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Original Research Article

Relationship between mitotic index and Ki67 expression in meningioma

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ABSTRACT

Background: Meningioma is the most common primary central nervous system (CNS) tumor which covers 36.4% of all CNS tumors. Two important factors determining the prognosis of patients with a diagnosis of meningioma are the percentage of tumor resection and the degree of tumor histopathology. Because there are limitations to routine histopathological examination in predicting tumor progressivity, several examination techniques have been developed including cytogenetics and use of immunohistochemical examination.

Method: Observational analytic study was carried out on 68 tumor samples in dr. Wahidin Sudirohusodo Hospital and the Makassar Pathology Diagnostic Center with diagnoses of meningioma from 2012-2018. The Sample size is determined by consecutive sampling method.

Results: Sample Size were 68 people from Dr. Wahidin Sudirohusodo Hospital and the Makassar Pathology Diagnostic Center with diagnoses of meningioma which fulfilled the inclusion criteria and consisted of 19(27.9%) men and 49 (72.1%) women with an average age of 42 years. The most common location of the tumor was in the convexity area with an incidence of 29.4%. Grade I meningioma was found 41.2%, grade II of 32.4% and grade III of 26.5%. In grade I, the mean mitotic index was 0.25, grade II was (7.4) and grade III was 22.75. In grade I, the mean Ki67 expression was 1.01%. The highest expression was obtained in grade III with a mean of 14.8% and the highest expression was 53%. The Spearman's rho test results between the mitotic index and Ki67 expression show that there is a positive correlation of 0.490, which means that there is a moderate correlation.

Conclusion: IHC expression of Ki67 increases proportionally to the degree of histopathology of meningioma. There is a positive correlation of 0.490 which means that there is a moderate correlation between the mitotic index and Ki67 expression.

Keywords: Immunohistochemical examinations, Ki67 expression, Meningioma, The mitotic index

INTRODUCTION

Data from the Central Brain Tumor Registry of the United States (CBTRUS), meningioma is the most

common tumor type of the primary central nervous system (CNS) tumor which covers one third or 36.4% of all CNS tumors. Its prevalence is estimated at 97.5/100,000 populations in the USA, with more than 170,000 individuals diagnosed with meningioma. The

incidence in women is higher than in men with a ratio of 2: 1 in intracranial meningioma and 10:1 in spinal meningioma. These tumors are often diagnosed in middle age.¹ Two important factors determining the prognosis of patients with a diagnosis of meningioma are the percentage of tumor resection and the degree of tumor histopathology. High-grade tumors, which do not reach total resection can cause recurrence. The WHO grade is the most useful recurrence predictor, in which benign, atypical and anaplastic meningiomas have a recurrence of 7-25%, 29-52% and 50-94% in 20 years, respectively.^{1,2} Since there are limitations to routine histopathological examination in predicting tumor progressivity, several examination techniques have been developed including cytogenetics and use of immunohistochemical examinations. However, those examinations havenot been carried out routinely on meningioma patients. Some significant immunohistochemical tests on meningioma include EMA, vimentinand cyokeratin. Ki-67/MIB-1 examination, which is a proliferative predictor, shows the results as a prognostic meningioma.¹⁻³

METHODS

This research is an analytical observational study. Samples analyzed were from 2012 to December 2018, in which 239 cases of meningioma grade I, 52 cases of meningioma grade II and 20 cases of grade III meningioma were obtained. The samples were then analyzed based on inclusion and exclusion criteria. Exclusion criteria include: recurrent meningioma, Meningiomas that have received radiation therapy and patients with a diagnosis of other cerebral tumor or tumor in different location.

The surgical specimens were fixed in 10% buffered neutral formalin, entirely sampled, and embedded in paraffin. A 5-µm sections were stained with haematoxylin and eosin (H&E). All histological and immunohistochemical (IHC) tumor slides were evaluated by two pathologist and regrouped as benign, atypical, and anaplastic meningiomas according to the WHO classification of tumors of the central nervous system.

