**Plasma vascular endothelial growth factor as a marker for early vascular damage in hypertension**

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**ABSTRACT**

Elevation of plasma VEGF (vascular endothelial growth factor) has been noted in patients with hypertension or atherosclerosis. VEGF has been regarded as a marker for endothelial dysfunction. However, the role of VEGF in hypertension-induced vascular injury and its relationship with endothelial function have not been studied. This study included 20 untreated hypertensive men with grade 1 or 2 hypertensive retinopathy, 10 untreated hypertensive men without hypertensive retinopathy and 10 healthy controls. None of the hypertensive patients had diabetes, renal impairment or overt vascular diseases. Plasma VEGF and adhesion molecules were measured using ELISAs. Endothelial function was measured by FMD (flow-mediated vasodilation) of the brachial artery. Plasma levels of VEGF, excluding adhesion molecules, were significantly higher in hypertensive patients with retinopathy when compared with patients without retinopathy (152.4 ± 80.8 pg/ml versus 104.7 ± 27.2 pg/ml, P = 0.035) or controls (152.4 ± 80.8 pg/ml versus 98.9 ± 23.7 pg/ml, P = 0.025). Levels of FMD were significantly lower in hypertensive patients than controls, but there were no significant differences between patients with or without retinopathy. Degrees of FMD were inversely correlated with VEGF levels (r = −0.351, P = 0.031). Elevation of plasma VEGF was associated with hypertensive retinopathy. Plasma VEGF could be used as a marker of early vascular damage induced by hypertension.

**INTRODUCTION**

VEGF (vascular endothelial growth factor) is known to be a multifunctional peptide capable of inducing receptor-mediated endothelial cell proliferation and angiogenesis [1]. Recently, a link between VEGF and cardiovascular diseases has been established. Elevation of VEGF has been noted following acute myocardial infarction [2,3]. It has also been associated with coronary artery disease and peripheral vascular disease [4], and has been noted after coronary artery bypass surgery [5]. VEGF is also increased in acute heart failure [6], and could be a factor for poor prognosis in acute coronary syndrome [7]. Even patients with coronary risk factors, such as hyperlipidaemia [8] and hypertension [9], showed association with increased VEGF. Impaired regulation of vascular growth has been noted in hypertension [10]. Previous studies have demonstrated that VEGF and its soluble receptor Flt-1 increase in hypertension and can be reduced after antihypertensive treatment [9]. However, the importance of VEGF in the development of vascular damage in association with hypertension has not been fully elucidated.

**Key words:** adhesion molecule, flow-mediated vasodilation (FMD), hypertension, retinopathy, vascular damage, vascular endothelial growth factor (VEGF).

**Abbreviations:** FMD, flow-mediated vasodilation; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor.

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Increased expression of adhesion molecules, such as ICAM-1 (intercellular adhesion molecule 1) and VCAM-1 (vascular cell adhesion molecule 1), has been noted when the endothelium is activated by inflammatory response [11]. Soluble ICAM-1 and VCAM-1 were increased in hypertension [12]. We hypothesized that VEGF and adhesion molecules could be early markers for the development of vascular damage in hypertension. As hypertensive retinal microvascular signs are important markers of microvascular damage of other organs due to elevated blood pressure [13,14], we used early changes in hypertensive retinopathy as an index of early vascular damage in hypertension and studied this hypothesis in the present study.

VEGF has also been regarded as an endothelial marker. Increased plasma VEGF concentration might simply reflect endothelial damage caused by hypertension [1]. In order to investigate whether VEGF was only an endothelial damage marker in the development of vascular damage in hypertension, we also measured FMD (flow-mediated vasodilation) in each subject to observe the relationship between VEGF and endothelial function.

