Ki-67(MIB-1) and Progesterone Receptor in Meningioma: An Immunohistochemical Study

Wahda Mohammed Taib Al- Nuaimy*, Jalal Ali Jalal, Banan Burhan Mohammed ***

ABSTRACT:

BACKGROUND:

In meningioma the tumor grade, subtype, and extent of surgical resection are strong prognostic factors, the growth of this tumor is still unpredictable, and additional prognostic markers are needed. Many studies have shown that the detection of the proliferative potential of meningioma by Ki-67 (MIB-1) and the detection of the progesterone receptor might predict the natural history of tumor and patient survival.

OBJECTIVE:

To evaluate the proliferative fraction Ki-67 (MIB-1) and progesterone receptor in meningioma, To correlate the expression of these two markers with various clinico- pathological parameters, To compare these results with other studies .

PATIENTS AND METHODS:

This is a prospective study, in which 50 cases of meningioma were collected in Nineveh province. These cases were examined immunohistochemically. The results of these two markers were correlated with various clinico- pathological parameters.

RESULTS:

The mean age of all cases was 46 years. There were 16 male and 34 female patients. According to the W.H.O 2000 classification, there were 84% grade I, 10% were grade II and 6 % were grade III. Only 14% of the patients had a history of recurrence. Progesterone receptor was positive in 72% of all cases. Significant relation was observed between the progesterone receptor and the patients age .It was significantly higher in female than male. A significant relationship was found between progesterone receptor with grade and histological types. Thirty three out of 43 cases without recurrence had positive progesterone receptor. The mean±Standard deviation (SD) of Ki-67 Labeling Index (Ki-67 LI) was 2.7±4.2%. No significant relation was found between the Ki-67 LI and patients age. The male patients had a higher Ki-67 LI than that of the female. Significant relation was found between Ki-67 LI and the grade of meningioma . The mean of Ki-67 LI in the recurrent cases was higher than in non recurrent cases. An inverse relationship was found between Ki-67 LI and progesterone receptor .

CONCLUSION:

Progesterone receptor showed a significant relation with the age and histological types. Ki-67 LI had a direct significant association with the grade and the recurrent cases., while inverse relation was observed between the progesterone receptor with the grade and the recurrent cases. An inverse relationship was observed between the progesterone receptor and Ki-67 LI.

KEY WORDS: meningioma, Ki-67 (MIB- 1), labeling index, progesterone receptor and immunohistochemical study.

INTRODUCTION:

Meningioma constitutes approximately 13%–26% of all intracranial tumors, although the cell of origin has not yet been proven, meningioma is probably derived from arachnoidal cap cells [(1)]. Meningioma was histologically classified by World Health Organization (WHO) 2000 into 3 different grades: most are benign (grade I), between 5% - 15% of meningiomas are atypical (grade II), while only 1-2% are anaplastic (grade III) [(2)] .They have a wide range of histopathological appearances. These subtypes share immunohistochemical and ultra-structural characteristics [(3)].

*Department of Pathology, College of Medicine, University of Mosul, Mosul, Iraq.

** Department of Pathology, College of Medicine, Hawler Medical University, Erbil, Iraq.

Predicting the behavior of meningioma based on histopathologic features continues to be problematic. While tumor grade, subtype and extent of resection are useful factors, recurrence has been noted in some meningiomas with no known aggressive parameters [4]. As many as 7 %-20% of benign (WHO Grade I) meningiomas are known to recur [5].

Studying the cell proliferation index may provide an objective method for assessing the tumor biology [4]. Evaluation of mitotic activity is the most commonly used and the fastest method. However, it is difficult to detect the proliferation potential of meningioma in routine slides as well as the cells in the M-phase of the cell cycle can only be detected by this method [6]. The Ki-67 antigen is a nonhistone protein expressed in proliferative phase of cell cycle (G1, S, G2 and M phases); it is considered to be the most reliable proliferative marker predicting tumor behavior and can be detected on formalin fixed paraffin embedded tissue sections [4]. The MIB-1 monoclonal antibody has been used frequently to stain Ki-67 antigen, in order to investigate the growth index of various systemic and intracranial neoplasm [3]. As MIB-1 LI (labelling index) would reflect tumor proliferating potential, the high level of this marker represent increased tumor proliferation [3] .

High MIB-1 LI is associated with a high recurrence rate in meningioma[3]. So assessment of the growth fraction in meningioma with Ki-67 LI could become an important tool in the prediction of the biological behavior of meningioma and the planning of adjuvant therapy [3].

It has been demonstrated that most meningiomas express hormone receptors on their cell membranes, although to a variable extent[7]. Actually, up to 90% of meningiomas express progesterone receptor (PR), while 30% - 48% express estrogen receptor [1]. The expression of PR by meningioma cells is a prognostically a favorable sign. While absence of PR expression would be accompanied by a more aggressive tumoral behavior [1].

