Association between ki67 & PR expression in grades of meningioma and its prognosis

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Abstract

Introduction: Meningioma is the very important type of tumor that occur in central nervous system (CNS) that represented 1/3 of whole tumor of CNS. In USA 97/100,000, persons recorded have meningioma. With 170,000 patients detected as meningioma. The aim of study is the aim of the present study is to evaluate the use of immunhistochemical expression of PR and ki67 for predicting the grades of meningioma, which are important in their prognosis, and line of treatment. **Method:** Ninety patients with meningioma were collected from the Neurosurgical Hospital-Baghdad, during the period from January 2017 to January 2020. The data for cases were collected to study the age, gender and grade of tumor. Staining hematoxylin/Eosin (H plus E) for histological inputting and classifying of the tumors and immunohistochemically workup for PR & Ki67 done. **Results:** Cross sectional study for 90 patients with age, mean (44 ± 12) years old, in current study 64% of patients was females and 36% of patients was males, strong positive PR expression represented 50% of patients, 79% of patients have low Ki 67, 15% of them have intermediate Ki67 and 6% of patients with high Ki67. Most of patients with age group 40-59 years old (51%). The mean and SD of Ki67 in (%) according to grades of meningioma I, II and III as following: (0.3 ± 0.07), (7 ± 5.9) and (38 ± 0.07) respectively. There is significant association between grades of meningioma and PR expression; 71% of grade I with PR expression strongly positive, 23% of grade II with PR expression were weakly positive and 100% of grade III with PR expression were negative. In addition, there is significant association between grades of meningioma and prognosis; 100% of grade I with good prognosis, 100% of

grade II with fair prognosis and 100% of grade III with poor prognosis. In current study, also there is significant association between grades of meningioma and Ki67; 100% of grade I with low level of Ki67, 100% of grade III with high level of Ki67 and 64% of grade II with intermediate level of Ki67. There is no significant association between age and gender with grades of meningioma. **Conclusion:** The mean Ki-67 was significantly more in meningioma grade III than in patients with grades II and I as WHO classification. There is strong expression of PR was found mainly in the grade I typical meningioma group. Utilization of markers for proliferation (ki-67 labeling index) and hormonal expression (Progesterone Receptor) in combination with histopathological features may help in the identification of biologically aggressive meningioma.

Key words: ki67 & PR expression, grades of meningioma, prognosis

Introduction:

Meningioma is the very important type of tumor that occur in central nervous system (CNS) that represented 1/3 of whole tumor of CNS. In USA 97/100,000, persons recorded have meningioma. With 170,000 patients detected as meningioma. Female to male ratio show females more than males 2:1 for meningioma intracranial and 10:1 for meningioma of spine all pervious tumor detected at middle age group [1]. Two important factors that affected on the prognosis of patients with meningioma: the ratio of resection of tumor and histopathological degree of tumor. Recurrence can occur when tumor with high-score not reach complete resection. Recurrence interpreter according to WHO guide, in which recurrence of meningioma that characterize as kind, unusual and anaplastic for 20 years is 7-25%, 29-52% and 50-94%, correspondingly [1,2]. Subsequently there are boundaries to repetitive histopathological checkup in expecting tumor progressivity, numerous scrutiny procedures established including cytogenetics and use of immunohistochemically checkups. However, those checkups have not been accepted as usually on patients with meningioma. Approximately important immunohistochemically tests on meningioma include EMA, vimentinand cytokeratin. Ki-67 checkup, which is a proliferative interpreter, displays the outcomes as a prognostic meningioma [1,3]. Therefore, the aim of study is the aim of the present study is to evaluate the use of immunhistochemical expression of PR and

ki67 for predicting the grades of meningioma, which are important in their prognosis, and line of treatment.

Method:

1-Materials:

Ninety patients with meningioma were collected from the Neurosurgical Hospital-Baghdad, during the period from January 2017 to January 2020. The data for cases were collected to study the age, gender and grade of tumor. Staining hematoxylin/Eosin (H plus E) for histological inputting and classifying of the tumors and immunohistochemically workup for PR & Ki67 done.

2-Specimens:

According to WHO classification the 90 cases classified into typical meningioma, atypical meningioma and malignant meningioma. From each formalin fixed paraffin embedded tissue, three sections of 5 micron thickness were obtained and stained by haematoxylin & eosin staining method and immunohistochemically by using monoclonal antibody for PR& ki-67.

