Original article

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Immunohistochemical Study Characterizing Estrogen and Progesterone Receptors Status in Meningiomas and Correlation with MIB-1 Labeling index

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Abstract

Background: Meningiomas constitute 13-26% of primary intracranial tumors and have predilection for females. Etiology is multifactorial including environmental causes. WHO classifies meningiomas into three grades based on various morphological criteria.

Method: This study was carried out to correlate ER, PR expression, MIB-1 LI and traditional morphological prognostic markers of meningiomas. Sixty seven consecutive cases of meningioma, operated at a tertiary care neurosurgical centre, were analyzed for grade using routine Hematoxylin-Eosin stain; estrogen and progesterone receptor (ER, PR) status by immunohistochemistry; and proliferation index using MIB-1 labeling.

Results: Majority were grade I neoplasms, with 11 being atypical and 05 anaplastic variants. The mean MIB-1 LI was 3.05%, 6.38% and 11.9% in grades I, II and III meningiomas respectively. ER expression was revealed in 20.89% cases with focal positivity, without any significance on correlation with grade, histology and gender. PR reactivity was found in 73% of the tumors. Higher positivity is found with low grade meningiomas, while high grade meningiomas are associated with negative PR expression. Unpaired t-test showed significant difference found in mean MIB-1 LI in PR positive and negative groups.

Conclusion: PR and MIB-1 LI immunohistochemical staining emerged as useful supplements of routine histopathological assessment and can be used to provide additional information regarding behavior and response to treatment.

Keywords: *Hormone receptor; Immunohistochemistry; Meningioma; Proliferation rate.*

Introduction

Meningiomas are composed of neoplastic meningothelial cells with epithelial and mesenchymal characteristics, resulting in diverse histologic appearances. An incidence of 6 per 100,000 persons constituting between 13 to 26% of primary intracranial tumors is seen. They occur more frequently in middle aged to elderly women. Radiation exposure, genetic factors, hormonal alterations and trauma have been implicated. The location of a meningioma dictates its clinical presentation. [1,2]

Application of the WHO criteria broadly stratifies meningothelial tumors into three tiers - meningioma, atypical meningioma, and anaplastic meningiomas (grades I, II, and III respectively). [3] A stronger association with PR status[4] and correlation with pregnancy, luteal phase of the menstrual cycle or coexistence with

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breast cancer has been seen. [5] Oral contraception pills (OCP) and hormone replacement therapy (HRT) administration showed that the grade of meningioma and likelihood of recurrence are related to PR expression, with negative tumors more likely to be of higher grade and to recur. [6] Wiegerts et al identified an increased relative risk of meningioma in postmenopausal women on HRT. [8] Since 1980, many case reports have been published in which individuals with diagnoses of both meningiomas and breast carcinomas suggested the association with common risk factors.[9]

Up to 20% of meningiomas are atypical and up to 2.8% are anaplastic (malignant) meningiomas. These show male predominance with higher proliferation indices. [10] Often, the invasive front of meningioma is discrete or proceeds along vessels, trapping islands of gliotic CNS parenchyma. [1,2,7,11] Meningiomas with indices >4% have increased risk of recurrence similar to atypical meningioma, whereas those with indices >20% are associated with death rates analogous to those with anaplastic meningioma. Perry et al, to predict tumor recurrence, said MIB-1 LI of at least 4.2% was strongly associated with decreased recurrence-free survival. [12] MIB-1 LI is correlated with regrowth potential. [13,14] In pediatric meningiomas, it ranged from 1.2 – 31.6% with significant difference between atypical or malignant tumors and between recurring and nonrecurring tumors. [9,15] Roser et al found significant correlation between negative PR status and high MIB-1 LI. [16] The absence of PR, and high mitotic index, as well as tumor grades, are significant factors in assessing disease-free survival. [17]

Multivariate analysis has shown that a three-factor interaction model incorporating a PR score of 0, a mitotic index >6 and malignant grade was a highly significant predictor of poor outcome. [18] Omulecka et al found 100% meningothelial, 95% transitional, 46% fibrous and 78% atypical meningiomas with PR expression. [5] Konstantinidou et al showed that ER expression is reduced in atypical meningiomas. [19,20] Roser et al showed comparable values for men and women for PR status. [21] There was no significant correlation between PR status and recurrence rates in grade I meningiomas. However, a combination of PR status and proliferation indices was shown to predict recurrence reliably. [21,22] Moderately high percentage of PR expression in grade I tumors has been found. [23] However, Kandemir et al did not find any such correlations. [24]

This endeavor was undertaken to study the expression of ER and PR in meningiomas and to compare the proliferation rate with the grade of these tumors.

Materials and methods

Sixty-seven consecutive meningiomas with relevant clinical data were selected from 2005 onwards. Serial 5 micrometer thin sections were stained by Haematoxylin and Eosin **(**H&E). Highest tumor grade sections were selected for ER, PR and MIB-1 IHC as described elsewhere. [25] Primary, ready-to-use monoclonal antibodies against ER and PR (Clones 1D5 and SP2, Neomarkers), and MIB-1 (Clone MIB-1, Dako) were used.

