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## RESEARCH ARTICLE

# THE EXPRESSION OF MATRIX METALLOPROTEINASE-9 (MMP-9) AND KI-67 LABELING INDEX IN INTRACRANIAL ASTROCYTOMA PATIENTS AT HAJI ADAM MALIK GENERAL HOSPITAL MEDAN 2017

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**ABSTRACT**

**Introduction:** Astrocytoma is one of neuroepithelial tumor and these tumors are graded in four scale based on the aggresiveness. Overexpressions of MMP-9 has been correlated to tumor's progresivity, poor prognostic and predictive marker in cancer, while Ki-67 is strongly associated with cell proliferation. **Method:** This study is being conducted to analyze the correlations between MMP-9 expressions and KI-67 in intracranial astrocytoma. This reprospective study is using cross-sectional method. The expression of MMP-9 and Ki-67 was evaluated, as well as its prognostoc value. The study is held at Haji Adam Malik General Hospital, Medan, North Sumatera with total samples of 19. **Results:** There is no significant correlation in astrocytoma classification with MMP-9 and Ki-67 Li expression (p=1,000). There is no significant correlation of MMP-9 overexpression with in mortality (p=0.117) however there is significant correlation of Ki-67 Li with mortality (p=0,005). **Conclusion**: There is no significant association between MMP-9 and Ki-67 Labeling Index in intracranial astrocytoma.



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## INTRODUCTION

Primary astrocytoma is one of neuroepithelial tumor and these tumors are graded in four scale based on the aggresiveness. WHO grading divides astrocytoma into four classification. However, etiology and pathophysiology is still unavailable. These days, angiogenesis is found as an important factor of invasion, metastatis, and prognosis. Several clinical studies have found that microvascular density is closely related to tumor malignancy and prognosis. In humans, it is known that there are more than 20 types of MMP genes and are divided into several subgroups based on their function and localization in tissues. Recent research suggests MMP plays a role in the development and aggression of tumor cells with angiogenesis, cell adhesion and migration, cell proliferation, apoptosis and growth factors. MMP-9 is part of a large group of proteases that have an important role in the degradation of extracellular matrix thus facilitating tumor invasion.The role of MMP-9 in GBM in mediating invasion and migration has been previously reported with histochemical staining which shows higher elevation in invasive tumors.

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Ki-67 is considered as the best marker to assess tumor cell proliferation and can be very helpful in cases where histology shows low grade astrocytoma while other parameters are more inclined towards malignancy.The proliferative index Ki-67 is a potential biological marker that can quantitatively estimate neoplasmic growth so that it can identify the prognosis. Several studies have been carried out to look for correlations between MMP-9 and Ki-67 expressions in several types of tumors. One of them is a study conducted by American Cancer Research in 2010, which showed matrix metalloproteinase (MMP) including MMP-2 and MMP-9 actively play a role in the invasive ability of gliomas and also correlates with histopathological features of malignancy. Not many studies have looked for an association between MMP-9 expression and Ki-67 in astrocytomas.

## MATERIALs AND METHODs

This study is a cross sectional study using analytical research methods to analyze the relationship between MMP-9 expression and Ki-67 in intracranial astrocytoma patients.The study was performed in the Neurosurgery Department of Adam Malik General Hospital Medan and Pathology Anatomy Department of Sumatera Utara Medical Faculty in Medan,

North Sumatra, and was conducted from January 2017 to December 2018. The number of subjects in this study were as many as 19 subjects. The subjects were all patients with astrocytoma who undergo surgical tumor removal at Adam Malik General Hospital between April 2019 to October 2019. The study subjects were determined by total sampling method with the determination of inclusion and exclusion criteria. Diagnosis was proven by histopathology and the subjects have met the inclusion criteria. Data of the subjects were taken from the medical record department of Neurosurgery Adam Malik General Hospital. Based on medical record number, we confirm the Anatomical Pathology Department to get the number of checks used to get the block of paraffin. MMP-9 and Ki-67 staining were performed in Anatomical Pathology Department by trained laboratory personnel and MMP-9 and Ki-67 expression analysis were performed semi-quantitatively by a specialist in anatomical pathology and clinical data without knowing the previous diagnosis. The independent variables in this study is MMP-9 expression; the dependent variable isKi-67 Labelling Index. Data was analyzed using the data processing software to determine research data characteristics. Categorical variables were analyzed in the form of frequencies and percentages presented in tabular form. Numeric variables are presented in the form of mean and standard deviation if the distribution is normal. If the distribution is not normal, grouping of data into group was used.

