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Circulating Levels of Interleukin-8 and Vascular Endothelial Growth Factor in Patients with Carotid Stenosis

Interleukin (IL)-8 and vascular endothelial growth factor (VEGF) are important factors that induce the migration and proliferation of endothelial cells, increase the vascular permeability, and the modulate chemotaxis of monocytes. These molecules have been found in human atherosclerotic plaques. However, it is not clear whether the circulating levels of IL-8 and VEGF correlate with the extents of carotid stenosis. In this study, we investigated the relationship between circulating levels of IL-8 as well as VEGF and the extents of carotid stenosis. Sera from 41 patients with carotid stenosis were assessed for concentrations of IL-8 and VEGF by enzyme-linked immunosorbent assay. The degree of stenosis of extracranial carotid artery was calibrated by carotid B-mode ultrasonography. The serum concentration of IL-8 ($r=-0.04733$, $p>0.05$) was not correlated with the degree of stenosis. However, the serum concentration of VEGF ($r=0.4974$, $p<0.01$) was significantly correlated with the degree of carotid stenosis. These findings suggest that increased serum level of VEGF might be a marker for higher degree of stenosis of extracranial carotid artery.

Key Words: *Atherosclerosis; Endothelial Growth Factors; Interleukin-8; Endothelium, Vascular; Carotid Stenosis*

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INTRODUCTION

The pathogenesis of atherosclerosis is a complex process, consisting of the accumulation of inflammatory cells, fibroproliferation, deposition of extracellular matrix, and angiogenesis, which lead to progressive fibrosis, calcification, and eventual luminal occlusion (1-3). Angiogenesis occurs in association with numerous conditions, including atherosclerosis, stroke, ischemic heart disease, diabetic retinopathy, and tumor metastasis (4). On the basis of experimental data, angiogenesis is induced by various chemical signals, which lead to DNA synthesis and mitosis of vascular cell (5).

Interleukin (IL)-8 is a chemokine related with chemotaxis and activation of neutrophils (6, 7). Recently, it has been reported that IL-8 regulates angiogenesis associated with atherosclerosis, non-small cell lung cancer, and idiopathic pulmonary fibrosis (8-10).

Vascular endothelial growth factor (VEGF) is a heparin-binding, 46-kDa dimeric, endothelial cell-specific mitogen and angiogenic factor that potently increases vascular permeability (11-13). This growth factor has been demonstrated to be involved in normal and several pathological processes including tumor progression, collateral vessel formation in ischemic tissues, and inflammation (14-16). The serum concentration of VEGF is elevated in patients with atherosclerosis or acute ischemic stroke (17, 18). Recently, it was suggested that the production of IL-8 and VEGF might contribute to the pathogenesis of atherosclerosis as well as to the angiogenesis (19). On the basis of the previous reports on the production of IL-8 and VEGF by mononuclear cells from carotid atherosclerotic plaques (20, 21), we hypothesized that one or both of these angiogenic factors may be correlated with the extents of carotid stenosis. To verify this, we investigated the relationship by measuring circulating levels of IL-8 and VEGF and the extents of carotid stenosis carotid B-mode ultrasonography.

MATERIALS AND METHODS

Subjects

Forty-one consecutive patients (25 men, 16 women, mean age 57.2±28.7 yr, range 29 to 79) with extracranial carotid stenosis were enrolled. Exclusion criteria for patients were presence of stenosis of vertebrobasilary or intracranial carotid arteries, history of recent vascular accident, for example stroke, angina, or peripheral vas-

cular disease (<1 month), recent infection, autoimmune disease, or malignancy. All patients gave informed consents for the use of their blood samples for an experimental study. The extent of extracranial carotid stenosis was measured by carotid B-mode ultrasonography on longitudinal section to demonstrate the most severe stenosis. We measured the distance from the near media-adventitia interface to the far media-adventitia interface (A) and the distance from the near intimal surface to the far intimal surface (B) at the most stenotic point of artery. The degree of carotid stenosis was calculated as (A-B)/A×100. According to stenotic degree of carotid artery, all patients were divided into three groups: mild (0-29%), moderate (30-69%), and severe stenosis (70-99%). Also, to reveal the extents of diffuse or bilateral carotid stenosis, we used carotid stenosis score (CSS). The visualized carotid artery was divided into the following three segments according to the modified Espeland classification (22): internal carotid artery (ICA), carotid bifurcation (BIF), and common carotid artery (CCA). The ICA segment was defined as the portion distal to the flow divider. The BIF segment extends from flow divider 8 mm proximally to the CCA. The CCA segment extends proximally from BIF segment. The most severe stenosis in each of these segments was then scored, with 0 point for no stenosis, 1 for 1 to 29%, 2 for 30 to 69%, and 3 for 70 to 99% stenosis of the segment. Score for each segment were added and therefore total score could range from 0 to 18 points. Relevant data, such as risk factors for atherosclerosis, were obtained from hospital records.

