Original Paper

Early Biomarkers of Renal Damage in Relation to Arterial Stiffness and Inflammation in Male Coronary Artery Disease Patients

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Key Words

Atherosclerosis • Inflammation • Coronary artery disease • Arterial stiffness • Pulse wave velocity • Novel kidney injury markers • L-FABP • KIM-1 • NGAL

Abstract

Background/Aims: Plasma neutrophil gelatinase-associated lipocalin (NGAL), urinary liver-type fatty acid-binding protein (L-FABP) and urinary kidney injury molecule-1 (KIM-1) have emerged as promising biomarkers for both acute and chronic kidney injury that also provide prognostic value for cardiovascular morbidity and mortality. Our aim was to evaluate their relationships with arterial stiffness and inflammation in coronary artery disease (CAD) patients and in clinically healthy controls. **Methods:** We studied 52 patients with CAD (age 63.2 ± 9.2 years) and 41 healthy controls (age 60.1 ± 7.2 years). Urinary L-FABP and KIM-1 as well as serum NGAL, adiponectin and resistin levels were measured using the enzyme-linked immunosorbent assay method. The technique of applanation tonometry was used for non-invasive pulse wave analysis and pulse wave velocity assessments. **Results:** Urinary L-FABP and KIM-1 were independent determinants of cf-PWV for the CAD patients ($R^2=0.584$, $P<0.001$) but not for the controls. Adiponectin correlated with log-KIM-1 ($r=0.31$, $P=0.028$) only for the patients, while NGAL correlated with WBC count ($\rho=0.29$, $P=0.038$; $r=0.35$, $P=0.029$) and resistin ($\rho=0.60$, $P<0.001$; $r=0.57$, $P<0.001$) for both the CAD and control groups, respectively. **Conclusion:** Our findings suggest that urinary L-FABP and KIM-1 may be independently associated with aortic stiffness in individuals with CAD.

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Introduction

Assessment of arterial stiffness provides useful information about the structural and functional alterations of the arterial tree. Stiffening of the aortic wall independently predicts all-cause and cardiovascular mortality in the general population, in chronic kidney disease (CKD) patients and in other high-risk groups [1-4]. Carotid-femoral pulse wave velocity (cf-PWV) is considered the “gold standard” measurement of aortic stiffness and might be a clinically useful tool to identify patients with high cardiovascular risk [5-6].

Plasma neutrophil gelatinase-associated lipocalin (NGAL), urinary liver-type fatty acid-binding protein (L-FABP) and urinary kidney injury molecule-1 (KIM-1) have emerged as promising diagnostic and prognostic biomarkers for both acute and chronic kidney injury in different clinical settings [7-14]. Moreover, several studies have shown that these markers may also provide a prognostic value for cardiovascular morbidity and mortality. Plasma NGAL has been independently associated with cardiovascular disease and mortality in community-dwelling older adults [15]. Higher NGAL levels also predict mortality in patients with myocardial infarction as well as in heart failure patients with and without CKD [16, 17]. Urinary L-FABP is emerging as a useful marker for renal and cardiovascular prognosis in type 2 diabetes mellitus [18]. In acute coronary syndrome patients, elevated L-FABP levels may help identify individuals at high risk for future cardiovascular events [19]. Recent prospective community-based studies in elderly men have suggested that urinary KIM-1 is independently associated with cardiovascular mortality and incidence of heart failure [20, 21]. Thus, the above findings suggest that these proteins may also be useful predictors of the development of cardiovascular disease. However, little is known about the potential pathophysiological mechanisms underlying the relationship between these novel kidney biomarkers and cardiovascular risk.

Since renal and cardiovascular dysfunctions are intertwined, alterations in vascular structure and function may be at least partially responsible for early renal structural damage. In the current study, we hypothesized that increased arterial stiffness might be associated with elevated levels of early kidney damage biomarkers independently of traditional markers of renal function in coronary artery disease (CAD) patients with an estimated glomerular filtration rate (eGFR) of more than 60 mL/min/1.73 m².

