Original Paper

Serum Neutrophil Gelatinase-Associated Lipocalin Levels and Aortic Stiffness in Noncritical Coronary Artery Disease

Korhan Soylu^a Gökyay Nar^d Gökhan Aksan^c Ömer Gedikli^f Sinan İnci^d
Serkan Yuksel^a Rukiye Nar^e Ayşegül İdil Soylu^b Okan Gulel^a
Mahmut Şahin^a

Departments of ^aCardiology and ^bRadiology, Faculty of Medicine, Ondokuz Mayıs University, and ^cDepartment of Cardiology, Gazi State Hospital, Samsun, Departments of ^dCardiology and ^eBiochemistry, Aksaray State Hospital, Aksaray, and ^fDepartment of Cardiology, Artvin State Hospital, Artvin, Turkey

Key Words

Aortic stiffness · Neutrophil gelatinase-associated lipocalin · Stable ischemic heart disease

Abstract

Aim: The aim of this study was to establish the degree of aortic stiffness and levels of neutrophil gelatinase-associated lipocalin (NGAL) in patients with stable ischemic heart disease. **Materials and Methods:** Patients who were found to have stable, noncritical lesions on coronary angiography were included in the study [noncritical coronary artery disease (CAD)]. The control group consisted of those patients who had similar risk profiles and metabolic parameters without atherosclerosis on angiography. **Results:** A total of 101 patients were included in the study of which 56 had noncritical CAD. Whereas the aortic strain (9.11 ± 3.4 vs. $14.01 \pm 4.1\%$, $p < 0.001$) and aortic distensibility ($3.98 \pm 1.9 \cdot 10^{-6} \text{ cm}^2/\text{dyn}$ vs. $6.33 \pm 2.3 \cdot 10^{-6} \text{ cm}^2/\text{dyn}$, $p < 0.001$) were lower in the noncritical CAD group, the aortic stiffness index was higher (6.34 ± 3.9 vs. 3.37 ± 2.4 , $p < 0.001$) as compared to controls. Serum NGAL levels were higher in the noncritical CAD group (79.29 ± 38.8 vs. $48.05 \pm 21.4 \text{ ng/ml}$, $p < 0.001$). NGAL levels were negatively correlated with aortic strain ($p < 0.01$, $r = 0.57$) and distensibility ($p < 0.001$, $r = 0.62$), but positively correlated with the aortic stiffness index ($p < 0.001$, $r = 0.72$). **Conclusion:** We show that in patients with noncritical CAD, the degree of aortic stiffness and NGAL levels are higher. These markers can be used as tools for further risk stratification of patients with noncritical CAD.

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Korhan Soylu, MD
Department of Cardiology, Faculty of Medicine
Ondokuz Mayıs University
TR-55139 Samsun (Turkey)
E-Mail korhansoylu@yahoo.com

Introduction

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kDa matrix metalloproteinase-9 (MMP-9)-bound glycoprotein that has initially been isolated from neutrophils [1]. It is found in cardiomyocytes, renal tubular cells, as well as the endothelial system [2, 3]. It has been suggested that NGAL is a marker for neutrophil activation and is involved in systemic inflammation and endothelial dysfunction leading to atherosclerotic plaque formation [4, 5].

Arterial stiffness is a measure of how the large arteries react to changes in blood pressure. Atherosclerosis and endothelial dysfunction has been associated with increasing arterial stiffness – as such the arterial stiffness has been used as a marker for atherosclerosis [6]. In addition, increased arterial stiffness has been associated with worse cardiovascular mortality [7, 8].

Studies focusing on NGAL and aortic stiffness have included patients with complex and advanced atherosclerotic disease. The reliability of these markers is less well-established in patients with earlier and milder forms of atherosclerosis. In this study, we aimed to study aortic stiffness and NGAL levels in patients with noncritical coronary artery disease (CAD) without critical coronary lesions.

