Int.j.pathol.2022;20(4): 141-146

Original Article

Correlation of Progesterone Receptor and Ki-67 Immunomarkers with Histopathological grades of Meningioma

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ABSTRACT

Back ground: Immunohistochemical markers such as PR and proliferative marker index Ki-67 have considerable prognostic and tumor predictive role in ambiguous cases of meningioma.

Objective: To assess Immuno-expression of Progesterone Receptor (PR) & Ki-67 in different histopathological grades of meningioma and to see any correlation between both of these Immuno-markers and WHO grades of meningioma.

Material and Methods: This Cross-sectional study was carried out in Pakistan Institute of Medical Sciences (PIMS), Islamabad from September 2017 to August 2018. All neurosurgical biopsies with clinical suspicion of meningioma were processed through various steps. Slides were prepared and stained with Haematoxylin & Eosin (H&E). Immuno-staining with PR & Ki-67 was done after numerous successive steps. Grading of meningioma and Immuno-expression of PR & Ki-67 in all slides were assessed. Data was analyzed using SPSS version 21.

Results: Total numbers of cases were 70. Female to male ratio was 1.33. Mean age was 44.34 years. Grade I cases were most common (n=55, 78.6%) followed by Grade II cases (n=12, 17.1%). Grade III cases were the least common (n=3, 4.3%). Among PR Immuno-positive cases, 58.3% were females while 41.4% were males. Overall, 68.6% PR positivity was seen (48/70). Highest PR positivity was seen in grade I cases (78.2%) followed by grade II cases (41.7%) Overall mean Ki-67 for all 70 cases was 5.56%. Lowest mean Ki-67 (3.3%) was seen in Grade I cases and it was higher (12.3%) for grade II cases, while the highest mean Ki-67 (19.7%) was seen in grade III cases.

Conclusion: A significant inverse correlation was found between PR and Ki-67 immuno-expression with histopathological grades of meningioma.

Key words: Meningioma, Grades, Progesterone Receptor (PR), Ki-67 labelling index (Ki-67LI).

Introduction

Meningiomas are the most prevalent benign brain tumors arising from the meningeal coverings of the brain and spinal cord. They comprise of more than one third of all primary CNS tumors (36.8%). [1] Brain tumors incidence in Pakistan is not well established. Ayub Medical College study in 2015 showed 28.3% incidence of meningiomas.[2]

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World Health Organization (WHO) classifies meningioma from grade I to grade III with grade I being the benign neoplasm, grade II the atypical ones and grade III being malignant meningiomas.[3] Grade I tumors are most prevalent meningiomas constituting about 69-81% tumors and grade II meningiomas are 20-25%. Grade III tumors being the least common ones comprising 1-6% of all meningiomas.[4] Meningioma is chiefly diagnosed in middle to old age. More than 90% of these tumors grow slowly with benign histology but some can show aggressive behavior like multiple recurrences, brain invasion and extension to adjacent areas of dura matter and bone. [5]The five-year survival rate of meningioma is less than 70% which deteriorate with age of the patient. Its five-year recurrence rate is

20% in radically resected benign tumors. Recurrence is associated with multiple surgeries and shortened life span of the individual suffering from the tumor. [6]

Histopathological parameters including WHO grades, expression of hormone receptor (PR) and proliferative marker (Ki-67) have prognostic and predictive significance in managing meningiomas. [7]The multiplex paradigm of grading of meningiomas is based on various criteria according to WHO 2016 classification. A neoplasm is considered grade I if mitosis is <4/10 high power field (HPF) and it includes various subtypes as meningiothelial, fibrous, transitional, psammomatous, angiomatous, microcystic, secretory and lymphoplasmacyte rich meningiomas. Meningioma is given grade II if mitosis is 4-19/10 HPF or there is increased cellularity, high nuclear to cytoplasmic ratio, and sheet like growth pattern, areas of brain invasion and foci of necrosis. Atypical, chordoid and clear cell meningiomas are subtypes of grade II. Grade III is given if mitosis is >20/10 HPF or there is loss of differentiated features and it includes subtypes as papillary, rhabdoid and anaplastic meningiomas. [3] Histology alone may reveal the malignancy of the tumor but it does not always correspond with the clinical outcome of the patient. 20% WHO grade I meningiomas recur after radical surgical resection while 71% grade II (atypical) and 50% grade III (malignant) neoplasms show benign behavior with no recurrence.[8] Irradiation of skull, genetic mutations and epigenetic factors may also cause recurrence. Another important factor is progression of the neoplasm to a higher WHO grade.[9]This uncertainty of tumor behavior is a substantial hazard in the management of meningioma as which patient is to receive adjuvant radiotherapy and which is to undergo close clinical follow-up. [4]