PATIENTS AND METHODS:

This is a prospective study. A total of 50 cases of meningioma specimens were collected from Al-Jamhori Teaching Hospital and some private laboratories in Nineveh province during a period extending from February 2009 to April 2010. Sections from paraffin embedded tissues were taken and stained with Hematoxylin and Eosin, then examined under the light microscope. They were categorized according to the WHO 2000 grading system as benign, atypical and anaplastic meningioma, WHO grades I, II and III respectively. Meningioma was categorized into subtypes according to the new WHO classification. Other information which included in this study (age and sex of the patient, and a history of recurrence) were retrieved from the medical reports.

The Ki-67(MIB-1) and progesterone receptors were assessed in these cases by immunohistochemical technique. The procedure followed the instruction provided by the manufacturer. The material for the procedure was obtained from Dakocytomation (Monoclonal Mouse Anti-Human Ki-67 Clone: MIB-1 Isotype: IgG1, kappa (Dako cytomation), Monoclonal Mouse Anti-Human Progesterone receptor (Dako cytomation) .The invision system was universal LSAB^{TM}+2 Kits.

For Ki-67 LI, each slide was scanned at power (X100) using a Leitz dialux microscope, and area that appeared to have the highest density of labeled nuclei (hot spot) was selected for counting at a high magnification (X400). An average of 1000 cells was counted and the Ki-67 LI was determined and expressed as the percentage of labeled nuclei. Section of lymph node was used as positive control and the mean of Ki-67 LI was derived for grade I, II and III [3].

For PR the breast cancer tissue was used as positive control .The PR status was determined by a semiquantitative

scoring scale with respect to staining intensity (intensity score) (graded as: 0, absent; 1, weak; 2, moderate; and 3, strong) and percentage of positive tumor cells (proportional score) (0, indicating the absence of positive nuclei; 1, the presence of <10% positive tumor nuclei; 2, an estimated 10–50% positive nuclei; 3, 51–80% positive tumor nuclei; and 4, >80% positive tumor nuclei). Total immunoreactive score (TIRS) was calculated for each case by multiplying the intensity score (IS) by the proportional score (PS), producing a TIRS which range from 0 to 12. Tumors with a TIRS of 2 or more were considered as PR positive [8].

TIRS= IS X PS ---------» (0-12) ---------» ≥2 positive.

Statistical analysis:

Labeling index values for Ki-67 were presented as the mean±SD and the association between Ki-67 LI and PR and with variable parameters was

assessed using Fisher exact test and t-test when indicated. P value of <0.05 was considered statistically significant.

RESULTS:

Fifty cases with meningioma were included in this study. The mean ages of all cases was 46 years ranging from 18 to 70 years; they were segregated into 5 groups (≤30, 31-40, 41-50, 51-60 & 61-70) years. The highest number of cases was found at age group 51-60, 13 cases (26%). There were 16 male and 34 female patients with a male to female ratio (1: 2.1). On reviewing the slides using the criteria recommended by the WHO 2000 classification, 42 cases (84%) were grade I (24 meningiothelial, 4 transitional, 4 fibroblastic, 3 angiomatois, 2 cases of secretory, lipomatous and metaplastic, and 1 case of mirocystic type) , 5 cases (10%) were grade II (atypical type) and 3 cases (6 %) were grade III meningioma (2 cases anaplastic and 1 papillary). Only 7 (14%) of the patients had a history of recurrence after a period ranging from nine months and 5 years , as shown in table (1).

The PR was positive in 36 cases which form (72%) of all cases and negative in 14 cases which form (28%). Significant relation was observed between the PR and patient age (P=0.021), the higher PR positivety (30.6%) was found in age group 41-50. The positive immunostaining rate for the PR was significantly higher in females than males, it was positive in 10 of the 16 male (27.8%) and in 26 of the 34 female patients (72.2%) (P =0.03), as shown in table (1). The frequency of positive receptors in patients with benign tumors (grade I) was significantly higher, than in the other two groups (grade II and grade III) (P=0.001), as shown in table (1) & figures (1,2,3,4,5).

Significant relation was found between PR positivety and the histological type (P=0.0023), PR was positive in 20 of 24 meningothelial cases, 3/4 cases of transitional, 2/3 cases of angiomatous, 1/2 cases of lipomatous, and metaplastic, all the fibroblastic and secretory cases were positive, 1 case was of microcystic which was also positive. In atypical type, PR was positive in 2/5 cases while all cases of anaplastic and papillary types were negative for PR. Thirty three out of 43 cases with no recurrence had positive PR, while 3 out of 7 cases with recurrence had positive PR (P = 0.003), as shown in table (1).

In all cases the mean ±standard deviation (SD) of Ki-67 LI was (2.7±4.2%) (ranging from 0.0% to 19.5%). No significant relation was found between the Ki-67 LI and patients age (P>0.05) with the higher mean of Ki-67 LI was observed in the age group (61-70). Regarding the sex, the male patients had Ki-67LI mean ±SD equal to 4.3±5.9 % while in females it was 1.9±2.9% (P= 0.005), as shown in table (2).

