OPEN ACCESS
Saudi Journal of Pathology and Microbiology
Abbreviated Key Title: Saudi J Pathol Microbiol
ISSN 2518-3362 (Print) |ISSN 2518-3370 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com/sjpm

Original Research Article

Immunohistochemical Expression of Ki-67, p53 and HER2/neu in Meningiomas

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DOI: 10.36348/sjpm.2020.v05i03.001 | **Received:** 22.02.2020 | **Accepted:** 02.03.2020 | **Published:** 07.03.2020

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Abstract

Introduction: Meningiomas are one of the most common primary intracranial tumours. Although most meningiomas are benign, the spectrum also includes atypical and malignant meningiomas. Clinical outcome of meningiomas is often difficult to predict. The stratification of risk on the basis of histomorphology alone remains problematic; thus additional biomarkers are needed. In this study biomarkers of prognostic and therapeutic interest like Ki-67, p53 and HER2/neu have been studied in correlation with clinicopathological parameters. ***Materials and methods*:** A retrospective study on histologically diagnosed meningiomas was undertaken. Slides were retrieved and reviewed. Clinical details were recorded from the files in the archives of the department. Immunohistochemical staining with markers Ki-67, p53 and HER2/neu were performed and the findings were interpreted. ***Results:*** The study included 17 cases with an age range of 16 to 73 years and a male: female ratio of 1.1:1.There were 11 cases (64.7%) of primary tumours and 6 cases (35.3%) were recurrent tumours. WHO Grade I meningiomas were maximum and accounted for 70.5% followed by grade II (17.5%) and grade III (12%) meningiomas. Ki-67 expression was seen in all cases with progressively increased expression in higher grades. p53 expression was observed in all the cases with higher levels (>10%) in Grade II and Grade III meningiomas compared to grade I meningiomas. The HER2/neu staining was negative in all cases studied. ***Conclusion:*** Grade I meningiomas appear to have low mitotic count on morphology but have higher proliferation rate on Ki-67 studies. Most of the recurrent tumours have higher p53 expression (>10%). Hence, adjuvant studies with biomarkers Ki-67 and p53 will be helpful in precise grading of meningiomas.

Keywords: Meningioma, Ki-67, p53, HER2/neu, WHO tumour grade.

INTRODUCTION

Meningiomas are common tumours of central nervous system (CNS), accounting for 15% to 25% of primary intracranial and intraspinal neoplasms. These are thought to arise from the cells of the arachnoid membrane covering the brain and spinal cord [1]. The peak incidence is found between the sixth and seventh decades of life, affecting females more often than males (2:1) [2]. Most of the patients present with neurological signs and symptoms, due to compression of the adjacent structures by the tumour. Radiological imaging techniques have limited ability to differentiate between different subtypes of meningiomas [3].

According to World Health Organization (WHO) meningiomas are classified as benign, atypical and malignant/anaplastic types. About 80% of meningiomas are slow-growing benign tumours of WHO grade I with many histological types. Complete resection of benign meningiomas (Grade I) is associated with 5-year recurrence rates of only 5%, whereas in the atypical meningiomas (GradeII), it has been found to be high (40%). Anaplastic meningiomas (Grade III) account for 1-3% of all meningiomas and have clinical characteristics similar to other malignant neoplasms [4].

Morphological parameters such as histological type, WHO grade, mitotic counts and brain invasion have been found to have prognostic value [5]. Histologically distinct subsets of meningiomas are associated with high risk of recurrence even after complete resection. The progression of meningiomas is difficult to predict clinically and the risk of such progression has been assessed mainly on histomorphological parameters with interobserver

variability. Currently there are only a few ancilliary techniques that are helpful in predicting the recurrence and prognostic behaviour of meningiomas [1]. The present study was undertaken to analyse the immunohistochemical expression status of Ki-67, p53 and HER2/neu in meningiomas in correlation with pathological parameters like WHO tumour grade and histological subtype.

