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Original Paper

Increased Plasma VEGF Levels in Patients with Cerebral Large Artery Disease Are Associated with Cerebral Microbleeds

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Keywords
Vascular endothelial growth factor · Cerebral microbleeds · Cerebral atherosclerosis

Abstract
Background/Purpose: Because atherosclerotic factors and antithrombotic agents sometimes induce cerebral microbleeds (CMBs), patients with cerebral large artery disease (CLAD) tend to have more CMBs than control subjects. On the other hand, VEGF contributes to the disruption of the blood-brain barrier, and it may induce parenchymal edema and bleeding. We conducted a study to evaluate the role of vascular endothelial growth factor (VEGF) in the occurrence of CMBs in patients with CLAD. ***Methods:*** CLAD is defined as stenosis or occlusion of either the carotid artery or the middle cerebral artery of 50% or more. We prospectively registered patients with CLAD who were hospitalized in our neurocenter. Biological backgrounds, atherosclerotic risk factors, administration of antithrombotics before hospitalization, and levels of cytokines and chemokines were evaluated. Susceptibility-weighted imaging or T2*-weighted MR angiography was used to evaluate CMBs. The Brain Observer MicroBleed Scale (BOMBS) was used for CMB assessments. Images were analyzed with regard to the presence or absence of CMBs. We also examined plasma VEGF concentrations using a commercial ELISA kit. Because more than half showed plasma VEGF levels below assay detection limits (3.2 pg/mL), the patients were dichotomized by plasma VEGF levels into two groups (above and below the detection limit). After univariate analyses, logistic regression analysis was conducted to determine the factors associated with the CMBs after adjustment for age, sex, the presence of hypertension, and administration of antithrombotic agents. A similar analysis with CMBs separated by location (cortex, subcortex, or posterior circulation) was also conducted. ***Results:*** Sixty-six patients (71.1 ± 8.9 years, 53 males and 13 females) were included in this study. Plasma VEGF lev-

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els were not correlated with age, sex, and atherosclerotic risk factors; however, patients with VEGF levels >3.2 pg/mL tended toward more frequent CMBs (60.0 vs. 32.6%, in the presence and absence of CMBs, $p = 0.056$). With regard to the location of CMBs, those in the cortex and/or at the gray-white junction were observed more frequently in the patients with VEGF levels >3.2 pg/mL after multivariable analyses (odds ratio: 3.80; 95% confidence interval: 1.07–13.5; $p = 0.039$). ***Conclusions:*** In patients with CLAD, elevated plasma VEGF might be associated with CMBs, especially those located in the cortex and/or at the gray-white junction.

Introduction

Cerebral large artery disease (CLAD), defined as stenosis or occlusion of the carotid artery (CA) or the middle cerebral artery (MCA), is known to be implicated in not only ischemic stroke but also cognitive impairment [1–6]. Ischemic stroke caused by CLAD leads to a decline in cognitive function; in addition, asymptomatic patients with CLAD also show reduced cognitive performance compared with control subjects [4]. A previous study indicated that in patients with isolated mild stroke there was usually no association between cognitive performance and the occurrence of ischemic stroke [6]. However, those with atherosclerosis risk factors, in addition to CLAD, may develop cognitive impairment after even mild stroke. On the other hand, atherosclerotic factors and antithrombotic agents sometimes induce cerebral microbleeds (CMBs), which may pose an increased risk of cognitive decline or cerebral hemorrhage [7, 8]. Few reports have described reliable risk factors that contribute to CMBs, although a report suggested an association between CMBs and the occurrence of ischemic stroke in patients with carotid stenosis [9].

Vascular endothelial growth factor (VEGF) is an angiogenesis factor that leads to the proliferation of vessels. VEGF is related to the development of collaterals in symptomatic patients with CLAD [10]. Because VEGF contributes to the disruption of the blood-brain barrier, it may induce parenchymal edema and bleeding [11]. Thus, it is conceivable that VEGF might exaggerate the formation of CMBs in CLAD cases.

The hypothesis of our study was that VEGF might affect the generation of CMBs in patients with CLAD.

Materials and Methods

CLAD is defined as stenosis or occlusion of either the CA or the MCA of 50% or more [6]. We prospectively registered patients diagnosed with CLAD who were hospitalized in our neurocenter. The degree of stenosis was measured by either CT angiography or carotid ultrasound. We excluded patients with either a modified Rankin Scale score of ≥2, or who fulfilled the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, dementia diagnostic criteria.