Most of benign meningioma (grade I) expressed low Ki-67 LI (mean ±SD =1.8±3.5%), in contrast to grade III meningioma, which had a high LI of Ki-67(mean±SD=10.6±6.4%). While atypical meningioma (grade II) had Ki-67 LI in range between grade I and grade III meningiomas, with mean Ki-67LI ± SD of 5.4±2.8%. Significant correlation was found between grade I and II (P=0.001), grade II and III (P=0.002) and grade I and III (P< 0.0001), as shown in table (2) & figure (1,2,3,4,5).

Ki-67 LI mean was higher in the histological types of grade III and grade II (mean±SD was 13±13% in anaplastic, 5.4±2.8% in atypical and the mean in papillary was 6%), as shown in figures (3,4,5). While the mean was <1.8% in the histological types of grade I (mean±SD was 1.8±2.5% in meningiothelial, 1.6±1.9% in transitional, 1.4±1.5% in fibroblastic, 0.7±0.5% in secretory , 0.4±0.6% in metaplastic, 0.15±0.07% in lipomatous and 1% microcystic) except for the angiomatous type, the mean±SD of Ki-67 LI was 6.6±11.2%, as shown in table (2).

The mean Ki-67 LI %±SD in the non recurrent group was 1.6±2.2% (range 0.0% - 10%) which was generally lower than those in the recurrent group mean±SD was 9.9±6.3% (range 4–19.5%); statistical significant difference was identified (P <0.0001),as shown in table (2).

Inverse significant relation (P=0.001) was found between Ki-67 LI and PR, that the mean±SD of Ki-67 LI in PR positive cases was 1.6±2.2% (range 0.0% - 9%) increased to 5.8±6.3% (range 0.0% - 19.5%) in PR negative cases, as shown in table(2).

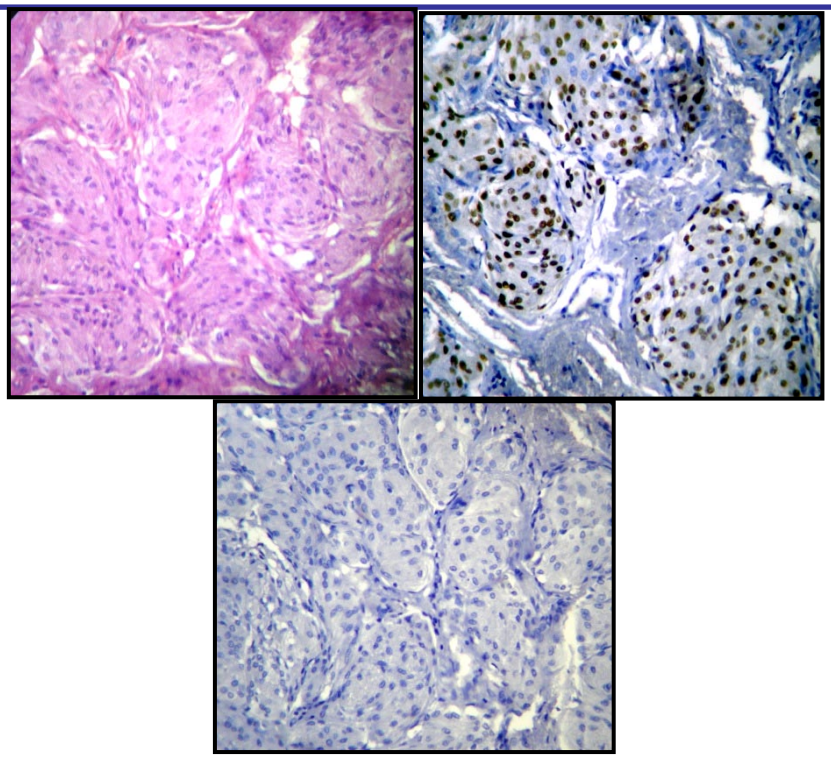
Table 1: Relation of PR With the Various Clinicopathological Parameters

		Ki-67 LI mean±SD%	Minimum Ki-67 LI %	Maximum Ki-67 LI %	P value
Age	≤30	2.3±2.2	0.1	5.3	P >0.05
	31-40	3.1±5.9	0.0	18	
	41-50	1.3±2.6	0.0	9	
	51-60	3.3±3.4	0.0	10	
	61-70	3.7±6.2	0.0	19.5	
Sex	Female	1.9±2.9	0,0	10	P=0.005
	Male	4.3±5.9	0.0	19.5	
Grade	Grade I	1.8±3.5	0.0	19.5	
	Grade II	5.4±2.8	2.5	10	
	Grade III	10.6±6.4	6	18	
Histolog. types	meningiothelial	1.8±2.5	0.0	9	
	transitional	1.6±1.9	0.1	4	
	fibroblastic	1.4±1.5	0.3	3.5	
	Angiomatous	6.6±11.2	0.0	19.5	
	secretory	0.7±0.5	0.3	1	
	lipomatous	0.15±0.07	0.1	0.2	
	metaplastic	0.4±0.6	0.0	0.8	
	microcystic	1	One case----	----	
	atypical	5.4±2.8	2.5	10	
	anaplastic	13±13	8	18	
	Papillary	6	One case---	---	
recurrence	Present	9.9±6.3	4	19.5	P<0.0001
	Absent	1.6±2.2	0.0	10	
PR	Positive	1.6±2.2	0.0	9	P=0.001
	Negative	5.8±6.3	0.0	19.5	

Table 2: Relation of Ki-67 LI mean with various clinico-pathological parameters and PR in meningioma.

