The association of plasma adiponectin levels with hypertensive retinopathy

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Abstract

Objective: Previous studies have demonstrated that low plasma adiponectin concentrations are associated with essential hypertension. It has also recently been shown that adiponectin plays an essential role in the modulation of angiogenesis. These data led us to hypothesize that adiponectin might contribute to end-organ damage in hypertension.

Methods: In the present study we have evaluated the relationship between plasma adiponectin concentrations and hypertensive retinopathy. One hundred and ten patients newly diagnosed with essential hypertension (EHT) (mean age, 46.79±5.0 years; body mass index (BMI), 26.47±2.23 kg/m²; male/female ratio, 58/52) and 57 healthy normotensive control subjects (NT) (mean age, 46.84±5.4 years; BMI, 26.66±2.65 kg/m²; male/female ratio, 33/24) were enrolled.

Results: Plasma adiponectin levels were significantly lower in EHT than in NT (P<0.001). In addition, adiponectin concentrations were strongly correlated with systolic and diastolic blood pressures in EHT (r=0.757, P<0.001; r=0.761, P<0.001) while there was no correlation in the NT group. Plasma adiponectin in patients with grade 0 hypertensive retinopathy (n=52) was significantly higher than that of the patients with grade 1 (n=30) and 2 (n=28) hypertensive retinopathy (P<0.001 for each). Plasma adiponectin in patients with grade 0 hypertensive retinopathy was also significantly lower than that in the NT group (P<0.001). The estimated threshold of plasma adiponectin concentration for hypertensive retinopathy was 17 μg/ml. This critical adiponectin level served largely to separate patients with retinopathy from those without.

Conclusion: Our results have shown that plasma adiponectin concentrations decrease progressively with higher grades of hypertensive retinopathy even after correction for other atherogenic risk factors, suggesting that a critical adiponectin level is needed for the development of retinopathy.

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Introduction

Adiponectin, expressed exclusively in adipocytes, is a novel protein product of the apM1 gene (1). The evidence indicates that adiponectin may be an important modulator for metabolic and vascular diseases. In vitro and animal studies and cross-sectional studies in humans have shown that hypoadiponectinemia is present in obesity, insulin resistance, type 2 diabetes and coronary disease (2–4). The reports about plasma adiponectin levels in essential hypertension (EHT) were formerly controversial (5, 6). However, recent data imply that plasma adiponectin levels are lower in EHT and negatively associated with blood pressures (7). Recent evidence indicates that low plasma adiponectin levels are associated with endothelial dysfunction both in hypertensive patients and adiponectin knock-out mice (8). Ouchi et al. (9) speculated that hypoadiponectinemia would soon be considered as an independent risk factor for coronary artery disease.

Hypertensive retinopathy (HR) is among the vascular complications of EHT (10, 11). It is known that the autoregulation of the retinal circulation fails as blood pressure increases beyond a critical limit (12). However, elevated blood pressure alone does not fully account for the extent of retinopathy. There are cases in which retinopathy was resolved despite the persistence of high blood pressure (13). In addition to the effect of high blood pressure, other humoral components probably take place in the pathogenesis of hypertensive retinopathy (14, 15). In our previous studies, we have established that another adipocytokine, leptin, was correlated with the extent of retinopathy both in essential hypertension and diabetes mellitus (16, 17). Yet again,
we established a strong negative association between plasma adiponectin levels and severity of retinopathy in diabetic patients (18). With regard to the overall data about the association of adipocytokines with the severity of the vascular complications, we decided to investigate whether there are any relationships between adiponectin levels and hypertensive retinopathy.

The study was designed to answer the following questions. (i) Is there any difference in the plasma adiponectin levels between patients with EHT and normotensive controls? (ii) Do plasma adiponectin levels change in hypertensive retinopathy? (iii) Is there any association between plasma adiponectin levels, blood pressure and the grade of hypertensive retinopathy. Subjects with known conditions which may affect plasma adiponectin levels were not enrolled into the following case-control study.

Subjects and methods

One hundred and ten newly diagnosed patients with essential hypertension (mean age, 46.79 ± 5.0 years; body mass index (BMI), 26.47 ± 2.23 kg/m²; male/female ratio, 58/52) were enrolled in the study. The subjects were selected from 177 patients who received a medical check-up at the outpatient clinics of the Gulhane School of Medicine. All selected patients had mild essential hypertension and none of them was taking any anti-hypertensive drugs.

