Prognostic Role of Matrix Metalloproteinase Expression in Meningioma: A Cross-Sectional Study.

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Research

Keywords: Matrix metalloproteinase-2, meningioma, WHO grade, angiogenesis, prognosis

DOI: https://doi.org/10.21203/rs.3.rs-127796/v1

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Abstract

Background: Meningioma is the most common intracranial tumor in adults. In addition to the extent of tumor surgical resection and WHO grade, angiogenesis is a prognostic factor that is influenced by MMP-2. Our study examined the association of these prognostic factors with MMP-2 expression in meningioma.

Methods: A cross-sectional study of patients diagnosed with meningioma between January 2008 and December 2017 was conducted. All samples were re-reviewed and subjected to immunohistochemical staining for Ki67, MMP-2, and CD34. Pearson's chi-squared test and Fisher's exact test were used to examine the association of MMP-2 expression with the WHO grade and microvascular density (MVD).

Results: The study included 99 patients aged 23–75. Most patients were female (73.7%). This study included 85 cases of low-grade meningioma (grade I) and 14 cases of high-grade meningioma (grade II, 11; grade III, 3). The most common subtypes were meningothelial, transitional, and fibroblastic. In total, 62 of 85 patients with low-grade meningioma and 10 of 14 patients with high-grade meningioma exhibited high MMP-2 expression, and the difference in the rates between the groups was not significant. Most patients in this study displayed MVD scores of 1+ (54/99) and 2+ (33/99). Of the 54 patients with an MVD score of 1+, 42 exhibited high MMP-2 expression. MMP-2 was expressed by all patients with meningioma.

Conclusion: In the future, more samples are required, in high-grade tumors, to prevent bias, and more specific immunohistochemical markers should be used to evaluate angiogenesis.

Background

Meningioma is derived from the meningothelial cells of the arachnoid layer, and it is generally a benign, slow-growing tumor that mainly affects women around the sixth decade of life (1). It is the most common intracranial tumor in adults, accounting for up to 20% of all primary intracranial tumors (2). Yusof et al. (3) reported that the incidence of brain tumors in North East Malaysia in 1996 was low (0.4 per 100,000 population), and meningioma was the second most common brain tumor after neuroglial tumor. In Hospital Universiti Sains Malaysia (HUSM), meningioma is the most frequent brain tumor, comprising approximately 36.4% of all brain malignancies between 2011 and 2014 (4).

Several risk factors contribute to the development of meningioma. Ionizing radiation is an established environmental risk factor, and the risk is higher for exposure during childhood than for adult exposure (5). The higher incidence of meningioma in women also suggests that female-specific hormones could play a role via estrogen and progesterone receptors (6). Several studies reported a possible association between meningioma and breast cancer (7) (8). Both malignances share risk factors such as endogenous and exogenous hormones as well as genetic predisposition (9). Michaud et al. (10) found a positive association between the risk of meningioma and body fatness as measured using BMI, waist circumference, and weight. The exact mechanism of this association is unclear, but possible mediators
could include hormonal factors, immunologic response, and the levels of insulin or insulin-like growth factors.

The diagnosis of meningioma is based on a histological assessment of tissue specimens obtained during surgery. According to the WHO Classification of Tumours of the Central Nervous System Revised 4th edition (5), meningioma is divided into three grades, namely grades I–III. The grading system is mainly based on histomorphological criteria and the number of mitoses. Approximately 90% of meningiomas are benign, slow-growing tumors (grade I), and their incidence increases with age (9). Grade I meningioma is associated with relative good outcomes (11); however, a significant number of benign lesions relapse (12). The most common subtypes are meningothelial, fibrous, and transitional meningioma. The remaining meningiomas are atypical (grade II) or malignant (grade III).

The prognosis of meningioma, including the risk of recurrence and survival rates, can be categorized using clinical and histopathological factors. The most significant clinical factor is the extent of tumor resection, which is determined by the tumor location, extent of invasion, and skill of the surgeon (5). Fernandez (13) reported that atypical meningioma, female sex, subtotal resection, and tumor size exceeding 4.5 cm were associated with significantly higher recurrence rates. Regarding histopathological factors, the most useful predictor for recurrence is the WHO grade. The recurrence rates for benign, atypical, and anaplastic meningioma are 7%-25%, 29%-52%, and 50%-94%, respectively (5).