MATERIALS AND METHODS

A retrospective study was conducted in the department of Pathology attached to a tertiary care hospital. The haematoxylin and eosin (H & E) stained slides were retrieved and reviewed. All relevant clinical details were recorded from the files available in the archives of the department. Meningiomas were classified into three groups, according to WHO classification and diagnostic criteria as follows [6]: classic meningiomas (WHO grade I), atypical meningiomas (WHO grade II), and anaplastic meningiomas (WHO grade III). Atypical meningiomas were defined as those with either a high mitotic rate (>4 per 10 HPFs) or the presence of at least 3 of these 4 features: small cell formation, macronucleoli, sheeting architecture, and hypercellularity. Anaplastic meningiomas were defined as those with either a high mitotic count (>20 per 10 HPFs) or with histologic features similar to those of carcinoma, sarcoma, and melanoma focally or diffusely [1, 6].

The best paraffin embedded block was selected and standard 4 micron sections were subjected to immunohistochemical study with appropriate positive and negative controls. Primary antibodies against Ki-67 (a mouse monoclonal antibody of GM001 clone, Pathnsitu), p53 (mouse monoclonal antibody of BP-53-12 clone, Pathnsitu) and HER2/neu (a rabbit monoclonal antibody of EP3 clone, Pathnsitu) were used. The Polyexcel HRP (non-biotin, micro-polymer based) /DAB Detection system was followed.

Interpretation of Immunohistochemical (IHC) result

Ki67 expression in tumour cells with brown nuclear staining was considered as positive. The number of positive cells in 10 high power fields (HPF) was observed. The scoring was done as score 1 (<4 positive cells/ 10HPF), 2 (4-19 positive cells/ 10HPF) and 3 (≥20 positive cells/ 10HPF) [6].

p53 expression in tumour cells with brown nuclear staining was considered positive and calculated as percentage of positive cells, by counting at least 1000 tumor cells in fields with the largest number of positive cells. The expression status was grouped into low (<10%) and high (≥10%) [4].

HER2/neu immunostaining is not usually found within arachnoid cells of normal meninges, hence its expression was always considered as an overexpression [1]. HER2/neu scoring was done similar to the breast carcinoma scoring system [5]. Score 0 (negative) – no staining observed or incomplete or barely perceptible membrane staining within ≤10% of tumour cells; 1+(negative) – incomplete membrane staining that is faintly/barely perceptible within ≥10% of tumour cells; 2+(equivocal) – incomplete and /or weak /moderate circumferential membrane staining within >10% of tumour cells or intense complete and circumferential membrane staining within ≤10% of tumour cells; 3+(positive) – complete and intense circumferential membrane staining within >10% of tumour cells. Scores of 0 or 1+ were considered negative for HER2/neu expression, scores of 2 + was equivocal and 3+ as positive for HER2/neu expression [5].

RESULTS

During the study period, 17 cases of meningiomas were found, out of which 11 (64.7%) cases were primary tumours and 6 (35.3%) cases were recurrent tumours. Summary of meningioma cases studied are shown in Table 1. The age of the patients ranged from 16 to 73 years, with a mean age of 46.41 years. A total of 9 (53%) patients were males and 8 (47%) patients were females with a male: female ratio of 1.1:1. Parietal region was the common location in this study. Grade I meningiomas accounted for 70.5%, grade II (17.5%) and grade III (12%) of the studied cases. In grade I meningiomas, the most common histologic type was meningotheliomatous 8 cases (66.6%), followed by transitional (16.6%) and psammomatous meningiomas (16.6%). In grade II meningiomas, both the cases were atypical meningiomas. Among the Grade III meningiomas there was one case each of rhabdoid and anaplastic meningioma. Photomicrographs of meningothelial and psammomamatous meningiomas shown in Figure 1 and 2.