3-Methods of staining procedures:

a) Deparaffinization: This has been performed by immersion in the following:

1. Xylene for 5 minutes.
2. Xylene for 5 minutes.
3. 99% ethanol for 5 minutes.
4. 99% ethanol for 5 minutes.
5. 99% ethanol for 5 minutes.
6. 95% ethanol for 5 minutes.
7. 70% ethanol for 5 minutes.
8. Distilled water.

b) Hematoxyline and eosin staining method:

1. Dewax sections (deparaffinization as above).
2. Stain in hematoxyline for 3-10 minutes.
3. Wash well in running tap water.

4. Remove excess stain by differentiating the sections in 1% acid alcohol (1% in HCL 70% alcohol) for 5-10 seconds.
5. Wash well with in tap water until sections regain their blue color.
6. Stain in eosin for 2-5 minutes.
7. Dehydrate slowly through increasing grades of alcohol (i.e.70%, 90% and 100%).
8. Clearing by xylene.
9. Mount wit DPX.

C- Immunohistochemical staining method: One tissue block with representative tumor was selected in each case for immunohistochemical staining. By using the Envision^{TM}Flex , staining was performed by manual method as following:-

1. Five microns section was obtained from the formalin fixed paraffin embedded tissue block and mounted on positively charged slide. The sections were dried for 1 hour at 60°C.

2. Deparafinization was done by incubating the section in an oven at 65°C for 20 minutes followed by two changes of xylene for each.

3. Target retrieval solution, PH 9 (Dako cytomation).

4. Incubate in water bath at 95°C for 20-30 minutes.

5. After cooling, wash in Envision^{TM} Flex wash buffer for 5 minutes.

6. Encircle tissue with Pap Pen. Wipe off buffer ½ cm above and below the tissue and draw a line with the Pap Pen.

7. Incubate with Envision^{TM}Flex Peroxide Blocking Reagent for 5 minutes.

8. Wash in Envision^{TM}Flex wash buffer.

9. Incubate with primary antibody for 20 minutes.

10. Wash in Envision^{TM}Flex wash buffer.

11. Envision^{TM}Flex/HRP secondary antibody (ready to use) for 20 minutes.

12. Wash in Envision^{TM}Flex wash buffer, incubate for 5 minutes.

13. Incubate with Envision^{TM} Flex-DAB+ chromogen (for 10 minutes).

14. Wash in Envision^{TM} Flex wash buffer.

15. Envision^{TM} Flex hematoxylin (ready to use) incubates for 5 minutes.

16. Wash slides in deionized water.

17. Wash in Envision^{TM} Flex wash buffer.
18. Wash slides in deionized water.
19. Dehydrate and mount the slides.

A- Ki-67 Scoring System: Immunohistochemistry for Ki-67 was carried out following the streptavidin–biotin–peroxidase method (Monoclonal Mouse, Anti-Human Ki-67 Antigen/FITC, Clone MIB-1, Manufactured by Dako, Denmark). The mouse monoclonal Ki-67 antibody was used at a dilution of 1:25. Breast cancer tissue was used as positive control, and negative controls were performed by omitting the primary antibody. The Ki-67 staining index was recorded as the percentage of positive-staining tumor cell nuclei in 1000 tumor cell nuclei evaluated. The determinations were based on high magnification areas with the most immunostaining.

B- Progesteron Receptor (PR): Immunohistochemistry for PR was carried out by using polyclonal rabbit antiprogesterone was used at dilution 1:100 as the primary antibody , goat anti-rabbit IgG, and rabbit peroxidase antiperoxidase complex . Breast cancer tissue was used as positive control, and negative controls were performed by omitting the primary antibody. PR expression recorded into group I to III as follows: Group I – high staining of nucleus, group II - Weak staining if nucleus and group III - Lacking staining of nucleus.

Statistical analysis done by SPSS 22 calculated mean and SD for age Ki67 and percentage, frequency for categorical variables. Chi square use for revealed association between categorical variables, significant association when P-value less than 0.05.

Results:

Cross sectional study for 90 patients with age, mean (44 ± 12) years old, in current study 64% of patients was females and 36% of patients was males, strong positive PR expression represented 50% of patients, 79% of patients have low Ki 67, 15% of them have intermediate Ki67 and 6% of patients with high Ki67. Most of patients with age group 40-59 years old (51%). The mean and SD of Ki67 in (%) according to grades of meningioma I, II and III as following: (0.3 ± 0.07), (7 ± 5.9) and (38 ± 0.07) respectively. As show in table 1 and 2.