Materials and Methods

Patient Selection

We assessed 606 consecutive patients with stable angina pectoris who underwent diagnostic coronary angiography in our institution between December 2012 and June 2013. Fifty-six study patients were selected from consecutive patients who underwent a coronary arteriogram for angina pectoris/signs of myocardial ischemia, and had angiographically noncritical coronary stenoses ($\leq 50\%$) in at least one of three coronary vessels. Forty-five age-matched individuals with angiographically proven normal coronary arteries served as controls. Those patients with $>50\%$ stenosis in any of the coronary vessels were excluded. Further exclusion criteria were significant stenosis by fractional flow reserve measurement, history of percutaneous or surgical revascularization, moderate to severe valve disease, left ventricular dysfunction (ejection fraction $<40\%$), chronic kidney disease, liver failure, atrial fibrillation, congenital heart disease, systemic conditions (ankylosing spondylitis, rheumatoid arthritis, Marfan's and Ehlers-Danlos disease), and aortic aneurysms.

The protocol was approved by the institutional review boards of the Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey, and adhered to the Declaration of Helsinki.

Biochemical Measurements

Blood samples were obtained in a fasting state from 8 a.m. to 10 p.m. These samples were centrifuged at 4,000 *g* for 10 min and the serum was analyzed for routine biochemical and hematologic parameters the same day. Biochemical parameters were measured with Abbott ARCHITECT c8000 (Abbott Laboratories, USA) using commercial kits. Hematologic parameters were measured using CellDyn 3700 (Abbott Laboratories) with laser and impedance methods.

Blood samples were obtained just after echocardiographic investigation for Lipocalin-2/NGAL. Serum Lipocalin-2/NGAL levels were measured with the ELISA method with the Triturus® ELISA analyzer (Grifols International, Barcelona, Spain) using the human Lipocalin-2/NGAL kit (BioVendor-Laboratorni medicina a.s., Brno, Czech Republic).

Echocardiographic Investigation

All patients underwent transthoracic echocardiography using Vivid-7, GE Vingmed Ultrasound with 2.5-MHz probes. Systolic and diastolic parameters were measured using the guidelines from the American Society of Echocardiography [9].

Aortic systolic and diastolic diameters were measured 3 cm distal to the aortic valve using the M-mode on the parasternal long-axis view. The systolic measurement was obtained at the time of full opening of the aortic valve and the diastolic measurement was obtained at the peak of the QRS. Simultaneous blood pressures were measured with careful assessment of systolic and diastolic pressures by calculating pulse

Table 1. Baseline clinical, laboratory and echocardiographic characteristics of the study population and comparison between groups

	Noncritical CAD group (n = 56)	Control group (n = 45)	p value
Demographics			
Age, years	57.4±10.2	56.4±10.2	0.632
Sex (female)	15 (27)	15 (33)	0.474
Body mass index	30.9±4.8	29.3±3.7	0.066
Heart rate, beats/min	76.6±10.2	77.0±10.0	0.848
Mean systolic blood pressure, mm Hg	130.5±9.1	127.3±8.5	0.075
Mean diastolic blood pressure, mm Hg	82.1±6.2	81.2±7.0	0.484
Concomitant diseases			
Diabetes	14 (25)	11 (24)	0.551
Hypertension	32 (57)	25 (56)	0.873
Cigarette smoking	24 (45)	22 (51)	0.581
Dyslipidemia	25 (45)	19 (42)	0.807
Chronic obstructive pulmonary disease	7 (13)	1 (2)	0.557
Peripheral arterial disease	1 (2)	1 (2)	0.876
Previous stroke	0	1 (2)	0.262
Laboratory			
White blood cell (×10 ³ /μl)	7.59±2.5	7.22±1.8	0.421
Hemoglobin, g/l	14.1±2.1	13.9±1.4	0.683
Serum glucose, mg/dl	105.9±22.6	100±26.7	0.234
Creatinine, mg/dl	0.80±0.20	0.75±0.17	0.183
HbA _{1c} , %	5.4±1.1	5.1±0.9	0.146
Triglyceride, mg/dl	201.5±120.2	167.2±66.5	0.089
Low-density lipoprotein cholesterol, mg/dl	131.4±32	126.4±27	0.400
High-density lipoprotein cholesterol, mg/dl	39.3±8.1	40.5±8.2	0.452
Total cholesterol, mg/dl	200.7±42.2	199.5±32.2	0.879
Statin use	6 (11)	3 (7)	0.727
Echocardiography			
Left ventricular ejection fraction, %	63.8±4.6	64.6±3.4	0.358
Left ventricle end-diastolic diameter, mm	48.8±4.8	47.6±3.6	0.168
Left ventricle end-systolic diameter, mm	29.3±4.7	29.8±4.0	0.594
E wave, m/s	0.61±0.1	0.62±0.1	0.768
A wave, m/s	0.72±0.2	0.69±0.2	0.406
E/A	0.89±0.3	0.95±0.3	0.260

