Relevance of IL-6 and MMP-9 to cerebral arteriovenous malformation and hemorrhage

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Abstract. The aim of the present study was to investigate the association of inflammatory cytokines and matrix metalloproteinase-9 (MMP-9) with cerebral arteriovenous malformation (AVM) and hemorrhage. A total of 31 AVM patients were divided into groups according to specimen sources; a ruptured group with 14 patients and an unruptured group with 17 patients. The control group comprised 30 epilepsy patients who underwent temporal lobectomy. Peripheral blood was obtained from the 30 control and all 31 AVM patients preoperatively. Tissue samples were removed from the AVM nidus during surgery or from the temporal lobes of patients undergoing surgical treatment for epilepsy. Enzyme-linked immunosorbent assay (ELISA) was used to measure plasma interleukin (IL)-6 levels. Western blot analysis was used to measure the levels of MMP-9 and transcription factors NF-κB and IκBα in the tissues. Immunofluorescence was used to measure tissue MMP-9 expression in each group. Gelatin zymography revealed the expression of activated MMP-2 and MMP-9 in the tissues. All the specimens were analyzed by routine hematoxylin and eosin (H&E) staining. IL-6 levels in the blood of the ruptured group were significantly higher compared with those of the unruptured and control groups (33.25±4.77 vs. 23.79±1.20, P<0.05; and 33.25±4.77 vs. 15.56±0.97, P<0.0001, respectively). NF-κB expression in the AVM ruptured group was significantly higher compared with that of the control group (5.00±0.12 vs. 2.36±0.33, P<0.05), but not with the unruptured group (5.00±0.12 vs. 2.96±0.69, P>0.05). The expression levels of IκBα in the ruptured and unruptured groups were similar to each other, but significantly less than those in the control group (0.12±0.02 vs. 1.27±0.06, P<0.001; and 0.45±0.15 vs. 1.27±0.06, P<0.01, respectively). MMP-9 protein expression levels in the unruptured group were increased compared with those in the control and ruptured groups (1.21±0.34 vs. 0.35±0.06, P<0.05; and 1.21±0.34 vs. 0.32±0.08, P<0.05, respectively). Gelatin zymography showed that the activity of MMP-9 was significantly higher in the ruptured compared with the unruptured and control groups (0.97±0.08 vs. 0.40±0.09, P<0.01; and 0.97±0.08 vs. 0.30±0.07, P<0.01, respectively). In the ruptured group, active MMP-2 expression levels were significantly higher compared with those in the other two groups (1.36±0.17 vs. 0.55±0.12, P=0.019; 1.36±0.17 vs. 0.36±0.09, P=0.006). The levels of IL-6 in the blood correlated with the tissue levels of activated MMP-9 (r=0.1691, P=0.0240). In conclusion, IL-6 expression levels were increased in the plasma of patients with cerebral AVM and this correlated with the activated MMP-9 levels of AVM tissues. Thus, plasma IL-6 levels are a potential predictor of hemorrhage risk in AVM patients.

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Introduction

Cerebral arteriovenous malformation (AVM) is a vascular disease that occurs primarily in the young. The most common clinical manifestations are hemorrhage, seizures and limb dysfunction. Hemorrhage is the main cause of death and disability in AVM patients and accounts for 30-86% of mortalities (1-4). At present, the causes and mechanisms of hemorrhage are unclear. In addition to hemodynamic factors, the inflammatory response is considered to be a major factor in hemorrhage. Recent studies on AVM have focused on matrix metalloproteinases (MMPs) and inflammatory factors (5-9). MMP-9 is a Zn^{2+}-dependent protease, the main functions of which are the degradation and remodeling of the extracellular matrix, which is closely associated with vascular remodeling and growth. MMP-9 expression levels increase in areas surrounding tumor-associated intracranial and cerebral AVM hemorrhages and with structural instability of the vessel walls in certain lesions, including cerebral and abdominal aortic aneurysms and carotid artery atherosclerosis. Excessive MMP-9 expression results in the degradation of the vascular matrix, which may weaken the vessel walls and cause blood vessels to rupture (10,11). Interleukin (IL)-6 has been associated with cardiovascular disease and its expression levels were shown to be significantly higher in hemorrhagic cerebral AVM tissues than in non-hemorrhagic tissues (5,6). In mouse brain tissues and human umbilical vein endothelial cells, IL-6 is

able to stimulate the expression and activity of MMP-9 (7). However, the association of plasma IL-6 and MMP-9 with hemorrhage in AVM has rarely been studied. Therefore, the present study aimed to explore the potential association of IL-6, NF-κB and MMP-9 with AVM hemorrhage.

