Original Research Article

The correlation of Ki-67 (MIB-1) proliferation index and progesterone receptor status with histological grade of meningioma: An observational study

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Received: 04-09-2021 / Revised: 09-10-2021 / Accepted: 16-11-2021

Abstract

Context: Five year rate for recurrence of symptoms regardless of method of treatment is 19.2% for benign tumors and 32.4% for malignant tumors. Behavior of meningiomas depends upon its histological type, grade, proliferation markers and PR status. **Aims:** To correlate the WHO grade, PR status and Ki-67 labeling index of meningiomas to correlate with other histologic and demographic parameters of prognostic significance. **Material and methods:** A total of 120 cases were taken over a period of 2 years. Routine H&E stain sections used for histological typing and grade of meningioma. Immunohistochemistry done for Ki-67 and Progestrone receptor using immunoperoxidase method. The various grades of meningiomas than correlated with Ki-67 labeling index and PR status and appropriate statistical calculations done. **Results:** The peak age distribution is between 31 to 50 years with mean age of 49.45 years, male to female ratio being 1:2.3. Maximum cases were of grade I (88.33%) followed by grade II(6.66%) and grade III(5%). There was no significant difference between mean age of different grades ($p>0.05$). The difference between mean Ki-67 labeling index between grade I and II, grade II and III and grade I and III all were significant ($p<0.0001$). The PR status difference between grade I and II tumor was significant ($p=0.0007$), however between grade II and III could not be calculated as none of these tumor showed PR positivity. The Pearson correlation coefficient between PR status with Ki67 labeling index came out to be -1.0, that shows highly significant negative correlation. **Conclusion:** This study shows an inverse correlation between WHO 2016 grades of meningioma and PR status while shows a linear relation with Ki-67 labeling index.

Keywords: Ki-67 labeling index, Meningioma, progesterone receptor, Recurrence, WHO grade

Introduction

Meningiomas are the most frequently reported primary intracranial tumors**[1]** arising from neoplastic meningothelial cells. Most are benign but their intracranial location and tendency to recur leads to morbidity and mortality[2]. Five year rate for recurrence of symptoms regardless of method of treatment (surgery/radiation) is 19.2% for benign tumors and 32.4% for malignant tumors. In patients whose benign tumors had been completely removed, 5 years rate of recurrence was 20.5% in a study by McCarthy et al[2]. Some studies have shown a good correlation between Ki-67(proliferative marker) expression calculated as Ki-67 Labeling Index and increasing grades of meningioma[3,4]. Others indicate that benign meningiomas that are Progesterone Receptor (PR) positive are less likely to recur, whereas aggressive meningiomas are associated with loss of PR and poorer prognosis[5,6]. Within same grade of meningioma, those with higher Ki-67 labeling index are more likely to recur[7].Evaluation of these parameters help in deciding aggressive treatment and frequency of follow-ups.

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This study was aimed to correlate the WHO grade, PR status and Ki-67 labeling index of meningiomas received in our institution and to correlate with other histologic and demographic parameters of prognostic significance.

Material and methods

Ethical approval for this study was taken from Ethical committee of Dr S N Medical College, Jodhpur (SNMC/IEC/2019/371-373 dated 26.04.2019). The study was a hospital based, retrospective, observational study conducted to assess the correlation of Ki67 index and progesterone receptor status with histological grades of meningioma diagnosed at Department of Pathology, Dr S N Medical College, Jodhpur from January 2017 to November 2019. The materials that provided data for this study included histology requisition forms accompanying resection specimen from neurosurgery department, patients case sheets, copies of histopathology reports, paraffin tissue blocks and slides archived.

Cases where case sheets and imaging reports were not available or where tissue blocks had been issued to patients are excluded from study. One hundred and twenty samples qualified for the study. The sample size was calculated at 95% confidence interval to verify an expected 38% proportion of PR positivity among Meningioma (as per findings of a previous study)[8,9] and taking 20% relative allowable error. Sample size was calculated using the formula for sample size for estimation of proportion –

$N=Za/2^2P[1-P]/E^2$

Where Za/2 = Standard normal deviate for 95% confidence interval (taken as 1.96); P = Expected proportion of PR positivity among Meningioma (taken as 38% as per reference article[8,9]); E = Relative allowable error (taken as 20%). Sample size was calculated to be

minimum 58 samples, but due to availability of cases, sample size was enhanced and rounded off to 120 patients of Meningioma.