Table-1: Summary of meningioma cases studied

Sl No	Age (Yrs)	Sex	WHO Grade of tumour	Histological type of meningioma	Primary or recurrent tumor	Ki-67 Score	p53 Score	HER2/neu Score
1	48	F	I	Transitional	Primary	1	Low	0
2	57	F	I	Transitional	Primary	2	High	0
3	53	M	I	Psammomatous	Primary	2	Low	0
4	57	M	I	Meningothelial	Primary	2	High	0
5	39	F	I	Meningothelial	Primary	2	Low	0
6	72	F	I	Psammomatous	Primary	3	High	0
7	26	F	I	Meningothelial	Primary	3	High	0
8	34	F	I	Meningothelial	Primary	3	High	0
9	66	M	I	Meningothelial	Primary	3	High	0
10	36	M	II	Atypical	Primary	3	High	0
11	50	M	III	Rhabdoid	Primary	3	High	0
12	32	M	I	Meningothelial	Recurrent	1	High	0
13	42	F	I	Meningothelial	Recurrent	2	Low	0
14	53	M	I	Meningothelial	Recurrent	2	High	0
15	35	M	II	Atypical	Recurrent	3	High	0
16	73	M	II	Atypical	Recurrent	3	High	0
17	16	F	III	Malignant	Recurrent	2	High	0

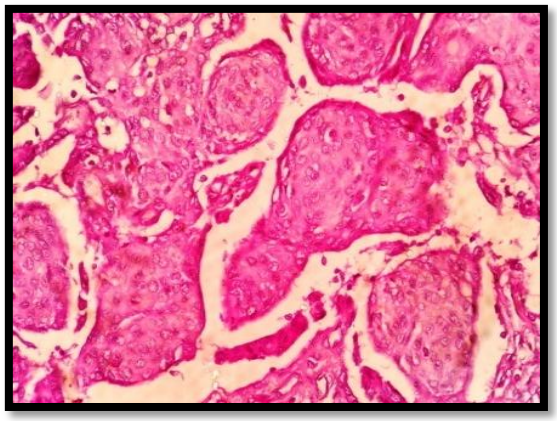

Fig-1: Showing meningothelial meningioma. (H&E, 10x)

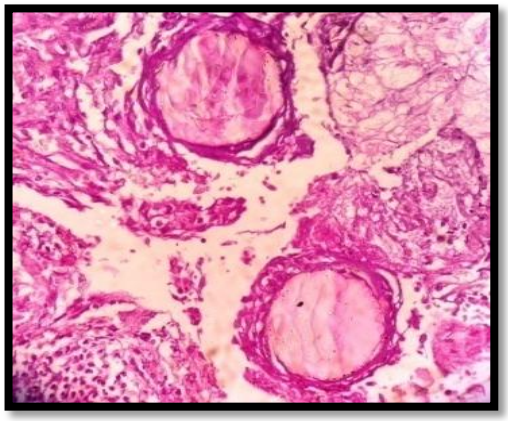

Fig-2: Showing psammomma bodies in meningioma. (H&E, 10x)

The Ki-67 expression was observed in all cases of meningiomas. In the primary grade I meningiomas (9/11 cases) there were one case of score 1, 4 cases each of score 2 and score 3 on Ki-67 study. One case each of primary grade 2 and grade 3 meningiomas showed score 3 on Ki-67 study. There were 3 recurrent grade I meningiomas wherein one case had score 1 and two cases score 2. The 2 cases of recurrent grade 2 meningioma showed score 3 and one case of recurrent grade 3 meningioma showed score 2 on Ki-67 labelling. The Ki-67 expression of various grades of meningiomas with their score is shown in Table 2. Photomicrographs of different scores of Ki-67 on IHC given in Figure 3.

Table-2: Ki-67 expression in primary and recurrent tumours in correlation with WHO grade

WHO Grade		Ki 67 Expression		
		Score 1	Score 2	Score 3
Primary Tumours (n =11)	Grade I (9)	1	4	4
	Grade II (1)	-	-	1
	Grade III (1)	-	-	1
Recurrent Tumours (n = 6)	Grade I (3)	1	2	-
	Grade II (2)	-	-	2
	Grade III (1)	-	1	-