We investigated medical records for atherosclerotic risk factors including age, sex, hypertension, diabetes mellitus, hyperlipidemia, current smoking habits, and current drinking habits, according to the definitions of atherosclerotic risks [6]. Years of education and any neurological deficit occurring within 120 days were also noted. Bilateral lesions were defined when patients had CLAD ≥50% on both sides, and CLAD was categorized as right, left, or bilateral. Also, the presence of tandem lesions was determined in patients with both CA and MCA lesions on either side. Cerebral perfusion was measured by Tc-99m ECD SPECT. The SPECT scan began 5 min after the administration of 600 MBq of Tc-99m ECD, and data were collected for 20 min using a triple-head gamma camera.

Brain MRI was performed using either a 3.0-T scanner (GE Healthcare) or a 1.5-T scanner (Philips Healthcare). The sequences included noncontrast two-dimensional fluid-attenuated inversion recovery (FLAIR) images and T2*-weighted MR angiography (SWAN) on the 3.0-T scanner, while FLAIR and susceptibility-weighted imaging (SWI) was obtained using the 1.5-T scanner. In terms of white matter lesions, deep white matter hyperintensity and periventricular hyperintensity were evaluated using FLAIR. The severity of deep white matter hyperintensity and periventricular hyperintensity was stratified according to methods described by Shinohara et al. [12]. In addition, SWI or SWAN was recorded for the evaluation of CMBs. The Brain Observer MicroBleed Scale (BOMBS) was used for the assessment of CBMs [13]. With regard to the presence or absence of CMBs, the images were analyzed by a neurologist (T.O.) and a neuroradiologist (K.T.) who were blinded to the patients' clinical characteristics. If an opinion differed between the two observers, a consensus would be built by discussions.

Biomarker Measurements

To evaluate the association of CMBs with VEGF, we measured plasma VEGF concentrations in the participants using a commercial ELISA kit (MILLIPLEX MAP Multiplex Assay Kit; Merck Millipore, Billerica, MA, USA) according to the manufacturer's instructions. In cases with acute stroke, blood samples were not collected within 14 days of stroke onset. Because the plasma VEGF levels of 39 patients were below the ELISA detection limit (VEGF = 3.2 pg/mL), the patients who participated in this study were dichotomized by VEGF plasma levels (≥3.2 or <3.2 pg/mL) for statistical assessments. Although we measured 29 cytokines and chemokines, VEGF most closely correlated with the presence of CMBs.

Statistics

Crude analyses were conducted to assess the differences in patients' background characteristics, the characteristics of the vascular lesions, and imaging findings between the participant groups with or without detectable plasma levels of VEGF. The prevalence of CMBs was also compared between the two groups defined by VEGF. Logistic regression analysis was conducted to determine the factors associated with the CMBs after adjustment for age, sex, the presence of hypertension, and administration of antithrombotic agents. A similar analysis, with CMBs separated by location (cortex, subcortex, or posterior circulation), and defined by the BOMBS criteria, was also conducted [13]. These data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 22.0, and a p value of 0.05 was considered statistically significant.

Results

Sixty-six CLAD patients were included in this study. Table 1 shows the background characteristics, atherosclerotic risks, and characteristics of CLAD for patients with or without detectable plasma VEGF levels. Thirty-nine patients (59.1%) exhibited a plasma VEGF level below the detection limit of the assay (3.2 pg/mL), while the number of those with VEGF ≥3.2 pg/mL was 27 (40.9%). There were no significant differences in these factors between the patients with VEGF ≥3.2 pg/mL and those with VEGF <3.2 pg/mL. Fifty patients were evaluated for the presence of CMBs by means of SWI, while SWAN was conducted on 10 patients. CMBs were present in 20 patients (30.3%). Among these, the numbers of patients with posterior, deep, and lobar CMBs were 12, 13, and 15, respectively. Table 2 shows the association between plasma VEGF levels and the frequency of CMBs. The presence of CMBs tended to be associated with VEGF ≥3.2 pg/mL. Meanwhile, the frequency of detectable plasma levels

Table 1. Patients' characteristics, risks of atherosclerosis, and characteristics of CLAD

	VEGF ≥3.2 pg/mL (*n* = 27)	VEGF <3.2 pg/mL (*n* = 39)	*p* value
Age, years	73 (63–78)	73 (66–77)	0.661
Sex	23 (85%)	30 (77%)	0.534
Symptoms within 120 days	9 (33%)	15 (38%)	0.796
Hypertension	20 (74%)	32 (82%)	0.544
Diabetes mellitus	11 (41%)	18 (46%)	0.802
Dyslipidemia	17 (63%)	27 (69%)	0.607
Smoking	8 (30%)	9 (23%)	0.578
Drinking	11 (41%)	9 (23%)	0.174
ICA lesion	25 (93%)	36 (92%)	1.000
Side			
Right	9 (33%)	19 (49%)	0.311
Left	18 (67%)	20 (51%)	
Bilateral	3 (11%)	6 (15%)	0.727
Antithrombotic agents	22 (81%)	27 (69%)	0.391
Degree of stenosis, %	73 (61–82)	80 (69–90)	0.140
CBF of MCA area of ipsilateral side, mL/100 g/min	32.6 (31.3–33.6)	32.6 (28.4–35.3)	0.749
CBF of MCA area of contralateral side, mL/100 g/min	33.5 (31.2–35.7)	33.1 (30.4–35.6)	0.814

CLAD, cerebral large artery disease; VEGF, vascular endothelial growth factor; ICA, internal carotid artery; CBF, cerebral blood flow; MCA, middle cerebral artery.

