Association between Serum Adiponectin Levels and Arteriolosclerosis in IgA Nephropathy Patients

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Abstract

Objective Adiponectin has attracted great attention because of its anti-atherogenic properties; however, to date the relationship between serum adiponectin and arteriolosclerosis has not been reported. In our study, we aimed to examine whether or not serum adiponectin levels are associated with arteriolosclerosis in patients with IgA nephropathy which is the most common form of chronic glomerulonephritis.

Materials and Methods We enrolled 35 patients aged 35.0±14.6, who underwent renal biopsy from August 2004 to February 2006 in our hospital, and were confirmed to have IgA nephropathy. We examined serum adiponectin, high-sensitive C-reactive protein, total cholesterol and triglyceride level, urinary protein excretion, body mass index (BMI), and the presence of arteriolosclerosis in the renal specimens. Since the serum adiponectin level is strongly affected by renal function, we classified the patients by creatinine clearance.

Results Multiple regression analysis showed the associations of adiponectin with creatinine clearance (p<0.001), BMI (p<0.001), serum triglyceride (p=0.001) and urinary protein excretion (p=0.001). We observed a positive relation of adiponectin with urinary protein excretion and an inverse relation of adiponectin with creatinine clearance, serum triglyceride, and BMI. We could not detect any relation between the presence of arteriolosclerosis and adiponectin in the IgA nephropathy patients as a whole; however, in patients whose creatinine clearance was 90-120 ml/min/1.73 m², the serum adiponectin level of patients with arteriolosclerosis was lower than in those without arteriolosclerosis (p=0.025).

Conclusion The serum level of adiponectin was related to arteriolosclerosis in IgA nephropathy patients whose renal function was almost normal. Adiponectin may prevent renal arteriolosclerosis.

Key words: kidney, chronic glomerular nephritis, creatinine clearance, glomerulonephritis, chronic kidney disease

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teriolosclerosis. Immunoglobulin A (IgA) nephropathy is the most common form of primary glomerulonephritis worldwide, and since biopsies are performed frequently, IgA nephropathy may be an ideal subject for assessing their relationship. A previous study showed an inverse relationship between serum adiponectin values and creatinine clearance in essential hypertension and an increased adiponectin level in patients with a combination of reduced renal function and hypertension (9). The results of another study indicated that the estimated glomerular filtration rate is independently associated with increased adiponectin serum concentrations in type 1 diabetic patients with nephropathy (10). In the present study we classified IgA nephropathy patients according to their renal function and investigated whether or not their serum adiponectin levels were associated with arteriolosclerosis.

**Materials and Methods**

**Study population and data collection**

We enrolled 35 patients aged 35.0±14.6, who underwent renal biopsy between August 2004 and February 2006 in our hospital. IgA nephropathy was diagnosed on the basis of the clinical manifestations as well as the renal biopsy findings. None of the patients had diabetic mellitus.

Peripheral blood samples were obtained immediately before the renal biopsy. Serum adiponectin, high-sensitivity C-reactive protein (hs-CRP), total-cholesterol, and triglyceride levels and urinary protein excretion and creatinine clearance were measured.

Routine chemistry profiles were determined by standard methods. The serum was separated and stored at -20°C until analyzed for adiponectin. The serum adiponectin was measured with a human adiponectin ELISA kit (Otsuka Pharmaceuticals, Tokyo). Body height was measured on the day of admission and body weight was measured on the day of the biopsy. Body mass index (BMI) was calculated as the body weight in kilograms divided by body height in meters squared. Since the serum adiponectin level is greatly affected by renal function, we classified the patients according to their creatinine clearance.

Hypertension was defined as a systolic blood pressure of 140 mmHg or more or diastolic blood pressure of 90 mmHg or more and/or the current use of antihypertensive drugs. Patients currently taking an angiotensin-converting enzyme (ACE) blocker or angiotensin II receptor blocker (ARB) to protect renal function but whose blood pressure was normal before they started to use drugs were not considered to have hypertension.