Archived routine Hematoxylene-eosin (H&E) stained slides of all cases confirmed as meningioma were retrieved, reviewed and again classified according to WHO 2016 criteria[10]. Immuno-histochemistry for Ki67 and Progesterone receptor (PR) was done on fully automated immunostainer. Primary antibodies used were mouse monoclonal antibodies from Dako. All the immunostained sections of different grades were scanned randomly at 100X Magnification for the most densely labeled areas. Then for Ki67, nuclear counting is performed at 400X. A total of 1000 nuclei are counted in the most densely labeled microscopic fields. Vascular endothelial cells, lymphocytes and necrotic areas if present were excluded from counts. The Ki-67labeling index is expressed as the percentage of Ki-67 immunolabelled tumor nuclei[11]. For PR staining, allred score was followed as: Proportion score 0 for no staining in any nuclei; 1 for < 1% nuclei stained; 2 for 1-10% nuclei stained, 3 for 11-33% nuclei stained; 4 for 34-66% nuclei stained; 5 for 37-100% nuclei stained. An intensity score of 1 given to weak nuclear staining; 2 for moderate staining and 3 given to strong staining. The proportion and intensity were then summed to produce total scores of 0 to 9. A score more than 3 was regarded as positive[12].

Observation and results

One hundred and twenty cases met the criteria and are included in the study. The peak age distribution is between 31 to 50 years with mean age of 49.45 years(Table I).

Table 1:Age and sex distribution in meningioma patient (n=120)

Age (Yr)	Male cases	Female Cases	Total
11-20	0	4	4
21-30	4	6	10
31-40	8	18	26
41-50	8	18	26
51-60	4	18	22
61-70	10	16	26
71-80	2	4	6
Total	36	84	120
Male to female ratio	1:2.3		

Thirty six cases occurred in males, while 84 in females giving male to female ratio of 1:2.3. Two cases were in pediatric age group, both being females. Distribution of meningiomas according to grade were, Grade I being highest (88.33%), followed by grade II (6.66%) and grade III being least common comprising of 5% of cases(Table II).

Table2:Association of Mean age and Ki-67 index with different grade of meningioma

WHO Grade	No of cases	Male	Female	M:F ratio	Mean age (years)	Mean Ki-67 Labeling index(Ki-67 LI)		Standard deviation(Ki-67 LI) (SD)	Between grade	P value (among Ki-67 LI of different grades)	P value (among mean age of different grades)
Grade I	106	32	74	1:2.3	50.5	1.09		0.55	I and II	<0.0001	>0.05
Grade II	8	2	6	1:3	42	7.2		1.32	II and III	<0.0001	>0.05
Grade III	6	2	4	1:2	41	34.67		11	I and III	<0.0001	>0.05
Total	120	36	84	1:2.3	49.45	Male (Mean Ki-67 LI)	3.57	P=0.719 (NS)			
						Female (Mean Ki-67 LI)	3.01				

Meningiomas in spinal location comprised of 13.3% (16 cases), rest were in intracranial location (86.7%).

Frontal region was the region, most commonly affected by meningioma (8.33%) followed by sphenoid wing (5.8%). The mean age for grade I meningioma was 50.5 years, for grade II 42 years and for grade III it was 41 years. There was no significant difference between mean age of different grades (p>0.05)(Table II). Within grade I meningioma meningothelial subtype was most common (56.7%) followed by transitional type (15%). Psammomatous type comprised of 11.4%, angiomatous 7.6%, fibroblastic 5.7%, secretory and lymphoplasmacytic comprised of 1.8% each (not shown). The mean Ki67 labeling index for male and females of all grades of meningiomas were 3.57 and 3.01 respectively, the difference of mean Ki67 labeling index between sexes was not statistically significant (p=0.719)(Table II).

