Title

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Journal

Journal of Stroke and Cerebrovascular Diseases, 28(2)

ISSN

1052-3057

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Publication Date

2019-02-01

DOI

10.1016/j.jstrokecerebrovasdis.2018.10.004

Peer reviewed

HHS Public Access

Author manuscript

J Stroke Cerebrovasc Dis. Author manuscript; available in PMC 2020 February 01.

Published in final edited form as:

J Stroke Cerebrovasc Dis. 2019 February ; 28(2): 360–368. doi:10.1016/j.jstrokecerebrovasdis.2018.10.004.

Differential expression of vascular endothelial growth factor-A_{165} isoforms between intracranial atherosclerosis and moyamoya disease

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Abstract

Background—Vascular endothelial growth factor-A_{165} (VEGF-A_{165}) has been identified as a combination of two alternative splice variants: proangiogenic VEGF-A_{165}a and antiangiogenic VEGF-A_{165}b. Intracranial atherosclerotic disease (ICAD) and moyamoya disease (MMD) are two main types of intracranial arterial steno-occlusive disorders (ICASD) with distinct capacities for collateral formation. Recent studies indicate that VEGF-A_{165} regulates collateral growth in ischemia. Therefore, we investigated if there is a distinctive composition of VEGF-A_{165} isoforms in ICAD and MMD.

Methods—Sixty-six ICAD patients, six MMD patients, and five controls were enrolled in this prospective study. ICAD and MMD patients received intensive medical management upon enrollment. Surgery was offered to 9 ICAD patients who had recurrent ischemic events, 6 MMD patients, and 5 surgical controls without ICAD. VEGF-A_{165}a and VEGF-A_{165}b plasma levels

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DISCLOSURES

None

were measured at baseline, within one week after patients having surgery, and at one, three, and six months after treatment.

Results—A significantly higher baseline VEGF-A_{165}a/b ratio was observed in MMD compared to ICAD (p=0.016). The VEGF-A_{165}a/b ratio increased significantly and rapidly after surgical treatment in ICAD (p=0.026) more so than in MMD and surgical controls. In patients with ICAD receiving intensive medical management, there was also an elevation of the VEGF-A_{165}a/b ratio, but at a slower rate, reaching the peak at three months after initiation of treatment (baseline vs. three months VEGF-A_{165}a/b ratio, p=0.028).

Conclusions—Our study shows an increased VEGF-A_{165}a/b ratio in MMD compared to ICAD, and suggests that both intensive medical management and surgical revascularization elevate the VEGF-A_{165}a/b ratio in ICAD patients.

Keywords

VEGF-A_{165}; Isoforms; Intracranial atherosclerosis; Moyamoya disease

INTRODUCTION

The vascular endothelial growth factor (VEGF) family of proteins are key angiogenic regulators in both physiological and pathological conditions. Among all the members of this family, VEGF-A is the best known and most potent angiogenic protein[1]. VEGF-A has different variants, among which VEGF-A_{165} is believed to be the dominant form binding to all receptors.[2] Previously, all VEGF-A isoforms were thought to be proangiogenic. Recently, VEGF-A_{165} has been identified as a combination of two alternative splice variants (isoforms $_{165}$a and $_{165}$b) [3] VEGF-A_{165}b is a powerful competitive innate inhibitor of the angiogenic effect of VEGF-A_{165}a.[4] To the best of our knowledge, no investigations of the role of these isoforms have been conducted in cerebrovascular diseases.

Intracranial arterial steno-occlusive disorders (ICASD) are some of the most common causes of stroke worldwide.[5, 6] Chronic progression of arterial stenosis may allow collaterals to develop over time, which could potentially offset the hemodynamic impairment induced by stenosis. Several recent studies show that collaterals dramatically alter stroke risk and functional outcome in ICASD.[7–9] Intracranial atherosclerotic disease (ICAD) is the most common subtype of ICASD. Unfortunately, the extent of collaterals in ICAD is limited. Only 20% of patients with ICAD stenosis of more than 50% have good collaterals.[10] This factor may be key in the worse prognosis of ICAD patients when compared to other stroke etiologies.[11–13] Conversely, another type of ICASD, moyamoya disease (MMD), is known for its pronounced collateral formation.[14]

Growing evidence indicates that ischemia-induced collateral growth in different pathologies is regulated by VEGF-A.[15, 16, 17] However, prior studies comparing plasma levels of VEGF between ICAD and MMD patients have not found differences.[18, 19] Of note, the role of VEGF isoforms has not been evaluated.

In the present study, we hypothesized that there is a distinctive pattern of the levels of VEGF-A_{165}a and VEGF-A_{165}b in patients with ICAD and MMD. In addition, we evaluated

the regulatory effects of intensive medical management and surgical revascularization on VEGF-A_{165} isoforms.