**Renal biopsy**

Needle biopsies were performed under ultrasound guidance, and one to three separate specimens were collected during each biopsy. The specimens were fixed in 10% phosphate-buffered formation (pH 7.2), embedded in paraffin, and cut into 2 μm sections. The specimens were examined after staining with hematoxylin-eosin, periodic acid Schiff stain, Masson trichrome stain, and periodic acid methenamine silver stain for light microscopy.

The specimens were evaluated histologically based on the following parameters: percentage of glomeruli exhibiting global sclerosis, crescent formation, glomerular tuft adhesion to Bowman’s capsule, grade of mesangial cell infiltration, fibrosis in the interstitium, arteriosclerosis and arteriolosclerosis. The severity of each histologic parameter was semiquantitatively evaluated and classified into following four grades: o, none; 1, mild; 2, moderate; and 3, severe. All morphologic evaluations were performed by two of the authors who were unaware of the protocol of the study.

**Statistical analysis**

Data are presented as means ± S.D. A multiple-regression analysis between the adiponectin concentrations and other clinical parameters was performed. Gender, rate of patients with hypertension and current treatment with an ARB were compared in the arteriolosclerosis group and non-arteriolosclerosis group by the chi-squared test. Fisher’s exact test was used when the criteria for the chi-squared test could not be applied. Statistically significant differences in clinical parameters between the arteriolosclerosis group and non-arteriolosclerosis group were assessed by means of Student’s t-test. All statistical calculations were performed with Stat View J 5.0 software. A P value less than 0.05 was considered statistically significant.

**Results**

**Background characteristics and laboratory data of the IgA nephropathy patients**

The clinical characteristics of the subjects are summarized in Table 1. Age at the time of the renal biopsy was 35.0±14.6 years; 16 subjects were male, and 19 were female. The BMI of almost all subjects was normal, and mean creatinine clearance was 103.1±22.3 ml/min. Proteinuria was detected in almost all patients, and mean urinary protein value was 0.8±0.6 g/day. The serum total-cholesterol and triglyceride values were slightly elevated. The serum hs-CRP values ranged from 0.0021 mg/dl to 0.5070 mg/dl (0.060±0.092 mg/dl), and the values of almost all patients were within the normal range. The serum adiponectin values tended to be higher in the females (8.58±4.03 mg/ml) than in the males (7.91±5.88 mg/ml), but the difference was not statistically significant. Hypertension was found in 20.0% of the patients. None of the subjects were taking ACE inhibitors but ARB; 25.7% of them were taking an ARB. Among them, 4 patients use losartan potassium, 4 candesartan cilexetil and 1 valsartan.
Correlations between serum adiponectin levels and other parameters

Multiple regression analysis showed associations between serum adiponectin values and creatinine clearance (p<0.001), BMI (p<0.001), serum triglyceride (p=0.0010) and urinary protein excretion (p=0.0013) (Table 2). A positive correlation was found between the adiponectin values and urinary protein excretion values and inverse correlations between the adiponectin values and creatinine clearance, BMI, and serum triglyceride values.

Pathology findings in the kidney

The renal biopsy findings are shown in Table 3. We could not obtain interlobular arteries in 6 patients. Typical arteriole with and without arteriolosclerosis are shown in Fig. 1. The serum adiponectin value was 8.36 μg/ml in the patient without arteriolosclerosis and 6.23 μg/ml in the patients with arteriolosclerosis.

Characteristics and laboratory data of the IgA nephropathy patients according to whether they had arteriolosclerosis

As shown in Table 4, age at the time of the renal biopsy and the urinary protein excretion, serum total-cholesterol, and serum triglyceride values was significantly higher in the arteriolosclerosis group than in the non-arteriolosclerosis group, and in the IgA nephropathy patients as a whole. The rate of patients with hypertension and current treatment with an ARB was significantly higher in the arteriolosclerosis group than in the non-arteriolosclerosis in the IgA patients as a whole. No correlation was found between the presence of arteriolosclerosis and adiponectin values in the IgA patients as a whole.