## RESULTS

In this study, the mean age of the study subjects was 31.37 ± 15.49 years. Most of the research subjects were 11 females (57.9%), while male subjects were 8 (42.1%) (Table 1).

**Table 1. Subject Characteristic**

| Characteristic | |
|---|---|
| Age (year) | 31.37 ± 15.49 |
| Gender (n/%) | |
| Male | 8 (42.1) |
| Female | 11 (57.9) |
| WHO Grade (n/%) | |
| I | 6 (31.6) |
| II | 8 (42.1) |
| III | 1 (5.3) |
| IV | 4 (21.1) |
| Total | 19 |

**Table 2. Association between Astrocytoma Classification and Gender**

| WHO Grade | Male n(%) | Female n(%) | p |
|---|---|---|---|
| I | 2 (25) | 4 (36.4) | 0.629[a] |
| II | 3 (37.5) | 5 (45.5) | |
| III | 1 (12.5) | 0 (0) | |
| IV | 2 (25) | 2 (18.2) | |
| Total | 8 (100) | 11 (100) | |

**Table 3. WHO Grading and Mortality**

| WHO Grade | Alive n(%) | Dead n(%) | p |
|---|---|---|---|
| I | 6 (40) | 0 (0) | 0.002 |
| II | 8 (53.3) | 0 (0) | |
| III | 0 (0) | 1 (25) | |
| IV | 1 (6.7) | 3 (75) | |
| Total | 15 (100) | 4 (100) | |

In table 2, after statistical tests, there were no significant differences in the degree of WHO between the groups of men and women (p = 0.629).

**Table 4. Ki-67 expression and Gender**

| Ki-67 LI | Male n (%) | Female n (%) | P |
|---|---|---|---|
| Negative | 2 (25) | 3 (27.3) | 0.912[a] |
| Positive | 6 (75) | 8 (72.7) | |
| Total | 8 (100) | 11 (100) | |

In this study found 3 subjects (75%) with grade IV astrocytoma died and 1 subject (25%) grade III astrocytoma died. After conducting statistical test it was found that there were significant differences between groups of living and dying based on WHO degrees (table 3). From the 19 study subjects, there were 5 subjects (25%) with Ki67 negative and 14 subjects (75%) with Ki67 positive. After statistical tests there were no significant differences between male and female subjects in terms of Ki67 expression (p = 0.912) (table 4). In the analysis of Ki-67 expression based on astrocytoma classification after statistical analyse it was found no significant correlation was found between the expression of Ki-67 and the degree of astrocytoma (r = 0,000; p = 1,000) (table 5). In this study it was found that the majority of living subjects had positive Ki67 expressions (11 people (73.3%)), while in the group of subjects who died the majority had positive Ki67 expressions (3 people (75%)). After statistical tests, it was found that there was a correlation between Ki67 expression and mortality (p = 0.050) (table 6).

Based on the results of MMP-9 examination in this study, the majority of study subjects (13 people (68.4%)) had positive MMP-9 expressions, while 6 people (31.6%) had negative MMP-9 expressions. After statistical tests, there were no differences in MMP-9 expression in the male and female groups (p = 0.224) (table 7). After statistical tests, a positive correlation was obtained between MMP-9expression and the degree of astrocytoma (r = 0.549; p = 0.015) (table 8). In this study, the majority of living subject groups had positive MMP-9 expressions (9 people (60%)), while all subjects who died had positive MMP-9 expressions. After statistical tests, there were no significant differences in mortality based on MMP-9 expression (p = 0.117) (table 9). After conducting statistical tests with the Spearman correlation, no correlation was found between the expression of MMP-9 and Ki-67 in this study (r = 0.149; p = 0.543) (table 10).

