Role of vascular endothelial growth factor and matrix metalloproteinase-9 in peritumoral brain edema associated with supratentorial benign meningiomas

Running Title: Peritumoral Brain Edema with Meningiomas

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Abstract

**Background:** Accumulating evidence indicates that vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9) play a central role in the development of peritumoral brain edema (PTBE) associated with human brain tumors. However, the roles of these proteins, particularly of MMP-9, in PTBE associated with benign meningiomas have not been elucidated.

**Objective:** We investigated the association between clinical features and biological factors, such as VEGF and MMP-9, and the incidence of PTBE and edema index (EI) in 60 patients with benign meningiomas.

**Methods:** In this study, supratentorial lesions were examined for evaluating the extent of PTBE in the surrounding normal brain tissue. VEGF and MMP-9 expression was immunohistochemically examined.

**Results:** Multivariate analysis revealed that the presence of pial blood supply (odds ratio, 12.250; \( P = .0096 \)) and VEGF (odds ratio, 7.683; \( P = .0155 \)), but not MMP-9 (odds ratio 1.178; \( P = .8113 \)), expression are significant factors that independently predict the incidence of PTBE and influence EI. VEGF (\( P = .0397 \)) and MMP-9 (\( P = .0057 \)) expression correlates with the presence of pial blood supply. Moreover, tumors with high VEGF and MMP-9 expression had higher EIs than those with high expression of either (\( P = .030 \)).

**Conclusion:** Our findings suggest indicate that MMP-9 expression was positively related to
VEGF expression and pial blood supply and promoted the occurrence of PTBE by inducing the disruption of the arachnoid membrane and formation of pial blood supply.

**Keywords:** matrix metalloproteinase-9, meningioma, pial blood supply, peritumoral brain edema, vascular endothelial growth factor.

**Introduction**

Approximately 60% human meningiomas exhibit peritumoral brain edema (PTBE), which may be responsible for clinical symptoms such as headache and vomiting, and may be related to clinical outcome.\(^1\) To date, various possible causes of PTBE have been reported, which includes tumor size,\(^1\) location,\(^1,4\) histological differentiation,\(^3-9\) vascular density,\(^10\) pial blood supply,\(^2,3,10,11\) tumor-related venous obstruction,\(^12\) sex hormone receptors,\(^13,14\) vascular endothelial growth factor (VEGF) expression,\(^10,11,15-18\) and its receptor.\(^19\) Recently, matrix metalloproteinase-2 (MMP-2) and MMP-9 expression in meningiomas has been well studied in relation to tumor invasiveness, PTBE, malignancy, and recurrence.\(^20-23\)

MMP-2 and MMP-9 are gelatinases that can degrade basement membrane-type collagen.\(^24-26\) At least 23 different MMPs are found in humans. These zinc-dependent endopeptidases degrade extracellular matrix components and are regulated on at least three levels: transcription, proteolytic activation of the zymogen, and inhibition of the active enzyme. Tissue inhibitors of metalloproteinases (TIMPs) are known to be the major endogenous regulators of MMPs in tissues.\(^24-26\) MMPs have been suggested to play an important role in tumor invasion and the metastasis of systemic cancers.\(^27,28\) In brain tumors,
particularly in malignant gliomas, MMPs have been well studied.\textsuperscript{29-32} In recent studies, higher MMP-9 expression in meningiomas has been shown to correlate with tumor invasiveness.\textsuperscript{21} In PTBE associated with meningiomas, recent studies have shown that higher MMP-9 expression, but not MMP-2, correlates with PTBE.\textsuperscript{21,22}

In recent studies, higher MMP-9 (\textsuperscript{33} von Randow AJ) or VEGF (reference \textsuperscript{34}) expression has been shown to correlate with PTBE in meningioma with higher pathological grades. However, limiting to benign pathologies, meningiomas differ in their degree of PTBE.\textsuperscript{33,34}

While the expression of biological factors such as VEGF and MMP-9 has been well studied in relation to pathological grades and PTBE associated with meningiomas, how these factors, particularly MMP-9, influence PTBE in benign meningioma or other significant factors for PTBE has not been well completely elucidated. Therefore, in the present study, we analyzed the association between clinical features and biological factors in supratentorial benign meningiomas using tissues that were surgically resected from 60 patients.