Subjects and Methods

Study population

Fifty-two male patients with angiographically proven CAD were prospectively studied. The patients were recruited from the Department of Cardiology, University of Tartu, Estonia. The exclusion criteria for the CAD group were any comorbid acute or chronic inflammatory disease, diabetes mellitus, myocardial infarction, cerebrovascular events or revascularization operation during the preceding 6 months, unstable angina, cardiac arrhythmias, clinically significant heart failure or valvular disease, reduced kidney function (eGFR < 60 mL/min/1.73 m²), presence of cancer or endocrine pathology.

Forty-one age- and gender-matched apparently healthy controls were identified through local family physicians in the same geographical area. In the control group, the following exclusion criteria were applied: any comorbid acute or chronic inflammatory disease, CAD, cerebral or peripheral atherosclerotic disease, diabetes mellitus, cardiac arrhythmias, clinically significant heart failure or valvular disease, hypertension, reduced kidney function (eGFR < 60 mL/min/1.73 m²), presence of cancer, infectious disease or endocrine pathology, or regular use of any medication.

Study protocol

All subjects completed a lifestyle and medical history questionnaire. Venous blood samples were drawn from each subject and spot morning urine specimens were collected from 45 patients and 37 controls. All samples were obtained between 8am and 11am after an overnight fast and abstinence from tobacco.

co, alcohol, tea and coffee. Height and weight were assessed and body mass index (BMI) was calculated. The subjects were allowed to rest in a supine position in a quiet, temperature-controlled room for at least 10 minutes. Thereafter, brachial blood pressure and cf-PWV were assessed and pulse wave analysis was made. All hemodynamic measurements were taken in duplicate and averaged. The study protocol was approved by the Ethics Committee of the University of Tartu, and all subjects gave their written informed consent.

Arterial stiffness and central hemodynamics measurements

The cf-PWV was calculated from the pulse transit time using ECG-gated carotid and femoral artery waveform recordings over a known distance (SphygmoCor; AtCor Medical, Sydney, Australia) [22]. For calculation of cf-PWV, the distance from suprasternal notch over umbilicus to the femoral artery minus carotid arterial length was used. Central aortic waveforms and other hemodynamic parameters were evaluated non-invasively with the commercially available SphygmoCor device (AtCor Medical, Sydney, Australia) [22]. After 15 sequential high-quality radial waveforms were recorded at the patient's left wrist using arterial applanation tonometry, a validated generalized transfer function for calculation of the central aortic pressure waveform was used [23, 24].

Biochemical analysis

Venous blood samples were collected and centrifuged at room temperature. The serum was then pipetted into Eppendorf tubes and stored at -70°C until analysis. Serum myeloperoxidase (MPO), adiponectin and NGAL levels were measured using an enzyme-linked immunosorbent assay (ELISA) (Myeloperoxidase enzyme immunoassay test kit, BIOCHECK, Inc. Foster City, CA; Human Total Adiponectin/Acrp30 immunoassay, R&D Systems Europe, Abingdon, UK; Human Lipocalin-2/NGAL immunoassay, R&D Systems Europe, Abingdon, UK, respectively). The levels of serum resistin, ferritin and interleukin-6 (IL-6) were determined using The Evidence Investigator (Metabolic Syndrome Array-1, Randox Laboratories, Crumlin, UK). Urine samples were collected into sterile plastic collection cups, aliquoted into Eppendorf tubes and kept frozen at -70°C until analysis. The levels of urinary L-FABP and KIM-1 were measured using ELISA (Human Liver Fatty Acid Binding Protein-1 ELISA kit, BlueGene Biotech, Shanghai, China; HAVCR1 (Human), KIM-1, Abnova GmbH, Heidelberg, Germany, respectively). The plasma levels of glucose, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, white blood cell (WBC) count, high-sensitivity C-reactive protein (hs-CRP), serum creatinine, eGFR (calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [25]), cystatin C (CysC) and urinary creatinine were measured in the local clinical laboratory with automated analyzers using standard laboratory methods.