## DISCUSSION

Astrocytomas are the most common type of primary tumor in the central nervous system and the most frequent cause of cancer deaths and account for about 1.3% of all malignant cancers, with an incidence of 7 per 100,000 population worldwide. Although histopathological features are very helpful in determining prognosis, histopathological differentiation is sometimes unhelpful in some cases, especially in circumstances where only a small amount of tissue is obtained for biopsy. Proliferation index is a potential marker that can quantitatively estimate the growth of neoplasms and is very useful for determining the prognosis in patients with neoplasms. Ki-67 is quantitatively related to the mitotic index through differences in cell cycle time differences and can show differences in malignant grading in astrocytic tumors. Therefore Ki-67 is expected to be an important proliferation parameter to determine other prognostic factors. MMP-9 has an important role in cell immune function. In pathophysiological conditions, MMP-9 regulation increases during wound development and recovery, and also occurs in

**Table 5. Ki-67 expression and WHO Grade Astrocytoma**

| Ki-67 LI | *Grade* I | *Grade* II | *Grade* III | *Grade* IV | r | P |
|---|---|---|---|---|---|---|
| Negative | 1 (16.7) | 3 (37.5) | 1 (100) | 0 (0) | 0.000 | 1.000[b] |
| Positive | 5 (83.3) | 5 (62.5) | 0 | 4 (100) | | |
| Total | 6 (100) | 8 (100) | 1 (100) | 4 (100) | | |

**Table 6. Ki67 expression and mortality**

| Ki-67 LI | Alive | Dead | Total | p |
|---|---|---|---|---|
| Negative | 4 (26.7) | 1 (25) | 5 (26.3) | 0.005[a] |
| Positive | 11 (73.3) | 3 (75) | 14 (73.7) | |
| Total | 15 (100) | 4 (100) | 19 (100) | |

**Table 7. MMP-9 Expression and Gender**

| MMP-9 | *Male n(%)* | *Female n(%)* | *p* |
|---|---|---|---|
| Negative | 3 (37.5) | 3 (27.3) | 0.224[a] |
| Positive | 5 (62.5) | 8 (72.7) | |
| Total | 8 (100) | 11 (100) | |

**Table 8. MMP-9 expression and WHO Grade Astrocytoma**

| MMP-9 | *WHO Grade n (%)* | | | | Total | r | P |
|---|---|---|---|---|---|---|---|
| | I | II | III | IV | | | |
| Negative | 4 (66.7) | 2 (25) | 0 (0) | 0 (0) | 6 (31.6) | 0.549 | 0.015[b] |
| Positive | 2 (33.3) | 6 (75) | 1 (100) | 4 (100) | 13 (68.4) | | |
| Total | 6 (100) | 8 (100) | 1 (100) | 4 (100) | 19 (100) | | |

**Table 9. MMP-9 Expression and Mortality**

| MMP-9 | Alive n(%) | Dead n(%) | Total | p |
|---|---|---|---|---|
| Negative | 6 (40) | 0 (0) | 6 (31.6) | 0.117[a] |
| Positive | 9 (60) | 4 (100) | 13 (68.4) | |
| Total | 15 (100) | 4 (100) | 19 (100) | |

**Table 10. Correlation of MMP-9 and Ki-67**

| MMP-9 | Ki-67 | | r | P |
|---|---|---|---|---|
| | Negative | Positive | | |
| Negative | 1 (20) | 5 (35.7) | 0.149 | 0.543[b] |
| Positive | 4 (80) | 9 (64.3) | | |

