Plasma Levels of Soluble Vascular Adhesion Molecule-1 and Cholesterol Oxidation Product in Type 2 Diabetic Patients with Nephropathy

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Functional impairment of the vascular endothelium is an early event in the development of atherosclerosis, and soluble adhesion molecules in plasma are regarded as an indicator of the endothelial damage in diabetes mellitus. We compared the soluble vascular adhesion molecule levels in the patients with diabetic nephropathy in concerning with plasma 7-ketocholesterol levels, which is major cholesterol auto-oxidation products. Average value of plasma VCAM-1 in 31 patients with type 2 diabetes mellitus was 297.6±10.2 ng/ml (mean±SE), and the value was significantly higher than that in 8 age-matched healthy controls (231.9±15.0 ng/ml). Among the 31 diabetic patients, the group with macroalbuminuria (n=8) had the higher levels of plasma VCAM-1 (349.5±26.0 ng/ml) than the levels in the group with normoalbuminuria (n=15 ; 280.6±12.3 ng/ml). The levels of plasma 7-ketocholesterol in diabetes (26.9±1.5 ng/ml) or the patients with macroalbuminuria (31.4±3.3 ng/ml) were significantly higher than the control (22.5±1.8 ng/ml). The level of soluble VCAM-1 showed significant correlation between the values of 7-ketocholesterol (r = 0.42, p = 0.024), TC (r = 0.42, p = 0.024), TC (r = 0.42, p = 0.024), and LDL-C (r = 0.38, p = 0.044). However no correlation was demonstrated with HbA1c nor creatinine level. We conclude that soluble VCAM-1 in plasma may be an indicator of oxidative stress and vascular injury in diabetic nephropathy. J Atheroscler Thromb, 2001 ; 8 : 21-24.

Key words : sVCAM-1, Diabetic nephropathy, Oxysterol, 7-ketocholesterol

Introduction

An early event in the process of atherogenesis (1) is considered to be oxidative stress including accumulation of oxidized LDL (oxLDL) and dysfunction of the vascular endothelium. Lipid components of oxLDL such as linoleyl hydroperoxide and lysophosphatidyl-choline, augment the ability of vascular endothelial cells to express cytokine-mediated vascular cell adhesion molecule-1 (VCAM-1) (2). Oxysterols are also major components of oxLDL and known to have various biological activities (3). Expression of the adhesion molecules, VCAM-1 and intercellular adhesion molecule-1 (ICAM-1) (1), on vascular endothelial cells are known to play an important role in atherosclerosis in diabetes mellitus. Accelerated atherosclerosis is the most important complication of diabetes mellitus. In particular, Otsuka et al. (4) have found an increased plasma levels of soluble VCAM-1 (sVCAM-1) in patients with type 2 diabetes mellitus and suggested the importance of endothelial dysfunction. In addition, plasma cell adhesion molecules have been reported to be implicated in the development and progression of the diabetic retinopathy (5) and of peripheral neuropathy in diabetes mellitus (6). Diabetic nephropathy was shown to be associated with the sVCAM-1 concentration by multivariate regression analysis (7). However, the biological significance of soluble forms of these adhesion molecules is not fully understood.

We compared in this study, the sVCAM-1 levels in the patients with diabetic nephropathy in concerning with
plasma 7-ketocholesterol levels, which is major cholesterol auto-oxidation products.

**Methods**

We studied 31 patients with type 2 diabetes mellitus (63.9±11.3 years old) and age-matched healthy controls (60.0±12.3 y.o.). The patients were divided into three subgroups according to the degree of diabetic nephropathy as follows: 15 cases of the normoalbuminuria (urinary albumin (U.Alb) <30 mg/gCr), microalbuminuria (30<U.Alb<300 mg/gCr) and macroalbuminuria (U.Alb>300 mg/gCr). The patient characterization were summarized in Table 1. There were no difference in the age and sex ratio, HbA1c and the kinds of treatment among the subgroups, except serum creatinine which was higher in macroalbuminuria than that in normoalbuminuria. All of the patients with micro- and macro-albuminuria were administered with angiotensin-converting enzyme (ACE) inhibitor as a renoprotective agent (8).

