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□ ORIGINAL ARTICLE □

The Relationship between Vascular Endothelial Growth Factor (VEGF) and Microalbuminuria in Patients with Essential Hypertension

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Abstract

Objective The existence of microalbuminuria (MAU) in patients with essential hypertension is a strong indicator of microvascular damage. Although endothelial dysfunction and increased vascular permeability both have a role in the development of MAU, its ethiopathogenesis in hypertensive patients is not yet clearly understood. Vascular endothelial growth factor (VEGF) is the most important regulator of pathological or physiological angiogenesis and it additionally leads to increased vascular permeability. This study aims to assess the relationship of serum VEGF levels to MAU in non-complicated, newly-diagnosed essential hypertensive patients (EHs).

Methods This study included 30 newly-diagnosed EHs with MAU, 46 newly-diagnosed EHs without MAU and 46 healthy controls. None of the EHs had diabetes, renal impairment or atherosclerotic diseases. Serum VEGF levels were measured using the ELISA method.

Results Serum levels of VEGF were significantly higher in EHs with MAU when compared with patients without MAU (225.15±109.34 pg/mL versus 166.78±114.35 pg/mL, p: 0.04) or controls (225.15±109.34 pg/mL versus 144.91±96.60 pg/mL, p: 0.007). On the other hand, no significant difference was observed between the non-MAU and control groups. In the univariate analysis, serum levels of VEGF, were positively correlated with systolic blood pressure (R: 0.253 p: 0.001), diastolic blood pressure (R: 0.162 p: 0.04), mean arterial pressure (R: 0.239 p: 0.002), creatinine clearance (R: 0.172 p: 0.04) and MAU (R: 0.338 p: 0.002). In the multiple linear regression analysis, VEGF levels were independently related to MAU (β: 0.248, p: 0.02).

Conclusion VEGF levels are higher in EHs in the presence of MAU. These high values may be important in the early diagnosis of vascular damage in EHs. Additionally, VEGF may increase glomerular permeability and lead to MAU in EHs.

Key words: hypertension, microalbuminuria, VEGF

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Introduction

The existence of microalbuminuria (MAU) in patients with essential hypertension is a strong indicator of microvascular damage (1). MAU is also known to be closely related to cardiovascular risk factors and cardiovascular morbidity (2). Although endothelial dysfunction and increased vascular permeability both have a role in the development of MAU (1), its ethiopathogenesis in hypertensive patients is not yet clearly understood (1).

Vascular endothelial growth factor (VEGF) is a multifunctional glycoprotein which is mitogenic for endothelial cells (3). It has a high affinity with endothelial cells in the macro and microvascular vessels (3). It is the most important regulator of pathological or physiological angiogenesis and addi-

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tionally leads to increased vascular permeability (3). On the other hand, VEGF inhibition by bevacizumab, a VEGF inhibitor, produces hypertension and proteinuria (4).

Recent studies have claimed that essential hypertensive patients (EHs) have increased serum VEGF levels (5, 6). This increase is particularly significant in patients with vascular damage such as hypertensive retinopathy, implying that VEGF may be important in the early diagnosis of vascular damage when hypertension is present (6). However, an adequate number of studies conducted especially on EHs with no diabetes mellitus (DM) do not exist in the literature.

The relationship between MAU and VEGF has primarily been examined in studies conducted on diabetic patients (7-12). In DM patients, it was suggested that increased VEGF levels as a result of high glucose may have a role in the development of MAU (7, 12-16). Such findings were also obtained in larger scale studies such as that of Asselbergs and colleagues (2). However, it is worth noting that Asselbergs et al conducted their study on patients with significant atherosclerosis as well as hypertensive patients (2). The close relationship between VEGF and atherosclerosis may be responsible for increased VEGF levels. Therefore it may be important for the relationship between VEGF and MAU to be observed in non-complicated hypertensive patients who have not yet received any treatment.

This study aims to assess the relationship of serum VEGF levels to MAU in non-complicated, newly-diagnosed EHs.