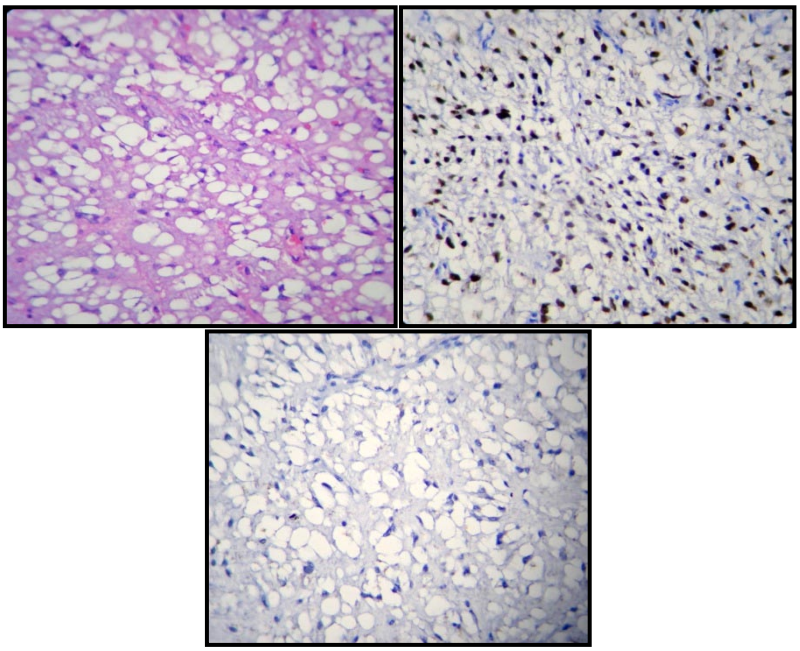
		PR +ve No.(%)	PR -ve No. (%)	Total no.(%)	P value
Age	≤30	3(8.3%)	4(28.6 %)	7(14%)	P=0.021
	31-40	7(19.44%)	2(14.3%)	9(18%)	
	41-50	11(30.6%)	1(7.1%)	12(24%)	
	51-60	8(22.22%)	5(35.7%)	13(26%)	
	61-70	7(19.44%)	2(14.3%)	9(18%)	
	Total no. (%)	36	14	50(100%)	
Sex	Female	26 (72. 2%)	8 (57.1%)	34(68%)	P= 0.03
	Male	10 (27.8%)	6 (42.9%)	16(32%)	
	Total no. (%)	36	14	50(100%)	
Grade	Grade I	34(97.1%)	8(57.3%)	42(84%)	P=0.001
	Grade II	2(2.9%)	3(21.4%)	5(10%)	
	Grade III	0(0.0%)	3(21.4%)	3(6%)	
	Total no. (%)	36	14	50(100%)	

Histolog.type	meningiothelial	20(55.55%)	4(28.6%)	24(48%)	P=0.0023
	transitional	3(8.3%)	1(7.14%)	4(8%)	
	fibroblastic	4(11.1%)	0(0.0%)	4(8%)	
	angiomatous	2(5.55%)	1(7.14%)	3(6%)	
	secretory	2(5.55%)	0(0.0%)	2(4%)	
	lipomatous	1(2.8%)	1(7.14%)	2(4%)	
	metaplastic	1(2.8%)	1(7.14%)	2(4%)	
	microcystic	1(2.8%)	0(0.0%)	1(2%)	
	atypical	2(5.55%)	3(21.42%)	5(10%)	
	anaplastic	0(0.0%)	2(14.28%)	2(4%)	
	Papillary	0(0.0%)	1(7.14%)	1(2%)	
	Total no. (%)	36	14	50(100%)	
recurrence	Present	3 (8.3%)	4(28.6%)	7(14%)	P=0.003
	Absent	33(91.7%)	10(71.4%)	43(86%)	
	Total no. (%)	36	14	50(100%)	



