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Plasma Vascular Endothelial Growth Factor Level Is Elevated in Patients with Multivessel Coronary Artery Disease

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Summary

Background: Vascular endothelial growth factor (VEGF) has been implicated in both angiogenesis and ischemia. However, the relationship between plasma VEGF level and coronary artery disease remains unknown.

Hypothesis: Plasma VEGF level may be associated with severe coronary artery disease and other cardiovascular risk factors.

Methods: We examined plasma VEGF concentration and coronary risk factors in 73 patients who underwent coronary angiography and 70 apparently healthy control subjects. According to the number of the three major coronary vessels with significant (≥ 75%) stenosis, we divided the patients into two groups: the mild stenosis group (0- and single-vessel disease, n = 36) and the severe stenosis group (double- and triple-vessel disease, n = 37).

Results: The log VEGF value of the severe stenosis group was significantly higher than that of the mild stenosis ($p < 0.05$) and control groups ($p < 0.05$). Furthermore, there was a significant positive trend in the log VEGF value according to the number of vessels with significant stenosis ($p = 0.016$). However, there was no significant difference in log VEGF value between the mild stenosis and control groups. Soluble vascular cellular adhesion molecule, soluble intracellular adhesion molecule, and other coronary risk factors were found to be associated with the presence of vessel stenosis.

Conclusion: Unlike established coronary risk factors, the plasma VEGF level may be associated with only severe coronary ischemia such as multiple coronary vessel disease.

Key words: vascular endothelial growth factor, ischemia, atherosclerosis, severity, coronary artery disease, cytokine

Introduction

Vascular endothelial growth factor (VEGF) is a specific and potent mitogen of micro- and macrovascular endothelial cells derived from arteries and veins, as well as an angiogenic factor.[1–3] Its role in angiogenesis has been studied extensively both in vivo and in vitro.[3] Vascular endothelial growth factor expression is regulated by a variety of hormones, growth factors, and cytokines including interleukin-6, epidermal growth factor, transforming factor-β, prostaglandin E2, and nitric oxide.[4] However, hypoxia is the major inducer of VEGF expression;[3] VEGF expression is rapidly and reversibly induced by exposure to low pO_2 in a variety of normal and transformed cultured cell types.[5, 6] Ischemia caused by occlusion of the left anterior descending coronary artery results in a dramatic increase in VEGF mRNA level in the pig myocardium.[7]

To date, the plasma or serum level of VEGF in conditions including various cancers and inflammatory and gynecological diseases has been well documented.[8–13] However, little is known about the plasma VEGF level in patients with coronary artery disease (CAD), especially with regard to the severity of coronary ischemia or atherosclerosis.

Therefore, we examined the relationship between plasma VEGF level and severity of coronary ischemia in vivo using venous samples. The severity of coronary ischemia was expressed by the number of the three major coronary vessels with significant stenosis. In addition, the severity of atherosclerosis was expressed in terms of the Gensini score,[14] al-

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though these two assessments are closely related. As soluble vascular cellular adhesion molecule (s-VCAM-1), soluble intracellular adhesion molecule (s-ICAM-1), and hyperlipidemia have been shown to be associated with atherosclerosis,[15–19] we also compared the plasma VEGF level with these parameters along with other cardiovascular risk factors.

Patients and Methods

This study included patients who were scheduled to undergo coronary angiography at the National Defense Medical College Hospital, Saitama, Japan. The study was approved by the Institutional Review Board and all patients gave written informed consent. The control subjects were apparently healthy and underwent a routine medical checkup, that did not include exercise stress testing, at the Mitsukoshi Health and Welfare Foundation. Most patients who were scheduled to undergo coronary angiography showed objective evidence of myocardial ischemia on either treadmill exercise testing or thallium myocardial scintigraphy.

