

## Expression of Progesterone Receptor and Proliferative Marker ki 67 in Various Grades of Meningioma

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**Abstract-** Meningiomas are slow-growing neoplasms which recur locally, their morphologic grading is simple but do not always correlate with patient outcome. The aim of present study is to evaluate the status of progesterone receptor (PR) and proliferation marker Ki67 in various grades of meningioma in a group of Iranian patients. 78 cases of meningioma were selected from the file of a hospital university. All archival H&E stained sections were reviewed and regraded according to WHO criteria. Immunohistochemical analysis for PR and Ki67 was performed on formalin- fixed, paraffin- embedded samples. PR status considered positive if > 10% of tumor cell's nuclei were strongly immunoreactive, or if > 50% of nuclei were stained with medium intensity. The Ki67 labeling index (LI) is defined as the percentage area with strongest immunostaining. PR were positive in 61/63(96.8%) of grade I tumors, 2/10(20%) of grade II, and 0/5(0%) of grade III tumors. Ki67 LI was  $2.98 \pm 2.27$  in grade I tumors,  $9.30 \pm 5.79$  in grade II tumors and  $34.00 \pm 5.47$  in grade III tumors. For both markers, differences between grade I, II and III tumors were significant ( $P < 0.001$ ). There was a reverse relationship between mean of Ki67 LI and PR status, with increasing grade of tumor. Evaluation of PR status and Ki67 LI together with conventional histologic evaluation can help in providing more information about the biologic behaviour of meningiomas, especially for those that histological grading is not straightforward.

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**Key words:** Meningioma; receptors, progesterone; mitotic index; Ki-67 antigen

### Introduction

Meningiomas which originating from the arachnoid cap cells are the most common primary CNS neoplasms and are estimated to constitute between 13 and 26% of all intracranial tumors. (1,2) Although >90% are slowly growing and histologically benign (3), some can display aggressive behavior such as invasion of the brain, dura, adjacent bone, and multiple recurrences. Grading of meningiomas has evolved from the simple "brain invasion= malignancy" approach to a multiplex paradigm that assigns grades on the basis of tumor subtype and histologic features (2). Majority of meningiomas have distinctive morphologic features that permit reliable diagnosis and classification (WHO grade I, II and III). While histologic features may indicate the malignant nature of the neoplasm, they do not always correlate with patient outcome, since 2.3% to 30% of histologically benign meningiomas (WHO grade I) recur

following microscopically complete surgical resection with removal of the involved dura and bone (4, 5).

In WHO grading system, tumor is considered grade II if there is: Brain invasion and/or four or more, but fewer than 20 mitoses/ 10 hpf and/or three or more of the following: increased cellularity; small cell change; prominent nucleoli; loss of lobular architecture and necrosis and/or chordoid or clear cell subtype. The meningioma is considered grade III if there is: 1) overt anaplasia, and/or 20 or more mitosis / 10 hpf, and/or rhabdoid or papillary subtype. Some of the diagnostic histologic criteria for atypical and anaplastic meningiomas are vague and are subject to considerable inter-observer interpretation (e.g. sheeting, small cell change) (6-17). High mitotic index has been generally considered to be a strong indicator of tumor recurrence (18). Also the preponderance of PRs and the scarcity of estrogen receptors in meningiomas are well known. The expression of PRs may relate to tumor grade and

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recurrence. The relation between sex hormone receptors and meningiomas has been the subject of several studies (19-25). The risk of recurrence and the biologic behavior of meningiomas can not be predicted by histology alone.

The aim of the current study was to investigate the correlation between various grades of meningioma with data obtained from evaluation of proliferative and hormonal (progesterone) markers.

## Patients and Methods

A total of 78 meningioma cases were available from the surgical pathology files at the Rasoul-e-Akram Hospital, Tehran, Iran from 2001-2006. The slides were reviewed and regraded according to the WHO classification (2). Immunohistochemical staining was performed on 4-5  $\mu$ m thick, formalin fixed, paraffin-embedded tissue sections that were mounted on poly-L-lysine precoated slides. In short, sections were deparaffinized and stained with monoclonal mouse antihuman PR and Ki67 (Dako, Copenhagen, Denmark). Heat induced antigen retrieval using autoclave method was applied. Negative controls were run at each staining session, as were positive control specimens using breast