Figures are either means ± SD or numbers with percentages in parentheses.

pressure. These measurements were repeated 3 times and mean values were accepted to minimize measurement error. The following formulas were used for aortic parameters [10]:

(1) Aortic strain (%) = (systolic aortic diameter – diastolic aortic diameter) × 100 / diastolic aortic diameter

(2) Distensibility (cm²/dyn) = 2 × (aortic strain) / (systolic blood pressure – diastolic blood pressure)

(3) Aortic stiffness index = ln (systolic blood pressure / diastolic blood pressure) / [(aortic systolic diameter – aortic diastolic diameter) / aortic diastolic diameter]

An intraobserver variability study on distensibility and aortic stiffness measurements among 10 volunteers showed coefficients of variation of 4.8 and 4.5%, respectively. Between observers, this coefficient was 6.9 and 5.7%.

Statistical Analyses

All data were loaded to the SPSS 15 program. The normal distribution of the data was tested using the Kolmogorov-Smirnov test. Student's t test was used to compare two groups of values demonstrating normal

Fig. 1. Box plot diagrams of NGAL level (ng/ml) in the noncritical CAD group and control subjects. SIHD = Stable ischemic heart disease.

Table 2. Comparison of aortic elastic properties and NGAL levels between groups

	Noncritical CAD group (n = 56)	Control group (n = 45)	p value
Aortic strain, %	9.11±3.4	14.01±4.1	<0.001
Aortic distensibility, 10 ⁻⁶ cm ² /dyn	3.98±1.9	6.33±2.3	<0.001
Aortic stiffness index	6.34±3.9	3.37±2.4	<0.001
NGAL levels, ng/ml	79.29±38.8	48.05±21.4	<0.001

distribution, while groups of values without normal distribution were compared using the Mann-Whitney U test. Comparison of categorical values was carried out by the χ^2 test. Any correlation between data was tested with the Spearman and Pearson correlation analysis. While the continuous data were expressed as mean $\pm$ standard deviation, the categorical data were expressed as percentage values, and a p value of <0.05 was accepted as statistically significant.

Results

A total of 101 patients were included in the study of which 56 had noncritical CAD and 45 were in the control group without CAD. Demographic parameters, routine laboratory data, systolic and diastolic echocardiographic parameters were similar in both groups. While the BMI was elevated in both groups, this was not statistically significant in between groups ($p = 0.06$) (table 1).

Serum NGAL Levels

Serum NGAL levels were significantly elevated in the noncritical CAD group when compared to the control group (79.29 ± 38.8 vs. 48.05 ± 21.4 ng/ml, $p < 0.001$; table 2; fig. 1).

Aortic Elasticity

Aortic strain was found to be lower in the noncritical CAD group when compared to those with no atherosclerotic heart disease (9.11 ± 3 vs. $14.01 \pm 4.1\%$, $p < 0.001$). Patients in the

Fig. 2. Correlation between NGAL level and aortic distensibility.

Fig. 3. Correlation between NGAL level and aortic strain.

Fig. 4. Correlation between NGAL level and aortic stiffness index.

noncritical CAD group also had less distensible aortas ($3.98 \pm 1.9 \times 10^{-6} \text{ cm}^2/\text{dyn}$ vs. $6.33 \pm 2.3 \times 10^{-6} \text{ cm}^2/\text{dyn}$, $p < 0.001$) and higher aortic stiffness indices (6.34 ± 3.9 vs. 3.37 ± 2.4 , $p < 0.001$) when compared to the control group.