Materials and methods

Materials. Tissue samples were collected from 31 AVM patients who underwent surgical treatment. The criteria to be allocated to the ruptured group were as follows: i) A history of hemorrhage before surgery, confirmed by computed tomography or magnetic resonance imaging; ii) a consistent hemorrhage and AVM location; iii) blood clot or hematoma was observed in the tissue during surgery; and iv) post-surgical pathology confirmed hemosiderin deposition. The criteria to be allocated to the unruptured group were as follows: i) No preoperative hemorrhage symptoms; ii) radiographic evidence showed no hemorrhaging; iii) no blood clot or hematoma was observed during surgery; and iv) no pathological hemosiderin deposition was noted after the surgery. Samples for the control group (n=31) were obtained from epilepsy patients undergoing temporal lobectomy (23-24). Blood samples were collected from all 31 AVM and 30 control patients during a normal physical examination. Consent was obtained from the patients and their families for the collection of specimens and blood. Patient information is presented in Table I.

Antibodies against human MMP-9 (cat. no. 3852), NF-κB p65 (no. 3034), IκBα (no. 9242) and β-actin (no. 4967) were obtained from Cell Signaling Technology, Inc. (Danvers, MA, USA). The human IL-6 enzyme-linked immunosorbent assay (ELISA) kit was purchased from Beijing 4A Biotech Co., Ltd. (CHE0009; Beijing, China). The MMP zymography assay kit (for MMP-2 and MMP-9) was purchased from China Puli Lai Gene Technology Co., Ltd. (P1700; China).

Table I. Characteristics of the patients with AVM.

	No. of patients (%)
Gender	
Male	17 (55)
Female	14 (45)
Hemorrhage	
Yes	14 (45)
No	17 (55)
Location	
Temporal	12 (39)
Occipital	7 (23)
Frontal	10 (32)
Other	2 (6)
Symptoms	
Headache	14 (45)
Epilepsy	9 (29)
Limb dysfunction	2 (6)
Other	5 (20)
Size (cm)	
<3	6 (19)
3-6	21 (68)
>6	4 (13)
Drainage	
Single	10 (32)
>1	21 (68)
Spetzler-Martin G	
1	8 (26)
2	10 (32)
3	11 (35)
4	2 (7)
5	0 (0)
With aneurysm	1 (3)

Immunofluorescence. Slice preparation, fixation and blocking was achieved in 5% bovine serum albumin at room temperature for 10 min, followed by washing in PBS (three times, 3 min each). Primary antibody incubation and rewarming was followed by secondary antibody incubation. Fluorescein isothiocyanate-conjugated secondary antibody was added dropwise. The sections were incubated at 37˚C in the dark for 30 min and then washed with PBS three times for 3 min each. The coverslips were then sealed and images were captured.

ELISA. Sample kits were prepared by adding samples to a plate and incubating at 37˚C for 90 min, after which the plate was washed four times (Beijing 4A Biotech Co., Ltd., CHE0009). Biotinylated antibody (100 µl) was added to the incubation solution. The plate was incubated at 37˚C for 60 min and then washed. Enzyme conjugate working solution (100 µl/well) was added and incubated at 37˚C for 60 min. After washing, color reagent (100 µl/well) was added. The plate was incubated in the dark at 37˚C for 10 min. A stop solution (100 µl/well) was added, each well was mixed and absorbance was measured immediately at 450 nm.

Gelatin zymography. Firstly, the positive control and test samples were pretreated. SDS-PAGE was run at a constant current of 30 mA for 45 min. After washing and incubation, Buffer B was discarded and Coomassie blue was added to stain the protein. The mixture was agitated in a horizontal shaker for 120 min. Bleaching solution (25% methanol, 10% acetic acid) was applied to the gel for 60 min. Band scanning was carried out by placing the gel on the scanner and scanning with non-transmitted light.

