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Investigating The Expression of ER, PR, And Ki-67 In Different Grades of Meningioma

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Abstract

Introduction: Tumor grade is one of the significant factors in determining the prognosis of meningioma. Ki-67 is a marker expressed in the active phase of the cell cycle and indicates cell division and can be used as an auxiliary tool in determining the grade of meningioma. Steroid hormones are one of the factors that are effective in the pathogenesis of meningioma. This study evaluates the expression level of ER, PR, and ki-67 markers in different grades of meningioma. Materials and Methods: In a descriptive study, slides, and paraffin blocks of 80 meningioma patients who underwent surgery over 10 years at Besat Hospital, Hamedan, were extracted from the patient's medical records and were evaluated in terms of the expression of ER markers, PR and ki-67 after immunohistochemical staining. Results: In meningioma grades 1, 2, and 3, mean PR expression was 17.33, 11.66, and zero (P=0.033), respectively, and mean Ki-67 expression was 0.67, 5.22, and 10.50, respectively (P<0.001), and mean ER expression was zero in all three grades. There was an inverse and significant correlation between tumor grade and PR rate (p=0.04, r=-0.21). Conclusion: ki-67 expression can be used as an auxiliary method in meningioma grading, and more PR positivity indicates lower grades, and thus, better tumor prognosis.

Keywords: *Meningioma, PR, ER, Ki-67, Immunohistochemistry.*

Introduction

Meningioma is a tumor originating from arachnoid cap cells and accounts for 37.6% of primary brain tumors in adults. Its annual incidence rate is 8.83 per 100,000 people [1]. The incidence of meningioma increases with increasing age [2]. The mean age at diagnosis is 66 years. The prevalence of meningioma in females is 1.12 to 2.33 times that of males [3]. The World Health Organization (WHO) classified meningioma into three grades based on histopathological characteristics: Benign Meningioma, Atypical Meningioma, and Anaplastic Meningioma [4]. Additionally, 15 subtypes have been introduced for meningioma, the most important of which are Meningothelial, Transitional, and Fibroblastic [5]. The most significant factor in determining the prognosis of meningioma is identifying the factors related to the rate of tumor recurrence. For example, surgical resection rate, histological type, tumor grade, proliferative activity, and hormone receptor status are effective in tumor recurrence [6]. In this regard, tumor grade is of great importance, so the recurrence rate is between 7 and 25% in grade 1, 30 and 50% in grade 2, and 50 and 95% in grade 3 [7].

One of the problems facing meningioma is that histological criteria alone do not determine the biological behavior of the tumor [8]. Thus, further studies are valuable to find markers that predict well the biological behavior of the tumor [9]. Another problem in examining these tumors is the presence of

high interobserver bias in applying the criteria used to determine the grading, which causes the tumor to not be treated correctly [10]. Thus, finding markers that are expressed in meningioma is of great importance. Ki_67, ER, and PR are among these markers [11]. Ki-67 is a marker expressed in the active phase of the cell cycle and indicates cell division [12]. This marker in meningioma is an independent predictive factor that increases the risk of recurrence after surgery [13]. The higher occurrence of meningioma in women, the behavior of this tumor in pregnancy, and the increased risk of meningioma with the use of progestin such as cyproterone acetate indicate that steroid hormones are involved in the growth of meningioma [14, 15]. The expression frequency of Ki67, ER, and PR markers was investigated in this study. Examining such markers along with grading can predict the biological behavior of the tumor and thus provide physicians with valuable information about the possibility of recurrence or malignant transformation.

Materials and Methods

In this descriptive-analytical study, 80 meningioma patients who were diagnosed with meningioma from the beginning of 2011 to the end of 2021 and had paraffin block for immunohistochemistry were examined. All slides and tissue blocks fixed in paraffin were extracted for each sample. First, all slides were reviewed by a pathologist to confirm the diagnosis and grading of the tumor. From each sample, the paraffin block containing the tumor with the highest grade was selected and stained by the IHC method. In this study, mouse antibodies Estrogen receptor clone 6F11, Progesterone receptor [16] Ki67 (MM1) from Leica Company were used. All steps were performed based on the protocol in the kit. First, three-micron slices were prepared from the blocks. Ag retrieval steps were performed using an Ag retrieval buffer. After using endogenous peroxidase staining and blocking protein, we added primary antibodies (anti-ER, PR, Ki-67). Then, we performed chromogen staining by DAB (diaminobenzidine) and stained with hematoxylin. Breast invasive ductal carcinoma tumor was considered a positive control.