MATERIALS AND METHODS

Population and study design

Population: Patients diagnosed with ICASD in four hospitals in the Los Angeles metropolitan area between 2012 and 2014 were enrolled in this prospective cohort study. A diagnosis of severe stenosis (≥70%) of an intracranial large artery was confirmed by catheter, CT, or MR angiography. The eligibility of the patients was assessed according to the inclusion and exclusion criteria listed in Table 1. The diagnosis of ICAD or MMD was adjudicated based on the standardized criteria in Table 2. In order to include surgical controls without cerebrovascular disease, intracranial tumor, or infectious or inflammatory problems, a group of patients with epilepsy who would potentially undergo subdural grid or depth electrode placement were enrolled. The study was approved by the local institutional review boards, and informed consent of each participant was obtained.

Intervention: Immediately upon enrollment, all patients with ICASD received antiplatelet treatment. Patients with ICAD had a strict management of primary vascular risk factors (systolic blood pressure and low-density lipoprotein [LDL]) and secondary vascular risk factors (diabetes, non-high-density lipoprotein [non-HDL], smoking, obesity, and inadequate physical activity). If a patient with ICAD had events of stroke or transient ischemic attacks (TIA) despite intensive medical management, they were considered for surgical revascularization. All patients with MMD were offered surgical revascularization. All surgical patients were treated with encephaloduroarteriosynangiosis (EDAS) according to our formerly published protocols.[20, 21] Intensive medical management, including the use of aspirin, was continued without interruption during the perioperative period. All the patients underwent regular follow-up visits at one, three, and six months.

VEGF-A Isoforms Level Determination

For ICAD patients, blood samples were collected at baseline and then during follow-up visits at one, three, and six months. For patients who underwent surgery, additional blood samples were collected during the postoperative period, within one week after surgery. For MMD patients and surgical controls, blood samples were only collected at baseline and within one week after surgery. The sample collection and loss at each time point in specific groups was illustrated in detail via a CONSORT flow diagram (Figure 1).

Blood was collected in EDTA tubes at each time. Plasma was immediately separated by centrifugation at 3500 rpm for 15 minutes at 4˚C and stored in 2 cc cryogenic vials at −80˚C. Quantitative measurement was performed using an enzyme-linked immunosorbent assay (ELISA, Ciraplex® Aushon Biosystems, MA, USA) to detect the plasma level of VEGF-A_{165}a and $_{165}$b. Each sample was measured twice, with the mean of the two values reported as the plasma level. The mean intra-assay coefficient of variation (CV) was established with a threshold of less than 20% CV considered valid.

Statistics

Descriptive statistics were computed to summarize demographic data, medical history, and vascular risk factors in each etiology. The comparison of baseline characteristics was made by using the student's t-test for continuous variables and Fisher's exact test for categorical variables.

The VEGF-A_{165}a/b ratio was calculated by dividing the VEGF-A_{165}a plasma level by the value of VEGF-A_{165}b. Univariate analyses were applied to evaluate the association between the baseline VEGF-A_{165}a/b ratio and etiology, together with other clinical variables including age, gender, race, vascular risk factors, and coronary artery disease. The statistical significance of the association was determined by the Wilcoxon Rank Sum test (nonparametric comparison) and Kruskal-Wallis test (multiple nonparametric comparisons) for categorical variables and linear fit for continuous variables. Clinical variables found to be potentially associated with VEGF-A_{165}a/b ratio ($p < 0.05$) in univariable analysis but unequally distributed in the different etiology groups were further analyzed within each group. If a variable remained significant, then it was considered as a confounder, and the association between etiology and VEGF-A_{165}a/b ratio was further evaluated separately within each stratum defined by the confounding variable.

The effects of the surgical intervention on the VEGF-A_{165}a/b ratio were investigated in ICAD, MMD, and controls; the values at baseline and within one week in each group were compared using the Wilcoxon Rank Sum test. The levels of VEGF-A_{165}a, VEGF-A_{165}b, and the VEGF-A_{165}a/b ratio during follow-up posttreatment were analyzed in ICAD; the values at each time point under an identical treatment were compared in pairs via the Wilcoxon Rank Sum test.

Statistical analyses were conducted using JMP statistical package, version 13.0. All the tests used were two-tailed, and $p<0.05$ was considered significant.

RESULTS

Population characteristics

A total of 77 patients were enrolled in this study, including 66 in the ICAD group, 6 in the MMD group, and 5 in the control group. Demographic characteristics, vascular risk-factor profiles, and medical histories for each group are shown in Supplemental Table 1. The mean age of patients with MMD was 40.9±11.1 years, which is significantly younger than that of patients with ICAD (61.4±12.6 years, p=0.0013). The majority of patients with MMD were female (5/6, 83.3%), while the proportion of female patients in ICAD and controls were 47% and 60% respectively. The most common race in ICAD and controls was White. Asians represented 66.7% of MMD patients. Hypertension was significantly more prevalent in ICAD patients (p=0.0003).