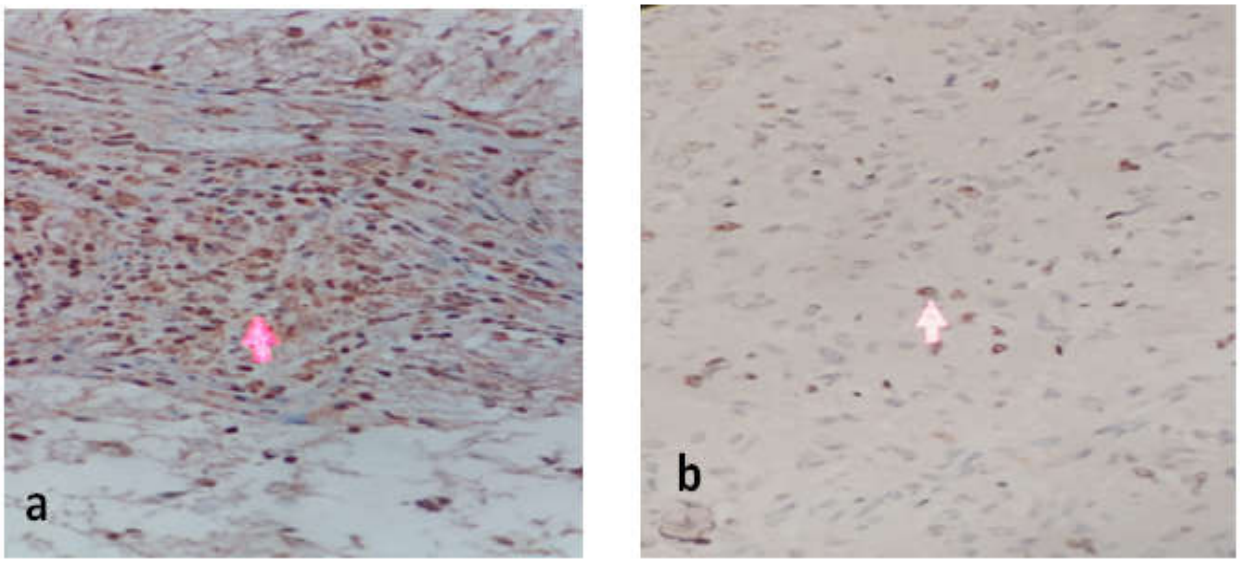


**Figure 1. (a) MMP9 positive expression on astrocytoma, (b) Ki-67 positive expression on astrocytoma**

pathological conditions that involve inflammatory processes, such as arthritis, diabetes, and malignancy. In this pathophysiological condition, the proteolytic component of MMP-9 contributes to stimulating an immunological response to the initiation of pathogenesis and exacerbation of the disease process. In this study, it was found that grade II astrocytoma based on WHO classification was the most astrocytoma type at 42.16%, followed by grade I (31.6%) and grade IV at 21.1%, and grade III at 5.3%. . These results are in accordance with previous studies in Thotakura 2014 where the highest incidence was found in grade II astrocytoma at 39.9% followed by astrositoma gr IV at 36.2%, grade III astrocytoma at 14.3% and grade I astrocytoma at 9.5%. A study by Chalood in 2012 also found that the highest incidence was grade II astrocytomas (43.1%) followed by grade IV (31.4%), grade I (13.2%) and grade III (11.8%). From the 19 samples in this study, there were 11 samples of women and 8 samples of men. If a comparison is made between the sexes, a ratio of a male: a female is equal to 1: 1,375, but no statistically significant differences are found in the two groups (p = 1,000). In contrast to a study conducted by Thotakura in 2004, it was explained that the incidence of astrocytoma was more common in men than in women with a male: female ratio of 1.84: 1. It also does not support the literature that previously mentioned that astrocytomas are more common in men than in women.This might be due to the relatively smaller number of samples compared to other comparative studies.

In this study it was found that the WHO classification had a role in mortality with a significance result of p = 0.002 (p <0.05). This shows that there is a significant relationship between WHO classification and patient mortality. Similar to the study conducted by Anvari et al, concluded that the degree of WHO classification of astrocytomas is related to mortality.
We found no significant relationship between the expression of Ki-67 Li to gender (p = 0.912). This is the same as the study conducted by Darweesh et al who concluded that no significant relationship was found between the expression of Ki-67 on gender (p = 0.481). It was also found that the expression of Ki-67 had no role in the classification of astrocytomas with a significance result of p = 1,000 (p> 0.05). This shows that there is no significant relationship between the percentage of Ki-67 with the classification of intracranial astrocytomas. This is not in accordance with previous publications which concluded that there is a significant relationship between Ki-67 and the degree of astrocytoma classification. The publication by Johannessen et al in 2006 explains that the value of ki-67 is increasing with the increasing degree of WHO classification. This is because Ki-67 can assess the proliferation activity of tumor cells so that the higher the Ki-67 value, the higher the degree of malignancy of the tumor.