Material and Method

The study was conducted between 2005 and 2006 in the Cardiology Clinic of Kırıkkale University School of Medicine and the Nephrology Clinic of Gazi University School of Medicine. A total of 76 newly-diagnosed untreated EHs and a total of 46 healthy volunteers, to be assigned to the control group, were admitted to the study. Patients with atherosclerotic heart disease, congestive heart failure, peripheral artery disease, DM, renal failure, secondary hypertension, cerebrovascular events, apparent proteinuria or any other inflammatory disease were not included in the study. The study was approved by the Ethical Committee on Studies Involving Human Beings at Kırıkkale University, Faculty of Medicine. Following this, all patients were informed and their signed consent was taken.

The blood pressure of all subjects was measured and their body mass index (BMI) was calculated by the formula (BMI: kg/m^2). Blood pressure values were based on the average of three different measurements taken after 15-minute resting periods; 12-derivation electrocardiograms were taken. When deemed necessary, transthoracic doppler echocardiogram and effort test were performed on subjects. Fasting blood glucose, blood urea nitrogen (BUN), creatinine (Cr), C-reactive protein (CRP), albumin, total cholesterol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol and triglyceride levels in blood samples drawn after a 12-hour fast were measured with the standard method whereas serum VEGF levels were measured by using the ELISA method. Oral glucose tolerance test was performed on subjects whose fasting blood glucose level was above 100 mg/dL. The 24-hour urinary protein (mg/24 h) and albumin (mg/24h) excretion of all subjects were measured using the calorimetric method and creatinine excretion was measured using the kinetic method. Their creatinine clearance (mL/mn) (Ccr) was calculated by the formula (24-hour urinary volume*urinary Cr/serum Cr*1440). MAU was defined as a urinary albumin excretione $\geq$30 mg/24 h and < 300 mg/24 h. Microscopies of the morning urine were examined. As a result, patients with abnormal glucose tolerance, DM, urinary tract infection, apparent proteinuria (>150 mg/day), renal dysfunction (serum Cr>1.5 mg/dL), and those who were diagnosed with ischemia or arrhythmia during rest or in effort electrocardiograms were excluded from the study.

Data are shown as mean ± SD or as percentages. Comparisons between groups were performed by student's t test, chi-square test or one-way analysis of variance (ANOVA), as appropriate. Univariate correlation was established by Pearson' correlation coefficient. To assess the influence of tested parameters on MAU as the dependent variable, multiple regression analysis was performed. All statistical calculations were performed using the Statistical Package for the Social Sciences (SPSS version 13). Significance was defined as a p value less than 0.05.

Results

MAU was detected in 30 of the 76 newly-diagnosed subjects with hypertension. The subjects were grouped into three: microalbuminuric EHs (n: 30), non-microalbuminuric EHs (n: 46) and the control group (n: 46).Table 1 shows the anthropometric measurements, demographic information and biochemical values of the groups. No significant difference was observed between groups regarding BMI, age, sex and smoking. VEGF levels were compared according to the existence of MAU; the VEGF level was 144.91±96.60 pg/mL in the control group; 166.78±114.35 pg/mL in the non-MAU group; and 225.15±109.34 pg/mL in the MAU group (Fig. 1). The VEGF levels were significantly different in MAU and non-MAU groups (p: 0.04). Those of the MAU and control groups were also significantly different (p: 0.007) (Fig. 1). However, no difference was found between the non-MAU and control groups (Fig. 1).

The univariate correlation analysis showed a significant correlation between serum VEGF levels and systolic blood pressure (r: 0.253 p: 0.001), diastolic blood pressure (r: 0.162 p: 0.04), mean arterial pressure (MAP) (r: 0.239 p: 0.002), Ccr (r: 0.172 p: 0.04) and MAU (r: 0.338 p: 0.002) (Fig. 2) (Table 2). When MAU was taken as a dependent variable and VEGF, MAP, CRP and Ccr as independent variables in the multiple linear regression analysis, VEGF was significantly associated with MAU (β: 0.248, p: 0.02).