Control subjects and patients were randomly selected from subjects who satisfied the following criteria: (1) absence of clinical and laboratory signs of acute or chronic inflammatory illness, (2) absence of overt neoplastic disease, (3) absence of other ischemic arteries, and (4) postmenopausal status for women. Women still undergoing menstrual cycles were excluded because the menstrual cycle and pregnancy are known to influence the plasma VEGF level.[10, 11] After recruiting, 73 patients who were scheduled to undergo coronary angiography (CAD group) and 70 control subjects remained after these exclusions. A fasting venous blood sample was collected in a tube containing ethylene diamine tetraacetic acid (EDTA). After being centrifuged at 3000 rpm at 4˚C for 15 min, the plasma sample was separated and stored at −80˚C until analysis.

Angiographic Analysis

The number of vessels with significant stenosis and the Gensini score were estimated using a computer-assisted cardiovascular angiography analysis system (Cardio-500, Kontron, Wanaque, N.J., USA) by an investigator blinded to the patient information as previously described.[20] Vessel edges were determined by computerized algorithms, and luminal diameters were measured with a dye-filled catheter of over 6F in diameter as a reference. The minimal lumen diameter, reference diameter, and percentage of stenosis were calculated from projections of the same view at the point of the most severe stenosis. Significant stenosis was defined as any stenosis ≥ 75% of the lumen diameter.

The 73 patients were divided into two ischemia groups according to the number of coronary vessels with significant stenosis among the three major coronary vessels. The mild stenosis group (n = 36) consisted of patients with one or no vessel with significant stenosis as well as patients with minor stenoses (25–70% stenosis in no more than two of the three major coronary vessels). The severe stenosis group (n = 37) included patients with significant stenoses in two or three vessels. We also categorized the 73 patients into the following two groups according to the Gensini score: the low Gensini score group (Gensini score 1–27, n = 45) and the high Gensini score group (Gensini score 28–140, n = 28). We chose 28 as the cut-off point of the Gensini score so as to maximize the difference in plasma VEGF level between the severe stenosis and control groups.

Measurement of Plasma Vascular Endothelial Growth Factor Level

The plasma VEGF level was measured using a commercially available competitive immunoassay kit (cytoKINE VEGF, ARP, Inc., Belmont, Mass., USA), which is designed to measure the total amount of VEGF within the plasma. This measurement includes free VEGF and VEGF bound to soluble receptors and binding proteins such as α_2-macroglobulin.[21] It has been shown that the major forms of VEGF are VEGF121 and VEGF165, while VEGF189 and VEGF206 are largely bound to cell membranes and do not represent measurable VEGF;[3, 21] VEGF121 has angiogenic and permeability effects on endothelial cells, as does VEGF165.[21, 22] Precoated goat antirabbit antibodies to plate were used to capture a specific cytokine complex in each sample, consisting of the VEGF antibody, biotinylated VEGF, and sample/standard. The assay sensitivity was 0.195 ng/ml, and the dynamic range was up to 50 ng/ml.

Measurement of s-VCAM-1 and s-ICAM-1

The s-VCAM-1 and s-ICAM-1 levels were measured by an enzyme immunoassay kit (CytoscreenTM, Biosource, Camarillo, Calif., USA). The VEGF, s-VCAM-1, and s-ICAM-1 concentrations in all control and patient samples were measured at the same time. The cholesterol and triglyceride levels were determined by enzymatic methods. The level of high-density lipoprotein cholesterol (HDL-C) was determined after precipitation of apolipoproteinB containing lipoproteins using phosphotungatate and magnesium.

Statistical Methods

Data were expressed as mean ± standard deviation. As the plasma VEGF levels were skewed to lower levels, they were also expressed as medians. The plasma VEGF concentrations were transformed into logarithmic forms for parametric statistical analyses because they were not normally distributed.