Angiogenesis is another prognostic marker for meningioma. Meningiomas are well known to be highly vascularized, as neoangiogenesis is stimulated by many angiogenic regulators including vascular endothelial growth factor (VEGF), caveolin-1, matrix metalloproteinase 9 (MMP-9), and endothelin-1 (14). Many studies have proven the important role of angiogenesis in tumor growth and metastasis to other sites such as the breasts, lungs, stomach, and uterus. Baressi (14) reported that meningioma with high neoangiogenic activity is significantly associated with a higher proliferation index. Neoangiogenesis is also a negative prognostic marker that is associated with shorter survival and higher recurrence rates.

MMPs comprise a superfamily of endopeptidases consisting of more than 20 enzymes. These enzymes are produced by various cells, including epithelial cells, fibroblasts, and inflammatory cells (15). MMPs have been identified as crucial mediators of both invasion and angiogenesis in brain tumors and angiogenesis. (16). Many studies of MMPs have been conducted, and the two most widely studied MMPs are MMP-2 and MMP-9 (17). In angiogenesis, MMPs degrade the basement membrane and other ECM components, allowing endothelial cells to detach and migrate into new tissues and release ECM-bound proangiogenic factors (e.g., VEGF, basic fibroblast growth factor, transforming growth factor-β) (18). The role of MMP-2 in the stimulation of angiogenesis has been proven in several studies.

The present study examined the significance of MMP-2 expression in the prognosis of meningioma, especially in relation to the WHO grade and angiogenesis. It is hoped that these findings could clarify whether an MMP-2 immunohistochemistry (IHC) test would be a useful practical assessment tool for pathologists that provides information on prognosis and predicts the outcome of treatment or recurrence in patients.
Methods

This cross-sectional study of intracranial meningioma was conducted at HUSM (Kubang Kerian, Kelantan, Malaysia). The study period was January 2008 to December 2017. The cases were retrieved from the computerized registry database (LIS and PATHOS system) of the Department of Pathology, HUSM. The clinical data were obtained by reviewing the patients’ medical reports. Ethical approval which was included the patients’ consent acquired from the Human Research Ethics Committee of Universiti Sains Malaysia ((USM/JEPeM/18010082), Director of Hospital Universiti Sains Malaysia (HUSM) and Head of Pathology Department, HUSM. In total, 216 cases of meningioma were identified, and 99 cases were included in the study after excluding those that did not fulfill the inclusion criteria. The sample size was calculated using Power and Sample Size Calculator, version 3.1.2. The sample size estimated was 90 cases after including a dropout rate of 10%.

IHC

Three immunohistochemical stains were used: 1) monoclonal mouse anti-human CD34 class III, 2) rabbit monoclonal anti-Ki67 antibody, and 3) rabbit polyclonal anti-MMP2 antibody. IHC was performed using a semi-automated method according to standard laboratory protocols and the manufacturer's guidelines. Tissue sections (3–4 µm thick) were cut and transferred to poly-l-lysine–precoated slides. This was followed by deparaffinization and rehydration. Antigen retrieval was performed using a heat-induced epitope retrieval method in a pressure cooker (pressure cooker WMF Perfect). The slides were incubated in antigen retrieval buffer (10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0) in a hot pressure cooker for 3 min. A peroxidase blocking agent was applied, followed by incubation for 5 min. For MMP2 IHC, the peroxidase blocking step was conducted after overnight incubation with the primary antibody. The slides were then incubated with a primary antibody (anti-Ki67, 1:200; anti-CD34, 1:100; anti-MMP2 antibody, 1:500) overnight at 4 °C using a Sequenza Immunostainer (Shandon Sequenza). Subsequently, after washing with Tris-buffered solution, the secondary antibody (labeled polymer-HRP, Dako Envision™+ Dual Link System-HRP, DAB+) was applied, and samples were incubated for 30 min at room temperature. The slides were then incubated with 3,3′-diaminobenzidine solution for 5 min. Finally, slides were counterstained with Harris’ hematoxylin for 5 s followed by dehydration, and a cover slip was placed on each slide using Cytoseal XYL mounting medium. For control tissue, neurofibroma was used for anti-MMP2 staining, whereas tonsil tissue was used for anti-Ki67 and anti-CD34 staining.