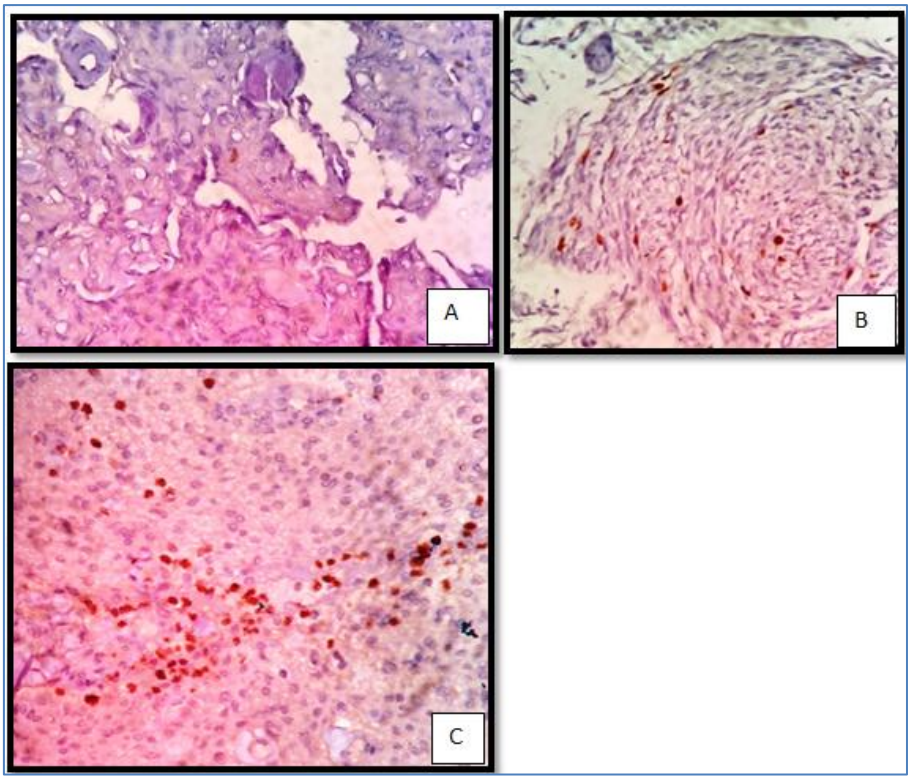

Fig-3: IHC with Ki-67 showing score 1 (A), score 2 (B), score 3 staining (C).

The p53 expression was scored as low and high expression. Out of the 9 primary grades I meningiomas 3 cases showed low expression and other 6 cases showed high expression. One case each of primary grade 2 and 3 meningiomas showed high p53 expression. In recurrent tumours, out of 3 grade 1 meningiomas, 2 cases had high p53 expression. All the grade 2 and grade 3 recurrent meningiomas showed high p53 expression. The p53 expression of various grades of meningiomas with their score is shown in Table 3. Photomicrographs of p53 score given in Figure 4.

Table-3: P53 expression in primary and recurrent tumours in comparison to WHO grade

WHO grade		p53 Expression	
		Low (<10%)	High (>10%)
Primary Tumour n =11	Grade I (9)	3	6
	Grade II (1)	-	1
	Grade III (1)	-	1
Recurrent Tumour n = 6	Grade I (3)	1	2
	Grade II (2)	-	2
	Grade III (1)	-	1

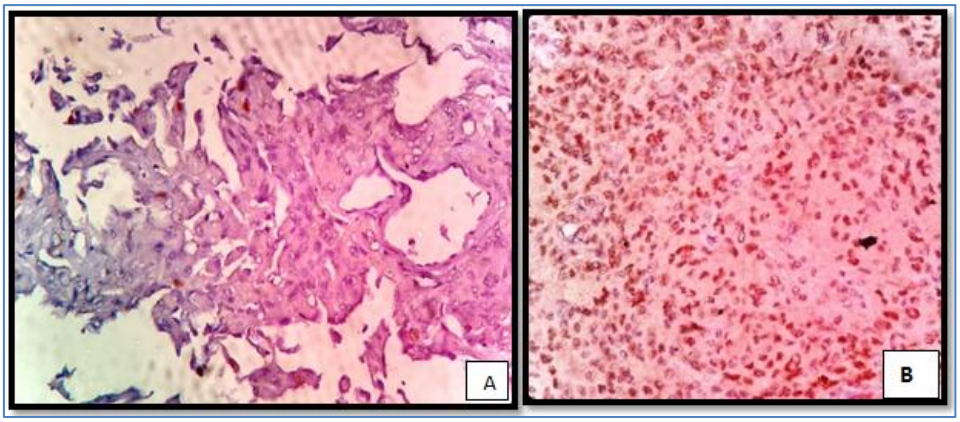

Fig-4: IHC with p53 showing low expression (<10%) in A and high expression (≥10%) in B

The HER2/neu expression in all the 17 cases did not show membranous positivity and were considered negative for HER2/neu (grade 0). Only faint cytoplasmic positivity was seen in some cases which were considered negative (Figure 5).