Etiology and the composition of VEGF-A isoforms

A total of 188 blood samples were collected from the enrolled patients at the times described in the Methods section. For each sample, the plasma levels of VEGF-A_{165}a and VEGF-

A_{165}b were measured, and the VEGF-A_{165}a/b ratios were calculated. In the univariate analysis, a significantly higher baseline VEGF-A_{165}a/b ratio was observed in MMD compared with ICAD (0.47±0.45 vs. 0.16±0.15, p=0.016). There was also a significantly negative association between the VEGF-A_{165}a/b ratio and age (p=0.004). However, age did not maintain significance in the within-group analysis, indicating that age was associated to etiology but not VEGF isoform levels. The complete results of the univariate analysis are summarized in Table 3. The total levels of VEGF-A_{165} ($_{165}$a plus $_{165}$b) were similar among the three groups, which were 348.3±162.8 (pg/ml) in ICAD, 271.4±89.3 (pg/ml) in MMD, and 327.4±77.1 (pg/ml) in controls (p=0.31). However, VEGF-A_{165}b levels in MMD were significantly lower than in ICAD (196.9±61.6 [pg/ml] vs. 308.2±20.2 [pg/ml], p=0.0006) and controls (196.9±61.6 [pg/ml] vs. 305.4±84.6 [pg/ml], p=0.007), and VEGF-A_{165}a levels were significantly higher in MMD than in controls (74.6±70.7 [pg/ml] vs. 20.6±15.4 [pg/ml], p=0.026) but not ICAD (74.6±70.7 [pg/ml] vs. 40.2±33.8 [pg/ml], p=0.092). The highest levels of VEGF-A_{165}b were found in the ICAD group (Figure 2).

Effect of surgical intervention on the VEGF-A_{165}a/b ratio

EDAS was conducted on nine ICAD patients and six MMD patients. Five control patients received intracranial surgery, as described in the Methods section. Postoperatively, patients treated with EDAS surgery were TIA and stroke-free for the lengths of their follow-ups. The VEGF-A_{165}a/b ratio was elevated within one week after surgery in all groups. However, statistically significant elevation was observed only in ICAD patients (0.10±0.06 at baseline vs. 0.27±0.23 within 1 week postsurgery, p=0.026, Figure 3). The VEGF-A_{165}a/b ratio within one week after surgery exhibited no significant difference between MMD and ICAD (0.35±0.35 in MMD vs. 0.27±0.23 in ICAD, p=0.82).

Effect of time and type of treatment on the VEGF-A_{165} isoforms in ICAD

Subgroup analyses by treatment showed distinct patterns of VEGF-A_{165}a, VEGF-A_{165}b, and the VEGF-A_{165}a/b ratio in ICAD over time (Figure 4).

For the patients with only intensive medical management, the VEGF-A_{165}a/b ratio gradually elevated until reaching its peak three months after treatment (baseline VEGF-A_{165}a/b ratio = 0.13±0.12, vs. three-month VEGF-A_{165}a/b ratio = 0.25±0.23, p=0.028). This is due to an increase in the VEGF-A_{165}a levels, which went from 31.36±24.84 (pg/mL) at baseline to 62.73±52.13 (pg/mL) at three months, p=0.015. The VEGF-A_{165}b levels did not change significantly over time (p=0.38).

For those patients who had EDAS surgery, the peak of the VEGF-A_{165}a/b ratio was achieved within one week after surgery (baseline VEGF-A_{165}a/b ratio = 0.10±0.06, vs. within-one-week VEGF-A_{165}a/b ratio = 0.27±0.23, p=0.026). Then, the ratio gradually declined by the six-month follow-up. In the surgical patients, the initial change in the VEGF-A_{165}a/b ratio was due to a significant decrease of VEGF-A_{165} b (baseline VEGF-A_{165}b = 401.09±314.52 [pg/mL], vs. within-one-week VEGF-A_{165}b = 232.27±68.54 [pg/mL], p=0.047).

DISCUSSION

The role of VEGF-A in vascular ischemic disease has been previously studied,[22–24.] but only a few studies have investigated the antiangiogenic action of VEGF-A_{165}b in peripheral artery disease.[25] To the best of our knowledge, the present study is the first investigation of the role of VEGF-A_{165} isoforms in cerebrovascular diseases.

We found that VEGF-A_{165} isoforms are distinct among different forms of cerebrovascular steno-occlusive disorders. Our study shows an increased VEGF-A_{165}a/b ratio in MMD compared to ICAD and controls. It also demonstrates a significant elevation of the $VEGF_{165}$a/b ratio within one week after surgical therapy for ICAD but not for MMD. In patients with ICAD, intensive medical management also produced an increase in the VEGF-A_{165}a/b ratio, but at a slower rate, with the ratio peaking at three months after initiation of treatment.