Statistical analysis

All statistical analyses were performed using the SPSS software for Windows, version 22.0. Dichotomous variables are expressed in percentages, whereas continuous variables are presented as mean and standard deviation or as median and interquartile range. The normality of the distribution of continuous variables was assessed using the Shapiro-Wilk test. The skewed distributions were normalized by logarithmic transformation. Comparisons between the two groups were performed by two-tailed Student's t-test and the Mann-Whitney U test, where appropriate. Univariate associations between predefined renal (L-FABP, KIM-1, NGAL, CysC, eGFR and creatinine), arterial stiffness (cf-PWV) and inflammatory parameters (WBC, IL-6, hs-CRP, resistin, adiponectin, MPO and ferritin) were analyzed using Pearson's r or Spearman's rho correlation, and multivariate analysis was applied to investigate the independent determinants of cf-PWV. The variables entered into the model were drawn from univariate analyses and from relevant published observations (mean age, BMI, MAP, L-FABP, KIM-1, NGAL, eGFR, hs-CRP, IL-6, glucose, cholesterol, triglycerides, smoking status, antihypertensive therapy and statin use). Multivariate analyses were performed using a stepwise (forward followed by backward) multiple linear regression. Statistical significance was defined as $P < 0.05$. A Fisher's exact test or chi-square test was used to compare differences in the proportions between the two groups. All statistical tests were checked for violations of assumptions.

Results

Table 1. Clinical and laboratory characteristics of the study groups

Characteristics of the study population

The clinical and laboratory characteristics of the CAD cases and the controls are presented in Table 1. There were no significant differences between the groups with respect to mean age, peripheral diastolic and central systolic or diastolic blood pressure, mean arterial pressure, current smoking status, total cholesterol, HDL-C, LDL-C, triglycerides, glucose, hs-CRP or ferritin levels. The two groups differed in BMI, height, peripheral systolic blood pressure, heart rate, cf-PWV, heart rate-corrected augmentation index (AIx@75), medication use, as well as in WBC count and