Methods

Patients and Specimens

Meningioma samples (n = 60, from 60 patients) were obtained from Okayama University Hospital (Okayama, Japan). The tumors were from patients who underwent surgery between 1989 and 2004. Informed consent was obtained in all cases. All specimens were from primary tumors that were classified and graded according to the 2007 World Health Organization (WHO) classification criteria (reference \textsuperscript{35}). The location of tumors
was limited to the supratentorial region and was categorized as convexity, falx, and parasagittal lesions for accurate estimation of the extent of edema and to ensure the uniformity of the surrounding tissues.

**Evaluation of Neuroradiological Imaging**

Before surgery, each patient’s tumor and any PTBE were assessed using magnetic resonance imaging (MRI). Tumor size was estimated from gadolinium-enhanced T1-weighted images. PTBE was evaluated on T2-weighted images. The tumor and PTBE were approximated from axial, coronal, and sagittal images as follows: the maximal perpendicular diameters (2a and 2b) of the tumor and PTBE were measured on axial scans, and the extent in the coronal direction (2c) was measured on coronal or sagittal scans. The total volume of the tumor and PTBE were then approximated using the formula for a spheroid \( V = \frac{4}{3} \pi abc \). The relationship between the tumor and PTBE volume, i.e., edema index (EI) was defined as follows: \( EI = \frac{V_{\text{edema}} + V_{\text{tumor}}}{V_{\text{tumor}}} \). The formula yields a value of 1 when edema is absent. Pial-cortical arterial supply was identified by angiography in the anteroposterior and lateral views of the external and internal carotid arteries (vertebral artery) without prior knowledge of the MRI findings.

**Immunohistochemical Analysis**

Tumor sections (4.5 \( \mu m \)) were deparaffinized in xylene, rehydrated through graded alcohols, and immersed for 15 min in phosphate-buffered saline (PBS). For antigen retrieval, all sections were microwaved in 0.01 M citrate-buffered solution (pH 6.0) for 15 min. Endogenous peroxidase was inactivated by treatment with 3% hydrogen peroxide in
methanol for 15 min. The sections were incubated in normal horse serum for 15 min to block non-specific staining. A mouse monoclonal antibody against VEGF (BD PharMingen, San Diego, CA) or MMP-9 (Daiichi Fine Chemical, Toyama, Jpn) diluted to 1:100 in DAKO Antibody Diluent (DAKO, Carpinteria, CA) was applied. Incubations were performed in a humidity chamber at 4 °C overnight. As a negative control, mouse non-immune IgG was applied as the primary antibody. After three rinses with PBS, diluted biotinylated horse anti-mouse antibody (Vector Laboratories, Burlingame, CA) was applied for 30 min. Following three additional washes with PBS, avidin–biotin complex (Vector Laboratories) was applied for 30 min, followed by 0.06% diaminobenzidine (Sigma, St. Louis, MO) with 0.01% hydrogen peroxide for 4 min. The sections were counterstained with hematoxylin.

VEGF and MMP-9 expression was qualitatively scored as follows: 0 for no staining (nearly 0% of cells were labeled), 1+ for a trace of positive cells (less than 30% of cells were labeled), 2+ for moderately diffuse staining or sparsely intensive staining (30%–60% of cells were labeled, and less than 30% of cells were labeled with the strong intensity), and 3+ for strongly diffuse staining (60%–100% of cells were labeled).

E.I. and S.O. classified the tumors according to 3 representative fields without prior knowledge of the patients’ clinical or radiological data. Staining of VEGF and MMP-9 was classified into two groups according to the scores. Lower 2 scores, 0 and 1+, were classified as the low-expression group and higher 2 scores, 2+ and 3+, were classified as the high-expression group.

Statistical Analysis
Database management and statistical analysis were performed using the SAS software. Univariate and multivariate logistic analyses tested for edema using the following predictive variables: age, sex, pial blood supply, and VEGF and MMP-9 expression. The results were expressed as estimated coefficients, $P$ values, and odds ratios (95% confidence intervals) for each variable. The tumor volume was analyzed by single linear regression analysis with EI as the dependent variable. The relationship between EI and clinical and biological variables, tumor volume, VEGF expression, and MMP-9 expression in relation to pial blood supply were evaluated by the Mann–Whitney U test. The relationship between VEGF expression and MMP-9 expression was evaluated using the Spearman rank correlation coefficient. The relationships between EI and histology, location, and the groups divided by the combination of VEGF expression and MMP-9 expression were evaluated by the Kruskal–Wallis test. $P < .05$ was considered statistically significant.