**EXPERIMENTAL**

**Subjects**
Twenty untreated hypertensive men (age, 38.8 ± 9.8 years) with grade 1 or 2 hypertensive retinopathy, 10 untreated hypertensive men (age, 31.7 ± 9.8 years) without retinopathy and 10 age-matched healthy men (age 32.0 ± 7.4 years) as controls were recruited for the present study. All patients were recruited from a hypertensive clinic. They had not been receiving any antihypertensive medication prior to recruitment. Blood pressure was measured using the standard sphygmomanometry method used in clinic. Hypertension was diagnosed if blood pressure > 140/90 mmHg on two separate occasions. None of the patients had other overt vascular diseases. Controls were recruited from healthy volunteers from our previous study [17], after careful evaluation for the risk factors. They were all non-smokers and did not show any risk factors. None of the subjects drank alcohol. Subjects with a fasting blood sugar more than 110 mg/dl or a body mass index greater than 25 kg/m² were excluded. For the retinopathy evaluation, direct or indirect ophthalmoscopy was performed on all hypertensive subjects after dilatation of the pupils. In order to reduce the possibility of misclassification, the fundoscopic examination was performed by a retinopathy specialist, who was a blind observer. The grade of hypertensive retinopathy was determined according to the Keith–Wagener classification [15]. Grade 1 (narrowing of the vessels) and grade 2 (pressure from the artery on the vein at arteriovenous crossings) retinopathies were regarded as early vascular damage.

Written informed consent was obtained from all of the subjects, and the study was approved by the Clinical Research Committee of the hospital.

**Measurement of plasma VEGF**
Fasting blood samples were drawn from the antecubital vein and the samples were treated with the anticoagulant trisodium citrate. All the blood samples were taken within 1 week after recruitment. After centrifugation at 1000 g for 20 minutes, the plasma was immediately separated and frozen to −70 °C until examination. Plasma concentrations of VEGF were assayed by ELISA (R&D Systems, Minneapolis, MN, U.S.A.). In brief, a monoclonal antibody specific for VEGF was pre-coated on to a microplate. Standards and samples were pipetted into the wells, and any VEGF present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specifically against VEGF was added to the wells. Following a wash to remove any unbound antibody–enzyme reagent, a substrate solution was added to the wells and the colour developed in proportion to the amount of VEGF bound in the initial step. Colour development was then stopped, and the intensity of the colour was measured using a microplate reader set at A450. The minimum detectable amount of VEGF was 5.0 pg/ml.

**Measurement of adhesion molecules**
Plasma concentrations of soluble ICAM-1 and VCAM-1 were measured by ELISAs (R&D Systems), as described previously [16]. In brief, a monoclonal antibody specific to ICAM-1 or VCAM-1 was pre-coated on to a microplate. Standards, samples, controls and conjugate were pipetted into the wells and any ICAM-1 or VCAM-1 present was sandwiched by the immobilized antibody and the enzyme-linked monoclonal antibody specifically against ICAM-1 or VCAM-1. Following a wash to remove any unbound substances or antibody–enzyme reagents, a substrate solution was added to the wells and the colour developed in proportion to the amount of ICAM-1 or VCAM-1 bound. The colour development was stopped, and the intensity of the colour was measured. The minimum detectable dose was 0.35 μg/l for ICAM-1 and 2.0 ug/l for VCAM-1.