There is a significant relationship between the expression of Ki-67 on mortality (p = 0.005). This is consistent with previous publications which have concluded that there is a significant relationship between Ki-67 and the prognosis of patients with astrocytoma. In 1994, Sallinen concluded that the best sensitivity and specificity were found at a Ki-67 value of 8% and Ki-67 was said to have the potential to assess a strong prognosis with a 15.3% as a limit. A study conducted by Di in 1997 concluded that a Ki-67 value <8% is related to a longer safety score of 5 or 10 years. Whereas in a study conducted by Reavey-Cantwell in 2001 stated that patients with astrocytoma with a Ki-67 value> 20% had a 2.2 times greater risk of death compared with patients with astrocytoma with a Ki-67 value <20 %. All of these publications support that Ki-67 has a prognostic value both for safety and recurrence. The results of this study indicate that there were no significant differences in MMP-9 expression in the male and female groups (p = 0.224). This is also the same as a study conducted by Li et al in 2016, found that there were no significant differences in MMP-9 expression in groups of men and women. There are many studies that emphasize the important role of MMP-9 ongrowth and invasive rate of gliomas. Hu et al reported that MMP-9 being silent either due to RNA or antisense RNA reduced tumor proliferation, growth and neovascularization both in vivo and in vitro. Treatment with microglia inhibitors causes a reduction in MMP9 expression and reduces the growth and degree of invasion of gliomas in mice. Many factors may be responsible so there are variations in the results of Ki-67 Li in several studies. The expression of Ki-67 Li may be influenced by the fixation process, immunohistochemical procedures, especially the treatment of antigens, and the interpretation of the staining results. Low Ki-67 Li expression in high degree astrocytomas can also occur due to errors in sampling and tumor heterogeneity.

The treatment of Ki-67 antigens is better when using hydrated autoclav compared to microwaves and can produce higher Ki-67 Li expression. This might occur due to the successful denaturation of better formalin fixation. Computer-assisted interpretation methods also seem to be lowering expression (up to 30%) than manual methods. Inter-observer variability can also influence the estimation of Ki-67 Li expression. In this study, no significant correlation was found between MMP-9 expression and Ki-67 Li (r = 0.149; p = 0.543). There are no studies that correlate MMP9 and Ki-67 Li directly in astrocytomas. However, a study conducted by Ozek et al in 2016 on meningioma patients concluded that there was a significant correlation between MMP9 expression and Ki-67 Li in meningioma. Concurrent expressions of MMP-9 and Ki-67 in cancer cells indicate the aggressiveness of the tumor.The difference in the results of this study in terms of the correlation of MMP-9 and Ki-67 Li expression with the results of previous studies may be caused by several things, namely the relatively smaller number of samples and there are no other studies in common with this study.

**Conclusion**

In this study no significant correlation was found between MMP-9 and Ki-67 Li expression in astrocytoma patients. No relationship was found between the degree of astrocytoma and sex (p = 0.629). A significant relationship was found between the degree of astrocytoma and mortality (p = 0.002). In this study, no relationship was found between the expression of Ki-67 Li and gender (p = 0.912). No significant correlation was found between the expression of Ki-67 Li and the degree of astrocytoma (p = 1,000). A relationship was found between the expression of Ki-67 Li and mortality (p = 0.005). No association was found between MMP9 expression and gender (p = 0.224). Significant correlation was found between MMP-9 expression and astrocytoma classification (p = 0.015). No association was found between MMP-9 expression and mortality in this study (p = 0.117).

## REFERENCES

Berger, Mitchel S., Chang, Susan., Sanai, Nader. 2011. Gliomas in Adult. *Journal of Neurosurgery.* 5(115).

Das, Basumitra., Vamsya Raj, Kurimella., Atla, Bhagyalakshmi., 2018. Clinicohistopathology Study of Astrocytoma Along with Ki-67 Proliferative Index. *International Journal of Research in Medical Science, 6(2): 665-670.*

Chaloob, Mohammed Kassim., Ali, Hussam Hason., Mohammed, Ahmed Salahaldeen., 2012. Immunohistochemical Expression of ki-67, PCNA and CD34 in Astrocytomas: A Climicopathological Study. *Oman Medical Journal Vol.27*, No.5:368-374.