**Results**

*Patient Characteristics*

Characteristics of the 60 patients included in this study are shown in Table 1. There were 16 males (26.7%) and 44 females (73.3%), with a mean age of 55.3 years (range, 22–81 years). All tumors were classified according to the WHO criteria; 14 meningothelial, 11 fibrous, 27 transitional, 2 psammomatous, 1 angiomatous, 3 microcystic, and 2 secretory meningiomas. The tumor locations were classified as supratentorial: 33 convexity, 16 parasagittal, and 11 falx.
Immunohistochemical Staining

The anti-human VEGF monoclonal antibody was immunoreactive, with evidence of cytoplasmic staining, in 48 cases (80%). Using our expression grading system, the tumor sections were classified as follows: 12 as 0, 27 as 1+, 16 as 2+, and 5 as 3+. The anti-human MMP-9 monoclonal antibody was immunoreactive, with evidence of cytoplasmic staining, in all cases. Using our grading system, the sections were classified as follows: 31 as 1+, 20 as 2+, and 9 as 3+ (Figure 1) (Table 2).

Univariate and Multivariate Logistic Regression Analyses

To evaluate the factors influencing the incidence of PTBE, univariate and multivariate logistic regression analyses were performed (Table 3). The presence of pial blood supply and high VEGF expression were independent predictors of PTBE. Tumor volume showed a tendency toward predicting the incidence of PTBE in univariate analysis, but was not a statistically significant factor in multivariate analysis. Age, sex, and high MMP-9 expression were not statistically significant ($P > .05$) predictors for the incidence of PTBE.

Correlation of Variables with Edema Index

We evaluated the development of PTBE using EI (Figure 2). Tumor volume was not significantly correlated with EI ($P = .1094$). Pial blood supply was associated with a higher EI (EI = 6.720 ± 3.120 versus EI = 2.672 ± 0.837; $P = .0012$). EI was significantly higher in the high-VEGF group than in the low-VEGF group (EI = 5.148 ± 1.607 versus EI = 3.683 ±
1.834; \( P = .0007 \)). The high-MMP-9 group showed a tendency toward a higher EI that was not significant (EI = 5.964 ± 2.532 versus EI = 2.542 ± 0.878; \( P = .062 \)). As potential factors, histology and location were also evaluated. No significant difference in VEGF and MMP-9 expression, pial blood supply, or EI was observed between histological subtypes (Table 4) (meningothelial meningiomas, EI = 6.142 ± 17.928; fibrous meningiomas, EI = 5.067 ± 8.189; transitional meningiomas, EI = 2.666 ± 6.031; psammomatous meningiomas, EI = 1.526 ± 0.744; angiomatous meningiomas, EI = 4.617; microcystic meningiomas, EI = 2.731 ± 2.922; secretory meningiomas, EI = 11.332 ± 6.172; \( P = .3062 \)). No significant difference was observed between locations (convexity, EI = 5.469 ± 12.841; parasagittal, EI = 2.986 ± 6.586; falx EI = 2.135 ± 2.164; \( P = .8349 \)). Furthermore, location over the convexity was classified into frontal, parietal, temporal, and occipital, where the tumor mainly attached. Between these locations, there was no significant difference (frontal, EI = 3.977 ± 11.389; parietal, EI = 4.388 ± 7.498; temporal, EI = 3.560 ± 4.765; occipital, EI = 5.212 ± 11.079; \( P = .7446 \)).