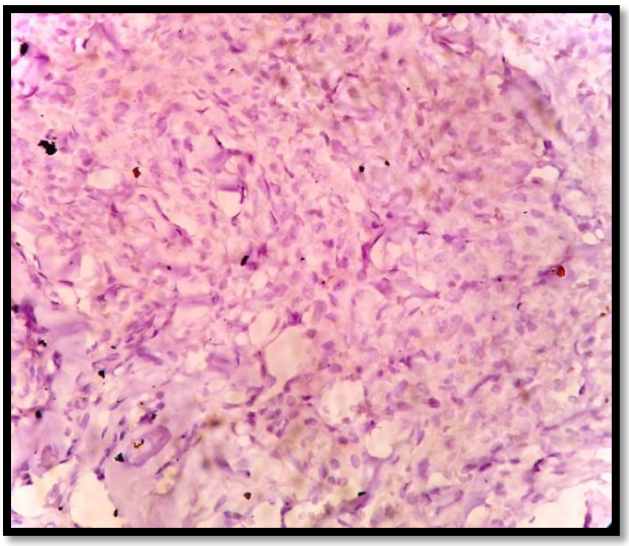

Fig-5: IHC with HER2/neu showing negative staining (score 0)

DISCUSSION

Meningiomas are one of the most common primary brain tumours with many morphological variants. WHO grade I tumors include histological types like meningothelial, fibrous, transitional, psammomatous, angiomatous, microcystic, secretory, lymphoplasmacytic rich and metaplastic meningiomas. Distinct subtypes like chondroid and clear cell meningiomas are classified as WHO grade II and the papillary and rhabdoid meningiomas are WHO grade III meningiomas [4]. There are morphological criteria that define different grades of meningioma; however, the distinction by morphology alone is difficult. There has been no highly reliable immunohistochemical marker that can separate the different WHO grades till date.

The differentiation between grade I and grade II meningiomas at times with assessment of mitotic figures alone would lead to interobserver variability [4]. Atypical and anaplastic meningiomas with active proliferation behave more aggressively with higher tendency for recurrence [4]. However, there exist a borderline and a group of cases that show clinically aggressive behavior despite being histologically benign (WHO Grade I). As many as 7-20% of these benign (WHO Grade I) tumours are known to recur [7]. In the recent years, interest in this clinically diverse group of tumours has intensified, leading researchers to investigate combinations of predictive and prognostic factors, with the aim to identify the patients who should be followed more closely for recurrence or treated more aggressively at the time of diagnosis [8].

In this context, adjuvant biomarkers are needed to facilitate the assessment of proliferation accurately. Thus, this study was undertaken to detect the immunohistochemical expression of Ki-67, p53 and HER2/neu in WHO grades I–III meningiomas.

In the present study the age of the patients ranged from 16 to 73 years, with a mean age of 46.41 years which was consistent with other studies [9, 10]. Our results were in accordance with the common agreement that most meningiomas are benign and grade I meningiomas constituted the majority of the tumours accounting for 70.5% of the studied cases [11]. With the tumour histological subtype, the most common subtype in the current study was meningothelial (66.6%), followed by transitional (16.6%) and psammomatous meningioma (16.6%). These findings fall within the figures given by the WHO classification of tumours of the central nervous system [6, 11]. Though recurrence is known to be more common in Grade II-III meningiomas, the present study had 3 cases (50%) of grade I meningiomas in the ones which had recurred. There was no significant relationship found between the tumour histological subtype and tumour grade, a finding that correlated with a study which highlighted that even though a vast range of meningioma subtypes exist, the clinical behavior and outcomes correlate with the WHO grade, and not on the histologic subtype [11].