Ki67, CD34, and MMP2 scoring was performed separately using different methods according to previous literature. For Ki67, the score was recorded as the percentage of positively stained tumor nuclei (brown staining) per 1000 tumor cells (19). Cells were counted in regions of maximum immunoreactivity (hot spot) under a high-power objective (×400). According to Perry et al. (19), the Ki67 index is categorized into three grades: WHO grade 1, <4%; WHO grade 2, 4–20%; and WHO grade III, >20%. However, because of the small numbers of grade II and III lesions in this study, we combined these grades and categorized the index as low (grade I) or high grade (grades II–III). MMP-2 expression was evaluated using IHC as the
sum of the frequency and intensity of cytoplasmic staining according to the scale described by Strojnik et al. (20).

Twenty representative fields were counted, and the IHC scores were determined. The total score included the sum of the frequency of positively stained tumor cells (negative staining, 0; 1%-29%, 1; 30%-60%, 2; and 61%-100%, 3) and the intensity of staining (negative, 0; weak, 1; moderate, 2; and strong, 3), as presented in Fig. 1. Scores of ≥ 4 indicated high expression, whereas lower scores denoted low expression. The assessment of micro vessels staining by CD34 was performed according to the procedure described by Weidner et al. (21). Three areas with the highest neovascularization (hot spots) were identified in a × 40 field, and micro vessels were then counted manually in each of these areas under × 400 magnification. Brown staining of single endothelial cells or clusters of endothelial cells with or without a lumen denoted an individual vessel. The mean number of micro vessels in these three fields was recorded as the micro vessel density (MVD). According to Weidner et al., the MVD was further categorized into four scores as follows: 0–33, 1+; 34–67, 2+; 68–100, 3+; and > 100, 4+ (Fig. 2). Larger vessels or thick-walled vessels were not counted.

**Statistical analysis**

All statistical analyses were conducted using Statistical Package for Social Sciences version 26. The clinicopathological data were analyzed using descriptive statistics. The associations of MMP-2 expression with different grades of meningioma and MVD were assessed using Pearson's chi-squared test and Fisher's exact test, respectively. Statistical significance was indicated by \( p < 0.05 \).

**Results**

**Clinicopathological data**

A total of 99 patients with meningioma were included, female (73/99) was more than male with the ratio of 2.8:1. The patients' age ranged from 23–75 years old (mean, 50.95 years). Majority of patients were Malay (96%) followed by Chinese (3%) and 1% was Orang Asli.

Eighty-five patients (85.9%) were low-grade meningioma and 14 patients (14%) with high-grade meningioma (11 and 3 patients with grade II (atypical and chordoid subtypes) and grade III lesions (anaplastic subtype), respectively. All histologic slides were re-reviewed, and the findings corresponded with previous findings. The most common subtypes were meningothelial (50.5%), transitional (15.5%), and fibroblastic (10%). The other subtypes included psammomatous (3%), microcystic (2%), angiomatous (2%), microcystic/angiomatous (2%) and secretory (1%). The most frequent tumor location was the frontal region (29.3%), followed by the parasagittal region (16.2%) and sphenoid wing (14.1%), whereas the remaining patients (40.4%) had tumors at other sites. Four patients had tumor recurrence.

**MMP-2 expression according to grade of meningioma**
Both low (73%) and high-grade lesions (71%) exhibited high MMP-2 expression, in which there was no statistical differences noted ($p < 0.950$, Pearson's chi-squared test) (Table 1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Grades</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>low</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>MMP-2</td>
<td>23 (27)</td>
<td>4 (29)</td>
</tr>
<tr>
<td>Low</td>
<td>62 (73)</td>
<td>10 (71)</td>
</tr>
<tr>
<td>High</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Pearson chi square test

**MMP-2 expression and angiogenesis**

Most patients had a low MVD score of 1+ (54 patients) or 2+ (33 patients), whereas six patients each had an MVD score of 3+ or 4+. All patients with an MVD score of 3+ or 4+ had low-grade meningioma. In total, 42 of 54 patients with an MVD score of 1+ and 21 of 33 patients with an MVD score of 2+ had high MMP-2 expression. Fisher’s exact test revealed no significant association between MMP-2 expression and angiogenesis (Table 2).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Angiogenesis score (MVD)</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1+</td>
<td>2+</td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>MMP-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>12 (22)</td>
<td>12 (36)</td>
</tr>
<tr>
<td>High</td>
<td>42 (77)</td>
<td>21 (64)</td>
</tr>
</tbody>
</table>