Table 1. Comparison of Clinical Characteristics between Microalbuminuria, Non-microalbuminuria or Control Group

	Hypertensive with microalbuminuria (n:30)	Hypertensive without microalbuminuria (n:46)	Control group (n:46)
Gender (F/M)	20/10	32/14	22/24
Smoking (Y/N)	9/21	14/32	9/37
Age (year)	47.74±10.05	48.33±10.13	47.43±6.57
BMI (kg/m^2)	26.20±3.19	27.62±3.05	28.11±3.96
Blood urea nitrogen (mg/dL)	31.05±8.25	27.82±8.25	29.26±9.07
Creatinine (mg/dL)	0.87±.23 **a**	0.81±.17	0.92±.22
Fasting blood glucose (mg/dL)	94.33±8.06	97.17±9.79	95.28±11.21
Albumine (g/dL)	4.98±.30	4.97±.31	5.00±.30
T-cholesterol (mg/dL)	195.19±53.37	208.52±45.83	214.52±48.92
LDL-cholesterol (mg/dL)	128.48±47.77	125.86±39.17	125.63±31.27
HDL-cholesterol (mg/dL)	51.31±14.10	46.73±10.36	47.36±8.44
Triglycerides (mg/dL)	160.05±68.85	176.38±108.59	190.55±82.95
Microalbuminuria (mg/24h)	96.74±61.46 **b**,**c**,**d**	11.86±8.18	7.13±5.70
Creatinine clearance (mL/mn)	102.47±47.01	101.27±31.38	91.23±56.70
Systolic BP (mmHg)	154.52±19.93 **e, f**	159.40±13.03	123.26±9.56
Diastolic BP (mmHg)	97.61±11.25 **h, j**	102.14±7.08	72.17±11.24
MAP(mmHg)	135.56±15.64 **k, m**	140.32±9.60	106.23±7.73
CRP (mg/dL)	3.11±4.77	2.58±2.69	2.95±4.22
VEGF (pg/mL)	225.15±109.34 **n,o**	166.78±114.35	144.91±96.60

BMI: Body Mass Index, MAP: Mean Arterial Pressure, CRP: C-reactive protein
a) Non-microalbuinuria vs control p:0.04, **b**) Microalbuminuria vs non-microalbuminuria p:0.000 **c**) Non-microalbuinuria vs control p:0.000 **d**) Microalbuminuria vs control p:0.000 **e**) Microalbuminuria vs control p:0.000 **f**) Non-microalbuinuria vs control p:0.000 **h**) Microalbuminuria vs control p:0.000 **j**) Non-microalbuinuria vs control p:0.000 **k**) Microalbuminuria vs control p:0.000 **m**) Non-microalbuinuria vs control p:0.000 **n**) Microalbuminuria vs non-microalbuminuria p:0.04 **o)** Microalbuminuria vs control p:0.007

Discussion

Our study found higher VEGF levels in patients with essential hypertension than in the control group. Additionally, it was more significant in EHs with MAU, than in those with non-MAU and the control group.

Recent studies report an increase in the VEGF levels of hypertensive patients (5, 6). Although the clinical significance of the increase is not yet clear, it is claimed to be an early indicator of vascular damage secondary to hypertension (6). In our study, although blood pressure levels in non-MAU group were higher than in normotensives (even slightly higher than MAU group) VEGF levels were found to be particularly elevated in EHs with MAU. However there was no difference between non-MAU and control groups. This result suggests that vascular damage may be responsible for the increased VEGF levels in EHs. Similarly, Tsai et al concluded in a study that VEGF levels were higher in the presence of hypertensive retinopathy which is a vascular damage finding similar to MAU (6). This corroborates the conclusion that VEGF may be an early indicator of vascular damage in EHs.