Finding of atypia on histology is highly subjective, with significant interobserver variability. Thus an objective method of counting positively stained nuclei on IHC provides a more reproducible and an accurate method for assessing aggressive behavior as an adjunct to histology [7]. MIB-1 is an anti-Ki-67 monoclonal antibody that has been found to be useful for the analysis of proliferative potential [11]. This has been widely used in many studies of meningiomas as a prognostic marker and adjuvant to histopathology for grading of meningiomas. A high Ki-67 index is associated with aggressiveness and poor prognosis for meningiomas [12]. Thus tumours that do not have any histological features of malignancy but have a high score should be reviewed carefully [7]. Precaution should be taken in interpreting Ki-67 positivity on IHC, as lymphocytes or other proliferative cells can show positivity for Ki-67 antigen. This error can be eliminated by meticulously comparing with the hematoxylin and eosin section (H and E). This reinforces the fact that IHC stains can only be used as an adjunct to histology and cannot replace them [13].

Various studied in the past have shown that proliferative index increases with the increasing tumour grade [13]. In this study Ki-67 marker was positive in all the cases. The cases of primary grade II and III meningiomas all revealed higher Ki-67 expression. Interestingly, even some of the primary grade I tumours showed higher Ki-67 scores of 2 and 3 which suggest

that though these grade 1 tumours may have low mitotic count in routine H & E sections but they might have high proliferation rate which can be detected by Ki-67 antigen as IHC marker. Similar finding of morphologically benign tumours with high proliferative index was also noted in another study [13].The morphological grading of meningiomas based on mitotic count alone is highly subjective, leading to low accuracy. Hence, Ki-67 can be used as routine IHC marker in all meningioma cases to categorise patients who has more chance of recurrence which helps in individualising the therapy for meningioma patients [7].

The p53 protein functions as a tumour suppressor wherein following irreversible DNA damage, the p53 protein induces cell apoptosis. The wild-type protein has an inhibitory effect on cell proliferation and transformation, but gene mutations alter its tumour suppressor activity. A high proportion of cells with mutant protein indicate increased tumour aggressiveness [2]. The positive p53 expression has been reported in a range of 10-88% [4]. One study reported an immunoreactivity for p53 (9.5% grade I, 72.7% grade II, and 88.9% grade III meningiomas), and also found increasing expression levels with higher grades [14]. In our study, only high p53 expression was observed in all the cases with higher levels in Grade II and Grade III meningiomas compared to grade I meningiomas which is consistent with other studies [14]. However, in another large study done, there was no significant association between p53 levels and tumour grade [4]. In our study out of 9 primary grade I meningiomas, 6 cases showed high expression and in recurrent tumours, out of 3 grade1 meningiomas, 2 cases had high p53 expression. Thus, grade I meningiomas can show higher p53 expression which may indicate aggressive behaviour of the tumour. High Ki-67 proliferative activity and p53 expression in recurrent cases was observed in this study which was consistent with the study done by Rao *et al*. [13].

The targeted therapy for HER2/neu is proving to be effective; researchers in the last decade have shown interest in the role of HER2/neu as a possible target for therapy in meningiomas also [15]. The HER2/neu expression in meningiomas is observed to be in a wide range of 2.5 to 67.3% across various studies [1, 16-18]. Although some investigators have refuted any association between HER2/neu expression and the recurrence of meningioma [16], others suggested that it was associated with a poor prognosis [1,19]. However, still other investigators concluded that the role of HER2/neu expression in the pathogenesis, progression, and treatment of meningiomas still needs investigation [5], and recommended that investigations should include a combination of biomarkers [18]. In the present study, HER2/neu was negative in all the cases studied. It could be due to wide variation in expression of the marker in different study population [11].

CONCLUSION

Though the morphological assessment of Grade I meningiomas appears to have low mitotic count, Ki-67 study can show higher proliferation rate. Hence precise assessment of Ki-67 needs to be done and should be included in diagnostic criteria as many of the cases seem to be “on the fence” with regard to tumour grade. Tumours with higher p53 expression levels (>10%) have been associated with higher grades, and recurrent tumours. Thus, we suggest that biomarkers Ki67 and p53 should be studied in all cases of meningiomas to improve the accuracy of the grading and to help in categorising higher risk patients thus formulating alternative individualised treatment choices for them.

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