*Fisher Exact Test

**MVD score by meningioma grade**

The MVD score according the grade of meningioma is presented in Table 3. Most patients with low-grade meningioma had a score of 1+ (53%) or 2+ (33%). Only 7% of patients each had a score of 3+ or 4+. Meanwhile, among patients with high-grade meningioma, 64 and 36% of patients had MVD scores of 1+
and 2+, respectively, and no patients had a score of +3 or +4. Pearson's chi square test revealed no significant association between the MVD score and grade of meningioma ($p = 0.773$).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Microvascular density (MVD) score</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1+ n (%)</td>
<td>2+ n (%)</td>
</tr>
<tr>
<td>Grade</td>
<td>45 (53)</td>
<td>28 (33)</td>
</tr>
<tr>
<td>Low</td>
<td>9 (64)</td>
<td>5 (36)</td>
</tr>
<tr>
<td>High</td>
<td></td>
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</tr>
</tbody>
</table>

* Pearson chi square test

**Discussion**

Meningioma is the most frequently diagnosed primary brain tumor, and it accounts for approximately one-third of all primary brain and spinal tumors (9). Despite its generally benign and slow-growing nature, the biological behavior of meningioma varies considerably, and it cannot be predicted using histomorphologicalclassification alone. Benign meningioma has a recurrence rate of 7–25% (5). According to previous studies, specific MMPs have been demonstrated to enhance angiogenesis (18), and they are predictive of the recurrence of meningioma (22). MMP-2 expression is reportedly higher in high-grade meningioma (22) (23). In this study, clinicopathological data, MMP-2 expression, and CD34 expression (MVD) were determined and analyzed in all cases.

HUSM is a referral center for brain tumors in the East Coast region, which explains the high number of brain tumors treated at this institution. In this study, the vast majority of patients had WHO grade I tumors, corresponding with previous findings that benign meningioma is more common atypical and anaplastic meningioma (24)(25)(26). The sex predilection of meningioma was established in several studies, and it is most likely explained by presence of higher levels of sex hormones in women (9)(27) (28). Meanwhile, the finding that most patients were of Malay ethnicity is explained by the fact that the Kelantan population predominantly consists of Malay people (29).

According to the WHO Classification of Tumours of the Central Nervous System (5), meningioma arises in intracranial, intraspinal, and epidural regions. The frequent locations within the intracranial region are the cerebral convexities, olfactory grooves, sphenoid ridges, para-suprasellar region, optic nerve sheath, petrous ridges, tentorium, and posterior fossa.

The role of MMPs, including MMP-2 in various neoplasms is well studied, and their involvement in tumor invasion and progression has been established (18). MMP-2 is more highly expressed in higher-grade
brain neoplasms such as glioblastoma and is associated with poor survival (30) (31). Our findings that most high-grade meningiomas in this study exhibited high MMP-2 expression are consistent with previous studies illustrating a significant increase in MMP-2 expression from lower-grade to higher-grade meningioma (22) (23).

We also observed that that most low-grade meningiomas also displayed high MMP-2 expression. Although this finding does not support our hypothesis, a few studies assessed MMP expression among different low-grade histological subtypes. Das et al. (32) noted a moderate or high level of MMP-2 in patients with transitional and meningothelial meningioma. Rooprai et al. (16) found that the fibroblastic subtype meningioma exhibited the highest MMP-2 expression, followed by the transitional and meningothelial subtypes.

In addition to incomplete surgical removal of low-grade meningioma, high expression of MMP-2 also is a prognostic factor for tumor recurrence (33). However, one study found no MMP-2 expression in patients with meningothelial meningioma, and its expression was only in patients with transitional, fibromatous, psammomatous, and angiomatous subtypes (23). An evaluation of 12 patients with grade I meningioma conducted by Kirches et al. (34) using zymography and reverse transcription PCR detected no MMP activity in any samples. These contradictory findings relative to our results may be attributable to the use of different laboratory methods and scoring systems for MMP-2 expression.