Variable	CAD patients (n=52)	Controls (n=41)	P-value
Age (years)	63.2 ± 9.2	60.1 ± 7.2	0.075
Body mass index (kg/m ²)	27.9 ± 3.5	26.0 ± 3.4	0.012
Height (m)	176.2 ± 6.4	179.3 ± 6.5	0.025
Peripheral SBP (mmHg)	135.0 ± 14.5	128.4 ± 13.2	0.027
Peripheral DBP (mmHg)	78.5 ± 8.0	77.9 ± 7.3	0.706
Central SBP (mmHg)	122.7 ± 11.1	119.8 ± 15.1	0.303
Central DBP (mmHg)	79.7 ± 7.6	78.4 ± 6.7	0.378
MAP (mmHg)	96.0 (91.6-101.4)	92.5 (89.5-99.5)	0.103
Heart rate (bpm)	62.8 ± 8.3	57.8 ± 7.1	0.003
WBC count (x10 ⁹ /L)	6.4 (5.2-7.9)	5.1 (4.7-5.8)	0.001
Total cholesterol (mmol/l)	5.1 ± 1.0	5.5 ± 0.9	0.080
HDL-cholesterol (mmol/l)	1.5 ± 0.4	1.6 ± 0.4	0.153
LDL-cholesterol (mmol/l)	3.2 (2.8-4.1)	3.7 (3.3-4.3)	0.058
Triglycerides (mmol/l)	1.2 (0.8-1.6)	1.0 (0.7-1.4)	0.311
Glucose (mmol/l)	5.8 (5.3-6.3)	5.8 (5.4-6.1)	0.858
hs-CRP (mg/l)	1.3 (0.7-2.8)	1.2 (0.7-2.1)	0.423
Myeloperoxidase (ng/mL)	58.4 (35.3-110.7)	45.2 (28.0-62.6)	0.014
Interleukin-6 (pg/mL)	1.33 (0.74-1.98)	0.95 (0.64-1.29)	0.048
Ferritin (ng/mL)	128.0 (68.6-202.0)	92.7 (54.7-190.7)	0.110
Adiponectin (ng/mL)	5701 ± 2890	7081 ± 3612	0.045
Resistin (ng/mL)	3.4 (2.6-4.5)	2.8 (2.4-3.5)	0.043
AIx@75 (%)	18.9 ± 7.2	15.1 ± 8.0	0.021
cf-PWV (m/s)	9.7 ± 2.6	8.2 ± 1.7	0.003
eGFR (ml/min/1.73m ²)	85 (73-97)	89 (79-93)	0.847
S-Creat (μmol/L)	83.2 ± 12.3	84.0 ± 11.2	0.750
S-Urea (mmol/L)	5.7 (4.7-7.0)	5.3 (4.5-6.0)	0.114
Cystatin C (mg/L)	0.93 ± 0.14	0.90 ± 0.10	0.278
NGAL (ng/mL)	74.6 (61.9-83.5)	73.7 (59.4-85.0)	0.892
L-FABP/Creat (ng/mmol)	169.0 (121.9-228.2)	187.2 (136.5-271.3)	0.244
KIM-1/Creat (ng/mmol)	79.6 (53.6-110.7)	85.8 (54.7-118.5)	0.636
Current smoking, n (%)	13 (25)	5 (12)	0.214
Medication, n (%)			
Antihypertensives	48 (92)	0 (0)	<0.001
Antiplatelets	34 (65)	0 (0)	<0.001
Statins	28 (54)	0 (0)	<0.001

The values are expressed as means (± standard deviation), medians (interquartile range), or prevalence (%). CAD, coronary artery disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; WBC, white blood cell count; HDL, high-density lipoprotein; LDL, low-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; AIx@75, augmentation index adjusted to a heart rate of 75 beats per minute; cf-PWV, carotid-femoral pulse wave velocity; eGFR, estimated glomerular filtration rate; Creat, creatinine; NGAL, neutrophil gelatinase-associated lipocalin; L-FABP, liver-type fatty acid-binding protein; KIM-1, kidney injury molecule-1.

serum MPO, IL-6, adiponectin and resistin levels. There were no significant differences in eGFR, serum creatinine, urea, CysC, NGAL, urinary L-FABP or KIM-1 levels between the patients and the controls.

Association between arterial stiffness and markers of renal function and tubular damage

In a univariate analysis for the predefined variables, log-L-FABP (r=0.44, P=0.002; Figure 1), CysC (r=0.52, P<0.001), eGFR (rho=-0.36, P=0.012) and creatinine (r=0.33, P=0.021)

correlated positively with cf-PWV for the CAD group but not for the controls (data not shown). Also, log-KIM-1 showed a borderline significant relationship ($r=0.29$, $P=0.062$) with aortic stiffness for the patients. In multivariate analysis with cf-PWV as the dependent variable, L-FABP and KIM-1 were the only renal markers that remained significant after adjustment for potential confounders (mean age, BMI, MAP, NGAL, eGFR, hs-CRP, IL-6, glucose, cholesterol, triglycerides, smoking status, antihypertensive therapy and statin use) ($R^2=0.58$; $P<0.001$; Table 2). In the post hoc subgroup analyses based on eGFR levels, L-FABP ($r=0.6$, $P=0.002$) showed a strong and CysC ($r=0.4$, $P=0.043$) a moderate correlation with cf-PWV for the subgroup of CAD patients with eGFR between 60 and 89 mL/min/1.73 m².

Serum NGAL was not correlated with cf-PWV or any other measure of arterial stiffness for either CAD patients or controls (data not shown).

