

Immunohistochemical expression of matrix metalloproteinase -9 in invasive meningioma

**Samar U. Hasan^a, Rasha M. Abdelkareem^c, Ahmed A. S. A. ELhakeem^d,
Ali R. Hamdan^b, Eman M. S. Muhammad^c**

^a Pathology Department, Faculty of Medicine, South Valley University, Qena, Egypt.

^b Neurosurgery Department, Faculty of Medicine, South Valley University, Qena, Egypt.

^c Pathology Department, Faculty of Medicine, Sohag University, Sohag, Egypt.

^d Pathology Department, Faculty of Medicine, AL-Azhar University, Asuit, Egypt.

Abstract:

Background: Meningioma is a common intracranial tumor arising from the meningotheial (arachnoid) cells. Meningiomas are divided into 15 histological subtypes and three grades, including benign (grade I), atypical (grade II) and anaplastic (grade III). It is important to correctly decide whether a meningioma is brain-invasive or not. Tumor invasion can be described as a stepwise process involving the degradation of the extracellular matrix (ECM), tumor cell adhesion to resident cells or components, increased proliferation, and cell migration into new intracellular/ECM space. It is believed that expression of Matrix metalloproteinases (MMPs) can be related with this process, especially the matrix metalloproteinase-9 (MMP9).

Objectives: Investigation of matrix metalloproteinase-9 expression in different grades of meningioma and correlate this expression with brain invasion.

Patients and methods: Matrix metalloproteinase-9 immunostaining was studied in 50 specimens of meningioma using avidin-biotin peroxidase method.

Results: The percentage of brain invasive was 5% as regard to MMP9 score 1, 5% as regard MMP9 score 2, 20% as regard MMP9 score 3, 70% as regard MMP9 score 4. A significant positive correlation was found between MMP9 score and brain invasion ($p < 0.001$).

Conclusion: Increased MMP9 expression is correlated with brain invasion.

Keywords: Matrix metalloproteinase-9, Meningioma, Invasion.

Introduction:

Meningiomas represent the second most common primary central nervous system tumors. Persistent risk of meningiomas recurrence is a compelling reason to seek adjuvant therapies to decrease the rates of relapse (*Norden et al., 2010*). Meningiomas as defined by the World Health Organization (WHO) are "meningotheial (arachnoid) cell neoplasms, typically attached to the inner surface of the dura mater," and these tumors fall into WHO grades I, II, and III (*Apra et al., 2018*). In Egypt, according to *Zalata et al. (2011)* the relative frequency of central nervous system (CNS) tumors in Delta region, meningiomas formed the next most frequent histological type in

the study as it constituted 25.6% of all studied CNS tumors and 27.2% of primary CNS tumors. Tumor invasion can be described as a stepwise process involving the degradation of the extracellular matrix (ECM), tumor cell adhesion to resident cells or components, increased proliferation, and cell migration into new intracellular/ECM space (*Fathi and Roelcke, 2013*). MMP9 are able to degrade proteins of ECM and basement membranes, playing the defining role in invasion and metastasis. Correlation between MMP-9 and brain invasion in meningiomas is of particular interest because strong expression of MMP-9 is correlated with higher grade, increased

invasiveness, and poorer survival (*Utsuki et al., 2005; and Fathi and Roelcke, 2013*).

Patients and methods:

Tissue samples:

Formalin-fixed paraffin-embedded brain tumor tissue blocks from fifty patients selected prospectively from specimens that were delivered to Pathology Laboratory from Neurosurgery Department, Faculty of Medicine, South valley University during the period from 2015 to July 2019. The meningioma was graded in accordance with the 2016 WHO classification.

Immunohistochemistry:

After evaluating (H&E) stained slides. Serial sections from each block were used for IHC. IHC of MMP9 carried out using avidin biotin peroxidase complex method. A dilution of 1:100 from Mouse monoclonal antibody against human MMP9(CloneVIIC2; Lab Vision Corp., Freemont, California, USA) was used.

Scoring of immunoreactions and statistical analyses:

MMP9 expression appeared as brownish cytoplasmic staining. The immunoreactive score (IRS) was determined by multiplying an estimate of the percentage of the immunoreactive cells (Proportion score; PS) with an estimate of the staining intensity (intensity score; IS) according to *VonRadow et al., (2006)*. A proportion score was defined as the percentage of positively stained cells: 0= negative, (1-25%) positive tumor cells= 1, (26–50%) positive tumor cells= 2, (51-75 %) positive tumor cells =3, (76 -100 %) positive tumor cells =4. An intensity score was defined as the staining intensity of positive tumor cells: No staining=0, Weak staining=1, Medium staining=2, Strong staining=3. An IRS of 1-2 was considered score1, 3-4 was score2, 6-8 was score3 and 9-12 was considered score 4.

Statistical analysis:

Data was analyzed using SPSS program version 17.0. Quantitative data was expressed as means ± standard deviation, median and range. Qualitative data was expressed as number and percentage. The data were tested for normality using ShapiroWilk test. The nonparametric Mann–Whitney test, Kruskal–Wallis test and Spearman's correlation

were used for data which wasn't normally distributed. P value less than 0.05 was considered statistically significant and less than 0.001 was considered highly significant.