**Correlation of the Presence of Pial Blood Supply with Other Variables**

Because the presence of pial blood supply was identified as a clinical factor that independently predicts the incidence of PTBE and influences the development of PTBE, we analyzed the correlations between the presence of pial blood supply and other factors (Figure 3). We observed that the presence of pial blood supply was significantly correlated with higher tumor volume (tumor volume = 38.014 ± 6.405 versus tumor volume = 16.756 ± 2.932; \( P = .0008 \)), high VEGF expression (VEGF score = 1.565 ± 0.207 versus VEGF score...
We present a representative case showing high EI in the large convexity meningioma having high VEGF and MMP-9 expression (Figure 4). A large, homogeneously enhancing tumor on gadolinium-enhanced T1-weighted images (Figure 4A) was found in 52 year-old female patients. A remarkable PTBE was seen on T2-weighted images (Figure 4B). Partial tumor staining from meningeal arteries was shown by left internal carotid angiogram (Figure 4C) and external carotid angiogram (Figure 4D). Pial blood supply from a branch of left anterior cerebral artery was also shown by left internal carotid angiogram (Figure 4C). Microscopic examination of hematoxylin and eosin staining revealed transitional meningioma (Figure 4E). Immunohistochemical staining for VEGF (figure 4F) and MMP-9 (Figure 4G) were positive. VEGF and MMP-9 score were 3+ and 3+, respectively.

Relationship between VEGF and MMP-9

We analyzed the relationship between VEGF expression and MMP-9 expression (Figure 5). MMP-9 expression showed a positive relation with VEGF expression \( (P = .0479) \) (Figure 5A). Furthermore, tumors with high VEGF and MMP-9 expression showed a higher mean EI than those with low expression of either. Significant differences were observed between each group \( (P = .030) \) (Figure 5B).

Discussion

In this study, VEGF and MMP-9 expression varied in 60 benign supratentorial...
meningiomas. Multivariate analysis revealed that the presence of pial blood supply and VEGF expression were significantly correlated with the incidence of PTBE and a higher EI. A trend towards correlation between MMP-9 and EI was found, although statistical significance was not reached. VEGF and MMP-9 expression was correlated with the presence of pial blood supply. Moreover, the probability of PTBE and a higher EI was greater in tumors with high VEGF and MMP-9 expression than in those with low expression of either. Although various possible causes of PTBE have been reported, such as tumor size, location, and histological subtypes, we could not detect other potential factors in this study. To address this, further studies with more samples of each group are needed.

We found that pial blood supply and high VEGF expression were significant factors that independently predicted the incidence of PTBE and significantly correlated with EI consistent with several previous studies. However, these factors could play a different role in PTBE formation associated with meningiomas when combined. Bitzer et al. and Sindou et al. showed a correlation between pial blood supply and tumor size. Vascular enrichment has been reported as a potent factor for the development of PTBE. Furthermore, Pitolesi et al. demonstrated that VEGF expression is related to MVD and pial blood supply. We demonstrated that MMP-9 expression correlates with the presence of pial blood supply. In previous studies, MMP-9 expression was higher in non-benign meningomas than in benign meningiomas and correlated with severe edema, brain invasion, and tumor recurrence. Several studies have indicated that MMP-9 expression in benign meningiomas varies irrespective of the scoring method used. Meningiomas are
located in the extra-axial space and separated from the brain parenchyma by the arachnoid membrane and pia mater. Since the mechanism of development of PTBE is vasogenic, breakdown of the blood–brain barrier or direct contact of the tumor with the brain parenchyma is essential for the development of PTBE. The presence of pial blood supply reflects the disruption of the arachnoid membrane in the brain cortex that is formed of two layers of cells interrupted by a layer of basement membrane. There are several possible explanations for the relationship between pial blood supply and MMP-9 expression. First, MMP-9 expressed in meningiomas could play a role in the invasion of meningioma cells into the arachnoid membrane and its disruption for the development of PTBE. The correlation between MMP-9 expression and focal invasion of benign lesions has been previously demonstrated. Mizoue et al. reported that MMP-9 expression contributed to the infiltration of the dura mater by meningioma cells. Nordqvist et al. analyzed the expression of MMP-9 in meningiomas associated with different degrees of brain invasion and edema. They demonstrated a relation between the extent of MMP-9 expression and arachnoid disruption and brain invasion. Second, MMP-9 could promote angiogenesis in brain parenchyma. The presence of pial blood supply could be the result of MMP-9-activated angiogenesis that is promoted by the invasion of the extracellular matrix by microvascular endothelial cells and the increased bio-availability of VEGF. Barresi et al. found that a trend towards correlation between MMP-9 and VEGF expression in 50 cases of meningiomas. We showed a positive correlation between VEGF expression and MMP-9 expression in meningiomas. In some cases of our series, the presence of pial blood supply and high MMP-9 expression with some degree of VEGF expression showed a high EI even in the small tumor.
On the other hand, in the larger tumor having low VEGF and MMP-9 expression, PTBE and pial blood supply were not observed. These findings imply that tumor having some degree of VEGF and MMP-9 expression may possess a high EI, regardless of tumor size. Moreover, the presence of pial blood supply, signifying the disruption of the arachnoid membrane, could be the visible result of high MMP-9 expression. These results were consistent with our statistical analyses.