Immunohistochemistry (IHC) has been in use of pathological diagnostic regime since decades. IHC markers such as PR and proliferative marker index Ki-67 have considerable prognostic and tumor predictive role in ambiguous cases of meningioma. [9] PR expression is found in 70-80% of all meningiomas having a negative correlation with proliferative index Ki-67. Also, with higher WHO grades of meningiomas, there is loss of differentiation marker (PR) and increase of proliferative marker (Ki-67). Losses of PR positivity and high Ki-67 index with higher grades of meningioma signifies their importance in grading and management of these tumors. [5]

Convexity tumors are most common and are easily resected surgically with low five-year tumor recurrence rate of 3%. On the other hand, para-sellar and sphenoid ridge meningiomas are not easily resected completely and they have higher five-year recurrence rate. Tumor recurrence rate for para-sellar tumors is 19% and 34% for sphenoid ridge neoplasms.[6]Adjuvant radiotherapy is also the treatment of choice in higher grade meningiomas but skull irradiation increases malignant transformation of these neoplasms. [9]In such cases target therapy with anti-progesterone drugs (Mifepristone) would be of special importance in slowing or may even inhibiting the growth of the tumor. Also, when meningiomatosis is compared with isolated meningiomas, they exhibit increased female dominance (90%) and higher PR expression and such cases are not ideal candidates for surgery. [10]

The rationale of my study is, PR expression status will provide an option of target therapy with anti-PR agents (Mifepristone). In patients who are either unfit for surgery or are candidates for multiple surgeries because of meningiomatosis, this target therapy will be a preference. This treatment plan will also avoid adjuvant radiotherapy which is a cause of malignant transformation of the tumor. Ki-67 will apprehend proliferative state of the tumor thus helping in timely intervention in aggressive and recurrent neoplasms of high Ki-67.

Materials and Methods

After approval from Ethical Review Board & Advanced Studies and Research Board of Shaheed Zulfiqar Ali Bhutto Medical University (SZABMU) Islamabad, this cross-sectional study was carried out in the Department of Histopathology, PIMS Islamabad from 1st September 2017 to 30th August 2018. Consecutive non-probability sampling technique was used. Neurosurgical biopsies and resection specimens (received in 10% formalin) of 70 patients of both genders and all ages with clinical impression of meningioma were included. Non-meningioma specimens and inadequate samples with specimen insufficient for making histopathological diagnosis were excluded from this study. After gross examination the tissues were processed in automatic tissue processor and blocks were prepared, followed by cutting, slide preparation and staining with hematoxylin and eosin (H&E) stain. Microscopy of all cases was carried out on Olympus CX 22 LED series microscope, by postgraduate resident along with supervisor and diagnosis on (H&E) slides was made. All cases of meningioma were graded according to WHO grading system 2016. [3] Immunohistochemistry

(IHC) of PR and Ki-67 immuno-markers was done. Distribution of PR staining is semi quantitatively scored by using immune-reactive score (IRS). [7]

The intensity of PR staining is interpreted as follows:

0: no staining, +1: weak staining, +2: moderate staining, +3: strong staining.

The percentage of positively stained tumor cells in entire section is scored as follows:

0: absence of positively stained nuclei, 1+ : <10% of cells stained, 2+: 10-50% of cells stained,

3+: 51-80% of cells stained, 4+ : >80% cells stained.