**Measurement of FMD**
FMD of the brachial artery was measured in a quiet, temperature-controlled room after 10 min of bed rest using a method described previously [17]. Briefly, FMD, in response to reactive hyperaemia, was measured in the left brachial artery. A high-resolution ultrasound machine (Hewlett-Packard Sonos 2500) equipped with a 7.5-MHz linear array probe was used for the present study. Arterial diameters were measured at the baseline and during reactive hyperaemia. The condition of reactive hyperaemia was induced by inflation of a pneumatic cuff.
Table 1  Basic data and measurements between groups
The results are expressed as the means ± S.D. or number of patients (%); ∗ P < 0.05. SBP, systolic blood pressure; DBP, diastolic blood pressure.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hypertensives with retinopathy (n = 20)</th>
<th>Hypertensives without retinopathy (n = 10)</th>
<th>Controls (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>38.8 ± 9.8</td>
<td>31.7 ± 9.8</td>
<td>32.0 ± 7.4</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.1 ± 1.0</td>
<td>23.1 ± 1.1</td>
<td>22.8 ± 1.1</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>182.4 ± 35.9</td>
<td>166.0 ± 28.7</td>
<td>119.8 ± 12.4∗</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>120.0 ± 23.0</td>
<td>112.3 ± 21.5</td>
<td>74.0 ± 6.6∗</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>75.5 ± 8.5</td>
<td>71.5 ± 7.4</td>
<td>69.8 ± 7.4</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>201.4 ± 31.8</td>
<td>176.1 ± 27.7*</td>
<td>214.4 ± 37.0</td>
</tr>
<tr>
<td>Triacylglycerol (mg/dl)</td>
<td>152.0 ± 73.7</td>
<td>171.4 ± 83.1</td>
<td>147.9 ± 65.6</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dl)</td>
<td>88.0 ± 6.6</td>
<td>84.0 ± 3.5</td>
<td>85.4 ± 9.8</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>1.0 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>VEGF (pg/ml)</td>
<td>152.4 ± 80.8∗</td>
<td>104.7 ± 27.2</td>
<td>98.9 ± 23.7</td>
</tr>
<tr>
<td>VCAM-1 (µg/l)</td>
<td>416.1 ± 76.3</td>
<td>384.1 ± 48.7</td>
<td>400.0 ± 92.5</td>
</tr>
<tr>
<td>ICAM-1 (µg/l)</td>
<td>198.1 ± 43.7</td>
<td>184.0 ± 29.7</td>
<td>193.4 ± 44.7</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>4.4 ± 1.7</td>
<td>5.1 ± 1.6</td>
<td>9.6 ± 2.1∗</td>
</tr>
<tr>
<td>Smoking</td>
<td>6 (30 %)</td>
<td>4 (40 %)</td>
<td>0</td>
</tr>
</tbody>
</table>

Statistics
The results are expressed as the means ± S.D. A Kruskal–Wallis statistical analysis followed by a Mann–Whitney rank-sum test were used for comparison of continuous variables between groups. A Spearman’s rank correlation test was used for assessment of the relation between FMD, VEGF and adhesion molecules. A P value < 0.05 was considered statistically significant. Analysis was performed using SPSS version 10.0 for Windows (SPSS, Chicago, IL, U.S.A.).

RESULTS
Table 1 shows the clinical characteristics and measurements in hypertensive patients with and without retinopathy and in controls. Blood pressure was significantly higher in hypertensive patients than controls, although on the forearm to a pressure above 250 mmHg for 4.5 min. The brachial artery was scanned in longitudinal sections 2 to 5 cm above the elbow. The arterial diameter was measured on B-mode images at the end-diastolic phase from one media–adventitia interface to the other at the clearest section for six times at baseline. The average of the six measurements was considered as the baseline data. We then measured three more times every 30 s after reactive hyperaemia. The average of the three consecutive maximal diameters was taken as the data after hyperaemia. FMD was calculated as the percentage change in diameter compared with the baseline. In our laboratory, two independent investigators performed the measurements. The intra-observer and inter-observer variations were 0.9 % and 1.4 % respectively. The true variability of the method was about 15–20 % in the present study.

there was no difference between patients with or without retinopathy. Current smoking status was similar in the two groups of hypertension. Cholesterol was significantly lower in hypertensive patients without retinopathy than in the other two groups. Plasma levels of VEGF, but not adhesion molecules, were significantly higher in hypertensive patients with retinopathy when compared with patients without retinopathy or controls. There were no differences in VEGF levels between grade 1 (n = 16) and grade 2 (n = 4) retinopathy. There were no significant differences in VEGF levels between patients without retinopathy and controls (Figure 1). Levels of FMD were significantly lower in hypertensive patients than in controls, but there were no significant
Degrees of FMD were inversely correlated with VEGF levels ($r = -0.351$, $P = 0.031$) in all subjects pooled. However, VEGF and FMD were not correlated with levels of adhesion molecules. VEGF was not correlated with blood pressure, lipid profiles or age. FMD was significantly correlated with blood pressure, but not lipid profiles or age (Table 2). Smoking status did not influence the levels of VEGF and FMD. After multivariate regression analysis controlling for age, blood pressure, smoking status, cholesterol and fasting blood sugar, VEGF levels were still significantly different between patients with or without retinopathy ($P = 0.013$).