Table 1: distribution of variables.

variables		frequency	percentage
gender	**female**	58	64.4
	male	32	35.6
PR expression	**negative**	39	43.3
	strong positive	45	50.0
	weak positive	6	6.7
Ki 67	**low**	71	78.9
	intermediate	14	15.6
	high	5	5.6
age	**15-39 years**	34	37.8
	40- 59 years	46	51.1
	more than 60	10	11.1

Table 2: mean and Std of Ki67 (%) according to grades of meningioma.

grades of meningioma	Mean	Std. Deviation	Maximum	Minimum
I	0.3	0.07	2	0
II	7	5.9	19	0
III	38	0.07	45	28

As in figure 1 and 2, the prognosis of meningioma is 70% of patients with good prognosis and 24.4% with fair prognosis. While the distribution of patients according to grade of meningioma is 70% of them in grade I , 24.5% in grade II and 5.5% in grade III.

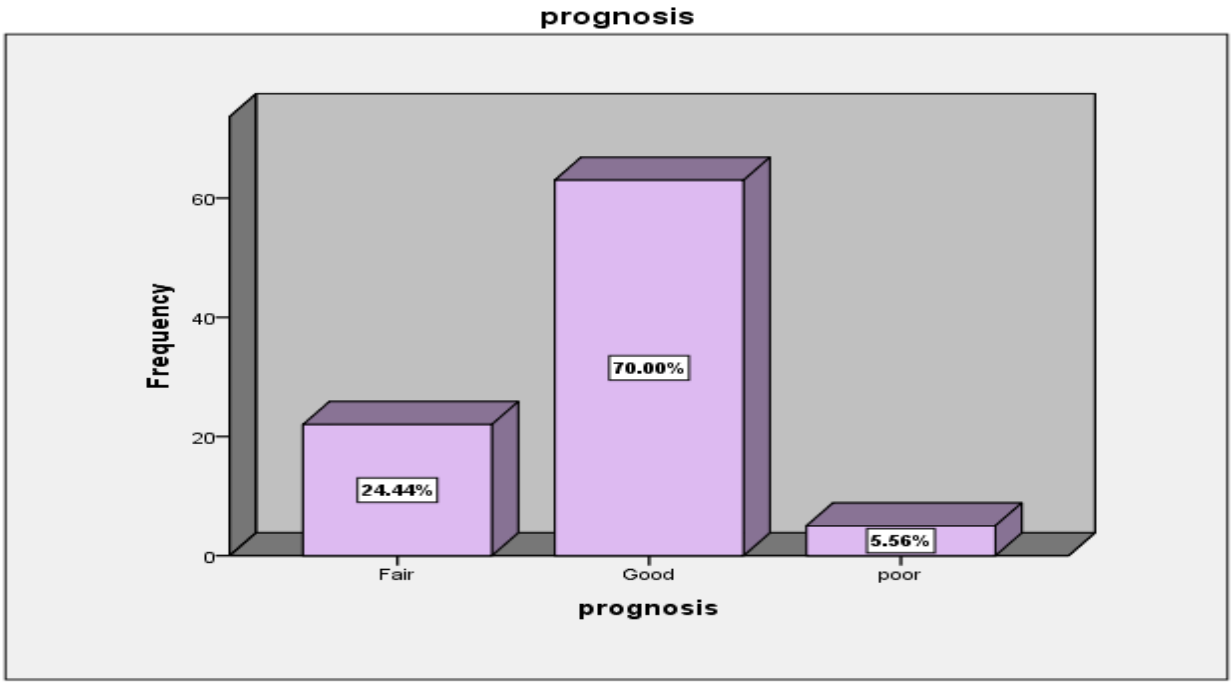

Fig 1: distribution of prognosis of meningioma.