Table 5 shows the characteristics and laboratory data of the IgA nephropathy patients whose renal function were almost normal (creatinine clearance 90-120 ml/min/1.73 m$^2$) grouped according to whether arteriolosclerosis was present. The mean age of the patients at the time of the renal biopsy was 28.1±14.6 years in non-arteriolosclerosis group, and they were younger than the arteriolosclerosis group (40.3±6.9, P=0.041). BMI was higher in the arteriolosclerosis group (23.3±3.2) than in non-arteriolosclerosis group (19.4±2.0, P=0.010). The urinary protein excretion, serum total-cholesterol, and serum triglyceride values tended to be higher in the arteriolosclerosis group, but the differences were not statistically significant. There were no statistically differences between the two groups in gender, creatinine clearance, or serum hs-CRP values. Rate of patients with hypertension and current treatments with an ARB tended to be higher in the arteriolosclerosis group, but the differences were not statistically significant.

No correlation was found between the presence of arteriolosclerosis and the serum adiponectin values in the IgA patients as a whole, but among the patients whose creatinine clearance was almost normal the serum adiponectin level of the patients with arteriolosclerosis was lower than in those without arteriolosclerosis (P=0.025).
Figure 1. Renal pathology in a typical case with and without arteriolosclerosis. a) Case without arteriolosclerosis: 27y male, Ccr 116 ml/min, serum adiponectin 8.36 μg/ml. b) Case with arteriolosclerosis: 31y male, Ccr 114 ml/min, serum adiponectin 6.23 μg/ml. Bright magenta staining was observed.

Discussion

The results of this study confirm the findings of other studies showing lower serum adiponectin levels in hypertriglyceridemia and higher serum adiponectin levels in subjects with renal dysfunction and severe proteinuria. We also found that among IgA nephropathy patients whose renal function was almost normal, the serum adiponectin level was lower in the patients with arteriolosclerosis than in those without arteriolosclerosis.

Women have been reported to have higher adiponectin levels than men (2, 11), and the women in our study also tended to have higher serum adiponectin levels (8.58±4.03 μg/ml) than the man (7.91±5.88 μg/ml), but the difference was not statistically significant. That may be due to the rather small number of subjects.

Poteinuria has been reported to be positively associated with the serum adiponectin levels of patients with the nephrotic syndrome (12). It was suggested that in the secondary forms of insulin resistance and hyperlipidemia, such as in nephrotic syndrome, in which the energy balance is often negative (12), stimuli that increase adiponectin synthesis predominate over inhibitory factors, and thereby lead to an appropriate, marked increase in the serum concentration of adiponectin. Even though the level of proteinuria (0.8±0.6 g/day) in this study was lower than in nephrotic syndrome, a positive association was observed in the IgA nephropathy patients. Significantly elevated adiponectin concentrations have been found with albuminuria in type 1 diabetic patients (13), and multivariate regression models have shown that the associations between adiponectin values and albuminuria and the glomerular filtration rate are independent of established risk factors (13). The results of the present study are consistent with those reports.

Arteriolosclerosis is a common vascular lesion and is characterized by accumulation of various serum proteins in the subendothelial space that often extends into the media, and when it does it is termed hyaline arteriolosclerosis (hyalinosis). Hyaline has a characteristic morphologic appearance. It stains bright magenta with periodic acid Schiff (PAS) and has a glassy texture. It is observed in many different conditions, including aging, hypertension, diabetes mellitus, and focal segmental glomerulosclerosis (FSGS).

Although the etiology of arteriolosclerosis has not been clearly demonstrated, endothelial cells are thought to be damaged by hypertension, hyperglycemia, or hyperlipidemia, which increases their permeability to plasma protein increases and results in the deposition of hyaline material in the subendothelial space.

In the present study, the serum total-cholesterol and serum triglyceride values were significantly higher in the arteriolosclerosis group than in the non-arteriolosclerosis group in the IgA nephropathy patients as a whole, and these findings are consistent with the results of the previous study (14). Moritz and Oldt first described an increase in the frequency of hyaline arteriolosclerosis with age (15). The patients in the present study were older and patients with hypertension were more frequent in the arteriolosclerosis group than in the non-arteriolosclerosis group, which is in accordance with their report.