MMP-2 is known to be involved in tumor angiogenesis, and it has been proven to play a critical role in the ‘angiogenic switch’ during the early development of vascularization (18). MMP-2 is also one of the most studied MMPs concerning its role in tumor angiogenesis (15) (35) (36) (37). To the best of our knowledge, no prior study examined the association between MMP-2 expression and angiogenesis in meningioma, prompting this study.

The lack of an association between MMP-2 expression and grade of meningioma in this study may be related to the small number of patients with high-grade lesions. We can conclude that meningioma of all grades expresses MMP-2 at varying intensity. Strong MMP-2 expression is associated with a high risk of recurrence and poor survival rates. However, more samples from patients with higher-grade meningioma are required to ensure the findings are not biased.

MVD is a measure of the vessel count per high-power field that is used as an independent prognostic indicator (38). Most patients in the present study exhibited a low level of angiogenesis, but the majority of patients with low-level angiogenesis exhibited high MMP-2 expression, contradicting our hypothesis. MMP-2 degrades the basement membrane and ECM components, thereby releasing proangiogenic factors, and it also directly binds to αvβ3, an integrin receptor on the endothelial surface, to induce integrin signaling, thereby contributing to endothelial survival and proliferation (15).

Angiogenesis is well studied in meningioma, and it has been reported to be associated with peritumoral edema, recurrence, and higher tumor grades (39) (14) (40). However, the antibodies used in IHC to
evaluate angiogenesis differed among prior studies, including VEGF, CD31, and CD105, but MMP-2 was not included.

In this study, we also analyzed the MVD score according to the grade of meningioma. According to Barresi et al., extensive neoangiogenesis is significantly associated with a high proliferation index in meningioma and thus a higher tumor grade, which we could not verify in this study.

Many other studies supported the findings of Barresi et al. despite the use of different immunohistochemical markers for angiogenesis, including (38) (40) (41), CD105 (42), and CD34 (43). However, a few studies reported similar results as our study (44) (45). The possible explanation for these contradictory results is most likely the use of different methods for quantifying angiogenesis and the use of IHC for CD34. CD34 is a pan-endothelial marker that is stained in both newly formed vessels and the endothelial cells of pre-existing vessels. In the future, it would be better and more accurate to use more specific IHC markers that only stain newly formed blood vessels, such as CD105 (endoglin), as suggested by Barresi et al.

**Conclusion**

In summary, our study did not identify a correlation between MMP-2 expression and the prognosis of meningioma, namely tumor grades and angiogenesis. No significant association was observed despite the presence of high MMP-2 expression in most tumors. Most patients also exhibited a low level of angiogenesis as evaluated using the MVD score, and most of these patients also had high MMP-2 expression.

In the future, more samples are required, especially from patients with high-grade tumors, to prevent bias in the analysis. A more specific immunohistochemical marker also should be applied to evaluate angiogenesis to achieve accurate scoring. Long-term follow-up is also required to assess whether patients with high MMP-2 expression and high MVD scores experienced recurrence. This could support whether MMP-2 and angiogenesis are prognostic factors for the recurrence of meningioma.

**Abbreviations**

HUSM
Hospital Universiti Sains Malaysia
MVD
Microvessel density
USM
Universiti Sains Malaysia
VEGF
Vascular endothelial growth factor
BMI
Declarations

- Ethics approval and consent to participate: Ethical approval which was included the patients’ consent acquired from the Human Research Ethics Committee of Universiti Sains Malaysia ((USM/JEPeM/18010082), Director of Hospital Universiti Sains Malaysia (HUSM) and Head of Pathology Department, HUSM.
- Consent for publication: Not applicable.
- Availability of data and materials: The datasets analysed during the current study are available from the corresponding author on reasonable request.
- Competing interests: The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.
- Funding: The study was supported by a grant from the “Research University grants account no: RUI. 1001.PPSP.8012222) from Universiti Sains Malaysia."
- Authors’ contribution:
  - MAS preformed the data collection, data analysis and draft the manuscript writing;
  - SETS supervised MAS and give guidance on how to conduct the study, edit the writing draft and finalise the writing process.
  - HJ also supervised MAS on research conduct.
  - All authors revised, edited and approved the final manuscript.

- Acknowledgements:
  - The authors would like to thank Enago (www.enago.com) for the English language review.
  - All pathology laboratory staffs who helped during the technical lab works

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