The exclusion criteria were as follows: stage 2 hypertension (according to the VIIth report of the Joint National Committee) with blood pressure > 180/110 mmHg, grade 3 and 4 hypertensive retinopathy according to the Keith Wegener Classification (as most of the patients had other complications which could interfere with the adiponectin results), BMI > 30 kg/m², microalbuminuria, coronary heart disease, heart failure, renal failure, dyslipidemia and fasting blood glucose > 110 mg/dl. Finally, the patients whose insulin resistance score (HOMA-IR) results were higher than the mean −1 S.D. of that of the control patients were also excluded in order to prevent the effect of insulin resistance on adiponectin levels.

Fifty-seven normotensive subjects (NT) (mean age, 46.84 ± 5.4 years; BMI, 26.66 ± 2.65 kg/m²; male/female ratio, 33/24) who were the healthy participants who had undergone the check-up program were used as the control group. The controls had similar BMI, age and sex distribution as the EHT group. They underwent a routine physical and laboratory evaluation to ascertain that they had no hypertension, metabolic, hepatic or renal diseases. In addition, the control subjects had no family history of hypertension and diabetes.

Patients were evaluated by standard physical and laboratory examinations for secondary causes of hypertension and accompanying metabolic, cardiac, hepatic and renal diseases. The diagnosis of hypertension was based on the criteria of the seventh report of the National Joint Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (19). After the measurement of high blood pressure, all patients were followed up for 3 weeks and regarded as hypertensive if their blood pressure was still high within this period. Arterial blood pressure was measured in the right arm by mercury sphygmomanometer three times in a resting condition in the morning, and mean values were calculated for diastolic and systolic pressures. Hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg.

For the retinopathy evaluation, direct and indirect ophthalmoscopy was performed in all subjects after dilation of the pupils. A single blinded observer performed the fundoscopic examinations. Grade of hypertensive retinopathy was determined according to the Keith Wegener classification (20). Only patients with grade 0, 1 and 2 hypertensive retinopathy were included in the study. All subjects gave informed consent for their participation in the study. The ethical committee of the Gulhane School of Medicine approved the study.

Laboratory procedures

After an overnight fast, venous blood samples were drawn and promptly centrifuged, and the plasma was stored at −20°C until adiponectin assay was performed. All samples were run in the same assay. Plasma adiponectin concentrations were measured in duplicate by RIA (human adiponectin RIA kit; Linco Research, Inc., St Charles, MO, USA). Total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were determined by an enzymatic colorimetric method with an Olympus AU 600 autoanalyzer using reagents from Olympus Diagnostics, GmbH (Hamburg, Germany). Low-density lipoprotein (LDL) cholesterol was calculated by the formula of Friedewald et al. (21).

The serum basal insulin value was determined by the coated tube method (DPC, Los Angeles, CA, USA). In particular, a HOMA-IR was computed with the formula: HOMA-IR = fasting plasma glucose (mg/dl) × immunoreactive insulin (µU/ml)/405 (22).

Sample size

Prior to beginning our study we estimated the standard deviation adiponectin level at 5 µg/ml according to our clinical experience. We calculated the number of the patients needed to have a power of 90% to detect a difference of 10 µg/ml on the adiponectin level at the 0.05 level of significance. The estimate was 21 patients in each group (23).

Data analysis

Results are reported as the means ± S.D. The Levene’s test was used to evaluate the distribution characteristics.
of variables. Differences between EHT and NT groups were tested for significance by $t$-test. Mann–Whitney U test and chi-square test. The relationship between variables was analyzed by Pearson’s correlation. The results were also analyzed by one-way ANOVA test and Bonferroni-adjusted Mann–Whitney U and $t$-test for comparison of subgroups. We used the odds ratio (OR) as a measure of association between outcome and exposure. We investigated the effects of the variables on hypertension and hypertensive retinopathy by calculating ORs in univariate analyses for all hypertension and hypertensive retinopathy patients. Variables for which the unadjusted $P$ value was $\leq 0.20$ in logistic regression analysis were identified as potential risk markers and included in the full model. We conducted multivariate analyses by using conditional logistic regression. We reduced the model by using backward elimination and we eliminated potential risk markers by using likelihood ratio tests. Receiver operating characteristic (ROC) curve analysis was performed to determine a cut-off value of adiponectin for hypertensive retinopathy. Differences and correlations were considered significant at $P < 0.05$.