A previous study showed an inverse relationship between plasma adiponectin levels and creatinine clearance in essential hypertensive patients (16); another earlier study showed a positive association between adiponectin levels and abnormal renal function in diabetes patients (17). The multiple-regression analysis in our study revealed that creatinine clearance had the most significant effect on the serum adiponectin level. The reason why adiponectin level is high in ESRD is unclear. Despite the favourable effects of adi-
Adiponectin on the vasculature and insulin resistance, the levels are increased in patients with end-stage kidney disease, in whom insulin resistance and atherosclerosis are prevalent. The paradox is not explained by the change in isoformic distribution, or by adiponectin receptor down-regulation or dysfunction (18).

Since the serum adiponectin level is strongly affected by renal function, we classified the patients according to their creatinine clearance and found that among the patients whose creatinine clearance was almost normal, the serum adiponectin level of the patients with arteriolosclerosis was lower than in those without arteriolosclerosis.

Since plasma hs-CRP levels are known to be negatively correlated with plasma adiponectin levels (19), adiponectin was thought to be involved in inflammatory processes and to have a protective role against arteriosclerosis. However, we were unable to find any relation between hs-CRP and adiponectin in the present study and hs-CRP was unrelated to arteriolosclerosis. This result may have been attributable to the relatively low hs-CRP levels (0.060±0.092 mg/dl) or the small number of subjects, and adiponectin may have a protective role against arteriosclerosis other than by lowering hs-CRP. Low levels of the serum adiponectin were observed in arteriolosclerosis in our study. Decreased adiponectin levels in diabetes may be an indicator of macroangiopathy (8). It has been suggested that adiponectin may suppress adhesion molecules but the exact mechanism has not been fully elucidated (8). In the arteriolosclerosis group, a high level of BMI and hyperlipidemia, which seemed to be causes of arteriolosclerosis, were observed. Hypoadiponectinemia may directly protect arteriolosclerosis or it may develop as a result of something, like hyperlipidemia, that caused the arteriolosclerosis.

Furuhashi et al. suggested that renin-angiotensin system

### Table 4. Characteristics and Laboratory Data in IgA Nephropathy

<table>
<thead>
<tr>
<th>Arteriolosclerosis</th>
<th>(-)</th>
<th>(+)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>14</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Age*</td>
<td>26.5 ± 6.14</td>
<td>40.7 ± 16.0</td>
<td>0.003</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>6 / 8</td>
<td>10 / 11</td>
<td>N.S.</td>
</tr>
<tr>
<td>BMI</td>
<td>20.5 ± 2.9</td>
<td>22.5 ± 2.7</td>
<td>N.S.</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min/1.73m²)</td>
<td>109.6 ± 17.0</td>
<td>98.8 ± 24.7</td>
<td>N.S.</td>
</tr>
<tr>
<td>Urinary protein excretion (g/day)</td>
<td>0.50 ± 0.48</td>
<td>0.99 ± 0.88</td>
<td>0.026</td>
</tr>
<tr>
<td>Serum total-cholesterol (mg/dl)</td>
<td>172.5 ± 39.0</td>
<td>207.6 ± 31.7</td>
<td>0.007</td>
</tr>
<tr>
<td>Serum triglyceride (mg/dl)</td>
<td>89.8 ± 29.2</td>
<td>142.6 ± 64.1</td>
<td>0.010</td>
</tr>
<tr>
<td>Serum hs-CRP (mg/dl)</td>
<td>0.069 ± 0.130</td>
<td>0.054 ± 0.059</td>
<td>N.S.</td>
</tr>
<tr>
<td>Serum Adiponectin (µ g/ml)</td>
<td>7.99 ± 3.00</td>
<td>8.47 ± 5.92</td>
<td>N.S.</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>0</td>
<td>36.1</td>
<td>0.012</td>
</tr>
<tr>
<td>Use of ARB (%)</td>
<td>0</td>
<td>75.0</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Mean ± SD: standard deviation
BMI: body mass index
ARB: angiotensin II receptor blocker
*Age at the time of renal biopsy

Patients as a whole grouped according to whether arteriolosclerosis was present.