Results:

The age range of the studied patients was wide 17-85 years, with mean ± SD was 53.74±14.81years, the median age was 55 years. The male to female ratio was1-1.7

The representative H&E stained sections of the collected 50 specimens of meningiomas were evaluated according to the WHO classification(*Perry et al., 2016*).into the following histological grades:

- Twenty-six 26/50 (52%) cases were grade I.
- Nineteen 19/50 (38%) cases were grade II.
- Five 5/50 (10%) cases were grade III.

MMP9 expression appeared as brownish cytoplasmic staining. MMP-9 high positivity was seen in 46.1% (12/50) of grade I meningiomas, and in 89.4 % (17/50) of grade II meningiomas, However, 100% (5/50) of grade III meningiomas were highly positive for MMP-9. There is significant value (P value= 0.011) is observed increase MMP9 expression with increase the grade of meningioma. Regard to brain invasion in the studied group, in grade I (100%) of cases were noninvasive, gradeII (78.9%) were invasive, while grade III (100%) of cases were invasive. it is obvious that there is significant correlation between the grades of meningiomas and brain invasion (P value=.001).

Table1.The relation between WHO Grade and tumor invasion.

Variable		Tumor invasion		
		Invasive	Non invasive	p-value
WHO grade	I	0 (0%)	26 (100%)	.001*
	II	15 (78.9%)	4 (21.1%)	
	III	5 (100%)	0 (0%)	

Table 2. Correlation between MMP- 9score and the studied clinicopathological parameters.

Clinicopathological parameter	MMP9 score				P- value
	1	2	3	4	
Male	3(16.7%)	3(16.7%)	3(16.7%)	9(50%)	.942
Female	4(12.5%)	6(16.7%)	4(12.5%)	18(16.7%)	
Age	60.71±10.63	48.33±17.197	61.57±14.97	51.7±14.158	.155
Grade I	6(23.1%)	8(30.8%)	3(11.5%)	9(34.6%)	.011*
Grade II	1(5.3%)	1(5.3%)	4(21.1%)	13(68.3%)	
Grade III	0(0%)	0(0%)	0(0%)	5(100%)	

* P- value was calculated by Kruskal Wallis Test

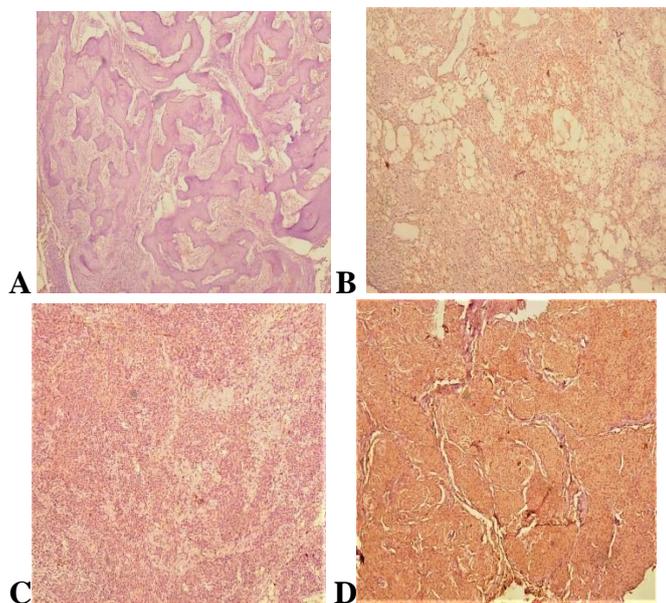


Figure 1. A) Score1 MMP9expression in metaplastic (osteogenic) meningioma Grade I (x200). B)Score2 MMP9expression in secretory meningioma Grade I(x200). C) Score3 MMP2expression in microcystic meningioma Grade I (x100). D) Score4 MMP9expression in atypical brain invasive meningioma Grade II (x200).

Discussion:

Our study included 50 specimens of meningiomas. These tumors were investigated for MMP9 expression. The IRS of MMP9 was detected in tumor tissue and was correlated with some clinicopathological variables (age, sex and tumor grade).

Regarding grade of meningiomas, MMP-9 high positivity was seen in 46.1% (12/50) of grade I meningiomas, and in 89.4 % (17/50) of grade II meningiomas, However, 100% (5/50) of grade III meningiomas were highly positive for MMP-9. Our results showed a significant positive correlation between MMP9 expression and increasing pathological grade of meningioma (p< 0.001). These results are in agreement with those previously reported by *Okada et al.(2004); Panagopoulos et al.(2008); Barresi et al.(2011)* and *Mahzouni et al.(2012)*.

Our study revealed that brain invasion in grade I was (0%) of cases, in grade II was (78.9%), while in grade III was (100%). it is obvious that there is significant correlation between the grades of meningiomas and brain invasion (p< 0.001). Higher MMP-9 activity in atypical and anaplastic meningiomas may be at the basis of the

higher invasive potential toward the brain parenchyma and bone of the seneoplasms. Besides, a correlation between MMP-9 and degree of brain invasion has been reported by (Nordqvist *et al.*, 2001).

Conclusion:

Increased MMP9 expression is correlated with, increasing grade, and brain invasion. prospects for further researches in this area MMP inhibitors may be combined to augment chemotherapy efficacy and to attenuate invasion.

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