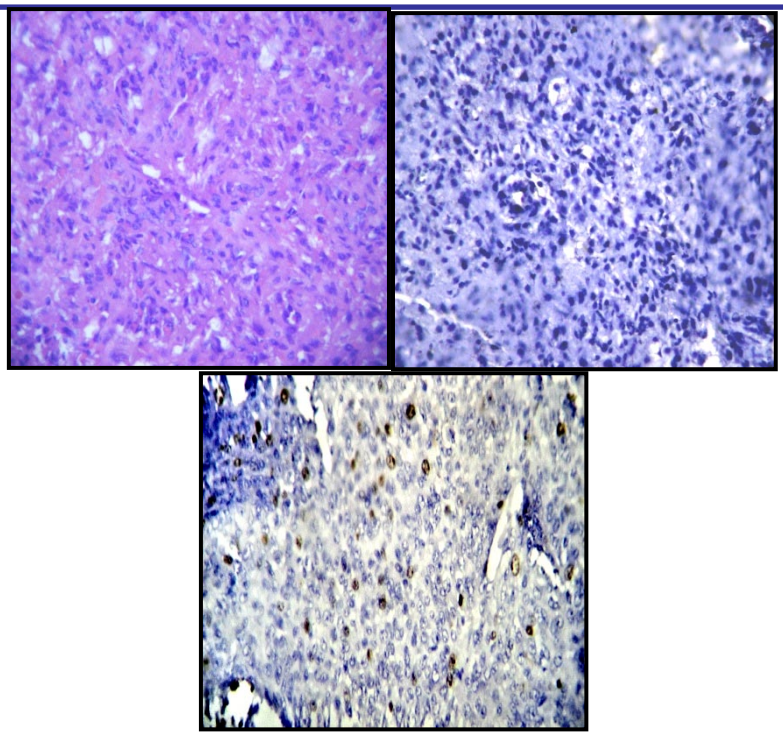
Left,(X 400) H&E; middle, (X 400) PR +ve; right, (X 400) Ki-67 LI=0.0%

Figure 1: Meningiothelial meningioma (grade I)



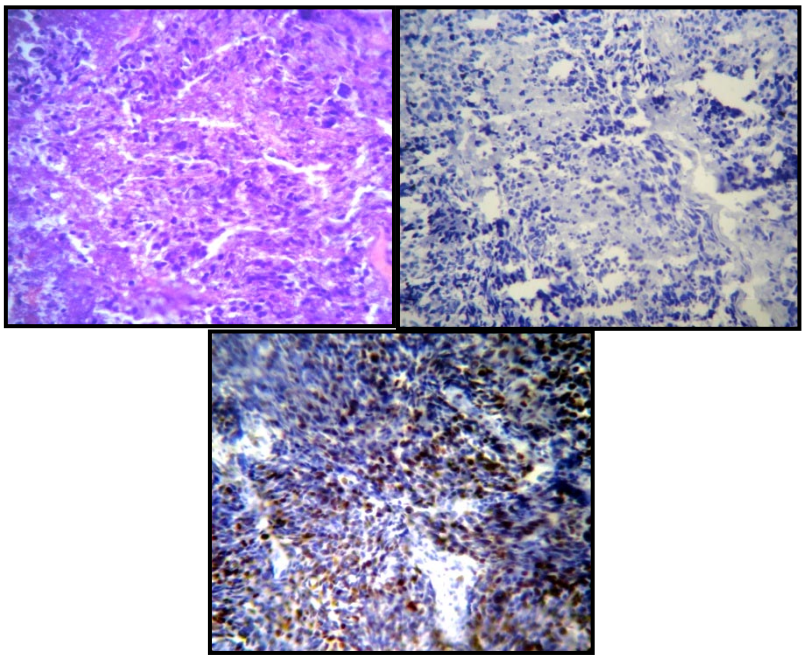
Left,(X 400) H&E; middle(X 400) +ve PR; right, (X 400) Ki-67 LI = 0.0%

Figure 2: Lipomatous meningioma (grade I)



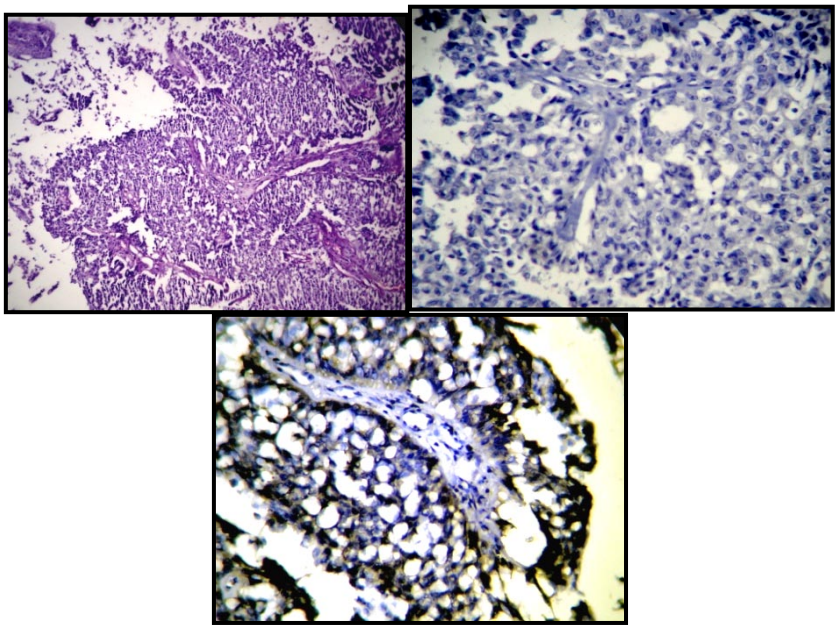
Left, (X 400) H&E; middle, (X 400) PR –ve; right, (X 400) Ki-67 LI =4%

