasma activity of HPA, which may better reflect the effects of HPA, was not evaluated in our study. Third, the stability of plaques was evaluated with conventional angiography, whereas there are better alternatives, such as optical coherence tomography or intravascular ultrasound. Fourth, only FBG could be evaluated regard-

ing the diabetes status of the study population; HbA_{1c} could not be evaluated. Furthermore, the effect of anti-diabetic drugs on serum levels of HPA is unknown. All the limitations mentioned above may have affected the results of our study. Therefore, there is a need for randomized, controlled trials involving a large number of patients with advanced plaque evaluation and long-term follow-up of diabetic conditions.

Conclusion

This study showed that patients with SCAD had lower serum levels of HPA compared to patients with normal coronary arteries, both in diabetic and nondiabetic populations. Diabetes and blood glucose levels were closely associated with serum levels of HPA. Low serum levels of HPA may predict SCAD in both diabetic and nondiabetic populations.

Statement of Ethics

All patients provided written informed consent, and the study protocol was approved by the local ethics committee of the hospital in accordance with the Declaration of Helsinki and good clinical practice guidelines.

Disclosure Statement

The authors declare no conflict of interest.

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