The stained slides were examined by a pathologist with a light microscope for the expression of Ki-67, ER, and PR markers. In the case of Ki-67, cells with brown nuclear staining were considered positive. Ki-67 Labeling Index was reported as a percentage of cells that were stained in a thousand cells counted. Counting was done in the area with the highest immunoreactivity and with high power. Ki-67 LI results were expressed as a percentage and then grouped as 0-4%, 4.1% to 7%, 7.1% to 11%, and above 11%. Lymph nodes were considered as a positive control.

In the case of ER and PR, the staining intensity and the percentage of stained cells were calculated. Tumors were classified as follows.

(0) or negative: none of the cells were stained

(1) Weakly positive: less than 10% of the cells were stained

(2) or moderately positive: between 10 and 50% of the cells were stained

(3) or strongly positive: more than 50% of the cells were stained

The presence of a paraffin block for immunohistochemistry was one of the inclusion criteria of the study. Incomplete data and insufficient or inappropriate paraffin block for immunohistochemistry were the exclusion criteria of the study. SPSS-26 software was used for data analysis. Descriptive information was summarized as frequency, percentage, mean, and standard deviation. In the analytical section, to compare the expression levels of PR and KI-67 according to different tumor grades, the non-parametric Kruskal-Wallis test was used quantitatively and Chi-square tests were used qualitatively. All analyses were performed at a significance level of 5%

Results and Discussion

The mean age of the patients was 60.3 years. In this regard, 73.8% were female and 26.3% were male. Regarding tumor grade, 63 cases (78.8%) were grade 1, 9 cases (11.3%) were grade 2, and 8 cases (10%) were grade 3. Histologically, the most common type was fibroblastic (27.5%), followed by meningotheliomatosis (21.3%) and transitional (16.3%) (Table 1).

Table 1: Frequency of histological types of meningioma tumors

Histological type	Frequency (%)	Histological type	Frequency (%)
Fibroblastic	22(27.5)	Psammomatous	7 (8.8)

Meningotheliomatosis	17 (21.3)	Angiomatosis	3 (3.8)
Transitional	13 (16.3)	Microcystic	1 (1.3)
Atypical	9 (11.3)	Enplaque	1 (1.3)
Anaplastic	7(8.8)	papillary	1 (1.3)

The mean expression of PR was 16.6 in total and it was 17.33 in grade 1, 11.66 in grade 2, and zero in grade three. The mean ki-67 was 2.16 in total, 0.67 in grade 1, 5.22 in grade 2, and 10.5 in grade 3. The expression of ER in total and all grades was zero (Table 2).

Table 2. Mean and standard deviation of PR, ki-67, and ER expression levels in different grades of meningioma

Grade	PR Mean ± Sd	ki-67 Mean ± Sd	ER Mean ±Sd
Grade 1	17.33 ± 27.26	0.67±0.44	0
Grade 2	11.66±25.94	5.22 ± 2.38	0
Grade 3	0	10.50 ± 4.10	0
Total	26.07±14.96	2.16±3.47	0
P.value*	0.033	>0.001	

The level of PR expression in different grades of meningioma was analyzed by the Kruskal-Walli's test. Its results revealed a significant relationship between PR expression and meningioma grade (p-value<0.025) so the level of PR expression is higher in lower grades

Table 3. PR expression levels in different grades of meningioma based on the rank classification

Grade	pr								N
	negative		weakly pos		moderately pos		strongly pos		
	n	%	n	%	n	%	n	%	
Grade 1	30	47.6%	12	19%	8	12.69%	13	20%	63
Grade 2	5	55.5%	3	33.3%	0	0%	1	11%	9
Grade 3	8	100%	0	0%	0	0%	0	0%	8
Total	43	53.7%	15	18.75%	8	10%	14	17.5%	80

The comparison of the expression levels of ki-67 in different grades of meningioma was analyzed with the Kruskal-Walli's test. Its results showed a significant relationship (p-value<0.000) so the expression level of ki-67 increases with the increase of the tumor grade.

Table 4. ki67 expression in different grades of meningioma based on the rank classification

Total	mib1li				Grade
	11-100%	7.1-11%	4.1-7%	0-4%	
63	0(0%)	0(0%)	0(0%)	63(100%)	Grade 1
9	0(0%)	2(2.2%)	5(55%)	2(22%)	Grade 2
8	2(2.5%)	6(7.5%)	0(0%)	0(0%)	Grade 3
80	2(2.5%)	8(10%)	5(6%)	65(81%)	

Table 5 shows the relationship between the expression level of PR and the histological type of tumor. Based on the result of the chi-square test, no significant relationship was observed between PR expression level and tumor histological type (p=0.068).

