پرونده‌های آموزشی مرکز اطلاعات علمی

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اصول تنظیم قراردادها

آموزشی مهارت‌های کاربردی در تدوین و چاپ مقاله
Association Between Visfatin Levels and Coronary Artery Disease in Patients with Chronic Kidney Disease

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Introduction. Visfatin (also known as pre-B cell colony-enhancing factor) is increased in patients with chronic kidney disease and has been linked with coronary atherosclerosis. Given that it has been reported that visfatin plays a role in endothelial dysfunction in chronic kidney disease patients, we examined associations between visfatin levels and several markers related to atherosclerosis.

Materials and Methods. The association between visfatin and atherosclerotic risk factors was studied in 173 chronic kidney disease patients (130 men and 43 women). Serum levels of visfatin were measured by the enzyme-linked immunosorbent assay.

Results. With increasing visfatin tertiles, patients proved to have a larger number of vessels with stenosis and a higher likelihood of coronary artery disease, as well as having incrementally lower estimated glomerular filtration rate and serum albumin and higher total leukocyte, neutrophil, and monocyte counts; high-sensitivity C-reactive protein; and brain natriuretic peptide levels. Visfatin showed significant positive correlations with low-density lipoprotein cholesterol, uric acid, blood urea nitrogen, creatinine, brain natriuretic peptide, E-selectin, total leukocyte count, neutrophil count, and high-sensitivity C-reactive protein, and a significant negative correlation with estimated glomerular filtration rate and albumin. Only E-selectin was independently associated with visfatin in multiple linear regression analysis.

Conclusions. This study indicates that plasma visfatin levels are significantly higher in the presence of coronary artery disease and are correlated with E-selectin levels, which suggest that increased plasma visfatin may be involved in the pathogenesis of coronary atherosclerosis in CKD patients.

Keywords. visfatin, chronic kidney disease, coronary artery disease, atherosclerosis, E-selectin

INTRODUCTION

Visfatin, also known as pre-B-cell colony-enhancing factor 1 (52 kDa to 55 kDa, middle molecule) or nicotinamide phosphoribosyltransferase (Nampt), is a ubiquitous adipokine that was first described by Fukuhara and colleagues1 in 2005. Previous studies have suggested that visfatin may be an important inflammatory protein and harmful factor in the setting of obesity-induced metabolic disturbance and associated with atherosclerosis, plaque destabilization in acute coronary syndrome, and carotid artery plaques.2-4 Furthermore, our recent study showed that plasma visfatin levels were associated with infarct-related artery occlusion,
and also found a close association between visfatin and coronary artery disease (CAD). The circulating levels of visfatin in patients with chronic kidney disease (CKD) have been reported to significantly increase and be correlated with endothelial dysfunction. Thus, visfatin may be associated with the progression of atherosclerosis.

Systemic inflammation has been strongly implicated in both atherosclerosis and cardiovascular morbidity. Patients with CKD are exposed to inflammation and endothelial dysfunction, with inflammation playing a pivotal role in all stages of atherogenesis, from foam cell to plaque formation to rupture and ultimately to thrombosis. Insight gained from previous basic and clinical data linking inflammation to atherosclerosis has yielded important diagnostic and prognostic information. Low-grade chronic inflammation as measured by high-sensitivity C-reactive protein (HS-CRP) predicts future risk of acute coronary syndrome independent of traditional cardiovascular risk factors. Circulating levels of brain natriuretic peptide (BNP), a cardiac hormone, reflect the severity of cardiac dysfunction and inflammation. An elevated total leukocyte count is a risk factor for atherosclerotic vascular disease, given that leukocyte-derived macrophages and other phagocytes are believed to contribute to vascular injury and atherosclerotic progression. Increased expression of E-selectin is found in the endothelium of human atherosclerotic lesions. However, an association of visfatin with atherosclerotic inflammatory markers and lipids in CKD patients has not yet been adequately investigated. In the present study, we examined the associations between visfatin levels and risk factors of coronary atherosclerosis.