Choe, Gheeyoung., Park, Junk K., Jouben-Steele, Lisa., 2002. Active Metalloproteinase 9 Expression is Associated with Primary Glioblastome Subtype. *Clinical Cancer Research* Vol 8, 2894-2901.

Habberstad, Andreas H., Gulati, Sasha., Torp, Sverre H., 2011. Evaluation of The Proliferation Markers Ki-67/MIB-1, Mitosin, Survivin, Phh3, and DNA Topoisomerase in Human Anaplastic Astrocytomas- An Immunihistochemical Study. *Diagn Pathol,* 6;43.

Hlobilkova, A. Et all. 2009. Analysis of VEGF, Flt-1, Nesyin and MMP-9 in Relation to Astrocytoma Pathogenesis and Progression. *Neoplasma* 56, 4, 2009.

Hu, Feng., Ku, Min-Chi., Markovic, Darko., 2014. Glioma-associated Microglial MMP-9 Expression is Upregulated by TLR2 Signaling and Sensitive to Minocycline. *Internationa Journal of Camcer:* 135, 2569-2578.

Jian-wei., Ren-ya, Zhan., Ming, Zhang., 2005. Expression of Endothelial Nitric Oxide Synthase and Vascular Endothelial Growth Factor in Association with Neovascularization in Human Primary Astrocytoma. *Journal of Zhejiang University Science* 6(7): 693-698.

Joseph, Justin V., Roosmalen, Ingrid van., Kruyt, Frank., 2015. Serum-Induced Differentiation of Glioblastoma Neurospheres Leads to Enhanced Migration/ Invasion Capacity That is Associated with Increased MMP9. *Public Library of Science*. 10(12): e0145393.

Johannessen, Al., Torp, SH., 2006. The Clinical Value of Ki-67/MIB-1 Labelling Index in Human Astrocytomas. *Pathology Oncology Research.* Vol 12:3.

Liu, Z., Li, L., Yang, Z., Luo, W., Li, X., Yang, H., Yao, K., Wu, B. and Fang, W., 2010. Increased expression of MMP9 is correlated with poor prognosis of nasopharyngeal carcinoma. BMC cancer, 10(1), p.270.

Liu, Ming-Fa., Yong-Yang, Hu., Hai-Xiong, Xu., 2015. Matrix Metalloproteinase-9/ Neutrophil Gelatinase-Associated Lipocalin Complex Activity in Human Glioma Samples Predicts Tumor Presence and Clinical Prognosis. *Disease Marker*, 138974.

Liu, Liping., Jueheng, Wu., Zhe, Ying., Han, A Liang., 2010. Astrocyte Elevated Gene-1 Upregulasi Matrix Metalloproteinase-9 and Induces Human Glioma Invasion. *Cancer Res,* 1;70(9):3750-9.

Louis, David N., Perry, Arie., Ohgaki, Hiroko., Wiestler, Otmar D., 2016. The 2016 World Health Organization Classification of Tumors of The Central Nervous System: a Summary. *Acta Neuropathologica,* pp 803-820.

Omuro, A., Deangelis, LM., 2013. Glioblastoma and Other Malignant Gliomas: A Clinical Review. *The Journal of The American Medical Association,* 310(17): 1842-50.

Ostrom, Quinn T., Bauchet, Luc., Barnholtz-Sloan, Jill S., 2014. The Epidemiology of Glioma in Adults: A State of The Science Review. *Neuro Oncol.* 16(7): 896-913.

Ozek, E. Et all. 2016. Matrix Metalloproteinase-9 Expression in Meningioma: Correlation with Growth Fraction and Role of Gender. A Pilot Immunohistochemical Study. *Neuro-oncology* Oct 2016.

Perry, Arie., Wesseling, Pieter., 2016. Histologic Classification of Gliomas. *Handbook of Clinical Neurology.* Vol 134: p71-95.

Ricci, Serena et.all. 2017. Evaluation of Matrix Metalloproteinase Type IV-Collagenases in Serum of Patients with Tumors of The Central Nervous System. *J Neurooncol* 131:223-232.