Table 5. Frequency of histological type according to PR expression

p-value	Chi-square value	Mean rank	histological type	Variable
0.068	15.69	36.66	Fibroblastic	PR
		48.03	Meningotheliomatosis	
		49.96	Transitional	
		52.14	Psammomatous	
		30.33	Angiomatosis	
		22	Microcystic	
		22	Plug	
		22	papillary	
		36	atypical	
		22	Anaplastic	

The results of Kendall's tau-b correlation test revealed an inverse and significant relationship between ki-67 and PR (p=0.04).

Table 6- The relationship between ki-67 and PR

Ki-67		Variables
sig	Correlation coefficient	
0.04	-0.21	PR

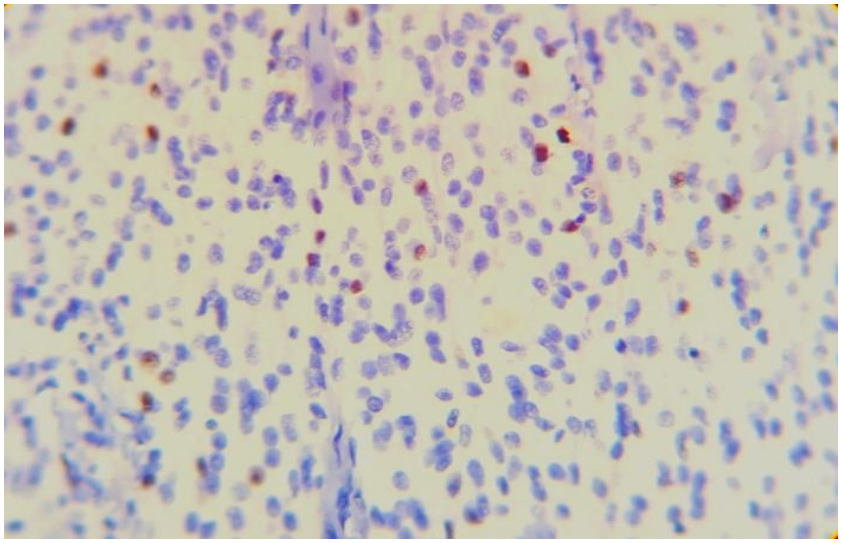

Figure 1. ki-67 =6% in grade 2 meningioma

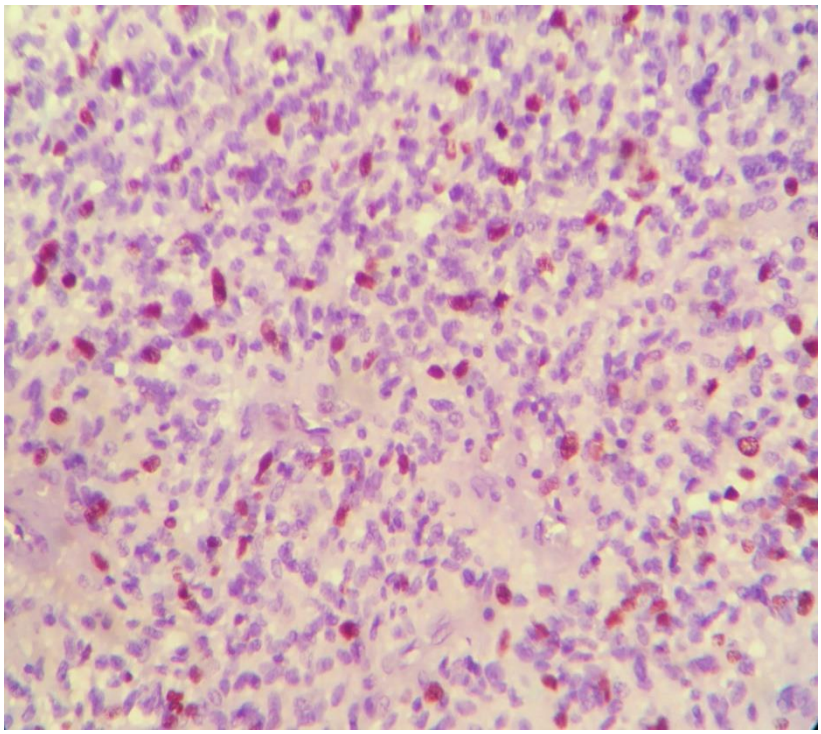

Figure 2. ki-67 =10% in grade 3 meningioma

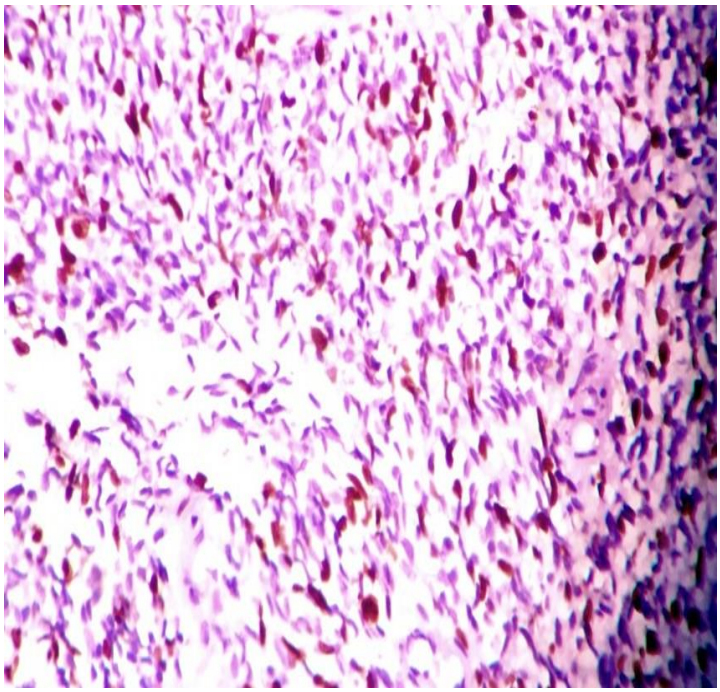

Figure 3. Grade 3 papillary meningioma, Ki-67=20%

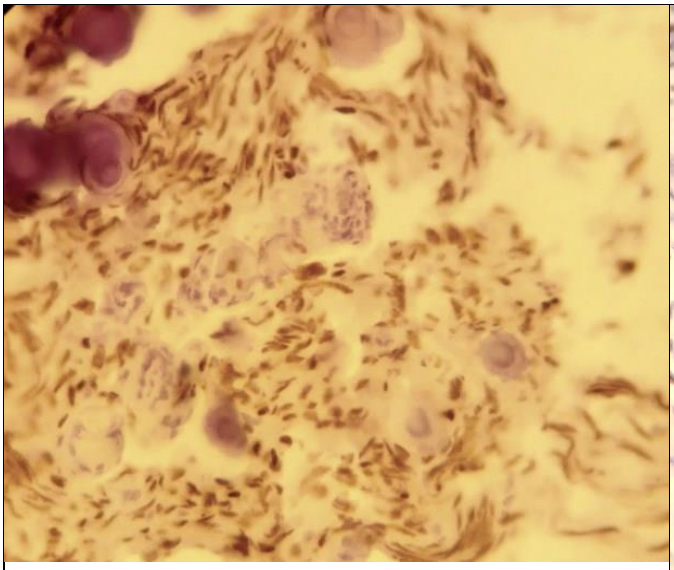

Figure 5 psammomatous meningioma (grade 1) PR IHC: strongly positive

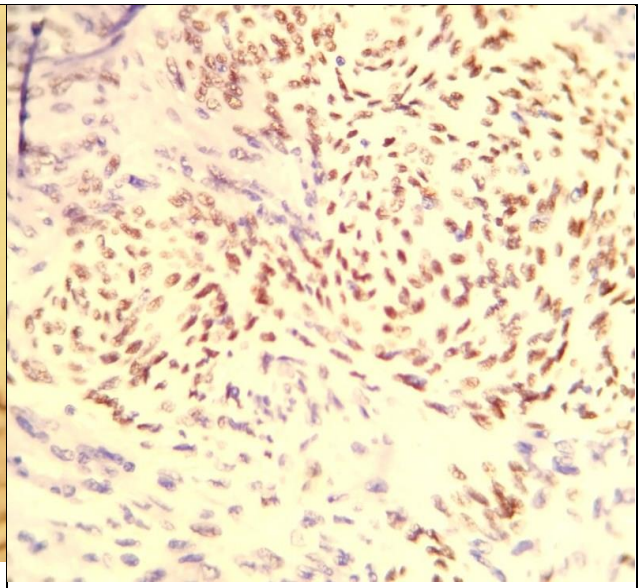

Figure 4. Grade I meningioma PR IHC: strongly positive

Discussion

One of the problems in examining these tumors is the existence of high bias among observers in the application of tumor grading criteria. This problem causes the tumor to not be properly treated [10]. Ki-67 is a biomarker expressed in the active phase (G1, S, G2, and M Phase) of the cell cycle and indicates cell division. The number of mitoses is a significant factor in determining the grade and prognosis of meningioma. However, it has been observed that counting mitosis in normal pathological slides (H&E) in grade 1 and grade 2 has a discordance rate of 20.9%. Hence, mitosis examination through the Ki-67 marker can help in determining the grade and behavior of the tumor [5]. The results of the present study revealed that the level of Ki-67 is significantly different in different grades of meningioma. With an increase in meningioma grade, the expression of Ki-67 also increased significantly. Consistent with the results of the present study, in the study by Ramesh et al. (2016), the mean of ki-67LI in different grades of meningioma (grade one 3.1%, grade two 7% and grade three 14.2%) had a significant difference [8]. In the present study, all grade one meningioma cases (63 cases) had Ki-67 expression less than 4%. In a study by Ning Liu et al. (2020), results showed that Ki-67 above 4% is a suitable cut-off for determining the prognosis of meningioma [17].

In the present study, in grade 2 meningioma, about 20% of cases had ki-67 less than 4%. This result is consistent with the results of a study by Ramrao et al. (2016), which showed that 26% of grade two meningiomas had ki-67 below 4% and the rest had ki-67 above 4%. In their study, 16% of Grade one meningiomas have a ki-67 higher than 4% and suggested a ki-67 level higher than 7% as a differentiating cut-off for grade one meningiomas from other meningiomas [18]. Another limitation of histological criteria in meningioma is that histological criteria alone do not determine the biological behavior of the tumor [8]. The higher rate of meningioma in females and the growth of this tumor in pregnancy have raised the hypothesis of a relationship between hormone receptors such as ER and PR and tumor grade and prognosis [14]. Thus, the present study investigated and compared the expression levels of PR in different grades of meningioma. Results showed that PR expression is significantly higher in lower grades of meningioma. The results of our study in this field are consistent with those of a study by Mukhopadhay et al. (2017), who showed that PR was expressed in 96.34% of grade 1 meningiomas, but not in any of grade 2 meningiomas [6].

Some studies have been conducted regarding the pathogenesis of hormones in the development of meningioma. For example, in a study of gene mutations in people with meningioma who had a history of long-term use of progesterone, results showed that PIK3CA mutations in these people are more than in other people [19]. Eduard H et al. (2021) also showed that Progesterone discontinuation has reduced tumor size in MRI [20]. Also, a prospective study revealed that patients using progestogen drugs have a higher risk of recurrence of grade 1 meningioma compared to estrogenic or combined drugs [16]. Thus, examining PR in people with meningioma can be a guide to prohibiting the use of hormonal drugs containing progesterone to prevent tumor recurrence in PR-positive people. Also, several studies related