MATERIALS AND METHODS

Patients

The study included 209 patients with mild-to-severe CKD (estimated glomerular filtration rate [GFR] between 89 mL/min/1.73 m² and 15 mL/min/1.73 m²) who underwent diagnostic coronary angiography for the examination of CAD due to typical and atypical chest pain at the Department of Cardiology of E-Da Hospital from June 2007 to December 2010. The GFRs were determined using the formula from the Modification of Diet in Renal Disease study immediately prior to the angiography procedure. All patients with a GFR between 15 mL/min/1.73 m² and 89 mL/min/1.73 m² were eligible. Exclusion criteria were: (1) coronary artery bypass graft surgery history; (2) inflammatory diseases (such as infection, sepsis, malignancy, liver disease, and collagen disease); (3) steroid use or surgery within 1 month prior to admission; and (4) severe congestive heart failure (New York Heart Association classes III–IV). A total of 36 patients were excluded from the study: 13 patients had a positive history for coronary artery bypass graft surgery; 9 patients had inflammatory diseases; and 14 patients had class IV congestive heart failure. All 173 remaining patients were included in the study. This study was approved by the Human Research Ethics Committee of our hospital, and informed consent was obtained from each patient.

Laboratory Measurements

Demographic data (age, sex, comorbidities, actual treatment, smoking status, body weight, and height) were collected before the angiographic procedure from the individual charts in the electronic hospital database. In the morning of the procedure day and after a 12-hour fasting period, blood samples were collected, stored, and analyzed by one laboratory. Serum levels of triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDLC), high-density lipoprotein cholesterol (HDLC), uric acid, albumin, creatinine, and glucose were measured by standard commercial methods on a parallel, multichannel analyzer (Hitachi 7170A, Tokyo, Japan), as in our previous reports. Peripheral leukocyte analyses included total leukocyte counts and differential percentages of neutrophils, monocytes, and lymphocytes using an automated cell counter (XE-2100 Hematology Alpha Transportation System, Sysmex Corporation, Kobe, Japan). The absolute count of a leukocyte subtype was calculated as the product of its respective differential percentage and total leukocyte count. To minimize the confounding effect of infection, subjects with a leukocyte count below 4.0 × 10⁹/L or greater than 10.0 × 10⁹/L were rechecked for analysis and examined extensively for possible occult of chronic infections. Any specimen with abnormal or atypical leukocytes were re-analyzed and excluded.

In addition, the concentrations of plasma visfatin and BNP were determined using a commercial enzyme immunoassay kit (Phoenix Pharmaceuticals,
Belmont, CA, USA). The concentrations of plasma C-reactive protein were measured using a high-sensitivity (HS-CRP) method (IMMAGE; Beckman Coulter, Immunochrome Systems, Brea, CA, USA). E-selectin levels were determined by commercial solid phase enzyme linked immunosorbent assay kits (B-Bridge International, Sunnyvale, CA, USA, and Phoenix Pharmaceuticals, Belmont, CA and R & D Systems Inc, USA, respectively). The intra-assay coefficients of variation were 2.4% to 2.7% for visfatin, less than 5% for BNP, 4.2% to 8.7% for HS-CRP, and 5.2% to 6.6% for E-selectin. Samples were measured in duplicate in a single experiment.

**Angiographic Definitions**

Coronary angiography images were obtained according to standard techniques, and the severity of stenosis was assessed using quantitative coronary angiography. Angiographies and quantitative coronary angiographic analysis were evaluated by at least 2 experienced interventional cardiologists blinded to clinical information and serologic parameters and were scored according to scoring system, the possible scores of this index ranged from 0 to 3 diseased vessels. The criterion for 1-, 2-, or 3-vessel disease was a greater-than-50% reduction in the internal diameter. The diameter of stenosis of the left main coronary artery could not exceed 50%.