Fasting blood samples were obtained using EDTA-2Na as an anticoagulant. Plasma levels of VCAM-1 were determined using specific ELISA kits (Biosource International, CA, USA). Plasma 7-ketocholesterol was measured by gas-chromatography/mass-spectrometry as described previously (9).

Statistical analysis was performed using analysis of variance and the least significant difference to compare differences among the groups and p <0.05 was regarded as significant. Data were expressed as mean±SE.

**Results**

The levels of plasma lipids and sVCAM-1 in diabetic patients and controls are summarized in Table 2. Plasma total cholesterol (TC) and LDL-C levels in diabetic patients were significantly higher than control group. However, plasma lipid levels among the patients with diabetic nephropathy showed no statistical difference.

Average value of plasma VCAM-1 levels in 31 patients (297.6±10.2 ng/ml) were significantly elevated than that in 8 control subjects (231.9±15.0 ng/ml). The average values of plasma VCAM-1 among the groups of nephropathy increased gradually with the degree of nephropathy. There was a significant difference between the control and the subgroups of normoalbuminuria (280.6±12.3 ng/ml), microalbuminuria (304.5±17.2 ng/ml), or macroalbuminuria (349.5±26.0 ng/ml) group (Fig. 1). The levels of soluble VCAM-1 in macro-albuminuria had a significantly higher value than that in normoalbuminuria group.

Plasma 7-ketocholesterol in diabetic patients (26.9±1.5 ng/ml) were significantly higher than control group (22.5±1.8 ng/ml). Plasma 7-ketocholesterol levels among the patients with diabetic nephropathy increased according to the progression of albuminuria, however statistical differ-

<table>
<thead>
<tr>
<th>Subject</th>
<th>No.</th>
<th>M/F</th>
<th>age (y.o.)</th>
<th>HbA1c (%)</th>
<th>Cr (mg/dl)</th>
<th>Treatment (Diet/OHA/Insulin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>3/5</td>
<td>60.0±12.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Type 2 DM</td>
<td>31</td>
<td>9/22</td>
<td>63.9±11.3</td>
<td>6.8±1.9</td>
<td>0.9±0.7</td>
<td>17/5/9</td>
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<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albuminuria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normo</td>
<td>15</td>
<td>4/11</td>
<td>63.5±2.5</td>
<td>6.2±2.3</td>
<td>0.7±0.2</td>
<td>9/3/3</td>
</tr>
<tr>
<td>Micro</td>
<td>8</td>
<td>2/6</td>
<td>61.4±10.9</td>
<td>7.0±1.4</td>
<td>0.7±0.1</td>
<td>4/1/3</td>
</tr>
<tr>
<td>Macro</td>
<td>8</td>
<td>3/5</td>
<td>64.3±13.1</td>
<td>7.3±1.5</td>
<td>1.2±0.9</td>
<td>4/1/3</td>
</tr>
</tbody>
</table>

Values are mean±S.E. *p<0.05 as compared with control
Normo: normoalbuminuria, Micro: microalbuminuria, Macro: macroalbuminuria, DM: diabetes mellitus
OHA: oral hypoglycemic agent

<table>
<thead>
<tr>
<th>Subject</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>7-ketocholesterol (ng/ml)</th>
<th>sVCAM-1 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>189.7±10.3</td>
<td>82.0±10.6</td>
<td>69.2±7.9</td>
<td>100.6±10.5</td>
<td>22.5±1.8</td>
<td>231.9±15.0</td>
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<tr>
<td>Type 2 DM</td>
<td>229.3±8.4</td>
<td>126.2±10.7</td>
<td>61.7±2.9</td>
<td>145.7±7.2</td>
<td>26.9±1.5</td>
<td>297.6±10.2</td>
</tr>
<tr>
<td>Total</td>
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<td></td>
<td></td>
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<tr>
<td>Albuminuria</td>
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</tr>
<tr>
<td>Normo</td>
<td>225.9±12.2</td>
<td>115.7±11.1</td>
<td>61.9±3.1</td>
<td>146.9±9.5</td>
<td>24.4±1.8</td>
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<tr>
<td>Micro</td>
<td>213.8±16.6</td>
<td>119.9±26.6</td>
<td>59.5±7.7</td>
<td>130.4±13.8</td>
<td>27.7±3.6</td>
<td>304.5±17.2</td>
</tr>
<tr>
<td>Macro</td>
<td>251.7±15.0</td>
<td>152.5±24.2</td>
<td>63.6±6.4</td>
<td>160.7±16.6</td>
<td>31.4±3.3</td>
<td>349.5±26.0</td>
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</tbody>
</table>