Western blot analysis. The vascular tissue total protein was isolated and an equal volume of 2X SDS sample buffer was added. After mixing, the sample was boiled at 100˚C in water for 10 min. After the samples were added, electrophoresis was carried out. Following gel-membrane transfer and blocking, primary antibody was used for an overnight incubation (dilution, 1:2000) at 4˚C. After incubation, the membrane was washed with PBS three times for 15 min each. Incubation with secondary antibody (1:1000 dilution) at room temperature for 1 h was followed by three 15-min washes in PBS. Band scanning was then carried out and all the specimens underwent routine hematoxylin and eosin (H&E) staining.

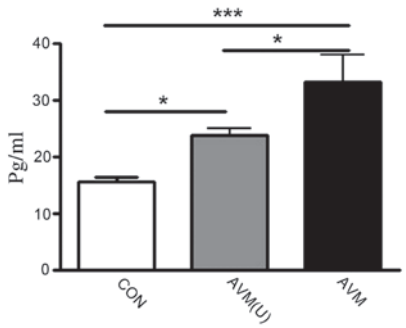

Figure 1. Plasma IL-6 ELISA results. IL-6 levels in the blood of the ruptured group were significantly higher than in the unruptured and control groups. *P<0.05; ***P<0.0001. CON, control group; AVM (U), unruptured group; AVM, ruptured group. AVM, arteriovenous malformation; IL, interleukin.

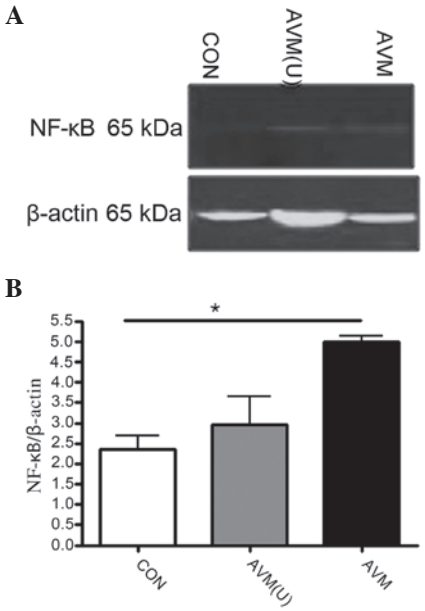

Figure 2. (A) NF-κB western blotting results. (B) NF-κB expression levels in the AVM ruptured group were significantly higher than in the control group. *P<0.05. CON, control group; AVM (U), unruptured group; AVM, ruptured group. AVM, arteriovenous malformation.

Statistical analysis. The results were expressed as the mean ± standard deviation. Experimental data were analyzed with GraphPad Prism 4.0 software. P<0.05 was considered to indicate a statistically significant difference.

Results

Plasma IL-6 level. Plasma samples were obtained during the surgical treatment of AVM patients and from healthy controls. In the plasma of the AVM ruptured group, the mean level of IL-6 was 33.25±4.77 pg/ml, which was significantly higher than that of the unruptured (23.79±1.20 pg/ml) and control groups (15.56±0.97 pg/ml; Fig. 1), indicating that the plasma IL-6 levels increased in the AVM ruptured group.

Tissue NF-κB and IκBα expression. Tissue samples were obtained from the surgical resection of AVM specimens. The specimens of the control group were obtained from the surgical resection of brain tissue vascular contusions in patients undergoing temporal lobectomy. The NF-κB levels in the AVM ruptured group (5.00±0.12 kDa) were not significantly different to those of the unruptured group (5.00±0.12 vs. 2.96±0.69; Fig. 2). The IκBα levels were similar in the ruptured and unruptured groups, however, they were significantly lower than those of the control group (0.12±0.02 vs. 1.27±0.06; and 0.45±0.15 vs. 1.27±0.06, respectively; Fig. 3). These data suggest that the nuclear transcription factor NF-κB was actively expressed in the AVM ruptured group.

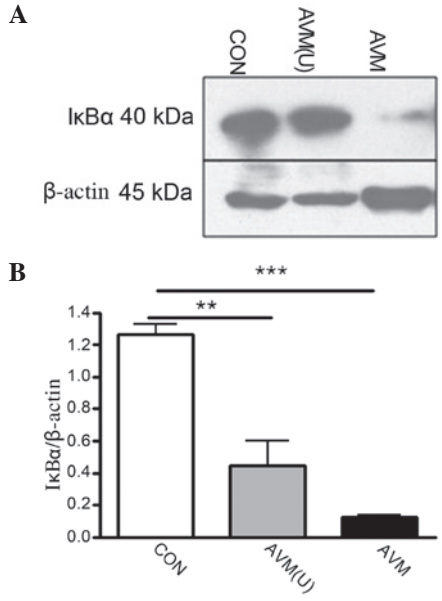

Figure 3. (A) IκBα western blotting results. (B) Expression levels of IκBα in the ruptured and unruptured groups were similar to each other, however, were significantly less than those in the control group. **P<0.01, ***P<0.001. CON, control group; AVM (U), unruptured group; AVM, ruptured group. AVM, arteriovenous malformation.