Blood sampling and measurement of IL-8 and VEGF

Venous blood from patients was collected into syringes by needle aspiration. Blood was kept at 4℃ for 40 min to clot and was centrifuged at 3,000 rpm for 15 min. Serum was stored at -70℃ until use. Concentrations of IL-8 and VEGF were measured by quantitative "sandwich" enzyme-linked immunosorbent assay (ELISA) technique. Briefly, standards and test samples were dispensed in duplicate into wells of 96-well microtiter plates, which had been pre-coated with monoclonal anti-human IL-8 (Endogen®, Woburn, MA, U.S.A.) and VEGF (Endogen®) antibodies. Then, horseradish peroxidase (HRP)-conjugated detection antibodies were added into the wells followed by HRP-conjugated streptavidin (Endogen®). For color reaction, 100 μL of 3,3',5,5'-tetramethylbenzidine substrate was added and the wells were incubated for 15 to 30 min. The absorption at 450 nm was determined using an automated ELISA microplate reader (Bio-Tech®, EL312e, Winooski, VT, U.S.A.).

Statistical analysis

All data are expressed as mean±standard deviation (SD). Difference between groups was analyzed by paired two-student t-test. Spearman's correlation test was used to assess relations between variables. p values less than 0.05 was regarded as stastiscally significant.

RESULTS

Clinical characteristics

Among the 41 patients with carotid stenosis, less than 30% stenosis on either side was found in 10, moderate stenosis (60 to 70%) of at least one carotid artery was present in 17, and the remaining patients showed severe stenosis of more than 70% unilaterally or bilaterally. Mean CSS was 5.5±2.5 in patients with mild carotid

Table 1. Baseline characteristics of study subjects

	Severe stenosis	Moderate stenosis	Mild stenosis
Number of cases	14	17	10
Sex (male)	13	10	2
Mean stenosis (%)	92.5	48.2	22.9
Mean carotid stenosis score	11.6	7.2	5.5
Hypertension	9	8	6
Diabetes mellitus	4	6	1
Smoking	9	8	5
Cholesterol (mg/dL)	211±48	218±45	198±40
Triglyceride (mg/dL)	157±55	145±62	147±58
LDL-cholesterol (mg/dL)	134±31	129±34	124±36
HDL-cholesterol (mg/dL)	39±10	37±12	40±8
Old myocardial ischemia	5	7	1
Use of aspirin	10	15	9
Lipid lowering agent	4	6	1

LDL, low density lipoprotein; HDL, high density lipoprotein

stenosis, 8.1±1.6 with moderate stenosis, and 11.6±2.0 with severe stenosis. The clinical characteristics of the patients in this study are given in Table 1.

IL-8 in patients with carotid stenosis

The mean serum level of IL-8 in patients with carotid stenosis was 16±12.5 pg/mL. There was no significant difference in the serum levels of IL-8 among the three groups according to the degree of carotid stenosis (Fig. 1A). Also, IL-8 levels were not correlated with the