Correlation Analysis

The correlation between serum NGAL levels and aortic elasticity parameters were studied. NGAL levels were negatively correlated with aortic strain ($p < 0.01$, $r = 0.57$) and distensibility ($p < 0.01$, $r = 0.62$), but positively correlated with aortic stiffness index ($p < 0.01$, $r = 0.72$; fig. 2–4).

Discussion

In our study, we show that in those patients with noncritical CAD, the aortic elasticity parameters and serum NGAL levels are significantly different from those patients with normal coronary arteries. In addition, we demonstrate that the serum NGAL levels strongly and meaningfully correlate with aortic elasticity parameters.

The adverse outcomes of patients with CAD relates to the complications that arise from the vulnerability of the atherosclerotic plaque. Clinical guidelines have focused on the prevention of coronary events; however, in clinical practice, patients with noncritical CAD receive less aggressive medical therapy. Yet, it has been shown that lesions that lead to coronary events are predominantly noncritical in severity ($<50\%$) [11]. Therefore, accurate risk stratification and assessment of plaque stability is of critical importance in patients with noncritical stenoses.

Arterial stiffness is an important marker for endothelial dysfunction and atherosclerosis [12–15]. In addition, the degree of arterial stiffness predicts the severity of atherosclerosis and its rate of progression [16, 17]. In a study of over 3,000 patients, it has been shown that carotid intima-media thickness positively correlates with arterial stiffness [18]. It has further been shown that arterial stiffness strongly correlates with the presence of coronary lesions of $>50\%$ severity [19]. There is also evidence that aortic stiffness is increased in patients with premature CAD [20]. Worsening aortic distensibility together with endothelial dysfunction might lead to hypertension, left ventricular hypertrophy, and increasing oxygen demand with further progression of atherosclerosis and vulnerable plaque [21, 22]. In our study, we show that even in patients with noncritical lesions, the aortic distensibility parameters are abnormal when compared to controls.

NGAL is a new inflammatory marker that is present in neutrophils and correlates with acute kidney injury [23–25]. More recently, NGAL has been found to play an active role in coronary atherosclerosis and heart failure [26, 27]. Its relevance in atherosclerosis has been shown to be related to the activity of MMP. Increased MMP-9 leads to modification of the coronary plaque and leads to rupture of the cap with subsequent myocardial infarction [28, 29]. NGAL binds to MMP-9 and protects it against inhibition [30]. Therefore, increased NGAL activity leads to active MMP-9 with subsequent increased vulnerability of the plaque and development of acute coronary syndromes [31]. In a recent study, increased NGAL levels were found to be an independent predictor of death and major adverse cardiac event outcomes in patients who received primary percutaneous coronary intervention for ST segment elevation myocardial infarction [26]. In addition, serum NGAL levels were found to be a useful predictor of the presence of CAD [32]. In this study, we describe for the first time the correlation of NGAL levels in patients with noncritical lesions.

The data on the levels of NGAL and its association with CAD are limited. Yet, the levels of NGAL have been found to correlate with the number of diseased vessels and the modified

Gensini score in patients with CAD [33]. In our study, we demonstrate that NGAL levels also meaningfully correlate with aortic elasticity parameters. These findings might be explained by the increased activity of MMP leading to degradation of elastin in the arterial wall [34]. Together with the aortic elasticity data, the presence of high levels of NGAL might inform us about the degree of atherosclerotic inflammation and might reflect plaque vulnerability.

Study Limitations

The main limitation of our study is that we have not studied clinical outcomes as they correlate with NGAL levels. Prospective studies that prognosticate noncritical CAD with the use of NGAL and aortic distensibility are necessary. Another limitation is the lack of use of intravascular ultrasound in the assessment of coronary atherosclerosis, which might have led to CAD not accurately diagnosed in the control group.

Conclusions

In this study, we show that patients with noncritical CAD have increased aortic stiffness and NGAL levels. These findings suggest that even in those patients with limited plaque burden, NGAL levels might inform us about the degree of aortic remodeling and inflammation. Therefore, NGAL levels and aortic stiffness might be a useful addition as patients are risk stratified for early-stage CAD.

Disclosure Statement

The authors have no conflicts of interest.

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