Results

Clinical and laboratory data for the patient and control groups are shown in Table 1. No significant difference in age, BMI, total cholesterol, HDL and LDL cholesterol, triglycerides, insulin levels and HOMA indexes was detected between EHT and NT groups. Although the adiponectin levels were higher in women than in men this difference was not statistically significantly either in patients or in controls ($P > 0.05$ for both). Adiponectin levels were not correlated with BMIs of both patients and controls ($r = -0.16, P = 0.836$ for patients; $r = -0.26, P = 0.632$ for controls). However, the plasma adiponectin concentrations were significantly lower in the EHT than in the NT group ($P < 0.001$). There was a significant negative correlation between plasma adiponectin and systolic and diastolic blood pressures ($r = -0.757, P < 0.001; r = -0.761, P < 0.001$) in the EHT group (Fig. 1). However, there was no correlation between the adiponectin levels and the systolic or diastolic blood pressures in the NT group. In addition, there was no correlation between the adiponectin levels and the HOMA indexes, insulin levels and the lipid parameters of the EHT and NT groups. As shown in Table 2 and Fig. 2, patients were subdivided into three groups according to the severity of the retinopathy. There were no differences between the subgroups according to age, BMI, insulin, HOMA index, serum total cholesterol, HDL and LDL cholesterol and triglycerides. Also the systolic and diastolic blood pressures were not different in both groups. However, plasma adiponectin in patients with grade 0 hypertensive retinopathy was significantly higher than that in patients with grade 1 hypertensive retinopathy ($P < 0.001$) or patients with grade 2 hypertensive retinopathy ($P < 0.001$). The adiponectin levels were not different between the grade 1 and 2 patients ($P = 0.585$). Plasma adiponectin in patients with grade 0 hypertensive retinopathy was also significantly lower than that of the NT group ($P < 0.001$). The effects of plasma adiponectin levels and gender on hypertension and hypertensive retinopathy were assessed by conditional logistic regression analysis, after adjustment for the other parameters by matching. By defining the presence of hypertension as the final variable in the conditional logistic regression model with the adiponectin levels as the continuous variable, gender and adiponectin were significantly involved in the model (adiponectin, $P < 0.001$; gender, $P = 0.023$) (Table 3). Adiponectin, as a continuous variable, was also a significant predictor for hypertensive retinopathy after being adjusted for systolic and diastolic blood pressures (OR $= 0.84$, 95% confidence interval (CI) $(0.78–0.90)$, $P < 0.001$).

### Table 1 Clinical and laboratory features of the patient and control groups. Values are means±s.d.

<table>
<thead>
<tr>
<th></th>
<th>Patients ($n = 110$)</th>
<th>Controls ($n = 57$)</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46.79±5.1</td>
<td>46.84±5.4</td>
<td>0.953†</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>58 (53%)/52 (47%)</td>
<td>33 (58%)/24 (42%)</td>
<td>0.525‡</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>151.61±6.21</td>
<td>120.33±13.17</td>
<td>&lt;0.001§</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>92.85±4.04</td>
<td>80.96±2.11</td>
<td>&lt;0.001§</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>183.60±33.14</td>
<td>176.82±22.67</td>
<td>0.125§</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>116.86±24.10</td>
<td>116.66±22.54</td>
<td>0.958§</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>104.75±25.55</td>
<td>109.45±25.88</td>
<td>0.266†</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>46.86±3.64</td>
<td>47.80±4.57</td>
<td>0.180†</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.47±2.23</td>
<td>26.66±2.65</td>
<td>0.641†</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>6.21±1.46</td>
<td>5.99±1.45</td>
<td>0.369†</td>
</tr>
<tr>
<td>HOMA</td>
<td>1.34±0.39</td>
<td>1.29±0.37</td>
<td>0.463†</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>16.90±6.46</td>
<td>37.67±7.99</td>
<td>&lt;0.001†</td>
</tr>
</tbody>
</table>

Bp, blood pressure (mmHg).

* These variables are matched for in analyses.

† $t$-test.

‡ Chi-square test.

§ Mann–Whitney U test.
while gender was not (OR = 1.09, 95% CI (0.40–2.96), P = 0.87). We used ROC curve analysis to find the cut-off value of adiponectin for the prediction of hypertensive retinopathy with the highest sensitivity and specificity. ROC curve analysis obtained a cut-off value of adiponectin for hypertensive retinopathy. The cut-off value of plasma adiponectin for hypertensive retinopathy was 17 µg/ml (sensitivity, 84%; specificity, 73%; area under the curve, 0.789; P < 0.001) (Fig. 3). When the cut-off value of adiponectin was taken as ≥17 µg/ml, adiponectin levels became a significant predictor of hypertensive retinopathy after adjustment for blood pressure while gender was not found to be significant (adiponectin, P = 0.001; gender, P = 0.55) (Table 4).