### Statistical Analysis

Data normality was analyzed using the Kolmogorov-Smirnov test. Continuous normally distributed variables are presented as mean ± standard deviation, and non-normally distributed variables as median (interquartile range). Statistical differences in variables were compared using a 1-way analysis of variance for normally distributed variables followed by the Tukey pairwise comparison. Categorical variables were recorded as frequencies and percentages, and inter-group comparisons were analyzed by the chi-square test. Since the distributions of plasma visfatin, HS-CRP, E-selectin, BNP, and triglyceride were skewed, logarithmically transformed values were used for statistical analysis. The Pearson correlation analysis was used to examine the correlations between plasma visfatin and the values of other parameters. To assess the influence of tested parameters, multiple linear regression analysis was used. Statistical significance was accepted if the P value was less than .05. All of the statistical analyses were performed using SAS statistical software, version 8.2 (SAS Institute Inc, Cary, NC, USA).

#### RESULTS

**Patients’ Characteristics**

Table 1 shows the clinical characteristics of
173 CKD patients (male, 75.2%; female, 24.9%) stratified by visfatin. The mean visfatin level in the study was 28.5 ng/mL. The whole cohort median values of plasma visfatin levels were 13.4 ng/mL (interquartile range, 8.3 ng/mL to 26.0 ng/mL). The patients were divided according to tertiles of visfatin, as follows: low visfatin (≤ 10.2 ng/mL), n = 57; medium visfatin (10.2 ng/mL to 20.5 ng/mL), n = 58; and high visfatin (≥ 20.5 ng/mL), n = 58. Higher visfatin was associated with an increased prevalence of CAD and a larger number of stenosed vessels. Data presented in Table 2 show that with increasing visfatin tertiles, there was not only a significant decrease in albumin concentrations and GFR values, but also a significant increase in total leukocyte, neutrophil, and monocyte counts, HS-CRP, and BNP concentrations.

There were 71 patients with diabetic nephropathy, 48 with hypertensive nephropathy, 18 with chronic glomerular diseases, and 36 with unclassified causes of kidney failure. We also analyzed visfatin levels in patients stratified by these diseases. The mean plasma visfatin levels were 32.4 ± 63.8 ng/mL, 29.9 ± 50.7 ng/mL, 23.1 ± 20.8 ng/mL, and 25.0 ± 24.9 ng/mL in patients with diabetic nephropathy, hypertensive nephropathy, chronic glomerular diseases, and unclassified causes of kidney failure, respectively. There was no significant difference in visfatin levels among the patients with different etiologies of CKD.

**Correlations Between Visfatin and Clinical And Biochemical Characteristics**

The correlation analysis for circulating visfatin and relevant parameters are included in Table 3. Visfatin was positively correlated with LDL-C, uric acid, blood urea nitrogen, creatinine, total leukocyte, neutrophil counts, HS-CRP, E-selectin, and BNP, and was negatively correlated with GFR and albumin. No association was found for body mass index, systolic and diastolic blood pressure, fasting blood glucose, hemoglobin A1c, total cholesterol, triglyceride, HDLC, hemoglobin, monocyte count, and lymphocyte count. In addition, multiple linear regression analysis was performed using visfatin as the dependent variable, and LDL-C, uric acid, creatinine, albumin, total leukocyte and neutrophil counts, HS-CRP, E-selectin, and BNP as independent variables. Only E-selectin was independently associated with visfatin (Table 4).