The chi-square test was performed for comparison of categorical variables among the three groups (control, mild stenosis, and severe stenosis). One-way analysis of variance (ANOVA) was used to compare other parameters, including the plasma VEGF level, according to the severity of coronary stenosis and Gensini score. Post hoc analysis was undertaken using Scheffe's test. The Mann-Whitney U test was used to

TABLE I Characteristics of the three groups

	Control group	Mild stenosis group	Severe stenosis group
Number	70	36	37
Age, years	58.9 ± 8.2	62.6 ± 10.9	61.8 ± 8.6
Body mass index, kg/m^2	22.8 ± 2.8	24.1 ± 3.7	22.8 ± 3.0
Male, % (n)	58.6 (41)	58.3 (21)	78.4 (29)
History of hypertension, % (n)	18.6 (13)	50.0 (18) [a]	45.9 (17) [a]
Current smoker, % (n)	27.1 (19)	30.6 (11)	18.9 (7)
Prior angioplasty, % (n)	0	16.7 (6)	45.9 (17) [b]
Prior MI, % (n)	0	8.3 (3)	37.8 (14) [b]
100% occlusion, % (n)	—	11.1 (4)	54.1 (20) [b]
Gensini score	—	8.9 ± 16.7	45.3 ± 33.8 [b]

Variables are expressed as mean ± standard deviation or percentage (number) in each group.
[a] $p < 0.001$ vs. control group.
[b] $p < 0.001$ vs. mild stenosis group.
Abbreviation: MI = myocardial infarction.

compare variables between the control and CAD groups and to evaluate the impact of cardiovascular risk factors in the subjects on the plasma VEGF level. P values of < 0.05 were considered to be significant.

Results

The characteristics of the control, mild stenosis, and severe stenosis groups are summarized in Table I. There were no significant differences in age, body mass index, gender, and percentage of current smokers among the three groups. The severe stenosis group had a significantly higher percentage of patients with prior angioplasty, patients with prior myocardial infarction, and patients with 100% occlusion than the mild stenosis group ($p < 0.001$, $p < 0.001$, and $p < 0.001$, respectively).

The log VEGF value increased in a stepwise fashion according to the number of vessels with significant stenosis (p for trend = 0.016; Fig. 1). As shown in Table II, the log VEGF value in the severe stenosis group (0.54 ± 0.26) was significantly higher than that in the mild stenosis group (0.42 ± 0.21; $p = 0.04$) and control group (0.43 ± 0.17; $p = 0.03$). However, there was no significant difference in log VEGF value between the mild stenosis and control groups. The s-ICAM-1 values showed a pattern similar to VEGF among the three groups. Although the control group had significantly lower s-VCAM-1 and triglyceride levels and a higher HDL-C level than the mild stenosis and severe stenosis groups, there were no significant differences in these parameters between these groups.

Furthermore, we compared various parameters in the CAD and control groups. There were significant differences in the s-VCAM-1, s-ICAM-1, triglyceride, and HDL-C levels between the CAD and control groups. However, there was no significant difference in the log VEGF value between the CAD (0.48 ± 0.24) and control groups (Table II).

When the severity of coronary atherosclerosis in the patients was evaluated using the Gensini score, plasma VEGF levels were 3.19 ± 1.65 ng/ml, median, 2.89 ng/ml for the lower Gensini score group (Gensini score < 28), and 4.06 ± 2.73 ng/ml, median, 2.99 ng/ml for the higher Gensini score group. However, we obtained similar results for all variables as shown in Table II, except for the log VEGF value (Table III), namely, they showed significant associations with the Gensini score. There was no statistically significant difference in log VEGF value among the three groups. However, there was a trend in the higher Gensini score group toward higher log VEGF values compared with the control group ($p = 0.096$).

To assess the impact of cardiovascular risk factors on plasma VEGF level, we examined plasma VEGF concentrations according to old age (> 60 years old), male gender, history of hypertension, high s-VCAM-1 level (> 800 ng/ml), high s-ICAM-1 level (> 250 ng/ml), and dyslipidemia in the sub-

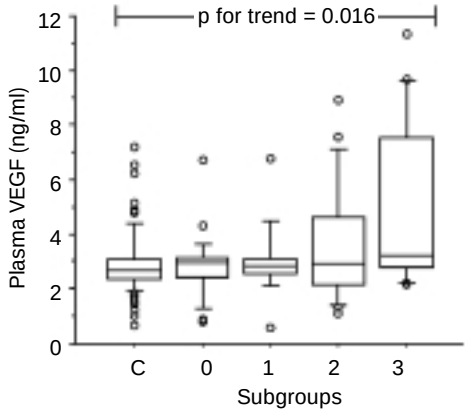

FIG. 1 Vascular endothelial growth factor (VEGF) levels (mean ± standard deviation) in plasma samples obtained from control subjects and patients who underwent coronary angiography. C = Control group, n = 70; 0 = patients with no vessels with significant stenosis, n = 21; 1 = patients with one vessel with significant stenosis, n = 15; 2 = patients with two vessels with significant stenosis, n = 21; 3 = patients with three vessels with significant stenosis, n = 16.