As recommended for breast cancer, an immuno-reactive score (IRS) is calculated for each case of meningioma by multiplying the staining intensity with the indicator for positive tumor cells producing an IRS range from 0 to 12. Meningioma cases having an IRS of 2 or more are considered positive. [7]

Ki-67 immunostaining was done and the entire section was screened to find the region with maximum positive nuclear staining of Ki-67 (hot spot areas). The percentage of positively stained nuclei was scored in the region using 40X objectives, in 100 tumor cells. Quotients (positively stained tumor cells / total counted tumor cells) were calculated as percentage after multiplication by 100 & rounded off to nearest integer. This is then called as Ki-67 Labelling Index (LI). [4]

All data were analyzed by using SPSS version 21. Mean and standard deviation were calculated for quantitative variables like age and immuno-histochemical scores. Frequency and percentages were calculated for qualitative variables like histopathological subtypes and grades of tumor. Pearson correlation test was used to compare histopathological grade with both immuno-histochemical markers (PR & Ki-67 LI) separately and then with both. P value of <0.05 was taken as significant.

Results

Out of the total 70 cases of meningioma, 57% (40/70) were females while 43% (30/70) were males. This gives female to male ratio of 1.33. The ages ranged from 7 years to 76 years with a mean age of 44.34 years and median age of 46 years. Among the female gender, 72.5% (29/40) were in age group 40-59 years. The vast majority of cases were WHO grade I (n=55, 78.6%) followed by grade II cases (n=12, 17.1%) while the least common were grade III cases (n=3, 4.3%). Meningiothelial meningioma was the most common histological subtype (n= 28) overall (40%) as well as in grade I cases (51%). In grade II cases, atypical meningioma exhibited the most common subtype (n=9, 75%). Because of the presence of brain invasion, one meningiothelial subtype was given the diagnosis of WHO grade II. All grade III cases were of anaplastic subtype (n=3, 100%). PR immuno-expression was seen in 68.6% cases (48/70). As far as gender variation regarding PR expression is concerned table 1 well elaborates it.

Table 1: PR expression with respect to gender and Age (n=70).

PR expression	Gender		Total
	Male	Female	
Negative	10 (33.3%)	12 (30%)	22
Positive	20 (66.7%)	28 (70%)	48
Total	**30**	**40**	**70**

Age (in years)	PR expression		Total
	Negative	Positive	
1-19	1	2	3
20-39	5	12	17
40-59	13	29	42
60-79	3	5	8
Total	**22**	**48**	**70**

More-over when PR immuno-expression regarding age group distribution is concerned, most frequent expression of this marker was found in age group 40-59 years as described in detail in table 1.

PR immuno-expression with respect to WHO grades illustrated a statistically significant trend of decreasing PR positivity in successive grades of meningioma (P= 0.035). Table 2 illustrates this trend.

Table 2: PR expression in WHO grades of meningioma (n=70)

PR expression		WHO grade			Total	P-Value
Status	**Score**	**1**	**2**	**3**		
Negative	0	6	3	1		
	1+	6	4	2		
	Total (A)	12 (21.8%)	7 (58.3%)	3 (100%)	22 (31.4%)	
Positive	2+	14	2	0		***0.035***
	3+	10	3	0		
	4+	19	0	0		
	Total (B)	43 (78.2%)	5 (41.7%)	0 (0%)	48 (68.6%)	
A+B		12+43 = 55	7+5 = 12	3+0 = 3	70	

Overall mean Ki-67 LI for all 70 cases was 5.56% ± 7.05% with a range of 0-35%. No statistically significant correlation was found between Ki-67 LI and gender of the patients (P value 0.709). With respect to grades of meningioma, an increasing value of mean Ki-67 LI was seen with increasing WHO grades showing a significant correlation with P value 0.02. (Table 4)

With increasing WHO grades, PR immuno-expression decreased while mean Ki-67 LI increased depicting an inverse correlation with statistical significance. (P value = 0.02) (Table 3).

Table 3: PR expression & mean Ki-67LI in WHO grades of meningioma (n=70).

WHO Grades	PR expression	Mean Ki-67LI	P-Value
Grade I	43/55 (78.2%)	3.31±2.94(%) (range 0-12%)	**0.02**
Grade II	5/12 (41.7%)	12.33±10.36(%) (range 1-30%)	
Grade III	0/3 (00%)	19.67±13.28(%) (range 12-35%)	
Total	**48/70 (68.6%)**	**5.56 ± 7.05(%) (range 0-35%)**	

Discussion

Meningioma is the brain tumor that arises from the cap cells of the arachnoid membrane mostly occurring in middle to old age and predominantly in female gender. It displays a wide range of histo-morphological picture and generally being categorized into benign, atypical and malignant. Most of these neoplasms are benign but some types exhibit aggressive behavior and recur even after complete removal. [11]The incidence rate of meningioma is increasing with age in United States. [12]In France, an overall increase of CNS tumors is noted particularly for meningiomas and especially in females.[13]