Another conclusion of the current study has been the independent effect of VEGF on MAU. Studies in the literature about the relationship of MAU and VEGF have mostly focused on patients with DM or complicated hypertensive patients with atherosclerosis (2, 7-16). The connection between

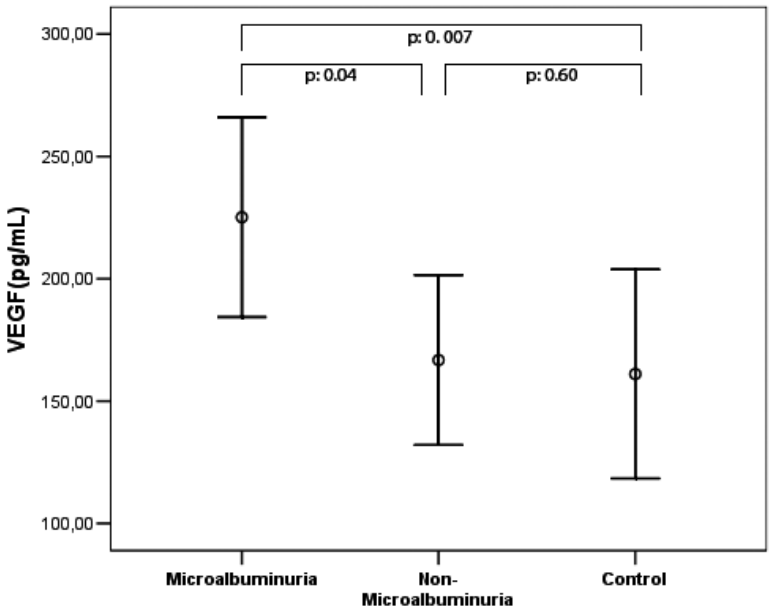

Figure 1. **Comparison of VEGF levels between hypertensive patients with microalbuminuria, without microalbuminuria or healthy subjects.**

Table 2. **Univariate Correlation Analyses of the Relation between VEGF and Clinical Parameters**

	R	p
Age (year)	0.086	0.28
BMI (kg/m^2)	0.069	0.47
Microalbuminuria (mg/24h)	0.338	0.002*
Creatinine clearance (mL/mn)	0.172	0.04*
Blood urea nitrogen (mg/dL)	0.018	0.82
Creatinine (mg/dL)	0.055	0.48
Fasting blood glucose (mg/dL)	0.091	0.25
T-cholesterol (mg/dL)	0.102	0.20
LDL-cholesterol (mg/dL)	0.132	0.09
HDL-cholesterol (mg/dL)	0.015	0.84
Triglycerides (mg/dL)	0.075	0.35
Systolic BP (mmHg)	0.253	0.001*
Diastolic BP (mmHg)	0.162	0.04*
MAP (mmHg)	0.239	0.002*
CRP (mg/dL)	0.011	0.89

BMI: Body Mass Index, MAP: Mean Arterial Pressure, CRP: C-reactive protein

atherosclerosis and VEGF has been described (17, 18). Therefore, echoing the study of Asselbergs et al, other factors may be responsible for the increase in VEGF observed in individuals with MAU and apparent atherosclerosis. At the same time, recent studies on diabetic humans and animals have yielded results implying that VEGF may have a role in the development of MAU (7, 11-14). However, the literature does not contain any other studies focusing on the