**Discussion**

The original finding of the study is the lower adiponectin level in hypertensive retinopathy than in the ones without hypertensive retinopathy. After adjustment for blood pressures, insulin levels, HOMA index and lipid parameters, plasma adiponectin is an independent predictor for hypertensive retinopathy. The predictive value of adiponectin for occurrence of hypertensive retinopathy was also calculated. The critical adiponectin level was in the range of 17 µg/ml. This level served to largely separate patients with hypertensive retinopathy from patients without retinopathy. The results also

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**Table 2** Characteristics of patients according to retinopathy grade. Values are means ± S.D.

<table>
<thead>
<tr>
<th></th>
<th>Grade 0 (n = 52)</th>
<th>Grade 1 (n = 30)</th>
<th>Grade 2 (n = 28)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46.88 ± 5.0</td>
<td>46.80 ± 5.4</td>
<td>46.60 ± 4.8</td>
<td>0.997</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>29/23</td>
<td>15/15</td>
<td>14/14</td>
<td>0.102</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>150.30 ± 6.98</td>
<td>152.46 ± 5.33</td>
<td>153.14 ± 5.17</td>
<td>0.053</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>92.55 ± 3.78</td>
<td>94.30 ± 2.85</td>
<td>91.85 ± 5.16</td>
<td>0.569</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>184.32 ± 34.32</td>
<td>181.93 ± 29.35</td>
<td>184.03 ± 35.77</td>
<td>0.858</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>117.01 ± 25.38</td>
<td>119.33 ± 23.25</td>
<td>113.92 ± 23.05</td>
<td>0.398</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>101.55 ± 24.76</td>
<td>106.20 ± 28.04</td>
<td>109.14 ± 24.29</td>
<td>0.936</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>46.76 ± 3.65</td>
<td>46.83 ± 3.66</td>
<td>47.07 ± 3.74</td>
<td>0.536</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.43 ± 2.12</td>
<td>26.97 ± 2.60</td>
<td>25.98 ± 1.93</td>
<td>0.430</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>6.28 ± 1.62</td>
<td>6.05 ± 1.29</td>
<td>6.24 ± 1.34</td>
<td>0.729</td>
</tr>
<tr>
<td>HOMA</td>
<td>1.35 ± 0.32</td>
<td>1.32 ± 0.38</td>
<td>1.34 ± 0.43</td>
<td>0.884</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>21.25 ± 5.85*</td>
<td>14.48 ± 5.30†</td>
<td>11.75 ± 4.73‡</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Bonferoni adjusted t-test, P < 0.001 for *vs † and *vs ‡; P = 0.585 for † vs ‡; P values were determined by one-way ANOVA.

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implied that plasma adiponectin levels are lower in non-insulin resistant EHT and negatively correlated with both systolic and diastolic blood pressures. The presence of low adiponectin levels in EHT and even lower levels in hypertensive retinopathy is worthy of further discussion.

The data so far imply that adiponectin seems to be a marker of endothelial function and acts as an endogenous biological modulator of vascular remodeling. Adiponectin attenuates excessive inflammatory responses in the vascular wall (24, 25). In a dose-dependent fashion, it suppresses tumor necrosis factor-α-stimulated adherence of monocytes to cultured human endothelial cells (24). This effect results from the inhibition of the expression of adhesion molecules, such as vascular cell adhesion molecule-1, E-selectin and intercellular adhesion molecule-1. Moreover, adiponectin inhibits specific binding of oxidized LDLs and their uptake by macrophages. In addition, it decreases the cholesterol ester content in macrophages by about 50% and inhibits transformation of macrophages to foam cells (25). In adiponectin knock-out mice, endothelium-dependent vasorelaxation is damaged (8) and extreme vascular remodeling response to injury is established (26, 27). The overall data indicate that adiponectin is an anti-inflammatory and anti-atherogenic protein. Hypertension is well associated with endothelial dysfunction and an increased tendency to atherosclerosis (28). Thus, the presence of low plasma adiponectin levels in EHT is likely to reflect impaired endothelial function due to chronic hypertension. From this point of view, much lower adiponectin levels in hypertensive retinopathy than in the patients who do not have hypertensive retinopathy can also be attributed to the extent of vascular disturbance.