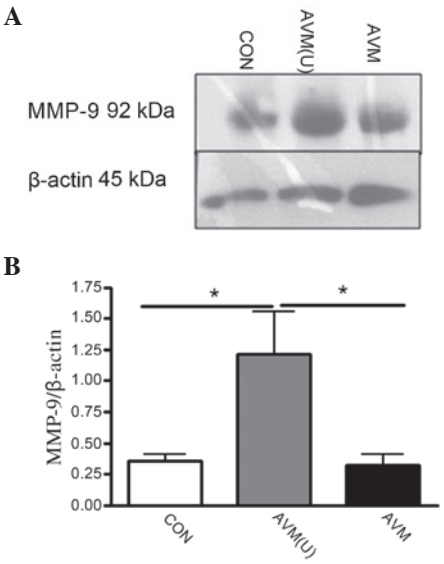

Figure 4. (A) MMP-9 western blotting results. (B) MMP-9 protein expression levels in the unruptured group were greater than those in the normal and ruptured groups. *P<0.05. CON, control group; AVM (U), unruptured group; AVM, ruptured group. AVM, arteriovenous malformation; MMP-9, matrix metalloproteinase-9.

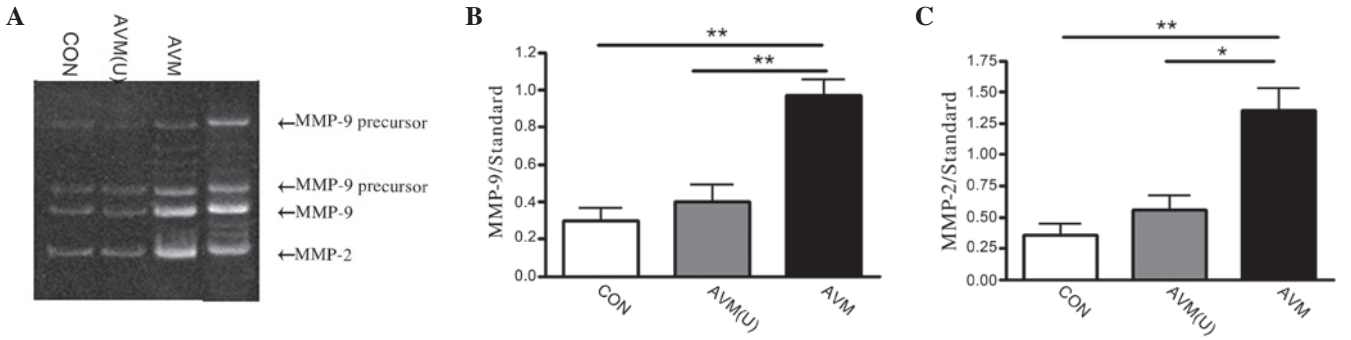

Figure 5. (A) MMP gelatin zymography results. (B and C) Gelatin zymography showed that the activity of MMP-9 was significantly higher in the ruptured than the unruptured and control groups. **P<0.01. CON, control group; AVM (U), unruptured group; AVM, ruptured group. AVM, arteriovenous malformation; MMP-9, matrix metalloproteinase-9.