Immunohistochemistry with anti-Ki-67 mouse monoclonal antibody (DAKO, clone MIB-1, dilution

1:500) was manually performed. The largest representative tumor tissue section available was selected for immunohistochemistry. Five µm thick FFPE tissue sections were obtained. Briefly, tissue sections were deparaffinized and hydrated in graded ethanol concentrations. Antigen retrieval was performed using citrate pH 6 solution followed by endogenous peroxidase blocking using phosphate-buffered saline (PBS), 30% hydrogen peroxide, and Tween 20 (0.1%). Tissue sections were incubated with primary antibody solution overnight at 4°C, followed by washes and incubation with HRP conjugated secondary antibody (DAKO) and detection of antibody complex by adding DAB chromogen (DAKO).

The immunohistochemical stains were independently scored manually by two pathologists with a double blind method. The highest area of proliferation was identified and Ki67 indices were calculated as number of mitotic figures or strongly positive nuclei, per 1000 and 100 tumor cells respectively. Positive expression will appear brown at the location of the antigen to be detected. Mitotic index was determined by counting the number of mitoses per 1000 tumor cells.

RESULTS

The samples consisted of 19 (27.9%) men and 49 (72.1%) women with an average age of 42 years (Table 1).

Table 1: Distribution of meningioma cases based on tumor location.

Location	Number	Percentage
Olfactory groove	7	10,2
Convexity	20	29,4
Falx/parasagital	12	17,6
Posterior Fossa	3	4,5
Sphenoid wing	16	23,5
Suprasellar	2	2,9
Retrobulbar	3	4,5
Spinal cord	5	7,4
Total	68	100

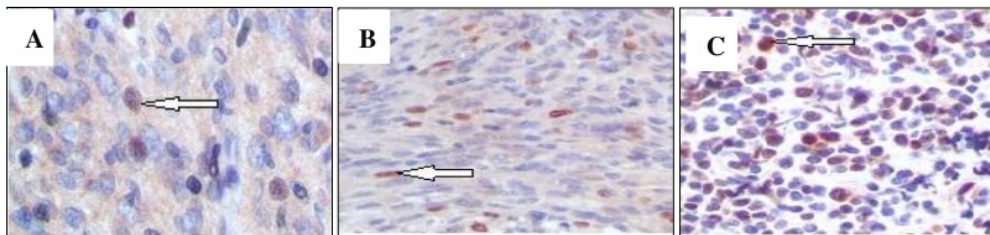


Figure 1: Ki67 expression was colored positively if brown cells were found after Ki67 antibody staining. (A) Grade I meningioma, (B) grade II meningioma, (C) grade III meningioma (arrow shows positive colored cells).

Table 2: Distribution of samples based on variations in pathology.

Meningioma Grade	Pathology	Number	Percentage
Grade I	Meningothelial Meningioma	8	11.8
	Fibroblastic Meningioma	3	4.4
	Mycrocystic Meningioma	3	4.4
	Mixed Meningioma	5	7.4
	Psammomatous Meningioma	6	8.8
	Transitional Meningioma	3	4.4
Grade II	Chordoid Meningioma	7	10.3
	Atypical Meningioma	15	22.1
Grade III	Papillary Meningioma	2	2.9
	Anaplastic meningioma	16	23.5
Total		68	100

¹¹ The most common location of the tumor is in the convection area with an incidence of 29.4%. Meningothelial meningioma ranks first in terms of the most tumor incidence in first-grade meningioma (Table 2).

Overall, anaplastic meningioma was the most type of tumor with 23.5% incidence (Table 3).

Based on the histopathological degree, grade I meningioma was found the most with 41.2% incidence, while grade III was 26.5% (Table 4).

Table 3: Distribution of samples according to histopathological degree based on the WHO category.

Grade	Number	Percentage
Grade I	28	41.2
Grade II	22	32.4
Grade III	18	26.5
Total	68	100

The results showed that the mitotic index in grade I was 0.25, grade II was 6.2 and grade III was 22.5.

Table 4: Mitotic index based on meningioma grade.

Mitotic Index	Minimum	Maximum	Median	Mean	SD
Grade 1	0	3	0	0.25	0.75
Grade 2	0	21	6	7.4	5.19
Grade 3	13	28	23.5	22.75	4.5

Table 5: Ki67 expression based on the type of tumor histopathology.