EHT causes various changes in the retinal vasculature of the eye (10, 11). Excessive intraluminal pressure in the retinal vessels breaks down auto-regulation in retinal vasculature and may lead to capillary microaneurysms and arterial or arteriolar macroaneurysms (12). However, the extent of retinopathy is not only related to high blood pressure itself but also to other factors such as endothelial dysfunction, the effects of inflammatory mediators and systemic or local actions of humoral agents (14, 15). Thus, the presence of low adiponectin levels in hypertensive retinopathy than in the patients who do not have hypertensive retinopathy can also be attributed to the extent of vascular disturbance.

Adiponectin in hypertensive retinopathy
of end-organ damage due to hypertension. As hypertensive complications such as microalbuminuria, peripheral or coronary heart disease were not involved in the study, we cannot anticipate whether apparent hypertensive vascular complications will accompany even lower adiponectin levels or not.

So far, the data about the relation between adiponectin and blood pressure have been highly confusing. A negative association was reported between the systolic blood pressure and adiponectin levels in healthy female adolescents (33). Adamczak et al. (6) described low adiponectin levels and a significantly negative correlation between adiponectin and blood pressure in hypertensive patients (6). However, this association no longer existed after adjustment for BMI and the authors did not measure insulin resistance. On the contrary, Mallamaci et al. (5) reported elevated plasma levels in hypertensive men while they did not establish any association in women. However, in this study, 60% of patients were under anti-hypertensive drug treatment which possibly caused divergent results. Although in our study plasma adiponectin levels were significantly lower and negatively associated with blood pressures in EHT, the recent findings of Iwashima et al. (7) imply that such a relationship is present only in normotensive subjects with or without diabetes mellitus and insulin resistance. Recently Furutashii and colleagues reported low adiponectin levels in insulin-resistant hypertensive patients (34, 35). The authors implied that insulin resistance might contribute to the low adiponectin levels in EHT. However, in our study, low adiponectin levels were present in hypertensive patients who were not insulin resistant. Moreover, we did not establish any association between the HOMA indexes, immunoreactive insulin levels and adiponectin levels. A possible cause of this lack of association is that the patients had narrow BMI ranges and were not insulin resistant. Ouchi et al. (8) reported that adiponectin knock-out mice do not exhibit metabolic disorders such as insulin resistance, diabetes mellitus and hypertension, unless they are fed with an atherogenic diet (8). This report implies that it is not necesssary to find an association between low adiponectin levels and insulin resistance in EHT. In a recent report (7), plasma adiponectin levels of EHT patients who were not under any medication were not correlated with HOMA indexes, BMIs and total cholesterol and triglycerides (7). There are also other hypotheses about the presence of low adiponectin levels in EHT. Several reports imply that β-adrenergic agonists inhibit adiponectin gene expression in adipocytes (36, 37). Adamczak et al. (6) hypothesized that sympathetic overactivity, which is one of the several mechanisms of EHT, may cause low adiponectin levels. Finally, accumulation of adiponectin was observed in the sub-endocardial space of an injured vascular wall (38). Whether hypertension-related vascular damage causes adiponectin deposition in the vascular wall is unclear. It is another speculation for the cause of low adiponectin levels in EHT.

This study has, however, some limitations. The study population was rather small. Thus large-scale studies would be helpful in order to make further comments on the relation between adiponectin and hypertensive retinopathy. Also the investigation of retinopathy was performed by a single observer. The data about the retinal examination would be more impartial if there were more than one observer. Finally, as the study is a case-control design, it is not easy to predict exactly whether the low adiponectin levels precede retinopathy or vice versa. Future cohort studies will be helpful in providing an answer.

In conclusion, our results show that plasma adiponectin levels are low in hypertensive patients who are not insulin resistant. Systolic and diastolic blood pressures are negatively related with plasma adiponectin levels. Adiponectin levels further decrease and correlate with the severity of hypertensive retinopathy. The data also suggest that a critical adiponectin level is needed for the development of retinopathy.

However, there are several questions to be answered. (i) What is the reason for low plasma adiponectin levels in EHT? (ii) Are low plasma adiponectin levels the cause or the result of hypertensive retinopathy? (iii) Is it an omen for the presence of hypertensive end-organ damage? We speculate that the presence of low adiponectin levels in EHT implies ongoing systemic vascular response to hypertension. Future studies may investigate associations between plasma adiponectin levels and other hypertensive complications such as microalbuminuria and left ventricular hypertrophy. Also upcoming studies will point out whether or not adiponectin is a good predictor of hypertensive vascular outcomes.

Acknowledgements

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