**DISCUSSION**

In the present study, we demonstrated in 173 CKD patients that visfatin showed significant positive correlations with LDL-cholesterol, uric acid, creatinine, BNP, E-selectin, total leukocyte

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**Table 2. Biochemical Characteristics, According to Tertiles of Visfatin in 173 Patients With Stages 2 to 4 of Chronic Kidney Disease**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Low Visfatin</th>
<th>Medium Visfatin</th>
<th>High Visfatin</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>57</td>
<td>58</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Fasting blood glucose, mg/dL</td>
<td>152.4 ± 69.3</td>
<td>147.6 ± 82.9</td>
<td>145.5 ± 75.1</td>
<td>.90</td>
</tr>
<tr>
<td>Hemoglobin A1c, %</td>
<td>7.5 ± 1.9</td>
<td>6.8 ± 1.9</td>
<td>6.7 ± 1.9</td>
<td>.13</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>168.6 ± 45.4</td>
<td>173.0 ± 41.1</td>
<td>174.3 ± 40.4</td>
<td>.76</td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>119.5 (86.0 to 216.0)</td>
<td>109.0 (73.0 to 160.0)</td>
<td>97.0 (74.5 to 197.0)</td>
<td>.24</td>
</tr>
<tr>
<td>HDLC, mg/dL</td>
<td>36.6 ± 8.8</td>
<td>40.9 ± 9.9</td>
<td>39.0 ± 11.6</td>
<td>.09</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>98.3 ± 35.7</td>
<td>107.9 ± 40.5</td>
<td>107.1 ± 36.6</td>
<td>.34</td>
</tr>
<tr>
<td>Uric acid, mg/dL</td>
<td>6.4 ± 1.8</td>
<td>6.5 ± 1.8</td>
<td>7.2 ± 2.6</td>
<td>.10</td>
</tr>
<tr>
<td>Blood urea nitrogen, mg/dL</td>
<td>18.7 ± 8.2</td>
<td>21.0 ± 11.4</td>
<td>23.5 ± 16.8</td>
<td>.14</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>1.3 ± 0.5</td>
<td>1.5 ± 1.0</td>
<td>1.6 ± 1.0</td>
<td>.09</td>
</tr>
<tr>
<td>GFR, mL/min/1.73 m²</td>
<td>62.3 ± 16.4</td>
<td>57.9 ± 19.2</td>
<td>54.2 ± 21.1</td>
<td>.047</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>13.6 ± 1.8</td>
<td>12.7 ± 2.1</td>
<td>13.3 ± 2.3</td>
<td>.06</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>4.0 ± 0.4</td>
<td>3.9 ± 0.4</td>
<td>3.8 ± 0.4</td>
<td>.03</td>
</tr>
<tr>
<td>Total leukocyte count, x 10⁶/L</td>
<td>7.848 ± 2.573</td>
<td>8.062 ± 3.017</td>
<td>10.240 ± 4.482</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Neutrophil count, x 10⁶/L</td>
<td>4901 ± 2460</td>
<td>5173 ± 2408</td>
<td>8136 ± 4210</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Monocyte count, x 10⁶/L</td>
<td>363 ± 130</td>
<td>461 ± 236</td>
<td>559 ± 415</td>
<td>.008</td>
</tr>
<tr>
<td>Lymphocyte count, x 10⁶/L</td>
<td>2137 ± 801</td>
<td>1983 ± 794</td>
<td>2025 ± 1340</td>
<td>.76</td>
</tr>
<tr>
<td>HS-CRP, mg/L</td>
<td>2.5 (1.2 to 8.4)</td>
<td>3.2 (1.0 to 7.3)</td>
<td>4.5 (1.4 to 25.1)</td>
<td>.04</td>
</tr>
<tr>
<td>E-selectin, ng/mL</td>
<td>24.6 ± 13.8</td>
<td>25.1 ± 11.9</td>
<td>27.5 ± 14.1</td>
<td>.58</td>
</tr>
<tr>
<td>BNP, ng/mL</td>
<td>4.6 (3.1 to 8.7)</td>
<td>6.4 (2.6 to 11.2)</td>
<td>5.4 (3.3 to 21.2)</td>
<td>.03</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± standard deviation or median (interquartile range). HDLC indicates high-density lipoprotein cholesterol; LDLC, low-density lipoprotein cholesterol; GFR, glomerular filtration rate; HS-CRP, high-sensitivity C-reactive protein; and BNP, brain natriuretic peptide.
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A significant negative correlation was found between visfatin and GFR and albumin. Further, a multiple regression analysis revealed that only E-selectin was independently associated with visfatin.