The receptor status was determined by a semi-quantitative scoring scale as described by Roser et al. [21] Staining intensity was graded as 0- absent, 1-weak, 2-moderate, 3-strong. With respect to percentage of positive tumor cells, they were scored as presence of 0 indicating absence; 1, few positive tumor nuclei <10% in the entire section; 2, 10-50% positive nuclei; 3, 51-80% positive tumor nuclei; and 4, >80% positive nuclei. As recommended for breast cancer,[21] and verified with meningioma tissue, [18] an immunoreactive score (IRS) was calculated. Tumors with IRS range of 2 or more were considered receptor positive. The area with highest density of labeled nuclei (hot spot) was selected for counting at high power magnification; 500 cells were counted and the ratio of positive staining nuclei to the total tumor cells was mean MIB-1 LI. [26]

Statistical analysis was performed using EPI Info software program window. The parameters were analyzed by the Student's t test, Chi Square 2 tailed p and Fischer exact test, and

Kruskas-Wallis test. Differences were significant at a p value of < 0.05.

Results

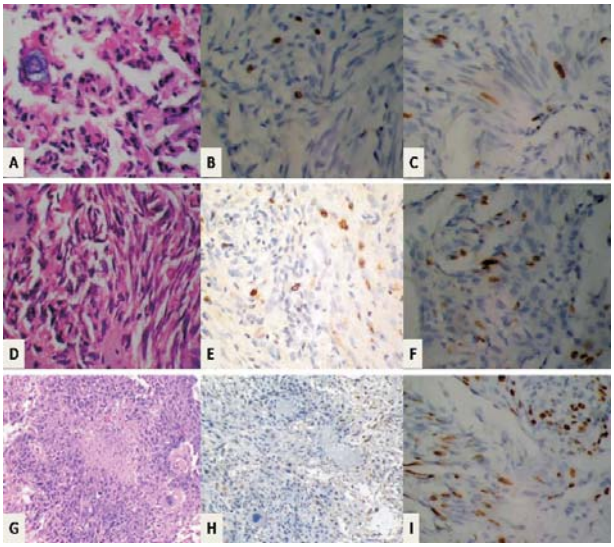
This study was conducted between May 2008-September 2010 and included sixty seven cases. Clinical information including age, gender, sites of tumor and neuroimaging was obtained. The patients, 38 females and 29 males with ages ranging from 9 to 89 years showed a peak incidence in the 40 to 60 years age. We failed to establish a correlation between the aggressiveness of meningiomas with age. The ratio of males to female was 1:1.3. No significant association of occurrence of meningiomas with gender was found.

Only three women had a past history of breast cancer and two women had used OCP. None had been exposed to HRT. Among the males, two had head injuries in the past and three had undergone irradiation to head and neck region. The frontoparietal region was the commonest site. To find out association between intracranial versus extracranial meningiomas with tumor behavior, Fisher exact test was carried out but was not significant.

The majority were grade I with 11 being atypical and 5 anaplastic meningiomas. The commonest histological subtype seen was meningothelial meningiomas. One was grade III, 3 of grade II and rest 22 of grade I. No particular relation to tumor site, gender or age was noted. Seven Fibroblastic grade I meningiomas were seen without relation with tumor site, gender and age. Thirteen transitional meningiomas seen were composed of meningothelial and fibroblastic components with Psammoma bodies and clusters of syncytial cells [Figure1A]. One each belonged to grade II and grade III category [Figure1D]. The presence of Psammoma bodies did not show any effect on tumor behavior. Five cases were of grade I angiomatous meningioma.

Four atypical meningiomas manifested between 4 to 19 mitotic figures in 10 high-power fields or had increased cellularity, small cells with high N:C ratio, prominent nucleoli,

Figure 1(A-I): Photomicrographs of Grade I meningioma showing Psammoma body (A: H&E x200); PR activity (B: PRx200); and low MIB-1LI (C: MIB-1 x200). Grade II meningiomas showing atypia (D: H&Ex200); PR reactivity (E: PRx200); and moderate MIB-1LI (E: MIB-1x200). Grade III meningiomas displaying focal necrosis, high grade atypia, increased mitosis (G: H&Ex100); low PR reactivity (H: PR200); and high MIB-1 LI (I: MIB-1x200)



patternless sheets, and foci of geographic necrosis. One spinal chordoid meningioma was found in a 16-year-old girl. No statistically significant association with tumor site, gender or age was seen. One papillary meningioma case from clivus was seen. Anaplastic meningioma includes either 20 or more mitotic figures per 10 HPF, or regions with anaplastic cytology resembling a sarcoma, carcinoma, or melanoma [Figure1G]. MIB-1 LI was high in most anaplastic meningiomas [Figure1I]. No significant association with clinical parameters was seen in the two anaplastic meningiomas. The mean MIB-1 LI was 3.05% in grade I meningiomas [Figure1C], 6.38% in grade II meningiomas [Figure1F] and 11.9% in grade III meningiomas. Kruskal-Wallis test showed a statistically significant difference in mean MIB-1 LI values of all three grades (p = 0.00).