Previous studies have failed to detect differences in circulating VEGF between MMD and ICAD.[18, 19] Rafat et al. found that the serum concentration of VEGF was increased in both MMD and ICAD patients compared to healthy controls but not different between the two conditions. However, circulating endothelial progenitor cells (cEPCs) were upregulated only in MMD, and the number of cEPCs was inversely correlated with the VEGF serum level.[18] This seems to contradict the conventional thought that the migration, adhesion, and proliferation of cEPCs are largely dependent on VEGF.[26, 27] In our study, the total concentration of the VEGF-A_{165} isoforms was also not different between MMD and ICAD. Nevertheless, the VEGF-A_{165}a/b ratio was significantly higher in MMD. This discrepancy between VEGF levels and cEPCs upregulation found by Rafat et al. could be explained by our findings. A higher proportion of competitive inhibitor VEGF-A_{165}b neutralizes the biological effect of VEGF-A_{165}a, impairing the recruitment and activation of cEPCs in ICAD patients. On the contrary, the high ratio of VEGF-A_{165}a/b in MMD represents a positive stimulus for EPCs, which could contribute to the increased collateral formation.[28]

The reason for the differences in the VEGF-A_{165}a/b ratio between MMD and ICAD is not clear. A possible explanation may reside in the role of insulin-like growth factor (IGF) and its ability to switch VEGF-A splicing from an antiangiogenic to a proangiogenic isoform in epithelial cell type.[29] Interestingly, a large number of studies have shown that lower levels of IGF-1 are associated with the presence of traditional vascular risk factors, including hypertension, hyperlipidemia, obesity, and type 2 diabetes mellitus.[30–34] ICAD patients are prone to have these vascular risk factors, which could lead to lower levels of IGF-1. This would impede the splicing to a proangiogenic phenotype during an angiogenic response to ischemia. Such inability could contribute to a distinct capacity for ischemia-induced collateral growth between ICAD and MMD. Additionally, as we found, the antiangiogenic phenotype in ICAD patients is gradually switched by intensive medical management, which targets correction of primary and secondary vascular risk factors. The switch to a less antiangiogenic profile may improve clinical outcomes in ICAD.[35] We speculate that proper control of these vascular risk factors by intensive medical management benefits the increased expression of IGF-1, which favors proangiogenic splicing.

In ICAD patients, we observed a rapid increase of the VEGF-A_{165}a/b ratio within one week after EDAS surgery. The surgery itself may be regarded as an angiogenesis-inducing condition since it causes the release of various proangiogenic factors.[36] However, the significant elevation in ICAD and MMD patients after EDAS compared to the controls who underwent intracranial surgeries with a similar degree of tissue manipulation indicates that the baseline hypoxic status with ICASD enhances a proangiogenic response after surgery. The observation in our study that the postoperative VEGF-A_{165}a/b ratios were not different between ICAD and MMD may help to explain the results observed by Gonzalez et al., who reported angiographic neovascularization after EDAS in patients with ICAD and MMD.[21] The postsurgical elevation of the VEGF-A_{165}a/b ratio was more prominent in ICAD. This can be explained in part by the initially higher VEGF-A_{165}a/b ratio in MMD, which may limit the proangiogenic induction in response to EDAS.

Until now, the ability of individuals to form sufficient collaterals remains a non-modifiable risk factor for stroke and only surgical techniques like indirect bypasses with EDAS surgeries provide interventions targeting the individual's ability to form collaterals. The markers we have evaluated in this study could potentially be used in the future to identify groups in whom aggressive surgical interventions could be indicated. However, the present study is an early-stage and explorative investigation of these isoforms' differential expression, and further confirmation in larger cohorts is necessary before delineating a definitive role for the VEGF-A_{165} isoforms as biomarkers of ICASD.

Some limitations of our study include a relatively small sample size, particularly in the MMD and control groups. However, this reflects the more common occurrence of ICAD and the relative rareness of MMD in the United States. Some clinical covariates among the three groups cannot be exactly matched due to distinct disease features. To account for the potential confounding effects driven by these covariates, instead of using regression analysis, which is limited by the asymmetric sample size of our cohort, we conducted a within-group evaluation. The additional variables found potentially significant in the univariate analysis (age and hypertension) did not remain significant in further analysis. Second, among patients undergoing medical management there was loss of samples due to loss of follow up in 62.7%, hemolysis in 15.7% and invalid measurements in 21.6% of the 51 samples that were not collected. All patients had baseline samples and therefore the missing data would not influence the result reported on the effects of etiology on the VEGF-A_{165} isoforms, which was done using exclusively the baseline data. In the same manner, the effects of surgical intervention on the groups of ICAD, MMD, and controls are not affected, as there was no sample loss at baseline and within 1 week after surgery. The expected effect of the sample loss in the patients with ICAD after 3 months postsurgery is minimal as only 2 samples were lost due to hemolysis. In the ICAD patients that were medically treated, the attrition in sample number may impact the significance of our observation that medical treatment gradually elevates the VEGF-A_{165}a/b ratio. However, the ICAD patients with missing samples showed no difference on demographic and clinical characteristics compared to those with all scheduled sample measuring, suggesting missing at random and consequently limited influence on VEGF-A_{165} isoform levels (Supplemental Table 2). Finally, the plasma levels of VEGF-A_{165} could be influenced by various factors such as diseases, medication, and individual variance. Several efforts have been made to address this limitation, including

a comprehensive inclusion and exclusion criteria, application of a homogeneous protocol of medical and surgical treatment for each disease, duplicate evaluations of VEGF-A_{165} isoforms at sequential time points examined simultaneously, and strict intra-assay coefficient of variation for the measurements of VEGF-A_{165} isoforms.