The mean Ki67 labeling index for different grades of meningioma (Table II). The difference between mean Ki67 labeling index between grade I and II, grade II and III and grade I and III all were significant (p<0.0001)Among different histological types of grade I meningiomas, mean Ki67 labelling index was higher for transitional and secretory subtypes than other subtypes but this difference was not significant. Mean Ki67 labeling index for both subtypes of grade II meningioma (Atypical and chordoid) was similar i.e. 7.2 and 7.1 respectively. With category of grade III meningioma, mean Ki67 labeling index for anaplastic meningioma was significant higher than for papillary type (p<0.05).The Progesterone receptor (PR) status of different grades of meningioma (Table IV).

Table 3:Association between PR status and Ki-67 Labeling index

PR Status	No. of Cases	Ki-67 LI		
		Mean Ki-67 LI	SD	P value
Positive	74	0.99	0.53	0.0001
Negative	46	6.7	11.79	

69.8% cases of grade I tumor showed PR positivity while none of grade II and III tumor were positive for PR immunohistochemistry. The PR status difference between grade I and II tumor was significant (p=0.0007)(Table 4).

Table 4: Progesterone receptor status in meningioma in relation to grade

WHO Grade	PR negative	PR positive	Grand Total	Between grade	P* value
I	32	74	106	I and II	0.0007
II	8	0	8	II and III	Could not be calculated
III	6	0	6	I and III	0.0007

*P value between two percentages

The PR status between grade II and grade III tumors couldn't be calculated as none of these tumor showed PR positivity.Out of 120 cases, ten cases showed a mitotic index of more than 4/10HPF with mean Ki67 labeling index of 1.51 and rest showed a mitotic index of less than 4/10HPF with mean Ki67 labeling index of 18.48. This difference is also significant (p<0.0005).Out of 120 cases, 112 cases showed a mitotic index of less than 4/10HPF with PR positivity in 66% and rest eight cases showing a mitotic index of more than 4/10HPF with all showing loss of progesterone receptor expression (PR negative). This difference is also significant (p<0.0005).Out of 84 female patients of all grades of meningioma, PR was positive in 54 cases (64.3%) while 20 out of 36 male patients showed PR positivity (55.5%). The difference is not significant (p=0.368)(comparison of proportions).Table IV shows association of PR status with Ki67 labeling index. The Pearson correlation coefficient value came out to be -1.0, that shows highly significant negative correlation.

Discussion

The study entitled "The correlation of Ki-67 (MIB-1) proliferation index and progesterone receptor status with histological grade of meningioma: An observational study" was conducted in the Department of Pathology. S.N. Medical College, Jodhpur in the year 2017 to 2019 on the 120 consecutive clinically and radiologically detected and histo-pathologically confirmed cases of meningioma obtained from the Department of neurosurgery.

Meningiomas are generally benign neoplasms that are often treated by surgical resection. Most patients are cured by surgery alone and remain recurrence free. Some tumors behave in a more aggressive fashion with development of local recurrence and metastasis.

The overall mean age of the patients in our study was 49.45 years with (age range of 13-80 years) which was similar to study by Shayanfar N et al[13](Mean age 49 yrs). Maximum cases were noted between 31 and 50 years. Only two (1.66%) of the cases occurred in the age group of 11 to 15 years, this is supported by Ferrante et al[14]. Studies from all over the world have shown that meningiomas are seen more commonly in females than males as in present study with male to female ratio of 1:2.3. They also occurred at a lower age group in this study as compared to western population[2,15]. In this study the most common location was frontal which was similar to study done by Abramovich et al[3]. The mean age in our study did not show any significant difference between the grades. This finding was similar to Bruna et al[15].According to histological grade we found 106 cases of Grade I (88.33%), 8 cases of Grade II (6.66%) and 6 cases of Grade III(5%). These findings were similar to Jaaskelainen et al[16]. Grade III cases comprised of 5% cases in our study which was similar to results of several other studies[17,18]. However Grade II cases comprised only 6.66 % cases in our study which was similar to Jaaskelainen et al (4.7%)[17]; Maier et al (7.2%)[16] but deviated from Willis et al(20.4 %)[18]