Meningioma Grade	Pathology Type	Number	Mean of Ki67 expression (%)
Grade I	Meningothelial Meningioma	8	0
	Fibroblastic Meningioma	3	1.3
	Mycrocystic Meningioma	3	0.6
	Mixed Meningioma	5	1.4
	Psammomatous Meningioma	6	1.1
	Transitional Meningioma	3	3.0
Grade II	Chordoid Meningioma	7	3.6
	Atypical Meningioma	15	2.1
Grade III	Papillary Meningioma	2	0.5
	Anaplastic meningioma	16	16.6

The mitotic index increases in accordance with histopathological grade. In grade I, the mean was 0.25, grade II was (7.4) while grade III was 22.75.

²⁰ Ki-67 antigen was detected using MIB-1 antibody. (Abry et al., 2010), in which ¹⁴ the Ki-67 index/MIB-1 was calculated in the form of the percentage of the tumor cell

nucleus that were positively colored by the total number of tumor cell nuclei (Figure 1).

Ki67 expression was found to be highest in anaplastic meningioma, reaching 16.6%. Ki67 was not found in meningothelial meningioma. IHC expression of Ki67 increases proportionally to the degree of histopathology of meningioma. In grade I, the mean Ki67 expression was

1.01%. The highest expression was obtained in grade III with an average of 14.8% and the highest expression of 53% (Table 5).

According to the results of the examination, it was found that the mean percentage of Ki67 expression in grade I was 1.01%, grade II was (2.4%) and grade III was 16.16% (Table 6 and Figure 2).

Table 6: Ki67 expression based on meningioma grade.

Ki67 Expression	Minimum	Maximum	Median	Mean	SD	p*
Grade 1	0	9	0	1.01	2.0	0.002
Grade 2	0	12	1.5	2.4	3.3	0.002
Grade 3	0	53	10.8	16.6	18.8	0.002

*Kruskal-Wallis test.

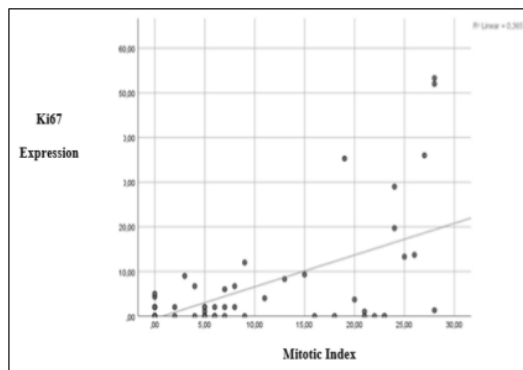


Figure 2: Distribution of the mitotic index and Ki67 expression in meningioma.

DISCUSSION

Based on gender, meningiomas were found more women than men with a ratio of 3:1. Data

from the Central Brain Tumor Registry of the United States (CBTRUS) demonstrates a more than twofold higher incidence among females [age-adjusted incidence rate (per 100,000 person years) of 8.36 and 3.61 for females and males, respectively].¹ Based on the research by Pratik and Dhaval, the incidence of meningioma in women was higher with a ratio of 2.1:1 compared to men.² This comparison was reversed in prepubertal-age meningiomas. In atypical and anaplastic meningioma cases, it was more dominant in men.¹ The cause of the distribution is still unclear. Several studies have shown a positive relationship between the use of hormonal therapy used in women with the development of meningioma.

The convexity area was the most common location of meningiomas, followed by posterior fossa and sphenoid area.^{3,4} In the type of atypical meningioma, several

studies showed that the non-skull base was the most common location for meningioma.⁴ The relationship between the incidence of tumor degree/grade and the location of the tumor is unclear, but it is thought to be related to embryological origin of meninges.

Meningothelial meningioma ranks the first in terms of the incidence of first-grade meningiomas. This was also found in the study of Nasrin & Sayed with the incidence of meningothelial meningioma of (65.1%). Different results found by Thomas B.G et.al with the results of transitional meningiomas having the highest incidence rate (40%) compared to meningothelial meningioma (17%) in terms of grade I.⁵

One of the criteria for determining the degree of histopathological meningioma based on the WHO category is mitotic activity, in which mitotic activity of 4-19 is categorized as grade II, while mitotic activity of more than 20 is classified as grade III.⁶ The results of our study show that mitotic index in grade I is 0.25, grade II is 6.2 and grade III is 22.5. The results of the Thomas B.G et.al study showed that the average mitotic index in grade I was 1.1, grade II was 4.4 and in anaplastic meningioma (grade III) was 28.5.⁵

Takahashi et al. found an association between mitotic and tumor progressivity. Patients without mitotic (group A) showed a tumor progressivity rate of 7% after 5 years with symptom-free period of 148 months in average. Group B (mitotic of 1-4) and group C (mitotic of more than 4) showed a 5-year recurrence rate averaging 87% and 90% with shorter symptom-free period (43 and 16 months in average).⁷ Determination of tumor histopathology degrees is useful for clinicians for further management and determination of risk factors for tumor recurrence.