TABLE II Levels of log vascular endothelial grwoth factor (VEGF) values and other variables in the four groups

	Control group	Mild stenosis group	Severe stenosis group	CAD group	p Values	
VEGF, ng/ml	2.66	2.85	3.07	2.92		
Log VEGF	0.43 ± 0.17	0.42 ± 0.21	0.54 ± 0.26	0.48 ± 0.24	C vs. M C vs. S M vs. S C vs. CA	0.95 0.03 0.04 0.06
s-VCAM-1, ng/ml	601 ± 167	771 ± 220	819 ± 477	795 ± 370	C vs. M C vs. S M vs. S C vs. CA	0.02 0.002 0.03 < 0.0001
s-ICAM-1, ng/ml	215 ± 68.3	225 ± 97.9	287 ± 135	256 ± 121	C vs. M C vs. S M vs. S C vs. CA	0.89 0.002 0.03 0.04
Triglyceride, ng/ml	101 ± 45.1	141 ± 78.0	157 ± 58.6	149 ± 69.0	C vs. M C vs. S M vs. S C vs. CA	0.005 < 0.0001 0.52 < 0.0001
HDLcholesterol, ng/ml	68.0 ± 18.9	47.8 ± 13.3	38.7 ± 11.2	43.5 ± 13.4	C vs. M C vs. S M vs. S C vs. CA	< 0.0001 < 0.0001 0.06 < 0.0001

Values are expressed as mean ± standard deviation.
Plasma VEGF levels are expressed as median.
Abbreviations: C = control, M = mild stenosis group, S = severe stenosis group, CA = CAD group, CAD = coronary artery disease, s-VCAM-1 = soluble vascular adhesion molecule, s-ICAM-1 = soluble intracellular adhesion molecule, HDL = high-density lipoprotein.

TABLE III Levels of log vascular endothelial grwoth factor (VEGF) values and other variables in the lower and higher Gensini score groups

	Lower Gensini score group	Higher Gensini score group	p Values	
Number	45	28		
VEGF, ng/ml	2.89	3.00		
Log VEGF	0.45 ± 0.23	0.53 ± 0.25	C vs. L C vs. H L vs. H	0.92 0.096 0.25
s-VCAM-1, ng/ml	762 ± 354	854 ± 388	C vs. L C vs. H L vs. H	0.02 0.001 0.41
s-ICAM-1, ng/ml	239 ± 102	282 ± 144	C vs. L C vs. H L vs. H	0.44 0.01 0.20
Triglyceride, mg/dl	148 ± 72.7	149 ± 63.3	C vs. L C vs. H L vs. H	< 0.0001 < 0.0001 0.99
HDL cholesterol, ng/ml	46.5 ± 13.7	38.7 ± 10.7	C vs. L C vs. H L vs. H	< 0.0001 < 0.0001 0.13

Values are expressed as mean ± standard deviation.
Plasma VEGF levels are expressed as median.
Abbreviations: C = control, L = lower Gensini score group (Gensini score < 28), H = higher Gensini score group (Gensini score = 28 or ≥ 28). Other abbreviations as in Table II.

jects studied. We defined the high s-VCAM-1 and s-ICAM-1 concentrations to be high based on previous reports.[16, 17] As shown in Table IV, the patients with a high s-VCAM-1 level had a significantly higher plasma VEGF level than those with a low s-VCAM-1 level ($p = 0.03$).