ER expression was revealed in 20.89% cases with focal positivity. No statistically significant relation was found with grade, histology and gender. PR reactivity was found in 73% of the tumors [Figure1B,E,H]. No statistically significant relation with age and gender was

seen. A statistically significant association was found with tumor grades. Higher positivity was found with low-grade meningiomas, while high-grade meningiomas were associated with negative PR expression [Figure1H], (p value = 0.00). Unpaired t-test showed significant difference found in mean MIB-1 LI in PR positive and negative groups. MIB-1 value was higher in PR negative groups. Mean MIB-1 value was 8.17% in PR negative with standard deviation (SD) of 5.26 and was lower with mean of 3.34% and SD of 3.1 in PR positives.

Discussion

This study was carried out to correlate ER, PR expression, MIB-1 LI and traditional morphological prognostic markers of meningiomas. Majority of our patients were in 40-60 years age group. The overall ratio of males to females was 1:1.3. The maximum number of meningiomas were located in the frontoparietal region (25%). We did not find any statistically significant association between the site of tumors with tumor grades, recurrence rate, age or gender.

Cancers with large female: male ratio seems to be influenced by female sex hormones. [27] Only two out of 38 patients had used OCP and none had ever used HRT, which is statistically insignificant. Wigerts et al[8] and Jhawar et al [28] found an elevated risk of meningioma among postmenopausal women who used HRT. A further investigation with larger sample size may have clarified the contradiction. In our study only three had breast cancer in the past. No statistically significant associations were seen unlike Custer et al. [5]

There were 51 classic (78%) grade I cases. These account for more than 90% of the cases in other studies. [7] Subjective methods such as mitoses, necrosis etc. are still used in determining the grade and proliferating activity. MIB-1 LI has been partially correlated with aggressiveness. [4,10,26] In our study, the mean ± SD MIB-1 LI was 3.05 ± 1.41 in grade I, and those of grade II and grade III meningiomas was 6.38 ± 3.38 and 11.9 ± 7.48 respectively. There was a statistically significant correlation with the grades, although MIB-1 LI was of different values than those seen in various studies. [13.16.26] No correlation between MIB-1 LI and histology of meningiomas was seen, although Ozen et al have reported highest value in fibrous and lowest in secretory meningiomas. [13] However, clear cell, chordoid and papillary variants, which have influence on tumor grades, showed a higher value of MIB-1 LI.

We, like Sandberg et al, found that mean MIB-1 LI for pediatric meningiomas without histological atypia did not differ significantly from adult meningioma without histological atypia. [15] Yamasaki et al reported that MIB-1 LI of the recurrent tumors were higher. [14] Similarly, our three cases of recurrent meningiomas revealed a higher MIB-1 LI of 3.0% - 8.0%. These are too small in number to draw any conclusion although a trend towards higher values can be seen. The problem with MIB-LI is the focal variability in tumor histology and grade. Tissue removed or section chosen may or may not represent the most proliferative area. Moreover, there appears to be overlap in terms of ranges of LI at the interphase between tumor grades. Despite these limitations, MIB-1 is useful for inferring the proliferative potential of the tumors.

Quantifying the hormonal status of the tumor may help to predict its biological behavior and provide options for further treatments. The higher incidence of meningiomas among women has led to the assumption that sex steroid hormones may influence the growth of meningiomas. [21] A significant correlation between tumor grades and PR expression status was observed. Lower tumor grades showed high PR expression while high tumor grades showed low PR expression. Eight WHO grade II (73%) and all grade III meningiomas in our study completely failed to express PR as seen by others [18]. Although not entirely unlikely, no association between PR status and age, tumor location, first time versus recurrence, and histology has been reported in the literature. [21-23] No significant association between the above-mentioned factors and PR status was seen.

All available data in literature point to higher expression of PR in female patients. [22] In our study, no significant association between

gender and PR expression was found after application of Fischer exact test. The result suggests that PR status is an important prognostic factor in meningioma, more so in combination with proliferative index. However, the PR status alone cannot be used to predict behavior in meningiomas and should not influence therapeutic strategies.

The absence of ER has been reported, despite high levels of PR expression. [23] In our study, only 17% of patients showed positive staining for ER without any significant relation with histology, PR, MIB-1 expression or gender. Our result is in agreement with others. [23] There was significant difference found in mean MIB-1 LI values in PR positive and PR negative groups. MIB-1 value was higher in PR negative group (8.17% with SD of 5.26). In PR positive group, MIB-1 LI value was lower (3.34% and SD of 3.1). The correlation in our study was perfectly in agreement with the WHO. [7] We did not find any statistically significant association between the cases studied and risk factors considered for meningiomas. The primary tumors were located predominantly intracranially.

Mean MIB-1 LI index showed linear correlation with tumor grades. Thus, PR and MIB-1 LI immunohistochemical staining have emerged as useful supplements of routine histopathological assessment and can be used to provide additional information regarding behavior and response to treatment. Further studies of a larger magnitude and prolonged follow up are required to prove our correlations more conclusively.

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