Values are expressed as mean±SE. *p<0.05; a vs Control, b vs Normo.
Normo: normoalbuminuria, Micro: microalbuminuria, Macro: macroalbuminuria, DM: diabetes mellitus
sVCAM-1 in Type 2 Diabetes with Nephropathy

Soluble VCAM-1 showed weak correlation with 7-ketocholesterol ($r=0.42$, $p=0.024$), TC ($r=0.42$, $p=0.014$) and LDL-C ($r=0.38$, $p=0.044$). However, no correlation was demonstrated between sVCAM-1 and HbA1c nor creatinine levels.

**Discussion**

The interactions between blood cells and the vascular wall have been regarded as an important triggering process in atherogenesis. Cell adhesion molecules play an important role for this interaction and VCAM-1 is one of the major molecules (10). The expression of VCAM-1 has been known to be enhanced in atherosclerotic lesions (11). The levels of sVCAM-1 in plasma may reflect its expression in endothelial cells and increased plasma VCAM-1 is regarded as an indicator of endothelial damage (4).

Oxidative stress is also concerning with endothelial injury especially in diabetes mellitus (12). Oxidatively modified LDL has been implicated as an important oxidative signal in the pathogenesis of atherosclerosis. Products of lipid oxidation such as fatty acid hydroperoxide or lysophosphatidylcholine (2) are already reported to enhance the expression and extracellular release of adhesion molecules (13, 14). Oxysterols are also revealed to be the important lipid components in oxidized lipoproteins (3). In our previous study, we reported the increased levels of plasma oxysterols in diabetic patients which may be reflected the oxidative stress under hyperglycemic condition. Recently, 7-ketocholesterol and other several oxysterols were demonstrated to enhance the expression of adhesion molecules and apoptosis in cultured endothelial cells (15). These observation suggested a possible functional link between oxidative modulatory signals (16) and the pathogenesis of diabetic complications (2).

In the present study, we found an increase in plasma VCAM-1 levels in patients with type 2 diabetes mellitus depending on the progress of nephropathy. The patients with macroalbuminuria showed significantly higher levels of sVCAM-1 than that in those with normalbuminuria.

Koga et al. reported the correlation of sVCAM-1 with diabetic nephropathy, and discussed that the elevation of circulating VCAM-1 level in diabetic nephropathy may result from underlying damaged renal tubular or glomerular epithelial cells and/or decreased renal clearance of this molecule, depending on the stage of nephropathy (7).

Actually Chen et al. reported that stimulation with oxidized LDL enhanced superoxide production by diabetic glomeruli (17).

The relation between plasma lipid components and VCAM-1 levels are obscure and under controversial (16, 18, 19). We evaluated plasma levels of 7-ketocholesterol, which is an auto-oxidation product of cholesterol and constitutes a part of oxidatively-modified lipoproteins (20). The levels of 7-ketocholesterol demonstrated a weak relation with the levels of sVCAM-1. This might indicate the causal relationship between oxidative stress and endothelial injury in diabetic nephropathy, however this may not reflect the direct relationship between these factors.

The patients with micro- and macro-albuminuria were administered an ACE inhibitor for renal protection. The levels of sVCAM-1 in plasma in these patients have been underestimated as ACE inhibitors are known as to counteract the activation of the endothelium (18). Under hyperglycemic condition, LDL may be glycated and glycated LDL, which is reported to augment TNF-α-induced VCAM-1 expression (10) has been known to be predisposed to further modification by oxidation (9, 21, 22). However, glycemic control was not implicated in determining plasma sVCAM-1 level in our subjects.

We have shown the possibility that plasma levels of sVCAM-1 may reflect as a marker of ongoing vascular dysfunction (13) linking the pathological condition of oxidative modulation and hyperglycemia (10) in the patients with diabetic nephropathy.

**References**

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