Statistically significant correlation of PR & Ki-67 IHC markers with grades of meningioma has been observed by many studies. Association of sex steroid hormones (PR) with meningioma is implied since decades. PR expression may correspond with the grade of the neoplasm and its recurrence. WHO grade I tumors (benign) reveal markedly higher PR expression as compared to the WHO grade II & III meningiomas (aggressive). Also, higher PR expression is observed in female patients and in meningiothelial subtypes.[14]Ki-67 has the most significant role in differentiating WHO grade I neoplasms from WHO grade III tumors. Mean Ki-67 is 0.7%, 2.1% and 11% for WHO grades I, II & III respectively. [15]

In our study, the incidence of WHO grade I, II & III was 78.6%, 17.1% and 4.3% respectively. This was in concordance with a study conducted in Lahore showing 80%, 16% & 4% incidence for Grade I, II & III respectively. [16] Regarding Immuno-expression of PR, 68.6% (48/70) cases showed PR positivity. This was in concordance with two Pakistani studies giving 78% PR positivity in meningioma by Tahir et al [17] and 60% PR positivity by Kanwar et al.[16] An Indonesian study showed >90% PR positivity after contraceptive use in patients of meningioma. [18]Study by Menke et al. conducted in California was also in concordance with our study showing PR Immuno-positivity of 77.3%. [19]

No significant gender variation of PR positivity was noticed in our study as illustrated by female versus male expression of 70% and 66.7% respectively. It was in harmony with a study carried out in Lahore. [16]Age group distribution regarding PR positivity in our study illustrated most common positivity (60.4%) in 40-59 years of age and least common positivity (4.2%) in age group 1-19 years. This was in concordance with

a study showing most common age group of PR positivity to be 40-50 years. [20]

PR Immuno-expression with respect to grade showed decreasing PR positivity in successive grades depicting 78.2% in grade I, 41.7% in grade II neoplasms and 0% in grade III tumors. Tahir et al depicted similar PR positive trends showing 89.8% in grade I, 46.6% in grade II and 0% PR positivity in grade III cases.[17] This was in concordance with an Iranian study depicting 96.8% PR positive grade I and 20% PR positive grade II tumors while none of the grade III neoplasm showed PR positivity. [5]Everson et al. study results in Texas were also in harmony with our study, showing gradual PR positivity decrease of 79%, 41% and 29% in grade I, II and III respectively.[21]

In our study mean Ki-67 LI for grade I, II and III meningioma successively increased with values 3.3%, 12.3% and 19.7% respectively. Shayanfar et al. suggested similar results with mean Ki-67 LI of 3%, 9.3% and 34% for grade I, II and III meningioma respectively. [5] Similar successive increase of mean Ki-67 LI with increase of grades were noticed in an Indian study. [22]

The significant inverse correlation of decreasing PR positivity and increasing mean Ki-67LI with successive WHO grades of meningioma (p value = 0.02) of our study was in harmony with Iranian, Nigerian & Indian studies. [5,7,20]

Conclusion

In conclusion, losses of PR positivity and high Ki-67 index have strong association with higher grades of tumor signifying their importance **in** grading and management of these tumors.

PR Immuno-status and Ki-67LI of tumors and their inverse correlation with successive WHO grades of meningioma could help identify patients in need of anti-PR target therapy and close follow-up. PR immuno-positivity could be beneficial in inoperable and recurrent cases of meningioma. Ki-67LI could also be a useful prognostic marker in deducing recurrence and invasive status of a tumor.

Limitations

Inter-observer variability in calculating Ki-67 LI was noticed. Follow-up of patients could not be done due to non-availability of clinical data. It was important in defining the prognostic value of Ki-67LI and PR among various WHO grades of meningioma. Lack of clinical history while submitting biopsy specimens limited access to valuable established risk factors of meningioma.

Conflict of Interest: Authors declare no conflict of interest.

Funding: No funding was received for this project

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HISTORY	
Date received:	17-07-2022
Date sent for review:	25-07-2022
Date received reviewers comments:	29-08-2022
Date received revised manuscript:	07-09-2022
Date accepted:	09-09-2022

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B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion