CLINICAL STUDY

Implication of lipocalin-2 and visfatin levels in patients with coronary heart disease

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Abstract

Objectives: Visfatin and lipocalin-2 are novel adipokines associated with insulin resistance (IR) and obesity-related metabolic disorders. We compared lipocalin-2 and visfatin concentrations between patients with coronary heart disease (CHD) and control subjects and evaluated their association with cardiovascular risk factors.

Methods: We examined serum visfatin, lipocalin-2 levels, and cardiovascular risk factors in 91 subjects (49 patients with angiographically confirmed CHD versus 42 age- and gender-matched control participants).

Results: Circulating lipocalin-2 levels were significantly higher in patients with CHD compared with the control subjects (82.6 ± 38.7 ng/ml versus 43.8 ± 27.8 ng/ml; $P < 0.001$). However, visfatin levels were not significantly different between patients with CHD and control subjects. Serum lipocalin-2 levels were positively associated with weight ($r = 0.26$; $P = 0.036$), fasting insulin ($r = 0.36$; $P = 0.003$), and IR ($r = 0.33$; $P = 0.007$), whereas these levels showed a negative correlation with high-density lipoprotein (HDL) cholesterol ($r = -0.30$; $P = 0.016$) after adjustment for gender and body mass index. However, visfatin levels were not associated with any variables of the metabolic syndrome. The multiple regression analysis showed that lipocalin-2 levels were independently associated with HDL cholesterol and IR ($R^2 = 0.199$). Furthermore, the multiple logistic regression analysis showed that systolic blood pressure, IR, and lipocalin-2 levels were independently associated with CHD.

Conclusions: Serum lipocalin-2 levels were significantly elevated in patients with CHD and were independently associated with CHD. The present findings suggest that the measurement of serum lipocalin-2 levels may be useful for assessing CHD risk.

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Introduction

Adipose tissue is an important endocrine organ; it secretes several hormones and cytokines that are involved in the metabolic syndrome (1). Adipokines, signaling proteins secreted by adipose tissue, have an important regulatory function throughout the body; they include leptin, tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), resistin, and adiponectin. Lower serum adiponectin levels have been reported in patients with coronary heart disease (CHD) (2, 3) and have been shown to be a risk factor for cardiovascular events (4). In addition, it has recently been suggested that IL-6, TNF- α , leptin, and adiponectin may not only be just markers of inflammation and cardiovascular (CV) risk but are also likely to play a pathogenic role in atheromatous plaque (5, 6).

Recently, lipocalin-2, also known as neutrophil gelatinase-associated lipocalin and 24p3, was reported

to be associated with obesity and insulin resistance (IR) in both mice and humans (7). In *db/db* obese diabetic mice, circulating lipocalin-2 concentrations were increased as well as the expression of lipocalin-2 in adipose and liver tissue compared with normal mice. In addition, serum lipocalin-2 levels were positively correlated with body mass index (BMI), hypertriglyceridemia, hyperglycemia, and IR, but negatively correlated with high-density lipoprotein (HDL) cholesterol in humans (7). Treatment with rosiglitazone markedly decreased lipocalin-2 expression in mice and circulating levels in both mice and humans (7). Furthermore, there was a recent report that expression of lipocalin-2 is increased in atherosclerotic plaques and myocardial infarction (8). Recently, Aigner *et al.* reported that lipocalin-2 regulates the inflammatory response during ischemia and reperfusion of the transplanted heart (9). Lipocalin families share a common tertiary structure formed by segments termed lipocalin folds (10).

One of the human lipocalins is retinol-binding protein 4, recently identified as an adipokine that may link obesity and IR (11, 12).

Visfatin is a novel adipokine that is preferentially produced in visceral adipose tissue; both its expression and plasma concentration increase with increasing levels of obesity (13). Visfatin treatment has exhibited insulin-mimetic activity resulting in a glucose-lowering effect (13), and increasing concentrations of visfatin were independently and significantly associated with type 2 diabetes (14). However, another study reported that there was no correlation between visfatin levels and various parameters of IR during euglycemic-hyperinsulinemic clamp experiments (15). Therefore, the previous studies evaluating the association between visfatin levels and diabetes, obesity, or dyslipidemia have yielded inconsistent results (16).

Although obesity and its associated conditions including the metabolic syndrome are closely associated with the development of CHD, there was no prior study reporting on an association between CHD and these novel adipokines. Therefore, the aim of the present study was to evaluate circulating lipocalin-2 and visfatin levels in patients with CHD and compare these levels with control subjects, and to determine their association with other cardiovascular risk factors for atherosclerosis.

Subjects and methods

Study subjects

The study group included 49 patients with an established diagnosis of CHD after coronary angiography (CAG) performed in cardiovascular centers of Korea University Guro Hospital. All clinical and angiographic data were stored and analyzed using CAG database system of Korea University Guro Hospital. Patients were divided into three groups: acute myocardial infarction (AMI), unstable angina pectoris (UAP), or stable angina pectoris (SAP). AMI was diagnosed on the basis of clinical symptoms, electro cardiogram (ECG) evidence of a >0.1 mV ST segment elevation in at least two leads (in cases of ST elevation myocardial infarction), and a greater than twofold increase in the level of serum creatine kinase-MB isoform concentration from the upper limit of the normal range. CAG confirmed the occlusion of a coronary artery with a TIMI grade flow of <3 . UAP was diagnosed on the basis of clinical symptoms (class IB, IIB, and IIIB in the Braunwald classification), a CAG finding of more than 70% stenosis in ≥ 1 coronary arteries, and no significant elevation in the level of serum creatine kinase-MB concentration. SAP was diagnosed based on typical chest pain during an exercise test and the CAG findings of more than 70% stenosis in ≥ 1 coronary arteries. The control group consisted of 42 healthy participants with normal routine

biochemistry evaluations, normal ECG, and matched by age and gender to the patients with CHD. The study exclusion criteria adopted were: a previous diagnosis or treatment of diabetes, major trauma or surgery, active infectious disease, malignant disease, and liver or renal dysfunction. Informed consent was obtained from all subjects before they participated in the study, which was approved by the ethical committee at our institution.

Measurements of risk variables

The BMI was calculated as weight/height² (kg/m²). Waist circumference was measured from the narrowest point between the lower borders of the rib cage and the iliac crest. Blood samples were obtained at least 3 months after an acute event. All blood samples were obtained in the morning after a 12-h overnight fast and were immediately stored at -80°C for subsequent assay. Serum triglycerides and HDL cholesterol were determined enzymatically using a chemistry analyzer (Hitachi 747). A glucose oxidase method was employed to measure plasma glucose, and a human insulin-specific RIA kit (Linco Research Inc., St Charles, MO, USA) was used to measure insulin levels. This kit had a reactivity of $<0.2\%$ with human proinsulin. IR was calculated by the homeostasis model assessment (HOMA) (17). Serum visfatin levels were measured using a Visfatin Human ELISA kit (Phoenix pharmaceuticals, Belmont, CA, USA), with an intra-CV of 4.4%. Serum lipocalin-2 levels were determined using a Human Lipocalin-2 Quantikine ELISA kit (R&D Systems, Minneapolis, MN, USA), with an intra-CV of 1.0%.

Statistical analysis

Data are expressed as means $\pm$ S.D. or median and interquartile range. Non-normally distributed variables were analyzed using log-transformed values. We used the Kolmogorov-Smirnov test to evaluate variables for normality. Differences between groups were tested using the unpaired Student's *t*-test or the Mann-Whitney *U* test. Categorical variables were compared by the χ^2 -test. The spearman rank correlation test was performed to determine the relationships between lipocalin-2, visfatin levels, and other cardiovascular risk variables. Multiple regression analysis was performed with lipocalin-2 concentrations as a dependent variable. Age, gender, smoking status, weight, BMI, waist circumference, systolic blood pressure, diastolic blood pressure, HDL cholesterol, triglyceride, fasting glucose, and HOMA-IR levels were employed as independent variables. The stepwise method was used for significant variable selection. Multivariate logistic regression analysis using the existence of CHD as a dependent variable was conducted to determine the relative contributions made by each variable to the outcome variable. Variables used in multiple regression analysis plus visfatin and lipocalin-2 levels were used as independent

variables. Significant independent variables were chosen using the backward: conditional variable selection method. Values of $P < 0.05$ were considered significant. Data were analyzed using SPSS for Windows (version 10.0; SPSS Inc., Chicago, IL, USA).

Results

Clinical and biochemical characteristics of the study subjects are presented in Table 1. Patients with CHD exhibited greater body weight, BMI, fasting glucose, fasting insulin levels, and HOMA-IR compared with the control subjects. However, age, gender, blood pressure, lipid profile, and smoking status were not different in comparisons between patients with CHD and control participants.