IHC expression of Ki67 increases proportionally to the degree of histopathology of meningioma. In grade I, the mean of Ki67 expression was 1.01%. The highest Ki67

expression was found in anaplastic meningioma, amounting to 16.6%. Ki67 was not found in meningothelial meningioma. The highest expression was obtained in grade III with a mean of 14.8% and the highest expression of 53%. The studies review by Allen et.al, found the average labeling index (values rounded off) for grade I was estimated to 3% with average range of values of 1-16%. For grade II tumors, the average value was 8% with average range of values of 2-20%. Grade III meningiomas had an average value of 17% with average range of values of 7-32%.⁹ The study by Ramesh BT et al. showed positive Ki-67 results in all cases of anaplastic, papillary, atypical and metaplastic meningiomas. Ki-67 expressions were 100% positive in grade III (100%) followed by grade II meningiomas (77.7%), very low in in grade I. The degree of meningioma follows the Ki-67 cut-off level.⁸ Ki-67 expression (MIB-1) is as an indicator to differentiate anaplastic meningiomas (grade III by WHO) (mean Ki-67: 11%) with WHO's grade I (mean Ki-67:0.7%).⁹ Meanwhile, atypical meningioma (WHO's grade II) showed an average of 2.1%.¹⁰ All studies showed a positive correlation between Ki-67/MIB-1 LI and tumor grade in human meningiomas.¹¹⁻¹³

Based on the Kruskal-Wallis test, there was a significant relationship between meningioma grade and the value of mitotic index (Asymp. Sig=0.000) and Ki67 expression (Asymp. Sig =0.002) with a value of <0.05.

The results of correlation calculation between mitotic index and Ki67 expression show that there is a positive correlation of 0.490, which means that there is a moderate correlation between mitotic index and Ki67 expression, the higher the mitotic index, the higher the Ki67 expression.

Table 7. Relationship between mitotic index and Ki67 expression.

		p	R
Spearman's rho	Mitotic Index	<0,0001	0,490**
	Ki67 Expression		

** Significant correlation.

This positive relationship was also found in mammary carcinoma. A study showed that Ki67 expression in mammary carcinoma ranged from 7-36.7%, increasing proportional to the degree of carcinoma.¹⁴ Ki-67 as a proliferation marker can be a predictor of meningioma recurrence. Concerning relapse, most studies reported higher labeling indices in recurrent meningiomas than in nonrecurrent (4% vs 2%).¹⁵⁻¹⁷

The results of univariate analysis conducted by Jordi Bruna et al.¹³ atypical and anaplastic meningiomas showed that Ki-67 labeling index and Karnofsky score after surgery were prognostic factors of tumor recurrence and life expectancy of patients.¹⁸

A combination of Ki67 and mitotic index is a quantitative method that is meaningful for predicting the symptom-free period in meningioma patients. The research conducted divided tumor patients into three groups: group A (Ki67 less than 1% and without mitotic), group B with Ki67 more than 5% and without mitotic, as well as group C with KI67 more than 5% and with mitotic. The limit value of Ki67 for the patient's outcome assessment was 1-5%. The results of the study showed that the combination of mitotic index and Ki67 expression is a better predictor for meningioma recurrence, compared to if only using the KI-67 indicator.⁷ In cervical carcinoma, KI-67 and IM correlated with the prognosis of patients receiving radiation therapy.¹⁹

Based on the aforementioned results, Ki67 expression can be useful as an indicator of proliferation in small biopsy specimen samples, which are difficult for mitotic index testing.

CONCLUSION

IHC expression of Ki67 increases proportionally to the degree of histopathology of meningioma. There is a positive correlation of 0.490 which means that there is a moderate correlation between the mitotic index and Ki67 expression.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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