In conclusion, we analyzed VEGF and MMP-9 expression and significant factors which may contribute to PTBE in benign supratentorial meningiomas. Our findings suggest that MMP-9 expression was positively related to VEGF expression and pial blood supply and promoted the occurrence of PTBE by inducing the disruption of the arachnoid membrane and formation of pial blood supply.

Acknowledgements

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References


16. Goldman CK, Bharara S, Palmer CA et al. Brain edema in meningiomas is associated


**Figure Legends**

**Figure 1.**
Representative immunostainings of VEGF (upper column) and MMP-9 (lower column). Each microscopic pictures show negative (-), weakly positive (+), moderately positive (2+), and strongly positive (3+) immunostaining of each proteins (200 × magnification). Scale bar = 100μm.

**Figure 2.**
The relationships between edema index (EI) and tumor volume (A), pial blood supply (B), VEGF expression (C), and MMP-9 expression (D). Both pial blood supply and high VEGF expression were significantly related to EI.

**Figure 3.**
The relationships between pial blood supply and tumor volume (A), VEGF score (B), and MMP-9 score (C). These factors significantly related to the existence of pial blood supply.

**Figure 4.**
A representative case of a meningioma having high tumor volume and marked edema. The gadolinium-enhanced T1-weighted image (A) and T2-weighted image (B) demonstrate a strong enhanced convexity meningioma with remarkable peritumoral brain edema. Lateral view of internal carotid (C) and external carotid (D) angiogram showed meningeal arterial supply to the center of convexity meningioma (arrow), and pial blood supply from anterior
cerebral artery to the rim of the tumor (arrowhead). Microscopic examination of hematoxylin and eosin staining revealed transitional meningioma (E). VEGF (F) and MMP-9 (G) expression was high in this tumor. Scale bar = 100μm.

Figure 5.

The relationship between VEGF expression and MMP-9 expression (A) and the relationship between edema index (EI) and the groups divided by the combination of VEGF expression and MMP-9 expression (B). The significant correlation between VEGF expression and MMP-9 expression was found. Moreover, the coexpression of VEGF and MMP-9 was positively related to EI.
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<thead>
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<td>Age</td>
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<td></td>
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<td>Sex</td>
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</tr>
<tr>
<td></td>
<td>Female 44 (73.3%)</td>
</tr>
<tr>
<td>Location</td>
<td>Convexity 33 (55.0%)</td>
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<td></td>
<td>Falx 11 (18.3%)</td>
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<tr>
<td></td>
<td>Parasagittal 16 (26.7%)</td>
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<tr>
<td>Histology</td>
<td>Meningothelial 14 (23.3%)</td>
</tr>
<tr>
<td></td>
<td>Fibrous 11 (18.3%)</td>
</tr>
<tr>
<td></td>
<td>Transitional 27 (45.0%)</td>
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<td></td>
<td>Psamommatous 2 (3.3%)</td>
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<td></td>
<td>Angiomatous 1 (1.7%)</td>
</tr>
<tr>
<td></td>
<td>Microcystic 3 (5.0%)</td>
</tr>
<tr>
<td></td>
<td>Secretory 2 (3.3%)</td>
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<tr>
<td>Tumor volume</td>
<td>Mean±SD 24.9±25.6</td>
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<td></td>
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<tr>
<td>Edema volume</td>
<td>Mean±SD 26.2±41.8</td>
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<td></td>
<td>Range 0-208.9</td>
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<tr>
<td>Pial blood supply</td>
<td>Negative 37 (61.7%)</td>
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<tr>
<td></td>
<td>Positive 23 (38.3%)</td>
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<td>Tumor and edema volume (cm³)</td>
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Table 2  Summary of immunoreactivity for 60 samples of benign meningiomas