Circulating lipocalin-2 levels were significantly higher in patients with CHD compared with the control subjects (82.6 ± 38.7 ng/ml versus 43.8 ± 27.8 ng/ml; $P < 0.001$; Fig. 1). However, visfatin levels were not different between patients with CHD and normal control subjects (45.2 ± 20.9 ng/ml versus 44.4 ± 21.0 ng/ml; $P = 0.761$). Interestingly, even when patients with CHD were compared with age-, gender-, and BMI-matched control subjects ($n = 26$, BMI 25.6 ± 2.1), serum lipocalin-2 levels were still significantly higher in patients with CHD (82.6 ± 38.7 ng/ml versus 44.6 ± 28.9 ng/ml; $P < 0.001$). Patients with AMI or UAP ($n = 11$) showed a tendency for increasing serum lipocalin-2 levels compared with the patients with SAP ($n = 38$), although this finding was not statistically significant (91.7 ± 32.7 vs 79.9 ± 40.3 ng/ml; $P = 0.326$). Lipocalin-2 concentrations from

Table 1 Clinical and laboratory characteristics of study subjects.

Variables	Control ($n = 42$)	CHD ($n = 49$)	<i>P</i>
Sex (M/F)	25/17	30/19	0.869
Age (years)	61.1 ± 7.8	60.6 ± 9.4	0.793
Height (cm)	161.3 ± 9.4	161.7 ± 7.6	0.801
Weight (kg)	63.2 ± 8.9	68.2 ± 8.3	0.007
Body mass index (kg/m^2)	24.4 ± 2.4	26.1 ± 2.6	0.002
Waist circumference (cm)	85.7 ± 6.6	88.0 ± 6.5	0.135
SBP (mmHg)	130.6 ± 12.9	128.0 ± 11.2	0.320
DBP (mmHg)	79.9 ± 9.8	78.9 ± 8.4	0.634
HDL cholesterol (mmol/l)	1.3 (1.2, 1.5)	1.3 (1.1, 1.6)	0.569
Triglyceride (mmol/l)	1.1 (0.8, 1.7)	1.2 (1.0, 1.7)	0.393
Fasting glucose (mmol/l)	5.1 ± 1.0	5.7 ± 1.0	0.003
Fasting insulin ($\mu\text{U/ml}$)	9.1 (6.7, 11.3)	13.5 (11.3, 18.8)	< 0.001
HOMA-IR	1.9 (1.6, 2.5)	3.4 (2.8, 4.6)	< 0.001
Smoking, <i>n</i> (%)	12 (28.6%)	15 (30.6%)	0.832

Data presented as means $\pm$ s.d. or median (inter-quartile range). SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; and HOMA-IR, homeostasis model assessment insulin resistance.

Figure 1 Serum lipocalin-2 concentrations in patients with coronary heart diseases and in control subjects. The box plot displays the 25th, median and 75th percentiles, and the minimum and maximum levels as horizontal lines outside the box.

men also exhibited higher levels compared with those from women (70.4 ± 40.1 vs 55.9 ± 36.3 ng/ml; $P = 0.091$).

In Table 2, results of partial correlation analysis adjusted for gender and BMI between serum lipocalin-2 and other cardiovascular risk factors are shown. Serum lipocalin-2 levels were positively associated with body weight ($r = 0.26$; $P = 0.036$), fasting insulin ($r = 0.36$; $P = 0.003$), and IR ($r = 0.33$; $P = 0.007$), whereas

Table 2 Partial correlation analysis adjusted for gender and body mass index.

	Lipocalin-2	
	<i>r</i>	<i>P</i>
Age	-0.06	0.645
Height	0.21	0.086
Weight	0.26	0.036
Waist circumference	0.18	0.161
Systolic blood pressure	-0.24	0.057
Diastolic blood pressure	-0.16	0.214
HDL cholesterol	-0.30	0.016
Triglyceride	0.07	0.578
Fasting glucose	0.00	0.975
Fasting insulin	0.36	0.003
HOMA-IR	0.33	0.007

HDL, high-density lipoprotein and HOMA-IR, homeostasis model assessment insulin resistance.

Table 3 Multiple regression analysis with lipocalin-2 concentrations as a dependent variable.