to the use of antiprogesterone drugs such as mifepristone in the clinical and radiographic improvement of patients with unresectable meningiomas have been conducted, which have provided conflicting results [21-23]. It may be due to unequal expression of hormone receptors in different grades and even in the same grade and in different people [24]. In our study, the level of expression also varied from zero to 80% in grade one meningioma. In the present study, all patients were negative for estrogen receptors (ER). These results were consistent with those of a study by Shanthi et al. (2017), in which none of the patients had ER-positive [25]. However, in the study by Mukhopadhyay et al. (2017), 3.7% of grade 1 meningiomas and all grade 2 cases were positive for ER [6]. In the present study, a significant and inverse correlation was observed between the level of PR and ki-67. This result is in line with the results of a study by Francisco et al. [26].

Conclusion

The result revealed a significant relationship between the expression level of PR and meningioma grade, so PR was expressed more in lower grades and negative in all grade 3 grades. Thus, it can be concluded that higher PR expression indicates a lower grade and, thus, a better prognosis of meningioma. Also, our study revealed that ki-67 is significantly different in different grades of meningioma. The level of Ki67 is higher in higher grades, so it can be helpful when the tumor grading based on H&E is difficult.

However, it is recommended to conduct a study with a larger sample size to determine the relationship between PR expression and histological types of meningioma and to determine the cut-off of ki-67 in different grades of meningioma.

Research limitations:

Acknowledgments

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