Table 2. Association between CMBs and VEGF

	VEGF ≥3.2 pg/mL (*n* = 27)	VEGF <3.2 pg/mL (*n* = 39)	*p* value
Presence of CMBs certain	12 (44%)	8 (21%)	0.056
CMBs at posterior fossa	6 (22%)	6 (15%)	0.528
Deep CMBs	7 (26%)	6 (15%)	0.353
Lobar CMBs	10 (37%)	5 (13%)	0.035

CMBs, cerebral microbleeds; VEGF, vascular endothelial growth factor.

of VEGF varied depending on the region of the CMBs; namely, lobar CMBs were significantly associated with patient plasma VEGF levels ≥3.2 pg/mL.

After adjustment for age, sex, hypertension, and prescription of antithrombotic agents, plasma levels of VEGF ≥3.2 pg/mL tended to be associated with the presence of CMBs (Table 3). Variation in the location of CMBs was still observed, and an increased level of VEGF was significantly associated with lobar CMBs.

Discussion

This study indicated the possible association of the presence of CMBs with an increased level of VEGF in patients with CLAD. Specifically, VEGF levels ≥3.2 pg/mL were strongly associated with the presence of lobar CMBs.

Table 3. Age- and sex-adjusted and multivariable analyses to elucidate the association between VEGF and the presence of CMBs

	Sex and age			Multivariable		
	OR	95% CI	*p* value	OR	95% CI	*p* value
Presence of CMBs	3.28	1.07–13.86	0.038	3.13	0.96–10.22	0.059
CMBs at posterior fossa	1.59	0.45–5.69	0.47	1.35	0.36–5.06	0.65
Deep CMBs	1.84	0.53–6.38	0.34	1.67	0.47–5.93	0.43
Lobar CMBs	4.02	1.17–13.86	0.028	3.80	1.07–13.49	0.039

VEGF, vascular endothelial growth factor; OR, odds ratio.

It is well known that VEGF induces angiogenesis. Thus, VEGF contributes to the proliferation of collateral formation in symptomatic patients [10], which aids in the enhancement of cerebral perfusion. In animal models of cerebral ischemia, however, VEGF might magnify the disruption of the blood-brain barrier, leading to edema and bleeding in the brain, which may result in the formation of CMBs [11]. This study supports the hypothesis that VEGF negatively affects brain function in patients with CLAD. Further studies may elucidate the positive and negative effects of VEGF. It is interesting to note that VEGF was significantly associated with the generation of CMBs with a lobar distribution, but not with deep CMBs or those at the posterior fossa.

That VEGF improves hypoperfusion [10] and supports the development of lobar CMBs could be explained by the possibility that VEGF exerts both positive and negative effects on lobar and leptomeningeal arteries, which follows the theory mentioned above. We sometimes encounter a large number of CMBs in the cortex of patients with amyloid angiopathy. Our findings suggest that VEGF may also play an important role in the development of amyloid angiopathy. Conversely, VEGF was not considered to be associated with deep CMBs or those located at the posterior fossa. While hypertension sometimes leads to the formation of CMBs in the deep perforating arteries, an increase in plasma VEGF may not be implicated in CMBs in these areas. These results indicate the possibility that VEGF does not influence the formation of all CMBs, and that different mechanisms for the formation of CMBs exist between lobar and deep brain regions.

There are some limitations to this study. First, the sample size was small. Second, there were a large number of patients whose plasma VEGF levels were below the detection limit of the assay (<3.2 pg/mL); thus, an accurate assessment of the VEGF concentration for these patients was not available. Further studies are required to fully elucidate the impact of plasma VEGF on CMBs.

In conclusion, the increase in plasma VEGF levels is important for the formation of collateral vessels in CLAD, especially in lobar regions. VEGF is associated with collateral formation, but these sometimes lead to the formation of CMBs.

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Statement of Ethics

The study protocol was approved by the Ethics Committee of Fukuoka University Hospital (IRB No.: 14-1-07). Informed consent was obtained from all the patients.

Disclosure Statement

The authors declare no conflicts of interest.

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