	Unstandardized coefficients		Standardized coefficients	
	β	S.E.M.	β	P
Constant	5.025	0.737		<0.001
HDL cholesterol	-0.036	0.011	-0.361	0.002
HOMA-IR	0.226	0.113	0.225	0.050

$R^2=0.199$. HDL, high-density lipoprotein and HOMA-IR, homeostasis model assessment insulin resistance.

they had a negative correlation with HDL cholesterol ($r=-0.30$; $P=0.016$). However, circulating visfatin levels were not significantly associated with variables such as obesity, blood pressure, lipid profile, and IR. The multiple regression analysis showed that lipocalin-2 levels were independently associated with HDL cholesterol and HOMA-IR ($R^2=0.199$; Table 3). Multiple logistic regression analysis was performed using the presence of CHD as a dependent variable. CHD was associated with systolic blood pressure, HOMA-IR, and lipocalin-2 levels independently from other cardiovascular risk factors (Table 4).

Discussion

The present study has shown for the first time that serum lipocalin-2 levels were significantly higher in patients with CHD than in age- and gender-matched control subjects. Furthermore, serum lipocalin-2 levels were independently associated with HDL cholesterol and IR. However, circulating visfatin levels were not significantly related to cardiovascular risk variables such as obesity, blood pressure, lipid profile, and IR and were not different in comparisons between patients with CHD and control subjects.

Chronic low-grade inflammation is now recognized to be a key mediator in the development of CHD (18). Epidemiologic studies have shown a consistent independent association between high-sensitivity C-reactive protein (hs-CRP) elevations and coronary risk (19). Wang *et al.* reported a strong positive association between lipocalin-2 concentrations and hs-CRP, independent of age, sex, and adiposity ($P=0.007$) and suggested that lipocalin-2 can be considered a marker of

obesity-related low-grade inflammation (7). Recently, Hemdahl *et al.* have shown that mice developing myocardial infarction exhibited increased lipocalin-2 expression in coronary atherosclerotic plaques (8).

Wang *et al.* exhibited that adipose tissue and liver are two principal sources contributing to increased lipocalin-2 levels in obese states. They also reported a significant positive correlation between lipocalin-2 levels and several variables associated with obesity-related metabolic disorders, including adverse lipid profiles, hyperinsulinemia, hyperglycemia, and IR (7). In the present study, we found that circulating lipocalin-2 levels were positively associated with body weight, fasting insulin, and HOMA-IR, whereas they were negatively associated with HDL cholesterol, consistent with the prior results. However, we could not find any relationship between BMI, waist circumference, triglyceride, fasting glucose, and lipocalin-2 levels. Of note is that our study included subjects with relatively narrow range of BMI compared with the prior study subjects (7).

Visfatin is a recently identified adipokine that is preferentially secreted by visceral adipocytes. Visfatin binds to and activates insulin receptors, and decreases IR (13). Chen *et al.* reported that visfatin concentration was elevated in type 2 diabetes mellitus even after statistical adjustment for known biomarkers (14). They also reported that plasma visfatin was associated with age, waist-to-hip ratio (WHR), fasting insulin, adiponectin levels, and HOMA-IR in a simple regression analysis, whereas in a multiple regression analysis, only WHR remained positively associated with plasma visfatin levels. However, we could not confirm these relationships in our study subjects. Our results are compatible with the recent observations based on investigation of a community-based sample; this suggested that circulating visfatin may not be a useful clinical biomarker for metabolic traits (16). Furthermore, there was no difference identified in circulating visfatin levels between subjects with CHD and control subjects in the present study, although a recent study reported increased expression of visfatin in macrophages of human unstable carotid and coronary atherosclerosis (20).

Because of the limitations of cross-sectional study in the present study, no causal relationship could be defined. It is not clear whether increased lipocalin-2 in patients with CHD is a causative factor or simply a bystander in the pathogenesis of atherosclerosis. In addition, normal ECG

Table 4 Multivariate logistic regression analysis with coronary heart disease as a dependent variable.

	B	S.E.M.	Wald	P	Exp (B)	95% CI	
SBP	0.091	0.059	2.364	0.124	1.095	0.975	1.229
HOMA-IR	5.293	1.965	7.253	0.007	199	4.225	9374
Lipocalin-2	0.045	0.023	3.835	0.050	1.046	1.000	1.094
Constant	-28.635	11.737	5.952	0.015	0.000		

SBP, systolic blood pressure and HOMA-IR, homeostasis model assessment insulin resistance.

in the control group does not completely exclude the presence of CHD particularly in elderly subjects.

In conclusion, circulating concentrations of lipocalin-2 were significantly higher in patients with CHD, and were independently associated with CHD. However, visfatin levels were not associated with cardiovascular risk factors for atherosclerosis. Further, prospective study with a large number of patients is needed to determine the predictive value of serum lipocalin-2 as a biomarker for metabolic and cardiovascular disease.

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