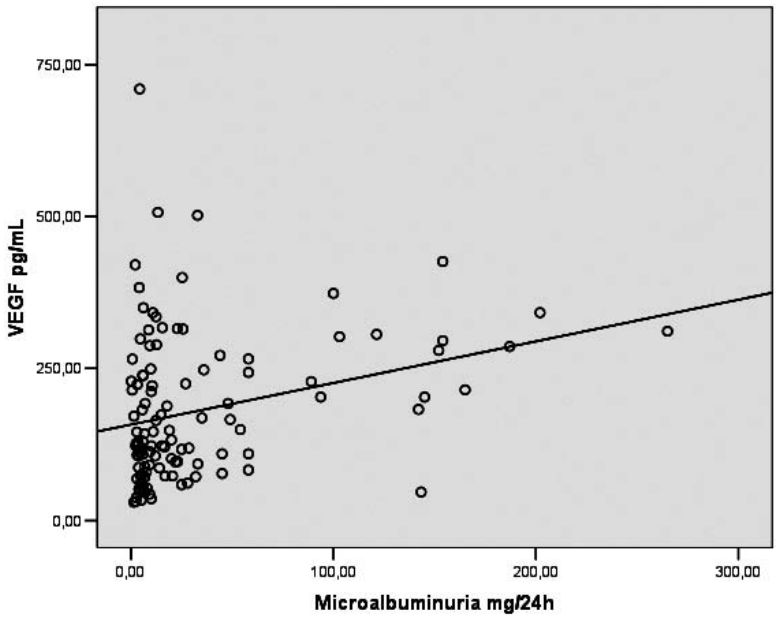

Figure 2. **Correlation of VEGF with microalbuminuria in hypertensive patients.**

relationship between VEGF and MAU in non-complicated newly-diagnosed EHs.

Renal tissue is a major source for the increase in vascular permeability and the production of VEGF which causes endothelial growth. It is known that VEGF synthesis occurs in renal tissue via podocytes in the glomeruli, via tubular cells in the tubulointerstitial area and via collecting channels in the medulla, and that VEGF receptors are present in glomerular endothelial cells (9). Recent *in vitro* studies have shown a significant increase in VEGF levels synthesized in the podocytes in renal tissue when high glucose is present (7, 15, 16). In addition, increased VEGF was found to be tied to the VEGF receptors in the glomerular endothelial cells (7). When tied to the endothelial cell receptors, VEGF may contribute to the development of microalbuminuria by increasing glomerular permeability through various mechanisms in diabetic patients (7). As a possible mechanism, it has been claimed that VEGF increases the glomerular filtration surface area through endothelial cell proliferation, induces endothelial fenestration, contributes to the opening of junctions by reducing occludin which is an endothelial junction protein, and disintegrates the endothelial basement membrane by stimulating the production of collagenase (7, 8).

It may be suggested that VEGF may also lead to MAU in non-DM hypertensive patients through such mechanisms. However, in these groups of patients, VEGF synthesis needs to be induced by other factors than high glucose. It is known in our day that, in addition to high glucose, VEGF synthesis is also induced by hypoxia, vasoactive hormones such as angiotensin II and vasopressin, mechanical strain such as vascular sheer stress, various cytokines synthesized in the endothelial system (TGF-β) and growth factors (fibroblast growth factor, platelet growth factor) (19). A positive correlation between VEGF levels and systolic and diastolic blood pressure was recorded in our study. Vascular sheer stress resulting from high blood pressure may lead to this relationship. Additionally, growth factors and various cytokines resulting from endothelial dysfunction in hypertensive patients may also lead to increased VEGF (2).

Microalbuminuria is an important indicator of widespread endothelial dysfunction (2). As in the present study, the presence of endothelial dysfunction may have a role in the development of higher VEGF levels in patients with MAU (2). Increased VEGF levels in hypertensive patients may thus be influential in the early diagnosis of vascular damage and in the glomerular permeability increase which occurs through various possible mechanisms.

The limitation of this study was that MAU was not specific for hypertension. Subclinical atherosclerosis contributed to the increased VEGF levels in the MAU group independent of hypertensive vascular changes. The other limitation of our study was that we did not evaluate retinopathy, which is known to be a reliable and simple way to detect endothelial damage. Prospective studies with more subjects are required to determine the relationship between VEGF levels and MAU in EHs.

As a result, VEGF levels are higher in EHs in the presence of MAU. These high values may be important in the early diagnosis of vascular damage in EHs. Additionally, VEGF may increase glomerular permeability and lead to MAU in hypertensive as well as diabetic patients.

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