In the control group, a significant inverse correlation was found between serum resistin levels and log-L-FABP ($r=-0.34$, $P=0.045$). Serum NGAL levels correlated with WBC count ($\rho=0.29$, $P=0.038$; $r=0.35$, $P=0.029$) and resistin ($\rho=0.60$, $P<0.001$; $r=0.57$, $P<0.001$) for the CAD and control groups, respectively, and with serum creatinine ($\rho=0.31$, $P=0.030$) only for the CAD group. Urinary log-

KIM-1 was linearly correlated with serum creatinine ($r=0.33$, $P=0.019$), adiponectin ($r=0.31$, $P=0.028$) and log-ferritin ($r=-0.32$, $P=0.022$) levels for the CAD group while no such correlations were found for the controls.

Fig. 1. Scatterplot showing linear correlation between carotid-femoral pulse wave velocity (cf-PWV) and logarithmically transformed urinary liver-type fatty acid-binding protein (log-L-FABP) for the coronary artery disease patients ($r=0.44$, $P=0.002$).

Table 2. Multiple regression model for the CAD group with cf-PWV as the dependent variable

Variable	Regression coefficient	Standard error	P value
Mean age	0.161	0.029	<0.001
L-FABP/Creat	0.011	0.003	0.001
Body mass index	0.197	0.077	0.014
KIM-1/Creat	0.013	0.006	0.023

$R^2=0.584$, $P<0.001$. Mean age, body mass index, mean arterial pressure, L-FABP/Creat, KIM-1/Creat, neutrophil gelatinase-associated lipocalin, cystatin C, estimated glomerular filtration rate, high-sensitivity C-reactive protein, interleukin-6, glucose, cholesterol, triglycerides, smoking status, antihypertensive therapy and statin use were entered as independent variables into the regression model. CAD, coronary artery disease; cf-PWV, carotid-femoral pulse wave velocity; L-FABP, liver-type fatty acid-binding protein; KIM-1, kidney injury molecule-1; Creat, creatinine.

Discussion

In the present study, we demonstrated correlations between aortic stiffness and markers of both renal function and tubular damage in the symptomatic CAD patients. The novel findings that urinary L-FABP and KIM-1 are independently associated with cf-PWV in CAD (Table 2) extend our current knowledge of these proteins and may partially explain their value as biomarkers for cardiovascular prognosis in different study populations [18-21].

L-FABP is involved in the fatty acid uptake, intracellular transport and metabolism [26, 27]. In the case of kidney damage, the expression of L-FABP in the proximal tubular cells is upregulated [28-31]. It has been previously shown that the estimated contribution of serum L-FABP to urinary L-FABP levels is only 3%, hence the L-FABP that is found in urine is produced primarily by the tubular cells [32, 33]. Interestingly, the mean urinary levels of this protein were moderately lower in the subjects with CAD compared to the controls in our study, however this difference was not statistically significant. Since L-FABP has been reported as a renoprotective endogenous antioxidant, it can be hypothesized that its lower levels may reflect reduced kidney protection in atherosclerotic patients without moderate to severe CKD [34-36].

In the presence of increased arterial stiffness, the kidneys are particularly susceptible to the damage resulting from high pulsatile pressure and flow [37]. We observed elevated values of cf-PWV and $AIx@75$ for the subjects with CAD. Furthermore, besides its independent association with L-FABP and KIM-1, cf-PWV was also correlated with serum creatinine, CysC and eGFR in these patients, but not in the healthy controls. Although the type of causality could not be determined, these findings support the view that increased arterial stiffness (and thus high pulsatile pressure and flow) contributes to renal dysfunction and propose that urinary L-FABP and KIM-1 may serve as novel biomarkers that reflect this relationship even in the early stages of renal damage. However, it should be noted that this relationship might be bidirectional and could also be affected by some other pathophysiological processes.