SUMMARY

Our results show that VEGF-A_{165} isoforms are distinct between MMD and ICAD. This study indicates that an increased VEGF-A_{165}a/b ratio is associated with MMD. It also indicates that both intensive medical management and surgical revascularization elevate the VEGF-A_{165}a/b ratio in ICAD patients. These findings justify future additional evaluations to better understand the mechanisms involved in the alternative splicing of VEGF-A in ICAD and MMD, and the potential roles of VEGF-A_{165}a and VEGF-A_{165}b in the outcomes after treatment of these patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

We are grateful to the Cedars-Sinai Medical Center and UCLA Stroke Center members for their support and continuous collaboration. Also, we warmly thank the research coordination team led by Antoinette Anderson.

SOURCES OF FUNDING

This work was supported by grants from the American Heart Association (14ISA20870000), NIH (K23 NS079477-01A1), and the Science and Technology Department of Zhejiang Province (2015C34007) and by the philanthropic support of the Margo and Irwin Winkler Family.

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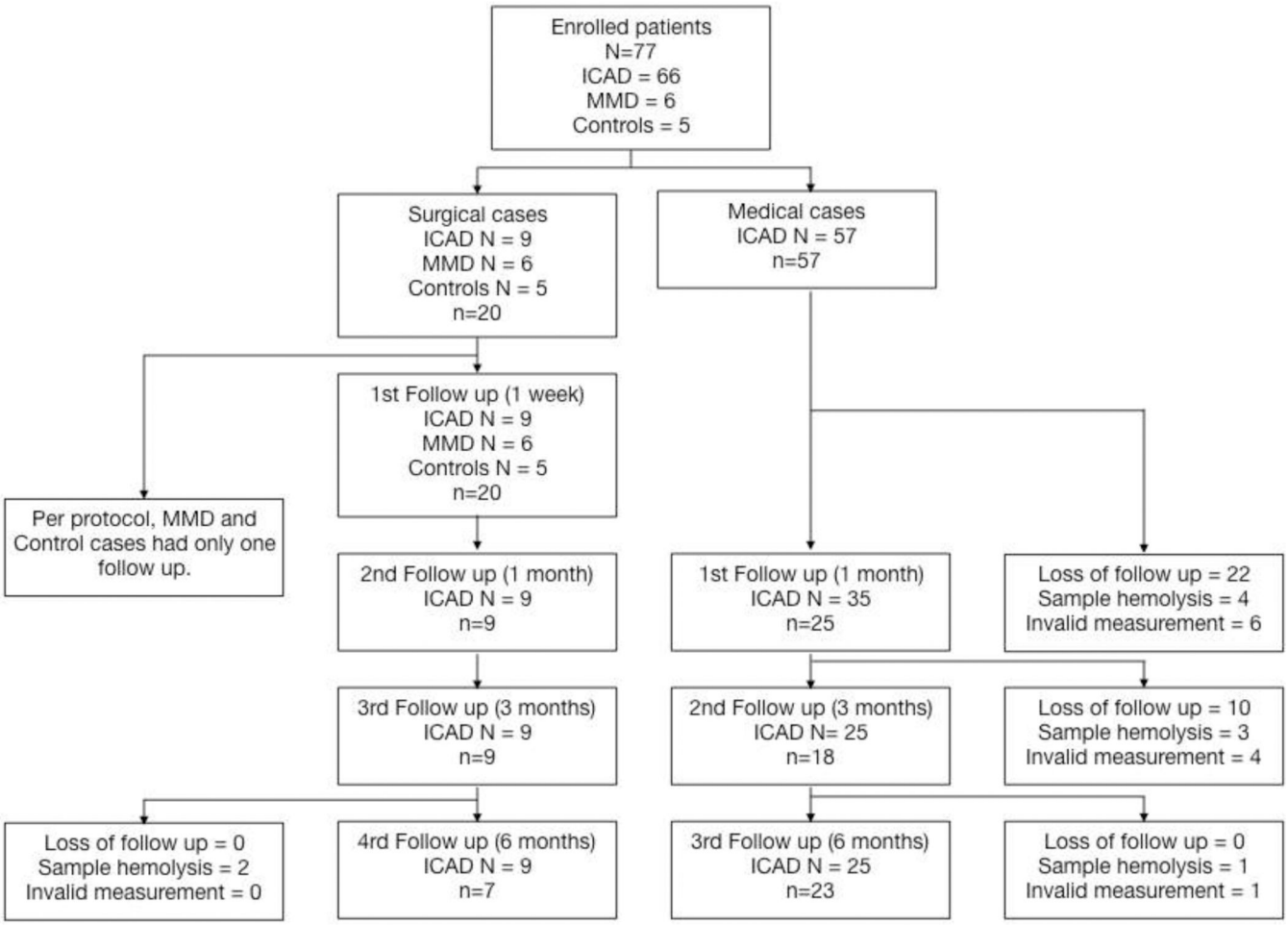

Figure 1.