### DISCUSSION

The present study showed that VEGF was significantly higher in hypertensive patients with retinopathy than in hypertensive patients without retinopathy or normotensive subjects. There were no significant differences in adhesion molecules between the groups. This result indicates that VEGF, rather than adhesion molecules, can be used as a marker for early microvascular damage in hypertension. Previous studies have demonstrated that VEGF is increased in hypertensive patients and decreased after control of blood pressure [9]. In our study, elevation of VEGF was noted among hypertensive patients only when vascular damage occurred in the retina. VEGF can be a marker for early microvascular damage in hypertension. Previous studies have shown that adhesion molecules are elevated in elderly hypertension [12]. Levels of VCAM-1 and ICAM-1 are associated with age and blood pressure [12]. However, adhesion molecules were not elevated in hypertensive patients in our study. This discrepancy is probably due to the fact that our patients were relatively young and in the early stage of hypertension. Adhesion molecules were not good markers for vascular damage in the early stage of hypertension. The relationship between blood pressure and adhesion molecules has also been inconsistent in previous studies [11].

There are several proposed mechanisms responsible for the elevation of VEGF in vascular diseases. First, in response to vascular damage, a wide array of growth factors, cytokines and other molecules are released, stimulating angiogenesis via VEGF, which is essential for the repair process [1]. Another possible mechanism is that elevation of VEGF may simply reflect endothelial cell damage apparent in hypertension [1]. In our study, FMD in hypertensive patients was significantly lower than in normal controls. It reflected macrovascular dysfunction in hypertension. The degrees of FMD were negatively correlated with the levels of VEGF. However, elevation of VEGF could only partially reflect endothelial cell damage caused by hypertension, since VEGF did not elevate in hypertensive patients without retinopathy. Association of VEGF and microvascular damage is possibly through other mechanisms. The fact that elevated VEGF is not solely related to endothelial damage has been supported by the lack of a correlation between VEGF and the von Willebrand factor [8]. Similar findings have also been reported in diabetes patients [4]. VEGF has been found to be raised only in diabetic patients with vascular disease, rather than diabetes alone. Elevation of VEGF is associated with atherosclerosis, but not diabetes itself [4]. A positive correlation between coronary artery collaterals and intra-coronary artery VEGF has been reported [18]. All of the evidence, as well as our observations, support the finding that elevation of VEGF levels is associated with vascular atherosclerosis caused by various risk factors.

The limitation of this study was that the retinal changes were not specific for hypertension. There was a trend for patients with retinopathy to be older and with higher blood pressure and cholesterol. These factors could have confounding effects on our results. The cumulative effects of age, smoking, cholesterol levels and fasting blood sugar probably contributed to atherosclerosis in the retinopathy group independent of hypertensive vascular disease.
changes. Age, blood pressure levels and cholesterol did not correlate with VEGF levels, and VEGF levels were still significantly different between patients with or without retinopathy after multivariable analysis controlling for age, blood pressure, smoking status, cholesterol and blood sugar. The effects of these factors in our study were probably small, and a large-scale study is needed for further investigation. The other limitation of our study was that we did not obtain urine for measurement of microalbumin. Microalbuminuria is known to be a reliable and simple way to detect endothelial damage.

In conclusion, our study demonstrates that plasma VEGF levels can be a useful marker for the detection of early microvascular damage in hypertension. Decreased levels of VEGF have been noted after treatment for hypertension or hyperlipidaemia [8,9]. However, there has been no direct evidence to indicate that decreased VEGF is associated with reduced atherosclerosis. The role of VEGF in the long-term prognosis for cardiovascular events in hypertension is worth further evaluation.

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