Rorive, Sandrime., Beryon, Alex., D'haene, Nicky., 2008. Matrix Metalloproteinase-9 Interplays with the IGFBP2-IGFII Complex to Promote Cell Growth and Motility in Astrocytomas. GLIA 56:1679-1690.

Saha, Rajdeep., Chatterjee, Uttara., Mandal, Sonali., Saha, Kaushik., 2014. Expression of Phospatase and Tensin Homolog, Epidermal Growth Factor Receptor, and Ki-67 in Astrocytoma: A Prospective Study. *Indian Journal of Medical and Pediatric Oncology,* 2(35): 149-155.

Scheithauer, Bernd., Rodriguez, Fausto., Jenkins, Robert B., 2008. Epithelial and Pseudoepithelial Differentiation in Glioblastoma and Gliosarcoma: A Comparative Morphologic and Molecular Genetic Study. *Cancer,* 113(10): 2779-2789.

Schroder, Roland., Feise, Klaus., Ernestus, Ralf-Ingo., 2002. Ki-67 Labeling is Correlated With the Time to Recurrence in Primary Glioblastomas. *Journal of Neuro-oncology,* 56(2), pp 127-132.

Sengupta, Subhalakshmi., Chatterjee, Uttara, Ghosh, Ashit., 2012. A Study of Histopathological Spectrum and Expression of Ki-67, TP53 in Primary Brain Tumors of Pediatric Age Group. *Indian Journal of Medical and Paediatric Oncology: Official Journal of Indian Society of Medical & Paediatric Oncology,* 33(1): 25-31.

Serao, Nicola., Delfino, Kristin., Southey, Bruce., Beever, Jonathan., 2011. Cell Cycle and Aging, Morphogenesis, and Response to Stimuli Genes are Individualized Biomarkers of Glioblastoma progression and Survival. *BMC Medical Genomics.* 4:49.

Shaffrey, Mark E., Farace, Elana., Schiff, David., 2005. The Ki-67 Labeling Index as A Prognostic Factor in Grade II Oligoastrocytomas. *Journal of Neurosurgery,* 6:102.

Sinceviciute, Ruta., et all. 2018. MMP2 is Associated with Glioma Malignancy and Patient Outcome. *Int J Clin Exp Pathol* 2018;11(6): 3010-3018.

Takei, Hidehiro., Bhattacharjee, Meenakshi., Rivera, Andreana., Dancer, Yeongju., 2007. New Immunohistochemical Markers in The Evaluation of Central Nervous System Tumors. *Arch Pathol Lab Med*, Vol 131.

Wirsching., Reifenberger, Guido., Hans-Georg., Christiane B, Kbobbe-Thomsen., 2016. Advances in The Molecular Genetics of Gliomas- Implication for Classification and Therapy. *Clinocal Oncology,* 14(7): 434-452.

Xu Cai., Jun-Jie, Qin., Hao, Shu-Yu., Li Huan., 2018. Clinical Characteristics Associated with The Intracranial Dissemination of Gliomas. *Clinical Neurology and Neurosurgery,* 141-146.

Xu Cai., Jun-Jie, Yang., Hao, Chen., 2017. Activation-Induced Upregulation of MMP9 in Mast Cells is a Positive Feedback Mediator for Mast Cell Activation. *Molecular Medicine Reports.* 15(4): 1791-2997.

Xue, Qiang., Cao Li., Chen, Xiao-Yan., Zhao, Jing., 2017. High Expression of MMP9 in Glioma Affects Cell Proliferation and is Associated with Patient Survival Rates. *Oncology Letters,* 13; 1325-1330.

Yabluchanskiy, Andriy., Ma, Yonggang., Lindsey, Merry. 2013. Matrix Metalloproteinase-9: Many Shades of Function in Cardiovascular Disease. American Physiology Society Nov; 28(6): 391-403.

Zhao, Jipei., Li, Gang., Zhao, Zhenwei., 2012. Matris Metalloproteinase-9 Expression is Increased in Astrocytic Glioma and Associated with Prognosis of Patients. *Japanese Journal of Clinical Oncology* 2012;42(11) 1060-1065.

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