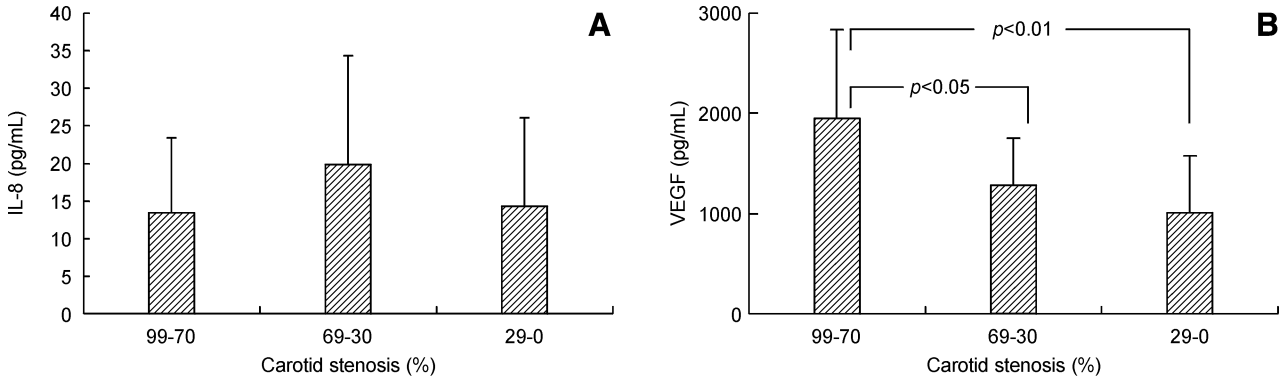

Fig. 1. A: Comparison of IL-8 levels between patients with severe, moderate, and mild carotid stenosis. There is no significant difference among the three groups. B: Comparison of VEGF levels between patients with severe, moderate, and mild carotid stenosis. Paired two-student test reveals significantly increased VEGF level in the severe versus moderate carotid stenosis ($p<0.05$) and in the severe versus mild carotid stenosis ($p<0.01$). IL-8, interleukin-8; VEGF, vascular endothelial growth factor.

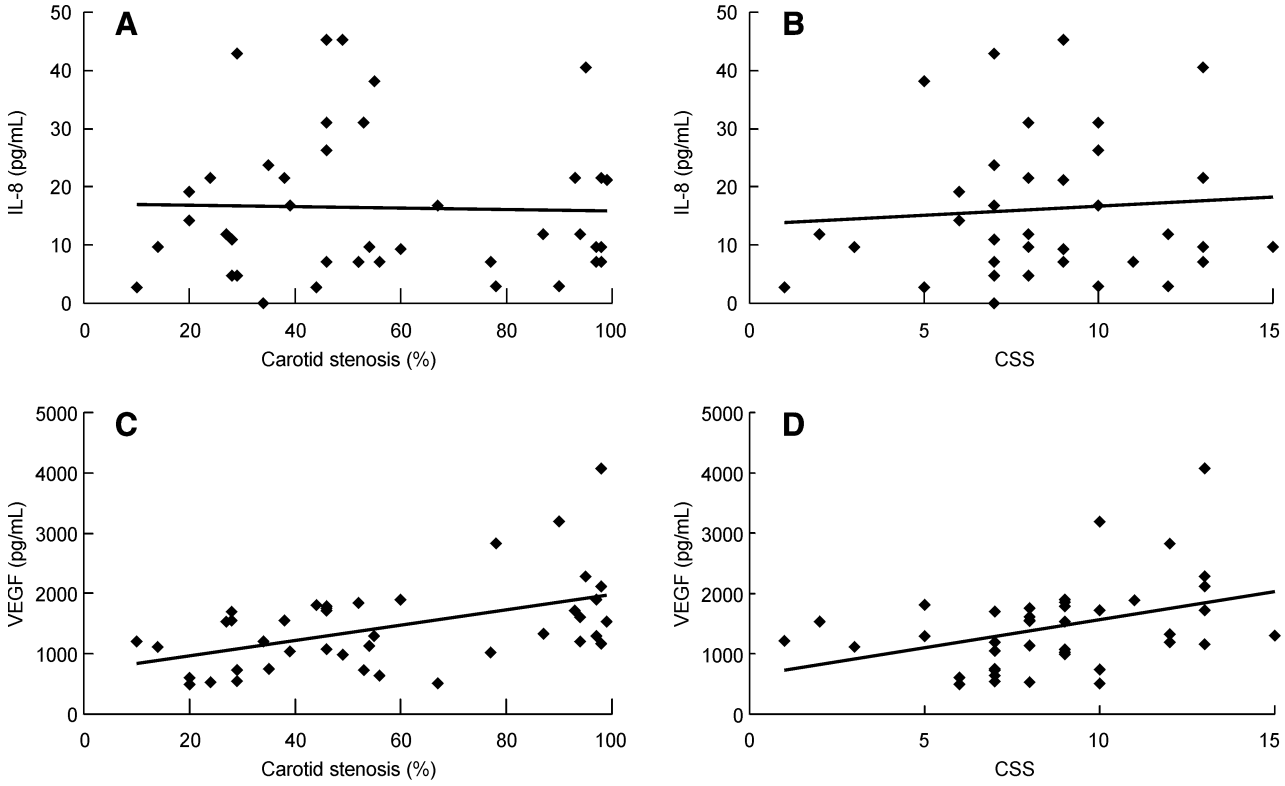

Fig. 2. Relationships between IL-8 and the extents of carotid stenosis (A), and CSS (B). IL-8-levels are not correlated with the extents of carotid stenosis and CSS. Relationships between VEGF and the extents of carotid stenosis (C), and CSS (D). VEGF levels are significantly correlated with the extents of carotid stenosis and CSS. IL-8, interleukin-8; VEGF, vascular endothelial growth factor; CSS, carotid stenosis score.

extent of carotid stenosis (r=-0.04733, $p>0.05$, Fig. 2A) and CSS (r=0.077, $p>0.05$, Fig. 2B).