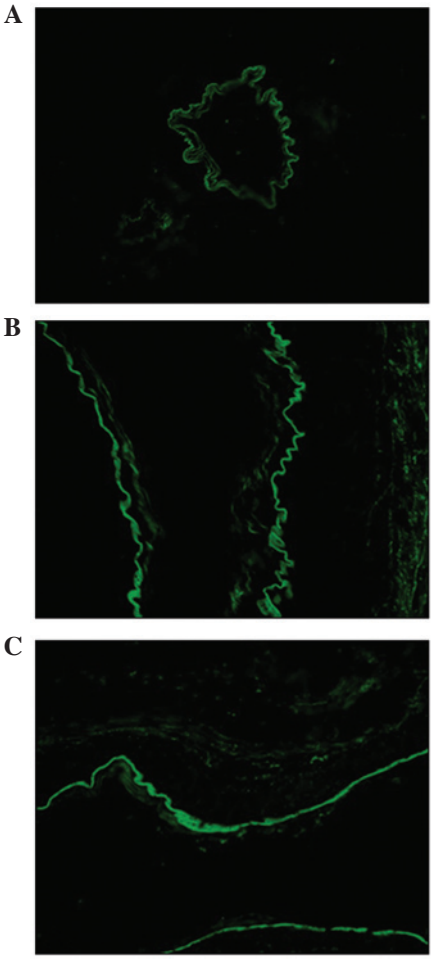

Figure 6. MMP-9 immunofluorescence results. (A) Control group, (B) AVM unruptured group and (C) AVM ruptured group. MMP-9 was expressed in endothelial cells, the extracellular matrix and vascular adventitia in the AVM ruptured and unruptured groups, whereas MMP-9 was only expressed in endothelial cells in the control group. MMP-9, matrix metalloproteinase-9; AVM, arteriovenous malformation.

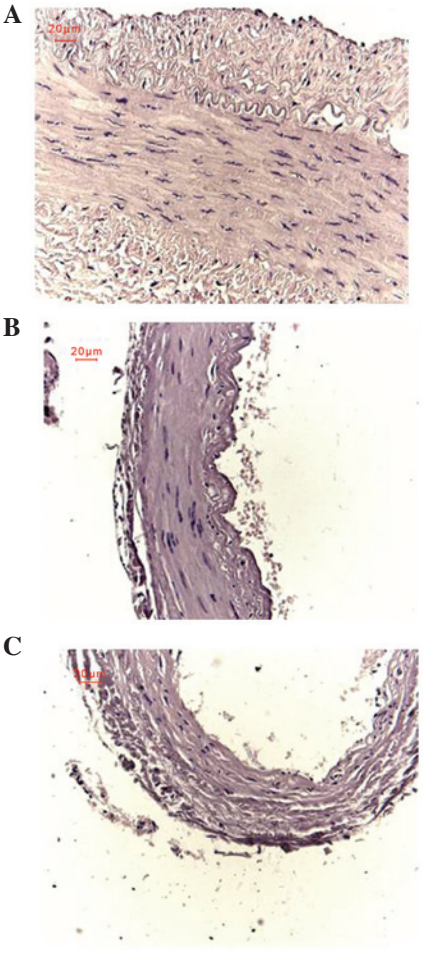

Figure 7. H&E staining results of the (A) control, (B) unruptured and (C) ruptured groups. The ruptured and unruptured AVM vessels had a disorderly structure of the smooth muscles, uneven wall thickness and partial/incomplete endothelial cells compared with the control vessels. H&E, hematoxylin and eosin; AVM, arteriovenous malformation.

MMP-9 protein level and activity. MMP-9 protein expression levels in the unruptured group were higher than those in the normal and ruptured groups (1.21±0.34 vs. 0.35±0.06; and 11.21±0.34 vs. 0.32±0.08, respectively; Fig. 4). The gelatin zymography assay indicated that MMP-9 activity in the ruptured group was significantly higher than in the unruptured and control groups (0.97±0.08 vs.0.40±0.09; and 0.97±0.08 vs. 0.30±0.07, respectively). The expression levels of MMP-2 in the ruptured group was significantly higher than those in the unruptured and control groups (1.36±0.17 vs. 0.55±0.12; and 1.36±0.17 vs. 0.36±0.09, respectively; Fig. 5), indicating that MMP-9 expression levels and activity

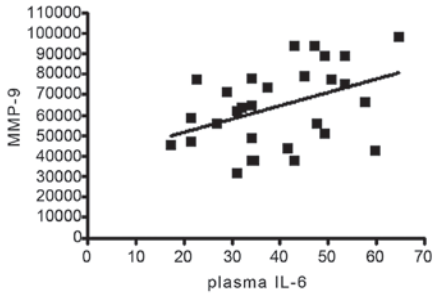

Figure 8. Plasma levels of IL-6 were correlated with the activated MMP-9 tissue protein levels, as demonstrated by zymography. The blood levels of IL-6 were associated with the tissue levels of activated tissue MMP-9 (r=0.1691, P=0.0240). IL, interleukin; MMP-9, matrix metalloproteinase-9.

are higher under AVM conditions than under normal conditions.