Out of the 120 evaluated cases 60 were meningothelial (50%), 16 transitional (13.3%), 12 Psammomatous (10%), 6 fibroblastic (5%), 8 were angiomatous (6.6%), two each of (1.66%) secretory, microcystic, chordoid and papillary, six were atypical (5%)and four case were anaplastic (1.66%), and this finding was similar to Gursan et al[18]. In other studies syncytial, fibroblastic and transitional variants were more common[2,20]. This is probably due to the lack of strict criteria for categorizing them into subtypes and it is also well known that categorizing grade I meningiomas into subtypes have no clinical significance[2,21]. This is further supported by the fact that there is no significant difference of Ki67 labeling index and PR status in different histological subtypes of grade I meningiomas in this study.The strongest criterion in determining the behavior of meningioma is histological grade. But even within same grade, behavior of different subtypes of meningioma vary, at the same time there are certain instances where all the criteria of grading are not met with to clearly label a grade to these tumors. Various studies have proposed the use of proliferative marker such as Ki-67 immunohistochemistry as an additional support to histology for predicting tumor recurrence and survival[3].Several studies have shown a good correlation between Ki-67 and grade in meningiomas[3,22]. In our study the mean Ki-67 labeling index showed increase with increasing grade of tumor. There is a significant difference in Ki-67 labeling index between grade I and II, Grade II and III, and between grade I and III (p <0.05) and these findings are corroborative with the previous studies[3,19,22]. There was no significant difference in Ki-67 labeling index between different histologic subtypes within grade I group and this finding correlated well to Mukherjee et al[13]. Tao et al reported that grade I meningiomas have a recurrence rate of 7-20%after surgical resection and within grade I tumors risk of recurrence is higher in those with high Ki-67 index[23]. Even after this observation by many authors Ki-67 labeling index have not been included in the grading criteria, probably due to the high inter-institutional and inter-individual variability with respect to their cut offs[20].We have found PR positivity in 61% of the cases which is similar to other studies[9,13]. However there are different other studies[18] which showed a little higher PR positivity (72%) as well as studies which showed lower PR positivity[8]. There is no significant gender difference in expression of PR in this study which is in line with few studies[8], while others showed a significant higher expression of PR in women than do men[13,19].Our Study included 8 cases of grade II and 6 cases of Grade III meningioma, none of them expressed PR .while 69.8%(74 out of 106) of Grade I tumors were positive for PR . In this study positive immunostaining rate for PR in Grade I meningiomas was significantly higher (p <0.05) than in high grade tumors which is similar to other studies[5,6,13,19].

Cahill et al[24] and Brandis et al[9] reported that malignant meningiomas are devoid of PR and ER. Reported associations for PR expression and histologic grade have been variable. Previous studies have stated the presence of significantly higher PR values in benign meningiomas[5,8,9,19] compared with WHO grade II or III tumors, and that atypical and anaplastic meningiomas frequently lack PR[8].

Previous studies have shown that PR negative tumors have higher Ki-67Labeling Index compared to PR positive tumors[8]. In the present study the Ki-67 Labeling Index was significantly higher in the PR negative tumors compared to PR positive tumors (p value <0.05) and this finding correlated well with the above studies. However Markwalder et al[25]., and Perrot et al., 1992[26] found no correlation between proliferation and PR status.

It is obvious from above national and international studies that the Ki-67 LI shows an increase with increasing grade of tumor while high grade tumors show loss of PR .Thus a combination of Ki-67 LI and PR status can be used as an adjunct to histological grading especially in benign borderline cases.Anti-progestin drug Mifepristone may be a theoretical possibility but Giulia Cossu et al[27] in their review article concluded that no clear evidence exists to recommend Mifepristone in inoperable meningiomas. Preliminary encouraging results were found in diffuse meningiomatosis.

Conclusion

This study shows an inverse correlation between WHO 2016 grades of meningioma and PR status while shows a linear relation with Ki-67 labeling index. There is no cut-off value for Ki-67 labeling index in different grades along with the fact that within same grade I tumors, different histologic subtypes have different Ki-67 labeling index and hence chances of recurrence. This study recommends that PR status and Ki-67 labeling index must be done in all cases of meningioma regardless of their grade that will identify a subset of patients with increased chances of recurrence who can be benefitted from a more customized treatment and follow-up.

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Source of support:Nil
Conflict of Interest:None