Flow diagram demonstrating the numbers of patients (N) and samples (n) in each group and time point, as well as the attrition quantities with their reasons at each step. In the surgical patients, only 2 samples were lost due to sample hemolysis at 6-month follow up. An inter-duplicate variance of ELISA measurements ≥20% was defined as invalid measurement and the data was excluded.

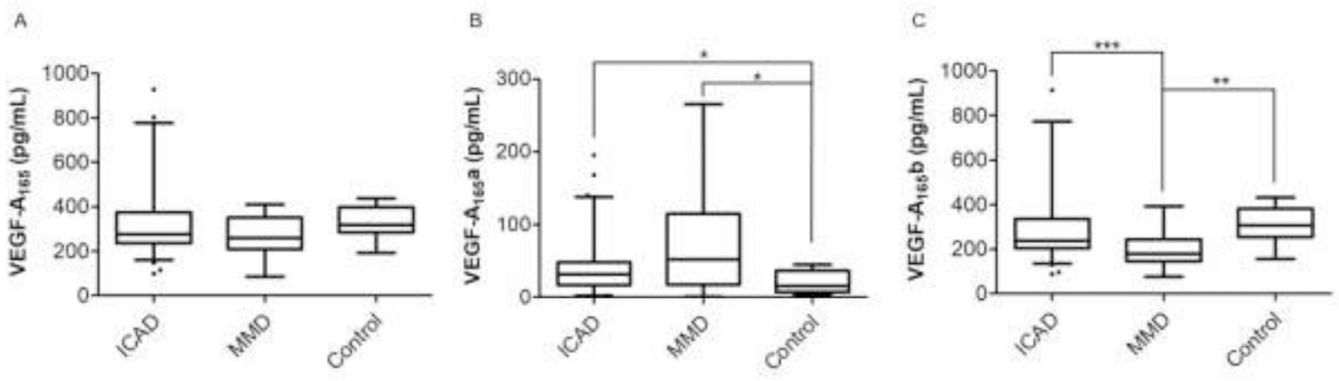

Figure 2.

Comparison of baseline VEGF-A_{165} (A), VEGF-A_{165}a (B), and VEGF-A_{165}b (C) levels in ICAD, MMD, and controls. The box plots graph shows that MMD patients have a significantly higher level of VEGF-A_{165}a compared to controls (p=0.026) and a significantly lower level of VEGF-A_{165}b compared to ICAD (p=0.0006) and controls (p=0.0070). The total levels of VEGF-A_{165} were not different between the three groups (p=0.31). The highest levels of VEGF-A_{165}a were observed in MMD, while the highest levels of VEGF-A_{165}b were seen in ICAD.

VEGF, vascular endothelial growth factor; ICAD, intracranial atherosclerotic disease; MMD, moyamoya disease; *, p<0.05; **, p<0.01; ***, p<0.001.