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<tr>
<th>Protein</th>
<th>Low expression</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
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<td>VEGF</td>
<td>12</td>
<td>27</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>MMP-9</td>
<td>0</td>
<td>31</td>
<td>20</td>
<td>9</td>
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Table 3
Univariate and multivariate analysis of the factors causing peritumoral brain edema associated with meningiomas

<table>
<thead>
<tr>
<th>Factors</th>
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<th>Multivariate</th>
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<tr>
<td></td>
<td>ORa</td>
<td>95%CIb</td>
<td>P</td>
<td>ORa</td>
<td>95%CIb</td>
<td>P</td>
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<td>Age (10 years)</td>
<td>1.081</td>
<td>0.751-1.557</td>
<td>0.6759</td>
<td>1.304</td>
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<td>Sex (female)</td>
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<td>0.244-2.556</td>
<td>0.6933</td>
<td>1.605</td>
<td>0.355-7.264</td>
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<tr>
<td>Tumor volume (10cm³)</td>
<td>1.291</td>
<td>0.989-1.685</td>
<td>0.0606</td>
<td>1.039</td>
<td>0.734-1.452</td>
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<tr>
<td>Pial blood supplyc</td>
<td>9.778</td>
<td>2.461-38.855</td>
<td>0.0012</td>
<td>12.250</td>
<td>1.839-81.603</td>
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<td>VEGFd</td>
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<td>1.960-30.757</td>
<td>0.0035</td>
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<td>MMP-9e</td>
<td>2.370</td>
<td>0.824-6.815</td>
<td>0.1092</td>
<td>1.178</td>
<td>0.307-4.526</td>
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aOdds ratios.- b95% confidence interval.-cpositive vs. negative.-dHigh vs. low.-eHigh vs. low.
<table>
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<tr>
<th></th>
<th>Total n=60</th>
<th>VEGF</th>
<th>MMP-9</th>
<th>Pialbloodsupply</th>
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<td>14</td>
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<td>11</td>
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<td>5.067±8.189</td>
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<td>Transitional</td>
<td>27</td>
<td>1.333±0.877</td>
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<td>8/19</td>
<td>2.666±6.031</td>
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<tr>
<td>Psamommatous</td>
<td>2</td>
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<td>2.000±0.000</td>
<td>1/1</td>
<td>1.526±0.744</td>
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<td>Angiomatous</td>
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<td>2.000</td>
<td>1.000</td>
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<tr>
<td>Microcystic</td>
<td>3</td>
<td>1.333±1.155</td>
<td>1.333±0.577</td>
<td>3/0</td>
<td>2.731±2.922</td>
</tr>
<tr>
<td>Secretory</td>
<td>2</td>
<td>2.000±0.000</td>
<td>1.500±0.707</td>
<td>1/1</td>
<td>11.332±6.172</td>
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P  0.5106  0.8165  0.1223  0.3062

Mean values are expressed as mean ± SD.
Kruskal–Wallis test was used.
Figure 1

(-)  (+)  (2+)  (3+)

VEGF

MMP-9
Figure 2

(A) Tumor volume vs. EL, with regression equation $Y = 6.262 - 0.083X$; $R^2 = 0.044$; $p = 0.1094$

(B) Comparison of pial blood supply: negative vs. positive, with $p = 0.0012$

(C) Comparison of VEGF: low vs. high, with $p = 0.0007$

(D) Comparison of MMP-9: low vs. high, with $p = 0.062$
Figure 3

A

![Graph A: Tumor volume vs. pial blood supply](image)

- **Tumor volume**
  - Negative: 15
  - Positive: 40
  - *p* = 0.0008

B

![Graph B: VEGF score vs. pial blood supply](image)

- **VEGF score**
  - Negative: 1.2
  - Positive: 1.8
  - *p* = 0.0397

C

![Graph C: MMP-9 score vs. pial blood supply](image)

- **MMP-9 score**
  - Negative: 1.5
  - Positive: 2.0
  - *p* = 0.0057
Figure 4
Figure 4
Figure 5

A

VEGF score

MMP-9 score

p=0.0479

1 2 3

B

VEGF and MMP-9 expressions

EI

0 1 2 3 4 5 6 7 8 9 10

both low either low both high

p=0.030