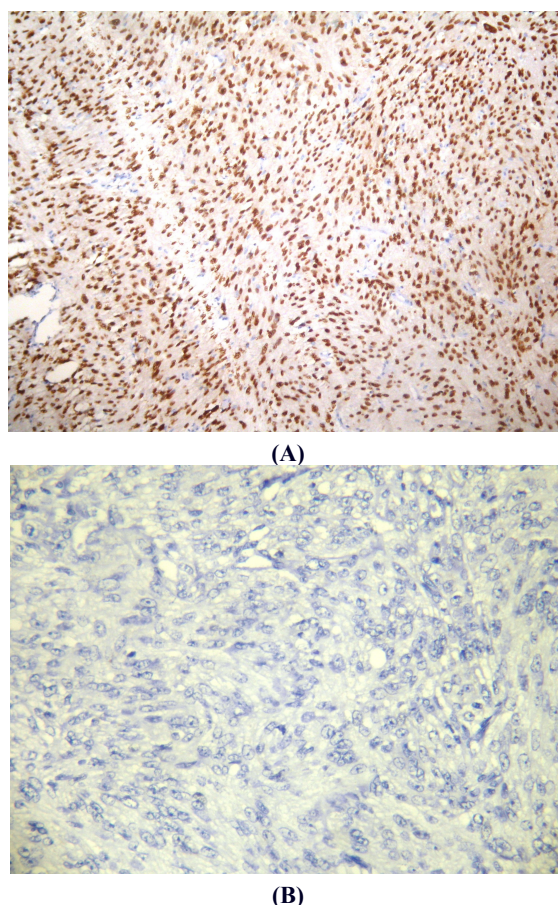
cancer tissue for PR and lymph node germinal center for Ki67. All slides were examined for positively stained tumor cell nuclei. For PR, the immunostained sections were graded semiquantitatively for intensity as well as extent of staining. Tumors with strong staining in at least 10% of nuclei or moderate staining in about 50% of nuclei were considered PR positive (32).

For Ki67 staining, proliferative index (PI) was expressed as a percentage of positively stained cells out of 1000 tumor cells counted in the most mitotically active areas. Patient demographic information such as age and sex was obtained from patient's medical record.

Statistical analysis of data was performed by chi2 test using SPSS (version 15.0) software program. A *P* value less than 0.05 was considered to be significant.

## Results

Among the 78 patients studied, 63 cases were grade I, 10 were grade II and 5 cases were grade III meningiomas. The mean age was 54 years (range, 22-82 years). 48 (76.2%) of grade I meningiomas were meningotheliomatous and transitional and 15 (23.8%) were fibromatous type.



**Figure 1.** Immunohistochemical stains for PR. (A) Grade I meningioma, (B) Grade III meningioma

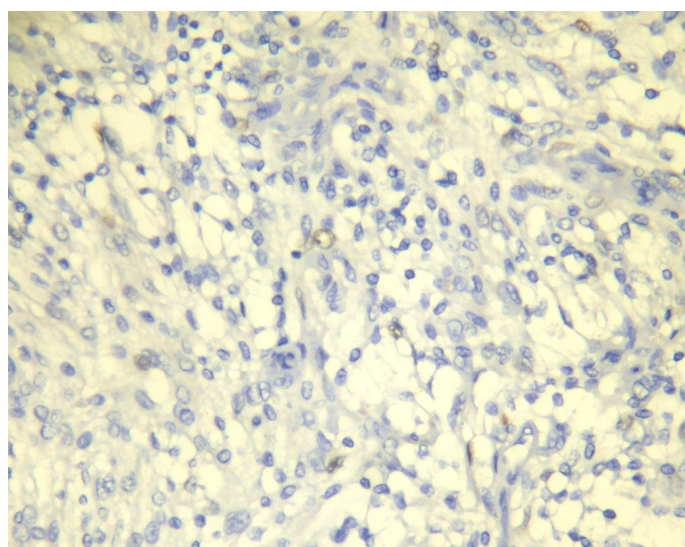
**PR and Ki67 expression in meningioma**

53(68%) were female and 25 (32%) were male (F/M ratio: 2.5/1). Nuclear immunostaining for PR was positive in 61/63 (96.8%) of grade I (Figure 1A), 2/10(20%) of grade II and none (0%) of grade III tumors (Figure 1B). The difference between groups was significant (Chi 2  $P < 0.0001$ ). Higher PR positivity were found in female than in male patients (86.8% vs 68%, chi 2  $P < 0.05$ ).