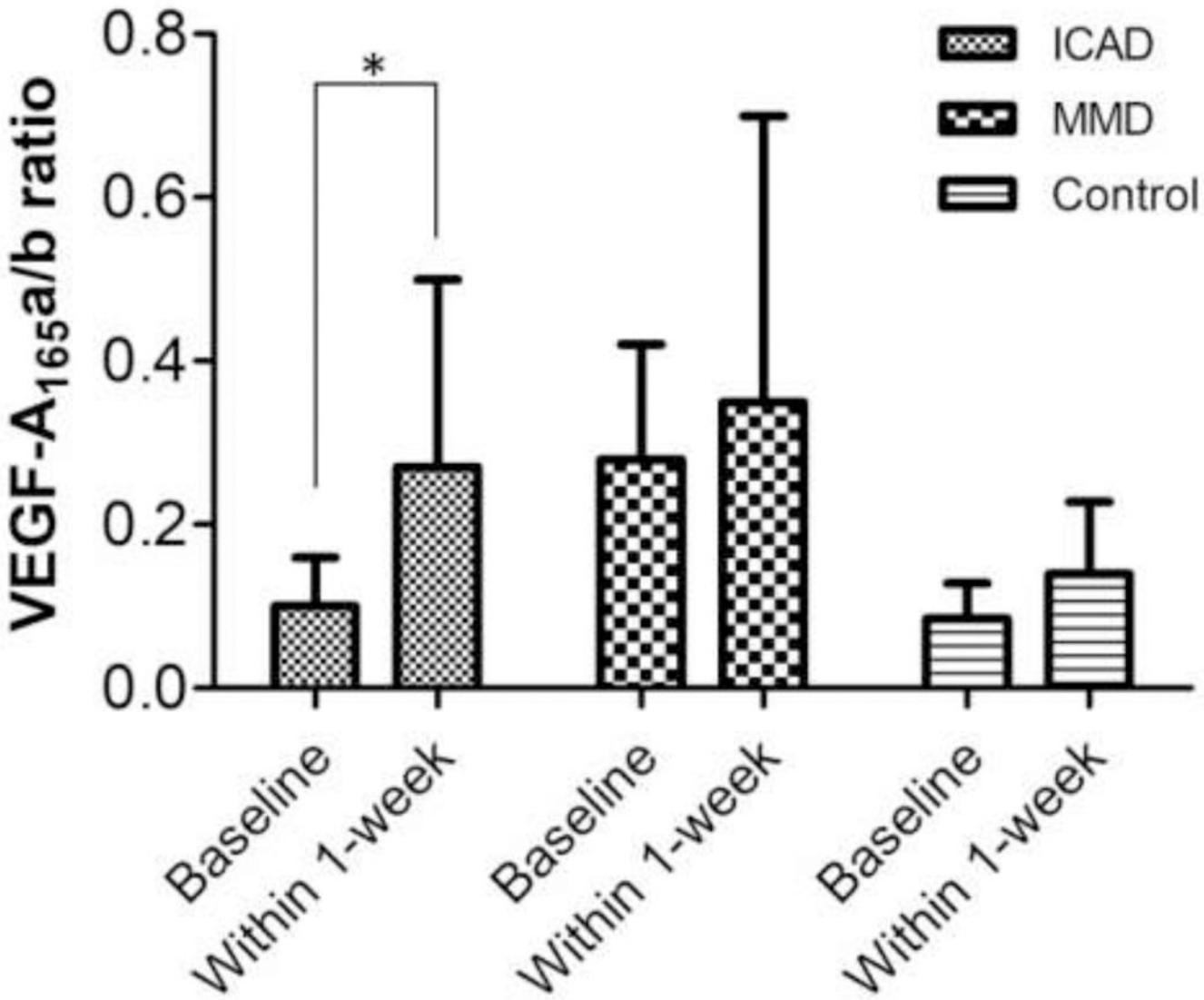

Figure 3.

Elevation of VEGF-A_{165}a/b ratio within one week after surgery according to etiology. The bar graph shows that the VEGF-A_{165}a/b ratio elevated in all the groups, but only in ICAD was the increase significant (p=0.026).

VEGF, vascular endothelial growth factor; ICAD, intracranial atherosclerotic disease; MMD, moyamoya disease; *, p<0.05.

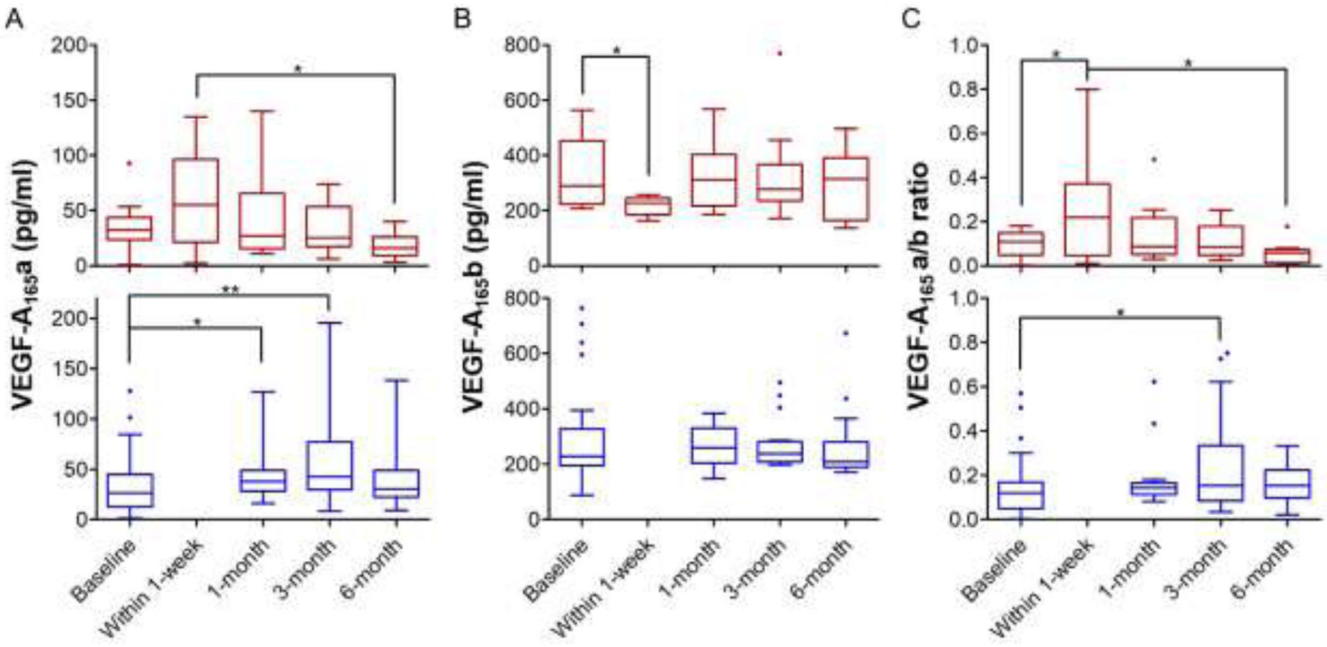

Figure 4.