We acknowledge that eGFR tends to be less accurate at levels above 60 mL/min per 1.73 m² [38]. However, the CKD-EPI creatinine equation used in the current work performs better than the Modification of Diet in Renal Disease Study formula, especially at higher GFR levels [25, 38]. When we performed post hoc subgroup analyses based on eGFR levels L-FABP showed a strong and CysC a moderate correlation with cf-PWV for the subgroup of CAD patients with eGFR between 60 and 89 mL/min/1.73 m², whereas none of the measured kidney biomarkers retained as significant correlates of aortic stiffness for the patients with eGFR ≥ 90 mL/min/1.73 m² (see the Results section). Although these findings are potentially interesting, larger studies with pre-specified subgroup analyses are warranted to explore these possible differences between CAD patients with eGFR ≥ 90 mL/min/1.73 m² and those with eGFR between 60 and 89 mL/min/1.73 m².

The lack of correlations between kidney biomarkers and cf-PWV in healthy subjects might be due to their better preserved vascular function and reduced inflammation compared to CAD patients. Indeed, decreased serum adiponectin, higher WBC count and elevated IL-6, MPO and resistin levels indicated systemic inflammation in the CAD group when juxtaposed with the healthy controls (Table 1). Previous findings suggest that elevated resistin may be especially undesirable in the case of reduced adiponectin levels [39, 40]. Although the true function of resistin still remains to be established, it seems to play significant regulatory roles in various biological processes including low-grade inflammation and atherosclerosis [41].

Urinary KIM-1 showed a borderline significant correlation with cf-PWV for the CAD patients. However, this relationship reached statistical significance after adjusting for potential confounders (Table 2). KIM-1 functions as a transmembrane phosphatidyl serine receptor that recognizes apoptotic cells and has also been suggested as an early marker for tubular damage [42, 43]. A recent study in mice demonstrated that by facilitating the phagocytic

process, KIM-1 might have a protective anti-inflammatory role in the early stages of acute kidney injury [44]. In our work, urinary KIM-1 correlated positively with serum adiponectin and inversely with ferritin for the CAD patients but not for the controls. Although the association between KIM-1 and aortic stiffness in the CAD group was rather weak, it reveals a novel aspect of this prognostic biomarker and deserves further study.

Several correlations also emerged between serum NGAL and indices of kidney function and inflammation. Positive relationships with WBC count and resistin for both groups are in line with the view that serum NGAL could serve as an inflammatory marker in a range of clinical settings [45-47]. This lipocalin is expressed by neutrophils and various epithelial cells and has received attention as an acute kidney injury marker because of its early upregulation in the damaged tubular cells [7, 48]. High expression of NGAL in atheromatous plaques and its role in inflammation might explain the previous finding that this biomarker could prove useful in discriminating between unstable and stable CAD patients [49, 50]. However, serum NGAL was not related to arterial stiffness in the current study. Further investigations will evidently determine its true role in inflammation and vascular damage.

Several limitations should be noted. First, the number of subjects in our cross-sectional study was relatively small and, thus, larger samples of CAD patients without moderate to severe CKD are needed to confirm our results. In addition, prospective longitudinal studies are required in these patients to determine the contribution of changes in arterial stiffness to renal damage as well as to clarify the role of urinary L-FABP and KIM-1 as potential early reflectors of this pathophysiological interplay. Third, only male participants were recruited to avoid the possible confounding effects of estrogen. Hence our findings cannot be fully extrapolated to women. Fourth, urine albumin-to-creatinine ratio was not determined in the current study [51]. Also, we could not withdraw the CAD patients from their medications to exclude their potential effects on hemodynamic parameters and on levels of biochemical markers. Finally, long-term health consequences of smoking cannot be ruled out.

Conclusion

We found independent associations between urinary L-FABP, KIM-1 and aortic stiffness for the CAD patients with eGFR above 60 ml/min per 1.73 m². Therefore, in these subjects, measurements of L-FABP and KIM-1 may help to further explore the interplay between early alterations in renal function and vascular damage.

Disclosure Statement

The authors declare no conflict of interest.

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