Water-soluble, protein-bound, and middle molecule uremic retention solutes were the three major groups of renal toxins. In a recent study, impaired renal filtration function was found to elevate circulating visfatin levels which belonged to the middle molecule uremic retention solute and coronary atherosclerotic inflammatory marker.6,20 The role of visfatin in nicotinamide adenine dinucleotide biosynthesis has been implicated in inflammatory states and this activity has been shown to be important for vascular smooth muscle cell maturation, indicating a possible involvement in vascular pathology.21,22 In the present study, we have demonstrated a significant positive correlation between visfatin and total leukocyte count, neutrophil count, and HS-CRP, which concurs with the prior studies and suggest that there is a potential link between visfatin and inflammation.23,24 Moreover, previous studies demonstrated that visfatin could activate human leukocyte expression of interleukin-1β, tumor necrosis factor-α, and especially interleukin-6,25 as well as increase monocyte matrix metalloproteinase-9 activity in monocytic Tamm-Horsfall protein 1 cells.4 Our previous study reported that plasma visfatin increased in patients with type 2 diabetes mellitus.26 Filippatos and colleagues27 also reported that plasma visfatin increased in patients with metabolic syndrome. Taken together, these important and intriguing results suggest that visfatin may be involved in the pathogenesis of atherosclerosis, diabetes and the metabolic syndrome, especially when these diseases are considered to be inflammatory processes.25,28

It is therefore reasonable to propose that visfatin may act as a pro-inflammatory cytokine and that it plays a role in chronic inflammation, thus contributing to the pathogenesis of atherosclerosis and cardiovascular disease.3

E-selectin, one of the specific endothelial adhesion molecules playing an important role in the initiation of coronary atherosclerosis and acute coronary syndrome, has been well-investigated.29,30 Endothelial dysfunction is one of the first hallmarks in the pathogenesis of atherosclerosis.31 Endothelial injury may result in the release of various factors that can be detected in the circulation and can be potentially used as markers of endothelial dysfunction. Several studies have suggested that circulating adhesion molecules may serve as markers of endothelial damage or atherosclerosis. Noshad and coworkers proposed tissue endothelin-1 level as the main predicting factor of atherosclerosis.32 The Atherosclerosis Risk In Communities study showed that E-selectin and intercellular adhesion molecule-1 are associated with carotid artery intima-
media thickness and are independent predictors of incident CAD.\textsuperscript{33} Squadrito and colleagues\textsuperscript{34} observed higher levels of circulating intercellular adhesion molecule-1 and E-selectin in patients with acute myocardial infarction. In the present study, E-selectin was significantly and positively correlated with visfatin, and was independently associated with visfatin in CKD patients by using multivariate analysis. Such a significant association between E-selectin and visfatin reiterates the findings of prior studies.\textsuperscript{35,36} Furthermore, our current study found plasma visfatin levels to also significantly correlate with renal function parameters (blood urea nitrogen, serum creatinine, GFR, and serum albumin), metabolic risk factors (LDL-C and uric acid) and BNP, which are strong independent and inverse predictors of cardiovascular events. This indicates and provides evidence that visfatin is synergistically increased with renal function deterioration and that both of these events contribute to coronary atherosclerosis.

CONCLUSIONS
This study found that high plasma visfatin levels were significantly higher in the presence of CAD and correlated with E-selectin levels, which suggest that increased plasma visfatin may likely be involved in the pathogenesis of coronary atherosclerosis in CKD patients.

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CONFLICT OF INTEREST
None declared.

REFERENCES


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