Tissue immunofluorescence. MMP-9 was expressed in endothelial cells, the extracellular matrix and vascular adventitia in the AVM ruptured and unruptured groups, whereas it was only expressed in endothelial cells in the control group. In the ruptured group, MMP-9 fluorescence was more intense and widespread than in the AVM tissues of the unruptured group (Fig. 6).

The ruptured and unruptured AVM vessels had a disorderly structure of the smooth muscles, uneven wall thickness and partial/incomplete endothelial cells compared with the control vessel (Fig. 7). The blood levels of IL-6 were correlated with the tissue levels of activated MMP-9 (r=0.1691, P=0.0240; Fig. 8).

Discussion

The present study has demonstrated that MMP-9 is activated in AVM tissues, which leads to abnormal vascular extracellular matrix degradation and increases the risk of cerebral AVM hemorrhage. Plasma IL-6 levels were significantly increased in AVM patients and this correlated with tissue-activated MMP-9 protein levels, indicating that plasma IL-6 concentration level changes may be an index for brain AVM hemorrhage.

Hemorrhage is a major cause of death and disability in cerebral AVM. H&E staining demonstrated that the ruptured and unruptured AVM vessels had a disorderly structure of the smooth muscles, uneven wall thickness and partial/incomplete endothelial cells compared with the control vessels (Fig. 7). Hemorrhages are caused by hemodynamic effects, however, the local inflammatory response and angiogenic processes are also important factors. The blood vessels near AVMs often experience inflammatory cell infiltration (8) and MMPs are important in local inflammatory responses.

AVM has historically been associated with angiogenic factors, including vascular endothelial growth factor (VEGF), CD34 and CD31 (12,13); however, MMPs have been the main focus in recent studies. MMPs are a group of Zn^{2+}- and Ca^{2+}-dependent proteases that modify the extracellular matrix. Their substrates include collagen, fibronectin, laminin and proteoglycans. Under normal circumstances, MMPs exist in an inactive zymogene form. After activation by plasminogens, they are rapidly degraded, which ensures a low level of active MMPs in tissues. MMP-9 and -2 are important in neovascularization and the remodeling and degradation of the extracellular matrix. Activated MMP-9 and -2 are able to degrade extracellular matrix components, including collagen IV, resulting in damage to the stability and integrity of blood vessels and a weakened blood-brain barrier, which leads to an increased risk of hemorrhage (9,14,15). MMP-9 or -2 activity also promotes a loose connection between vascular endothelial cells, causing damage to vascular smooth muscle and adventitia. We demonstrated that the MMP-9 total protein level in the unruptured group was significantly higher than that in the ruptured and control groups. Gelatin zymography showed that MMP-9 expression levels in the ruptured group were significantly higher than those in the unruptured and control groups, suggesting that more MMP-9 was activated and released in the form of active plasminogen in the ruptured group. The MMP-9 tissue immunofluorescence results showed that MMP-9 was expressed in the extracellular matrix and vascular adventitia in AVM patients, whereas it was only present in the endothelial cells in the control group. Furthermore, the distribution and fluorescence intensity of MMP-9 in the ruptured group were greater than in the unruptured group. This suggests that more MMP-9 was secreted and activated in ruptured AVM tissue, allowing it to degrade the extracellular matrix and basement membrane, resulting in damage to the stability and integrity of blood vessels and increasing the risk of AVM hemorrhage.