VEGF in patients with carotid stenosis

The mean serum level of VEGF in patients with carotid stenosis was 1439.6±736.4 pg/mL. Patients with severe carotid stenosis (1947.1±884.0 pg/mL) showed significantly higher VEGF levels than patients with moderate (1279.1±469.5 pg/mL, $p<0.05$) or mild carotid stenosis (1001.9±476.9 pg/mL, $p<0.01$), whereas no significant difference was noted between groups with moderate and mild carotid stenosis (Fig. 1B). VEGF concentrations were significantly related with the extents of carotid stenosis (r=0.4974, $p<0.01$, Fig. 2C) and CSS (r=0.388, $p<0.01$, Fig. 2D).

DISCUSSION

VEGF plays an important role in vasculogenesis, atherogenesis, and vascular remodeling in response to injury (23). This peptide stimulates endothelial cells to proliferate and to express collagenase in atherogenesis (24). Also called as vascular permeability factor, VEGF has the potential to induce extravasation of fluid and protein (25, 26). Clauss et al. (27) also documented that VEGF induces monocyte activation and migration. This data strongly suggest that VEGF play a role in the chemotaxis of monocytes/macrophages, which is crucial in inflammatory reactions of atherosclerosis. Also, VEGF could indirectly modulate the contraction and growth of smooth muscle cells (28). These facts raise the possibility that VEGF may relate with the progression of atherosclerosis. A previous report (19) showed that VEGF could contribute to aggravation of coronary atherosclerosis in vivo and in vitro. The results of this showed that the increased concentration of VEGF had a significant correlation with extents of carotid stenosis and CSS. Our observations are consistent with previous report by Martin et al. (17) who showed the relationship between the increased concentration of VEGF and the extents of coronary artery diseases. Particularly, Lazarous et al. (23) reported that VEGF administration exacerbated neointimal thickening after vascular injury in dogs. Therefore, our results along with others' findings might reflect that VEGF is an important factor to promote the carotid atheroma. However, we cannot exclude the possibility that the increased levels of VEGF according to the extents of carotid stenosis may be due to secondary vascular remodeling in response to injury on atherogenesis. Further studies will be needed. To the best of our knowledge, this is the first report on the relationship between serum levels of VEGF and carotid atheroma.

IL-8 is secreted from local endothelium and macrophages (29), and has several roles in atherogenesis, including chemotaxis, neovascularization, and proliferation of smooth muscle cells (6, 7). In the present study, serum levels of IL-8 showed no significant difference among the groups with different extents of carotid stenosis. Our observations are also consistent with previous reports, which showed no increase of serum level of IL-8 in patients with carotid stenosis (30) or stable angina (31). However, in patients with acute myocardial infarction (32), or acute stroke (33), increased serum levels of IL-8 have been reported. These findings are suggested that the serum levels of IL-8 might be related with acute events of vascular disorders. Considering IL-8 detected in coronary or carotid atheroma, we thought that the lack of increase of levels of IL-8 might be due to the dilution in peripheral blood. This interpretation is supported by other studies (34), which failed to detect proinflammatory cytokines in peripheral blood even after extensive inflammatory stimuli.

In this study, we had a limitation in the measurement of the severity of atheroma. Although we exactly estimated the severity of carotid stenosis, we could not express the whole severity of atherosclerosis, such as the degree of peripheral artery or coronary artery stenosis.

Taken together, this is the first study to demonstrate the correlation between VEGF levels and the extents of carotid stenosis. We think that circulating level of VEGF may reflect the progression of atherogenesis. Recently, VEGF has been used as a potential new therapeutic agent for occlusive vascular disease (35). Of course, totally occluded arteries may provide further rationale for such clinical trials. However, because our observation and other studies suggest that VEGF itself may aggravate the process of atherosclerosis, we should be cautious about the therapeutic use of VEGF on atherosclerosis.

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