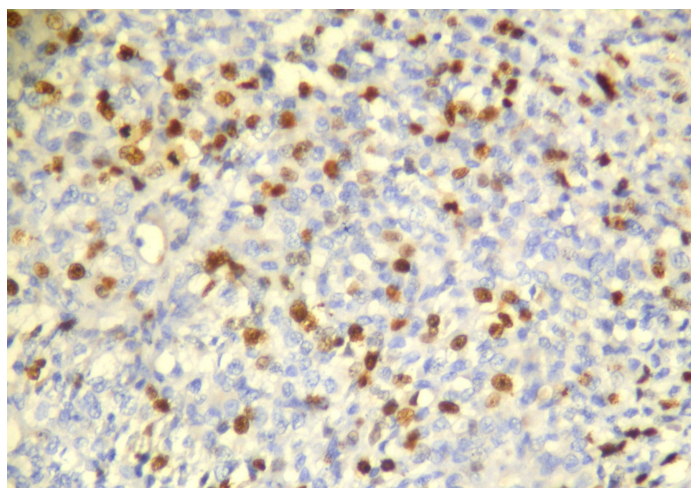
Mean ki67 LI was  $2.98 \pm 2.27$  (range, 0-15) among grade I tumors (Figure 2A),  $9.30 \pm 5.79$  (range, 2-20) in grade II and  $34 \pm 5.47$  (range,30-40) in grade III meningiomas (Figure 2B), (Table 1).

**Table 1.** Results of immunohistochemical staining for PR and Ki67 in various grades of meningioma

IHC marker	PR	Ki67
<b>Grade</b>		
Grade I	61/63(96.8%)	2.98±2.27 (range 0-15)
Grade II	2/10(20%)	9.30±5.79 (range 2-20)
Grade III	0/5(0%)	34±5.47 (range 30-40)
Total	78	



(A)



(B)

**Figure 2.** Ki67 labeling index staining.(A)Grade I tumor , (B)grade III tumor

Differences between three grades was significant (Anova  $P < 0.001$ ). No statistically significant difference of proliferation rate (MIB-1) was observed in male vs female gender.

A reverse correlation between PR positivity and mean ki67 LI was noted ( $R = -0.63$ ,  $P < 0001$ ).

Among histomorphologic subtypes of who grade I meningiomas, there was a trend to higher PR staining intensity in meningotheial tumors.

## Discussion

Recurrence after apparently complete resection is one of the most relevant problems of meningioma treatment. Meningiomas are considered to be potentially hormone-sensitive tumors. The role of sex hormones in the pathogenesis of meningiomas is yet not clarified, but there is growing evidence that progesterone might at least contribute to the growth of PR positive meningiomas and has also been correlated with recurrence of meningioma. The available data about relationship between expression of PR and proliferative activity in order to determine more precisely the potential of aggressiveness of meningioma is limited.

It is well known that in meningiomas the clinical behavior can not be predicted consistently based on the histomorphologic features alone. The histomorphologic features used for grading meningioma according to WHO classification included: Increased mitotic activity, loss of architecture (patternless sheeting), nuclear pleomorphism, prominent nucleoli, hypercellularity, high nuclear-cytoplasmic ratios (small cell changes), necrosis, and brain and adjacent structure invasions. Most of these criteria could be suggestive and /or may be present only in some areas of sections.

The aim of our study was the evaluation of the expression of PR and Ki67 LI in various grades of meningioma in a group of Iranian patients.

It has been reported that WHO grade II and III tumors had significantly fewer PR receptors than benign meningioma (grade I). Our results also showed a significant correlation between PR positivity and lower grade of meningiomas. Higher PR staining was also noted in female than in male patients and in meningotheial tumors than other types. The higher expression of PR in females has also been found by others (26).

There were an increasing mean ki67 LI with increase in meningioma grades. A reverse correlation was observed between PR positivity and mean Ki67 LI.

Maiuri *et al* showed that proliferative index and PR but not ER status have a strong predictive value, whereas the growth factors are not predictive (27). Whittle *et al* showed that PR negative meningiomas were biologically more aggressive than PR positive ones (28).

Subsequently Cahill *et al* and brandis *et al* reported that malignant meningiomas are devoid of PR and ER (29-31).

Roser *et al* confirmed the presence of significantly higher PR values in benign meningiomas compared with WHO grade II or III tumors. They found that PR status affected survival only in combination with the proliferation marker ki-67 (32). Finally Wolfsberger *et al* found that the highest PR index is observed in patients under the age of 50 years with WHO grade I meningiomas of the meningotheial subtype and low cell proliferation index (33). In conclusion, meningiomas are known to recur frequently, even after complete resection. The recurrence cannot be predicted by histomorphological features alone. Cell proliferation indices and hormone receptor status can be used as a guide in grading of meningioma and therefore in prediction the recurrence potential of them. Meningiomas with higher proliferation index and negative PR are very likely to be atypical (grade II) or malignant (grade III) and can potentially considered to be recurrent.

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