Figure 3: Atypical meningioma (grade II)



Left (X 400) H&E; middle (X 400) PR–ve ; right, (X 400) Ki-67LI = 18%

Figure 4: Anaplastic meningioma (grade III)



Left (X 100) H&E; middle, (X 400) PR –ve ; right, (X 400) Ki-67 LI =6%.

Figure 5: Papillary meningioma (grade III)

Table I: Comparison of the mean of Ki-67 L1 in different grades with other studies.

Study	Year	Region	Cases No.	Grade I mean Ki-67 LI%	Grade II mean Ki-67 LI%	Grade III mean Ki-67 LI%
Current study	2010	Iraq	50	1.8	5.4	10.6
Rao Sh. et al. (4)	2009	India	123	3.8	13.7	19.4
Uzum N.(6)	2008	Turkey	246	2.61	8.61	11.79
Karabali P.(3)	2006	Turkey	87	2.23	6.53	11.1
Kolles H.et al.(16)	2005	Germany	160	0.7	2.1	11
Roser F.et al. (17)	2004	Germany	600	3.28	9.95	12.18
Efrid H.(11)	2002	USA	57	0.75	3.2	6.o4
Amatya VJ. et al.(18)	2001	Japan	146	1.5	8.1	19.5

Table ii: Comparison of PR % expression with that of other studies

Study	Year	Region	No. of cases	PR +ve	%
Current study	2010	Iraq	50	36	72%
Takei H.[21]	2008	USA	57	50	87.7%
Claus EB. et al [13]	2008	Boston/USA	31	26	84%
Taghipour M. et al [14]	2007	Iran	51	35	68.8%
Applanat MP.et al[15]	2005	France	36	26	72%
Roser F et al [8]	2004	Germany	588	329	55.9%

DISCUSSION:

Tumor grade, subtype, and extent of surgical resection are strong prognostic factors in meningioma, the growth of this tumor is still unpredictable, and additional prognostic markers are needed [9]. Many studies have shown that the detection of the proliferative potential of meningioma by Ki-67 (MIB-1) and the detection of the progesterone receptor might predict the natural history of tumor and patient survival [10].

In the current study, Ki-67 LI was failed to show statistically significant relation with the age of the patients (P >0.05) , similar findings were reported by Roser F et al[10] , Efird H [11] and David SI et al [12] who did a study on meningioma in pediatric patients and concluded that Ki-67 LI for pediatric meningioma without atypia did not differ significantly from that for adult meningioma without atypia. Significant association was observed between PR and the age (P =0.021), while Roser F et al [10] and Claus EB et al [13] did not found such association between PR and the age, this may be due to variation in the age group included in the study and different antibody batches &/or different immunohistichemical technique used.

Significant relation was found between Ki-67 LI and the sex of the patients (P = 0.005); Efird H. et al [11] also found that Ki-67 LI is higher in males than females. While the positive immunostaining rate for the PR was significantly higher in females than males (P = 0.03), same result was found by Taghipour M. et al [14] and Applanat MP [15]. However Roser F et al[8]and Claus E B [13] found no relation of PR with the sex of the patients which may be due to different immunohistochemical technique &/or different antibody batches used .

Direct significant correlation was found between Ki-67 LI and the grade of the tumor that the Ki-67 LI increase with the increasing of the grade. Our results are comparable with data in the literature as shown in table (i)

This variation in the mean of LI may be due to differences in staining techniques, counting methods, and interpretation of results. In addition issues of tumor heterogeneity and sampling should also be considered.

In contrast Bruna J. et al [19] who did a study of Ki-67 LI on 28 cases of meningioma, found no relation with the grade; this may be due to small sample of his study and his study included atypical and anaplastic meningioma (grade II and III) only. Perry A. et al [20] did a large study

included 463 cases of meningioma suggested that an MIB-1 LI of 4.2% is an appropriate threshold value and found this cut off level strongly associated with decreased recurrence - free survival.

In the present study a total of 8 cases of grade I meningiomas showed a high Ki-67 LI > 4%, of which five cases presented with recurrence. All these cases were histologically WHO grade I and there was no evidence of brain invasion on surgical excision. The higher values of Ki-67 LI in the three non - recurrent low grade meningioma may be due to possible recurrence potential of the tumor [3], or it might be the focal area of histologically atypia may not be included in a biopsy attributed to sampling error [5].