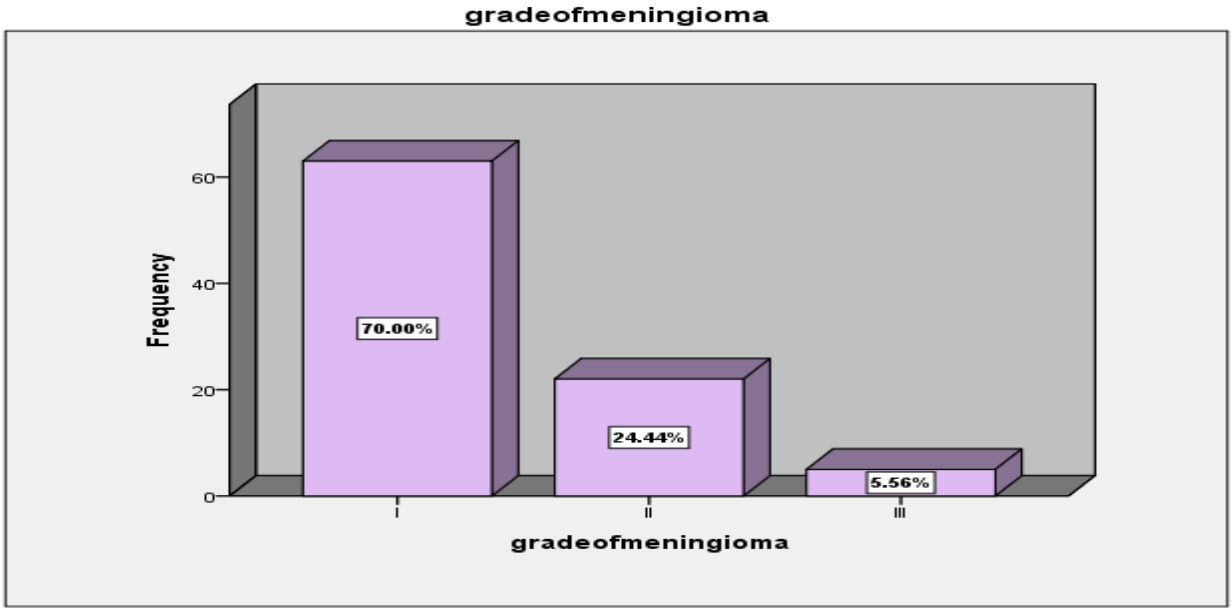

Fig 2: distribution of grades of meningioma.

In table 3: there is significant association between grades of meningioma and PR expression; 71% of grade I with PR expression strongly positive, 23% of grade II with PR expression were weakly positive and 100% of grade III with PR expression were negative. In addition, there is significant association between grades of meningioma and prognosis; 100% of grade I with good prognosis, 100% of grade II with fair prognosis and 100% of grade III with poor prognosis. In current study, also there is significant association between grades of meningioma and Ki67; 100% of grade I with low level of Ki67, 100% of grade III with high level of Ki67 and 64% of grade II with intermediate level of Ki67. There is no significant association between age and gender with grades of meningioma.

Table 3: association between variables and grades of meningioma.

variables	**grades of meningioma**			**P-value**
gender	I	II	III	
female	42	14	2	
	66.7%	63.6%	40.0%	0.48
male	21	8	3	
	33.3%	36.4%	60.0%	
PR expression				
negative	17	17	5	
	27.0%	77.3%	100.0%	**0.0001**
strong positive	45	0	0	
	71.4%	0.0%	0.0%	
weak positive	1	5	0	
	1.6%	22.7%	0.0%	
prognosis				
fair	0	22	0	
	0.0%	100.0%	0.0%	
good	63	0	0	
	100.0%	0.0%	0.0%	**0.0001**
poor	0	0	5	
	0.0%	0.0%	100.0%	
Ki67				
low	63	8	0	
	100.0%	36.4%	0.0%	
intermediate	0	14	0	**0.0001**
	0.0%	63.6%	0.0%	

high	0	0	5	
	0.0%	0.0%	100.0%	
age				
15-39 years	27	6	1	
	42.9%	27.3%	20.0%	
40- 59 years	30	13	3	0.62
	47.6%	59.1%	60.0%	
≥60	6	3	1	
	9.5%	13.6%	20.0%	

P-value less than 0.05 (significant).