### Table 5. Characteristics and Laboratory Data in IgA Nephropathy

<table>
<thead>
<tr>
<th>Arteriolosclerosis</th>
<th>(-)</th>
<th>(+)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>8</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Age*</td>
<td>28.1 ± 14.6</td>
<td>40.3 ± 6.9</td>
<td>0.041</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>3 / 5</td>
<td>6 / 3</td>
<td>N.S.</td>
</tr>
<tr>
<td>BMI</td>
<td>19.4 ± 2.0</td>
<td>23.3 ± 3.2</td>
<td>0.010</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min/1.73m²)</td>
<td>108.5 ± 10.2</td>
<td>104.5 ± 9.75</td>
<td>N.S.</td>
</tr>
<tr>
<td>Urinary protein excretion (g/day)</td>
<td>0.32 ± 0.47</td>
<td>0.81 ± 0.62</td>
<td>N.S.</td>
</tr>
<tr>
<td>Serum total-cholesterol (mg/dl)</td>
<td>172.1 ± 44.4</td>
<td>200.4 ± 24.8</td>
<td>N.S.</td>
</tr>
<tr>
<td>Serum triglyceride (mg/dl)</td>
<td>82.3 ± 31.1</td>
<td>143.8 ± 70.5</td>
<td>N.S.</td>
</tr>
<tr>
<td>Serum hs-CRP (mg/dl)</td>
<td>0.082 ± 0.17</td>
<td>0.067 ± 0.078</td>
<td>N.S.</td>
</tr>
<tr>
<td>Serum Adiponectin (µ g/ml)</td>
<td>9.19 ± 2.92</td>
<td>5.39 ± 3.31</td>
<td>0.025</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>0</td>
<td>44.4</td>
<td>N.S.</td>
</tr>
<tr>
<td>Use of ARB (%)</td>
<td>0</td>
<td>44.4</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Mean ± SD: standard deviation
BMI: body mass index
ARB: angiotensin II receptor blocker
*Age at the time of renal biopsy

Patients whose creatinine clearance were 90 – 120 ml/min/1.73 m² grouped according to whether presence of arteriolosclerosis was present.
blockade increases adiponectin concentrations and improves insulin sensitivity (20). None of the subjects in the present study were being treated with an ACE inhibitor, but 25.7% of the subjects were receiving an ARB. We could not assess the effect of ARB because of the small number of patients. Even though 44.4% of patients with normal renal function in the arteriolosclerosis group were being treated with an ARB, the adiponectin level was lower in the arteriolosclerosis group.

In patients without arteriolosclerosis, the negative relation between adiponectin and creatinine clearance was not statistically significant. The reason was not clear, but it may be due to the rather small number of subjects. Among the patients with normal renal function, a high level of adiponectin may prevent arteriolosclerosis and among the patients with decreased renal function, a high level of adiponectin may have a relatively slight influence on arteriolosclerosis.

Recently, high-molecular weight adiponectin was suggested to be the active form of adiponectin (21). Thus, might have been better to examine high-molecular weight adiponectin than total adiponectin in this study, but a close positive correlation between serum high-molecular weight and the total adiponectin concentrations has also been reported (21).

Hill et al have suggested that hyaline arteriolosclerosis is associated with impaired autoregulation (22). Since the extent of hyaline arteriolosclerosis is associated with the outcome of FSGS (23), interfering with the development of hyaline arteriolosclerosis may provide another approach to preventing the progression of renal disease (24). Adiponectin may therefore protect renal function by preventing the progression of arteriolosclerosis.

**Conclusion**

Serum adiponectin values were negatively correlated with the creatinine clearance, serum triglyceride, and BMI values of IgA nephropathy patients. A positive correlation was found between serum adiponectin values and urinary protein excretion in IgA nephropathy patients. The serum adiponectin levels seemed to be related to arteriolosclerosis in IgA nephropathy patients whose renal function was nearly normal. Adiponectin may prevent renal arteriolosclerosis.

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**References**

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