These findings draw our attention to the fact that biological behavior of meningioma and risk of recurrence in individual cases cannot be predicted by using conventional histological criteria alone, and recurrence may not be limited to meningioma with aggressive histological features [4]. This may suggest that Ki-67 may be of importance in identifying a subset of biologically aggressive morphologically benign meningioma. So the difference of MIB-1 LI was found among the subtypes of meningioma of the same grade. For this reason, the Ki-67 (MIB-1) LI cannot be used to grade the meningioma. It is therefore recommended that a phrase such as “with high proliferative activity” be added to the diagnosis of benign or atypical meningiomas if the labeling indices were conspicuously higher than expected for those entities. And these cases should be reviewed carefully [5]

Among the 50 cases which were included in the study, PR was positive in 36 (72%) cases. The result of this study was within the rate of detection found in other studies, ranging from 55.9% to 87.7%, as shown in table (ii).

The positive immunostaining rate for the PR in benign meningioma was significantly higher than that in non benign tumors (grade II and grade III) (P = 0.001). This is consistent with that found by Roser F et al [8] and Taghipour M. et al [14]. The reason for this relation is not clear, could probably be due to the higher incidence of mitoses in a tumoral cells in presence of low

number of PR and also angiogenesis increases in the absence of PR [14] .

Regarding the relation of Ki-67 LI with the histological type; Ki-67 LI mean was higher in the histological subtypes of grade III and grade II tumor, (mean±SD was 13±13% in anaplastic, 5.4±2.8% in atypical and 6% in papillary,). While it was <1.8% in grade I tumor. In angiomatous type the mean±SD of Ki-67 was 6.6±11.2%, this is because within grade I we had 1 case of angiomatous type which had a history of recurrence with the Ki-67 LI of 19.5%. Other wise these result were consistent with those reported by Karabali P [3] , who also found that Ki-67 LI in anaplastic meningiomas were higher than the other subtypes of meningioma, while Uzum N [6] found no relation of Ki-67 LI with the histological types of meningioma.

A significant correlating of PR immunostaining with histological type was observed in this study (P = 0.0023), that is PR positivity was more frequent in meningiothelial, transitional, fibroblastic than other types. While anaplastic and papillary had no PR immunostaining. However these results were differ from those found by Roser F et al [8] and Claus EB et al [13], who reported no relation between PR and histological types of meningioma. This may be due to difference in grade, histological types and variation in patient population included in their studies.

There was a significant relation between the Ki-67 and the recurrence of the tumor, that LI was higher in recurrent cases compared with non - recurrent cases (P< 0.0001), this is consistant with results found by Rao Sh. et al [4], Devaprasath A [5], Uzum N [6], Roser F. et al [17], Francesco M. et al [22] and Lanzafame S. et al [23], Who found that the Ki-67 is significantly higher in recurrent histologically benign meningiomas, as compared with benign meningiomas without recurrence.

While PR was highly positive in non recurrent cases than recurrent cases (P = 0.003). This result is similar to those observed by Roser F et al [8] , Torp SH. et al [9], Roser F.et al [17] and Francesco M. et al [22] . This may be attributed to that a lack of PR correlates with an accumulation of qualitative and quantitative karyotype abnormalities, a higher proportional involvement of chromosomes 14 and 22 in de novo tumors and an increasing potential for aggressive clinical behaviour, progression and recurrence of meningioma [1].So the detection of the presence of PR in meningioma is important. It was found that in some cases of meningioma such as aging of the patients, presence of medical problems, unaccessibility to tumor, incomplete removal, and recurrence or in malignant types in which surgery is not possible and will not be the only sufficient choice. In these cases, if positive PR exists, in addition to radiotherapy, hormonal manipulation can be used [14]. Even the administration of preoperative medroxyprogesterone in patients with tumors that have positive PR would result into better clinical response compared with patients with tumors negative PR [14].

Other authors [8,17,22] and current study showed a strong inverse correlation between Ki-67 LI and PR status (P = 0.001). Majority of cases with high Ki-67 LI were PR negative. However no significant association could be found in studies done by Claus E B et al [13] and Applanat MP et al [15].

Roser F [8] suggests that PR status alone cannot be used to predict prognosis in meningioma. But PR status in combination with the proliferative index can be useful prognostic factors in meningioma. Francesco M. et al [22] found that higher mitotic index and Ki-67 LI and PR negativity are predictive factors of recurrence of benign (WHO I) completely resected meningioma.

CONCLUSION:

The mean±SD of Ki-67 LI in meningiomas was 2.7±4.2% while the progesterone receptor was found in 72% of meningiomas. No significant relation was found between the Ki-67 LI and the age of the patients, while progesterone receptor showed a significant relation with the age. Both Ki-67 LI and progesterone receptors had a significant association with the sex of the patients. A direct significant relationship was found between the Ki-67 LI and the grade, while inverse relation was observed between the progesterone receptor and the grade. Significant relation was observed between the progesterone receptor and histological types. Ki-67 LI was higher in grade II and III than that of grade I. Significant relation was noticed between the Ki-67 LI and PR in recurrent cases. An inverse relationship was observed between the progesterone receptor and Ki-67 LI.