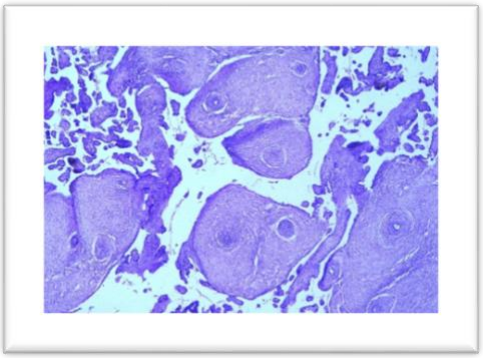

Fig 3: Benign meningioma H&E Stain X10

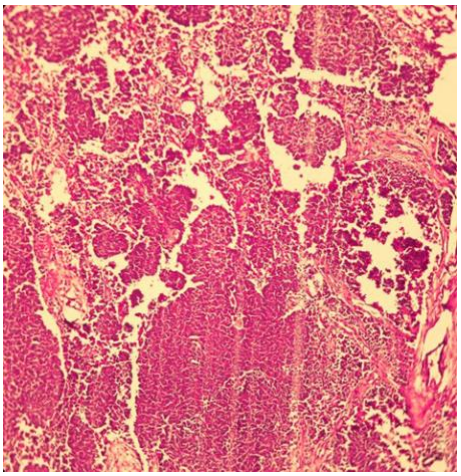

Fig 4: Anaplastic meningioma H& E stain X10

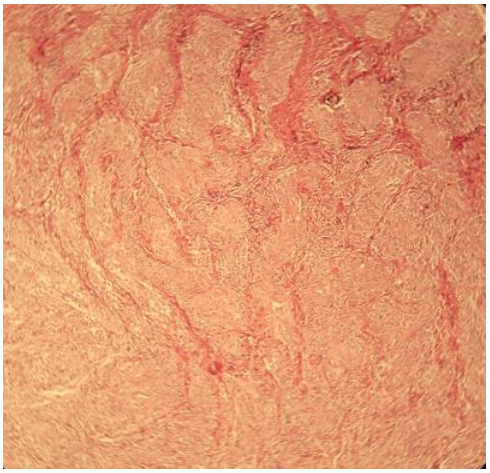

Fig 5: Atypical meningioma H& E stain X10

Discussion:

Meningioma explanation more than 30% of major intracranial tumors according to WHO histologically classification of nervous system tumors. Most grade I meningioma classify as benign tumor while grade II and grade III are associated with a advanced risk of reappearance and littler survival periods [4]. Human meningiomas unveil a heterogeneous histopathology, which may explain the repeated revisions of classification schemes. This study presents a review of 90 primary meningioma classified according to WHO classification of 2007. In current study, 64% of patients was females and 36% of patients was males this is similar to other results in other study that stated the incidence of meningioma in women was higher with a ratio of 2.1:1 compared to men [1,2]. This comparison was reversed in prepubertal-age meningioma. In atypical and anaplastic meningioma cases, it was more dominant in men [1,3]. 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. Age has no effects on grades of meningioma, but most cases in all three grades of meningioma occur between (40-59) years at distribution 47.6%, 59.1% and 60.00% in grade I, II, and III respectively. In current study the incidence of grade I is 70% which is usually associated with good prognosis, grade II is 24.5 and grade III is 5.5% which is poor prognosis while grade II is in between regarding incidence and prognosis, this is similar to other study that stated the incidence of grade I is higher than grade II and grade II is higher than grade III [5,6]. Several studies have been done to evaluate the title role

of Ki-67 and PR in meningioma and to assess their character as prognostic features in assessing the performance of meningioma but many studies have given different suggestions concerning the association of Ki-67 and PR expression with the organic performance of meningioma. In current study the expression of progesterone receptor is strong in 45(71.4%) in grad I, negative PR expression in all cases of grade III, with weak expression in grade II, so PR expression strongly associated with grade of meningioma as grade of meningioma increase PR expression will be decrease [7,8,9]. Ki-67 is a best indicator of cell proliferation eminence of the meningiomas. Ki-67 examines are progressively general because of their slight tissue necessities and appropriateness for regularly stable tissues [10]. In the current effort, the mean Ki-67 classification index was significantly advanced in meningiomas of WHO grade III than in patients with grades I and II, like to the literature [7,10]. Several studies in the past have demonstrated a proportionate increase in proliferative index and decrease progesterone receptor expression with increasing tumor grade. The assessment of proliferative activity and PR expression are a good indicator of tumor aggressiveness [11,12].

Conclusion

The mean Ki-67 was significantly more in meningioma grade III than in patients with grades II and I as WHO classification. There is strong expression of PR was found mainly in the grade I typical meningioma group. Utilization of markers for proliferation (ki-67 labeling index) and hormonal expression (Progesterone Receptor) in combination with histopathological features may help in the identification of biologically aggressive meningioma.

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