Findings of recent studies have shown that AVM patients with subarachnoid hemorrhage expressed higher levels of IL-6 protein in tissue compared with patients without rupture (6). IL-6 protein levels have been associated with the IL6 174GG genotype (16-20), which may be an upstream promoter in the angiogenic cascade (12,21-24). By contrast, other studies have shown that lower IL-6 levels exist in abdominal aortic aneurysm and an increased IL-6 level was associated with a lower incidence of ischemic events in giant cell arteritis (25-27). In the present study, we compared the plasma levels of IL-6 in the control, ruptured and unruptured groups and showed that IL-6 levels were significantly higher in AVM groups than in the control group. Additionally, IL-6 levels in the ruptured group were significantly higher than in the unruptured group. This result was consistent with previous observations that IL-6 may be associated with vascular disease. Chen *et al* (27) showed that IL-6 induced MMP-3 and -9 expression and activity in the mouse brain and increased the proliferation and migration of cerebral endothelial cells in AVM. IL-6 mRNA levels were associated with MMP-3 and -9 mRNA levels (27,28). These studies suggest an association between IL-6 and AVM. Therefore, to further investigate the association between IL-6 and hemorrhage in AVM, we examined the plasma IL-6 concentration and analyzed the correlation between the plasma level of IL-6 and activated MMP-9 in AVM tissues. Analysis of tissue MMP-9 protein levels and the plasma IL-6 level using linear regression identified that the plasma levels of IL-6 were correlated with the tissue levels of activated MMP-9 (r=0.1691, P=0.0240), as shown in Table I. This result was in collaboration the with outcome of a study by Chen *et al* (27), mentioned above. All these results appear to suggest plasma IL-6 level as a predictor

of hemorrhage risk. Results from the study carried out by Kim *et al* showed that tumor necrosis factor-α and IL-1 may also be associated with AVM hemorrhage (29,30). In this study, we also observed NF-κB protein expression in each group. NF-κB expression in the ruptured group was significantly higher than in the unruptured and control groups. IκBα, an inhibitor of NF-κB, was expressed at lower levels in the ruptured and unruptured groups than in the control group; this exhibits the same trend as IL-6.

Taken together, IL-6 levels in the plasma of patients with AVM showed similar increases regardless of whether they were in the ruptured or unruptured group and were significantly higher than those in the control group. Considering the hemorrhagic predicting characteristic of MMP-9, the correlation of plasma IL-6 and tissue MMP-9 and the significant differences in plasma IL-6 levels between the control, ruptured and unruptured groups, our study was consistent with the hypothesis that IL-6 is associated with the MMP-9 level and hemorrhage in AVM. Therefore, plasma IL-6 may offer a therapeutic intervention for cerebral AVM and is a possible predictor of hemorrhage risk.

There were limitations in the present study. Quantitative measures should have been carried out on the H&E staining and immunofluorescence to increase the relevance of results. Furthermore, future studies are required to explore the plasma levels of IL-6 in patients before surgery and after, for one day, one week and one month until three months, to define the variation in IL-6 levels during this time for AVM patients.