Of note, there were seven subjects with high VEGF concentrations (> 4 ng/ml) in the control group. They had significantly higher s-VCAM-1 levels (714 ± 147 mg/dl) than the 63 others (589 ± 165 mg/dl) in the control group ($p < 0.01$)

TABLE IV Plasma vascular endothelial growth factor (VEGF) concentrations according to cardiovascular risk factors

Cardiovascular risk factors	+	−	p Values
Age > 60 (n)	2.85 (92)	2.84 (51)	0.24
Male (n)	2.84 (91)	2.86 (52)	0.94
Hypertension (n)	2.86 (48)	2.78 (95)	0.12
Smoking (n)	2.70 (39)	2.90 (104)	0.11
s-VCAM-1 > 800 ng/ml (n)	3.02 (32)	2.77 (111)	0.03
s-ICAM-1 > 250 ng/ml (n)	2.76 (52)	2.88 (91)	0.52
Triglycerides > 150 mg/dl (n)	2.85 (37)	2.83 (106)	0.76
HDL cholesterol < 40 mg/dl (n)	2.84 (110)	2.85 (33)	0.69

Plasma VEGF concentrations are expressed as median.
Abbreviations as in Table II.

Discussion

This study was designed to evaluate the plasma VEGF level in venous samples from patients with CAD and to compare them with other risk factors and control subjects.

The high VEGF levels in some of the control subjects, most of which were accompanied by high-level s-VCAM-1, may have been due to the presence of silent ischemic lesions. It has been reported that silent coronary ischemia is common in apparently healthy subjects, especially when accompanied by coronary risk factors.[23, 24]

Although the plasma VEGF level is elevated in patients with multivessel disease, it was not associated with coronary atherosclerosis as defined by the Gensini score. With respect to the lack of association between plasma VEGF level and Gensini score, there is a possibility that the Gensini score itself is not suitable for evaluating coronary atherosclerosis and that the modified Gensini score[25] or extent score[26] may be better. However, the most likely reason is that plasma VEGF level may be associated with severe ischemia rather than with atherosclerosis.

With regard to the relationship between plasma VEGF level and atherosclerosis, it is unclear whether VEGF accelerates the progression of atherosclerosis.[27, 28] Given that VEGF may promote neovascularization in atherosclerotic plaque,[29] any cause–effect relationship between VEGF and atherosclerosis remains to be determined.

Multivessel coronary disease has several complex pathologies, including hypoxia, inflammation, atherosclerosis, and plaque formation, as well as angiogenesis and tissue repair. Recently, several investigators have reported that serum VEGF levels were increased after acute myocardial infarction (AMI).[30, 31] They suggested that the extent of myocardial damage contributes to the elevation of serum VEGF levels in AMI, and that VEGF plays a role as an endogenous activator of coronary collateral formation in the human heart. In addition, Servos *et al.*[32] postulated that VEGF provides vascular protection via increased nitric oxide and prostaglandin I2 production in the adult vasculature. Taken together, the plasma VEGF level in patients with multivessel disease might be elevated for the purposes of angiogenesis, tissue repair, or vascular protection, since micromyocardial infarction may occur even in the chronic stage.

Current results also suggest that the plasma VEGF level may be related to s-VCAM-1 and s-ICAM-1 levels. Melder *et al.*[33] reported that VEGF promotes VCAM-1 and ICAM-1 expression in endothelial cells. Furthermore, Swerlick and Swerlick[34] reported that a variety of cytokines and growth factors induce new blood vessel formation in vivo. Therefore, several complex associations between VEGF and cytokines are presumed to exist in terms of angiogenesis.

Conclusion

The current study indicates that plasma VEGF level is significantly associated with coronary ischemia. Unlike other coronary risk factors, plasma VEGF level may serve as a marker of severe coronary ischemia such as multivessel coronary disease. These findings may support angiogenesis and possibly tissue repair as the role of VEGF in CAD accompanied by significant ischemia. However, the degree of these associations, the underlying mechanism including the relationship to VEGF receptor, soluble Flt-1, and the source of elevated VEGF, as well as its etiologic role, remain to be determined.

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