REFERENCES:

1. Chargari C., Védrine L., Oliver Bauduceau O. Reapprasial of the role of endocrine therapy in meningioma management. *Endoc. -Related Ca*. 2008; 15: 931 -941.
2. Bruna J., Brell M., Ferrer I. et al. Ki-67 proliferative index predicts clinical outcome in patients with atypical or anaplastic meningioma .*Neuropath.* 2007;27: 114-20.
3. Karabali P. and Sav A. Proliferative Indices (MIB-1) in Meningiomas: Correlation with The Histological Subtypes and Grades. *J. Neuro. Sciences* 2006; 23: 279-286.
4. Rao Sh., Sadiya N., Doraiswami S. et al. Characterization of morphologically benign biologically aggressive meningiomas. *Neuro. India* 2009; 57 : 744-748.
5. Devaprasath A.and Chacko G. Diagnostic validity of the Ki-67 labeling index using the MIB-1 monoclonal antibody in the grading of meningiomas. *Neuro. India* 2003; 51:336-340.
6. Uzum N. and Omur Ataoglu G A. histological parameters with Ki-67 and bcl-2 in the prognosis of meningiomas according to WHO 2000 classification. *Tumori* 2008; 94:389-397.
7. Vankalakunti M., Vasishta R.K.., Radotra B.D. et al. MIB-1 immunolabeling: A valuable marker in prediction of benign recurring meningiomas *Neuropath.* 2007; 27: 407- 412.
8. Roser F., Nakamura M., Bellinzona M. et al. The prognostic value of progesterone receptor status in meningiomas. *J Clin Pathol* 2004; 57:1033-1037.
9. Torp SH., Lindboe CF., Gronberg BH. et al. Prognostic significance of Ki-67/ MIB-1 proliferation index in meningiomas.*Clin Neuropathol.* 2005; 24:170-4.
10. Roser F., Nakamura M., Ritz R. et al. Proliferation and Progesterone receptor status in benign meningiomas are not age dependent. *Ca* 2005; 104 :598-601.
11. Efird H. and Whyte H. MIB-1(Ki-67) index and transforming growth factor-alpha (TGFα) immunoreactivity are significant prognostic predictors for meningiomas. *J British Neuropathol Soci*.2002; 24:441 – 452.
12. David SI, Mark EA, Lother R. et al. MIB-1 Staining Index of Pediatric Meningiomas. *Neurosurg.* 2001; 48:590-597.
13. Claus E B, Park P J., Carroll R. et al. Specific Genes Expressed in Association with Progesterone Receptors in Meningioma. *Cancer Res* 2008; 68:314–22.
14. Taghipour M., Rakei SM., Monabati A. et al. The role of estrogen and progesterone receptors in grading of the malignancy of meningioma. *IRCMJ* 2007; 9:17-21.
15. Applanat MP, Groyer-Picard M. Th. and Kujas M. Immunocytochemical study of progesterone receptor in human meningioma. *Acta Neurochirurgica* 2005; 115: 20-30.
16. Kolles H., Niedermayer I., Schmitt CH. et al. Triple approach for diagnosis and grading of meningiomas:Histology, morphologyof Ki-67 and cytogenetics. *J. Acta Neuro chirurgica* 2005; 137: 174-181.
17. Roser F., Samii M., Ostertag H. et al. the Ki-67 proliferation antigen in meningiomas. Experience in 600 cases. *J. Acta Neuro chirurgica* 2004; 146: 37-44.
18. Amatya VJ., Takeshima Y., Sugiyama K. et al. Immunohistochemical study of Ki-67(MIB-1), p53, p21WAF1, and p27KIP1 expression in benign, atypical, and anaplastic meningiomas. *Human Pathol* 2001; 32:970-5.
19. Bruna J., Brell M., Ferrer I. et al. Ki-67 proliferative index predicts clinical outcome in patients with atypical or anaplastic meningioma. *Neuropath.* 2007; 27:114-20.
20. Perry A., Stafford S L., Scheithauer BW.et al. The Prognostic Significance of MIB-1, p53, and DNA Flow Cytometry in Completely Resected Primary Meningiomas *Ca* 1998; 82: 2262–9.
21. Takei H., Buckleair LW and Powell SZ. Immunohistochemical expression of apoptosis regulating proteins and sex hormone receptors in meningiomas. *Neuropathol* 2008; 28: 62-68.
22. Francesco M., Marialaura Del Basso C., Felice E. et al. recurrence of meningioma: predictive value of pathological features and hormonal and growth factors. J. *Neuro-Oncol* .2007; 82:63-68.
23. Lanzafame S., Torrisi A., Barbagallo G. et al. Correlation between histological grade, MIB-1, p53, and recurrence in 69 completely resected primary intracranial meningiomas with a 6 year mean follow up. *Pathol. Res. Pract.* 2000; 196:483-8.