References

1. ApSimon HT, Reef H, Phadke RV and Popovic EA: A population-based study of brain arteriovenous malformation: long-term treatment outcomes. Stroke 33: 2794-2800, 2002.
2. Crawford PM, West CR, Chadwick DW and Shaw MD: Arteriovenous malformations of the brain: natural history in unoperated patients. J Neurol Neurosurg Psychiatry 49: 1-10, 1986.
3. Fults D and Kelly DL Jr: Natural history of arteriovenous malformations of the brain: a clinical study. Neurosurgery 15: 658-662, 1984.
4. Martin NA and Vinters HV: Arteriovenous malformations. In: Neurovascular Surgery. Carter LP, Spetzler RF and Hamilton MG (eds). McGraw-Hill, New York, pp875-903, 1994.
5. Chen Y, Pawlikowska L, Yao JS, *et al*: Interleukin-6 involvement in brain arteriovenous malformations. Ann Neurol 59: 72-80, 2006.
6. Okazaki S, Furukado S, Abe Y, *et al*: Association of inflammatory markers and carotid intima-media thickness with the risk of cardiovascular events in high-risk patients. Cerebrovasc Dis 30: 180-187, 2010.
7. Chen Y, Zhu W, Bollen AW, *et al*: Evidence of inflammatory cell involvement in brain arteriovenous malformations. Neurosurgery 62: 1340-1350, 2008.
8. Fishman D, Faulds G, Jeffery R, *et al*: The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 102: 1369-1376, 1998.
9. Feiler S, Plesnila N, Thal SC, *et al*: Contribution of matrix metalloproteinase-9 to cerebral edema and functional outcome following experimental subarachnoid hemorrhage. Cerebrovasc Dis 32: 289-295, 2011.
10. Loftus IM, Naylor AR, Goodall S, *et al*: Increased matrix metalloproteinase-9 activity in unstable carotid plaques. A potential role in acute plaque disruption. Stroke 31: 40-47, 2000.
11. Chyatte D and Lewis I: Gelatinase activity and the occurrence of cerebral aneurysms. Stroke 28: 799-804, 1997.
12. Sandalcioglu IE, Asgari S, Wende D, *et al*: Proliferation activity is significantly elevated in partially embolized cerebral arteriovenous malformations. Cerebrovasc Dis 30: 396-401, 2010.
13. Choi EJ, Walker EJ, Shen F, *et al*: Minimal homozygous endothelial deletion of Eng with VEGF stimulation is sufficient to cause cerebrovascular dysplasia in the adult mouse. Cerebrovasc Dis 33: 540-547, 2012.
14. Terry CF, Loukaci V and Green FR: Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. J Biol Chem 275: 18138-18144, 2000.
15. Rundek T, Elkind MS, Pittman J, *et al*: Carotid intima-media thickness is associated with allelic variants of stromelysin-1, interleukin-6, and hepatic lipase genes: the Northern Manhattan Prospective Cohort Study. Stroke 33: 1420-1423, 2002.
16. Jones KG, Brull DJ, Brown LC, *et al*: Interleukin-6 (IL-6) and the prognosis of abdominal aortic aneurysms. Circulation 103: 2260-2265, 2001.
17. Brull DJ, Leeson CP, Montgomery HE, *et al*: The effect of the Interleukin-6-174G>C promoter gene polymorphism on endothelial function in healthy volunteers. Eur J Clin Invest 32: 153-157, 2002.
18. Acalovschi D, Wiest T, Hartmann M, *et al*: Multiple levels of regulation of the interleukin-6 system in stroke. Stroke 34: 1864-1869, 2003.
19. Hernández-Rodríguez J, Segarra M, Vilardell C, *et al*: Elevated production of interleukin-6 is associated with a lower incidence of disease-related ischemic events in patients with giant-cell arteritis: angiogenic activity of interleukin-6 as a potential protective mechanism. Circulation 107: 2428-2434, 2003.
20. Hashimoto T, Lawton MT, Wen G, *et al*: Gene microarray analysis of human brain arteriovenous malformations. Neurosurgery 54: 410-425, 2004.
21. Aoki Y, Jaffe ES, Chang Y, *et al*: Angiogenesis and hematopoiesis induced by Kaposi's sarcoma-associated herpesvirus-encoded interleukin-6. Blood 93: 4034-4043, 1999.
22. Gaudino M, Andreotti F, Zamparelli R, *et al*: The-174G/C interleukin-6 polymorphism influences postoperative interleukin-6 levels and postoperative atrial fibrillation. Is atrial fibrillation an inflammatory complication? Circulation 108 (Suppl 1): II195-II199, 2003.
23. Pola R, Flex A, Gaetani E, *et al*: Synergistic effect of -174G/C polymorphism of the interleukin-6 gene promoter and 469 E/K polymorphism of the intercellular adhesion molecule-1 gene in Italian patients with history of ischemic stroke. Stroke 34: 881-885, 2003.
24. Flex A, Gaetani E, Pola R, *et al*: The -174 G/C polymorphism of the interleukin-6 gene promoter is associated with peripheral artery occlusive disease. Eur J Vasc Endovasc Surg 24: 264-268, 2002.
25. Sternlicht MD and Werb Z: How matrix metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol 17: 463-516, 2001.
26. Brinckerhoff CE and Matrisian LM: Matrix metalloproteinases: a tail of a frog that became a prince. Nat Rev Mol Cell Biol 3: 207-214, 2002.
27. Chen Y, Pawlikowska L, Yao JS, *et al*: Interleukin-6 involvement in brain arteriovenous malformations. Ann Neurol 59: 72-80, 2006.
28. Hashimoto T, Wu Y, Lawton MT, *et al*: Coexpression of angiogenic factors in brain arteriovenous malformations. Neurosurgery 56: 1058-1065, 2005.
29. Achrol AS, Pawlikowska L, McCulloch CE, *et al*: Tumor necrosis factor-alpha-238G>A promoter polymorphism is associated with increased risk of new hemorrhage in the natural course of patients with brain arteriovenous malformations. Stroke 37: 231-234, 2006.
30. Kim H, Hysi PG, Pawlikowska L, *et al*: Common variants in interleukin-1-Beta gene are associated with intracranial hemorrhage and susceptibility to brain arteriovenous malformation. Cerebrovasc Dis 27: 176-182, 2009.