Box plots of VEGF-A_{165}a (A), VEGF-A_{165}b (B), and VEGF-A_{165}a/b ratio (C) at different time points after surgical revascularization (in red) and medical treatment (in blue) in ICAD patients. The upper halves of panel A, B, and C show the surgical effects on the variations of VEGF-A_{165}a, VEGF-A_{165}b, and the VEGF-A_{165}a/b ratio, respectively. Surgery significantly increased the VEGF-A_{165}a/b ratio within one week after surgery (p=0.026), with the ratio gradually declining to the lowest value at six months. Additionally, the VEGF-A_{165}b level within one week was significantly lower than at baseline (p=0.047). The lower halves of panel A, B, and C illustrate the medical effect on the VEGF-A_{165}a level, the VEGF-A_{165}b level, and the VEGF-A_{165}a/b ratio, respectively. The VEGF-A_{165}a/b ratio progressively increased and reached its peak after three months during intensive medical management (p=0.028). A significant difference between baseline and three months was also found in the VEGF-A_{165}a level (p=0.015). VEGF-A_{165}b levels did not change during follow-up (p=0.38).

VEGF, vascular endothelial growth factor; ICAD, intracranial atherosclerotic disease; *, p<0.05; **, p<0.01.

Table 1.

Inclusion and Exclusion Criteria

Inclusion criteria
1. ≥70% stenosis of intracranial large artery diagnosed by catheter, MR, or CT angiography.
2. Patient is willing and able to return for all follow-up visits.
3. Patient understands the purpose and requirements of the study, can make him- or herself understood, and has provided consent.
Exclusion criteria
1. Patients with any known vasculitic disease, viral vasculopathy, neurosyphilis, any other intracranial infection, any intracranial stenosis associated with cerebrospinal fluid pleocytosis, radiation-induced vasculopathy, fibromuscular dysplasia, sickle cell disease, neurofibromatosis, benign angiopathy of the central nervous system, postpartum angiopathy, suspected vasospastic process, or suspected recanalized embolus.
2. Patients with presence of any of the following unequivocal cardiac sources of embolism: chronic or paroxysmal atrial fibrillation, mitral stenosis, mechanical valve, endocarditis, intracardiac clot or vegetation, myocardial infarction within three months, dilated cardiomyopathy, left atrial spontaneous echo contrast, ejection fraction <30%.
3. Intracranial tumor or vascular malformation.
4. Any hemorrhagic infarct within 14 days before enrollment or any other intracranial hemorrhage (subarachnoid, subdural, or epidural) within 30 days.
5. Major surgery (including open femoral, aortic, or carotid surgery) within previous 30 days or planned in the next 180 days after enrollment.
6. Severe neurologic deficit that renders the patient not independent.

Table 2.

Diagnostic Criteria for ICAD and MMD

Diagnostic Criteria for ICAD
1. Imaging features of atherosclerosis such as calcification of the intracranial vessels, and 2. at least one of the following: a. insulin dependent diabetes for at least 15 years b. at least two of the following atherosclerotic risk factors: • hypertension (BP ≥ 140/90 mmHg or on antihypertensive therapy); • dyslipidemia (LDL > 130 mg/dl or HDL < 40 mg/dl or fasting triglycerides > 150 mg/dl or on lipid lowing therapy); • smoking history; • non-insulin-dependent diabetes or insulin dependent diabetes of less than 15 years duration; • family history of any of the following: myocardial infarction, coronary artery bypass, coronary artery angioplasty or stenting, stroke, carotid endarterectomy or stenting, peripheral vascular surgery in parent or sibling who was < 55 years of age for men or < 65 years of age for women at the time of the event; • history of any of the following: myocardial infarction, coronary artery bypass, coronary angioplasty or stenting, carotid endarterectomy or stenting, or peripheral vascular surgery for atherosclerotic disease; c. any stenosis of an extracranial carotid or vertebral artery, subclavian artery, coronary artery, iliac or femoral artery, other lower or upper extremity artery, mesenteric artery, or renal artery that was documented by noninvasive vascular imaging or catheter angiography and is considered atherosclerotic; d. aortic arch atheroma documented by noninvasive vascular imaging or catheter angiography; e. any aortic aneurysm documented by noninvasive vascular imaging or catheter angiography that is considered atherosclerotic.
Diagnostic Criteria for MMD
1. Bilateral intracranial arterial stenosis of the distal internal carotid arteries and the origin of the anterior cerebral and middle cerebral arteries, and 2. evidence of moyamoya collaterals in angiography, and 3. absence of any risk factors for atherosclerosis.

Table 3.

Univariate Analyses of the Factors Potentially Related with $VEGFA_{165}$a/b Ratio

Variable	P
Demographic factors	
Age	0.044*
Gender	0.98
Race	0.14
Medical history	
Coronary artery disease	0.84
Vascular risk factors	
Smoking history	0.89
Drinking history	0.80
Hypertension	0.078
Hyperlipidemia	0.10
Type 2 diabetes mellitus	0.78
Other factors	
Etiology	0.015*
Treatment	0.13
Time after treatment	0.15

*Statistically significant