Original Article

Prognostic Significance of Ki 67 Labelling Index and P63 Immunoreactivity in Intra Cranial and Intra Spinal Meningiomas

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ABSTRACT

Background: Meningiomas are indolent tumors and their grading is based on histopathological parameters which have inherent limitations. Adjunct immunohistochemical markers are often used to overcome these limitations.

Objective: To investigate the role of Ki67 and P63 immunoexpression in various grades of meningiomas.

Methods: A retrospective analysis of 144 biopsies of intracranial and spinal meningothelial and nonmeningothelial tumors operated from January 2011 to May 2016 was carried out. Intraoperative squash smears were stained by 1% aqueous toluidine blue and rapid H&E method. Ki67 and p63 immunohistochemical staining was performed on paraffin sections of 125 histologically proven meningiomas cases. Grading was done according to the modified 2016 World Health Organization classification of CNS neoplasms .The results were analysed using statistical methods.

Results: The total number of biopsy samples received was from144 cases. There was a female preponderance accounting for 68%of total cases. Positive cytohistological correlation was seen in 86.14% (125/144 cases). 112 out of 125 cases were found to be WHO grade I tumors out of which 20 showed a high Ki-67 LI and p63expression. 6 out of 7 cases of WHO Grade II meningiomas showed strong ki 67 and p63 nuclear expression whereas 3 anaplastic meningioma cases out of 6 WHO grade III meningiomas showed strong ki 67 and p63 nuclear positivity .

Conclusion: The expression of P63 is variable in different grades of meningiomas. There was statistically significant increase of P63 protein expression and Ki 67 LI between the grades I to II and grades II to grade III. In the present study it was observed that 20/112 grade I meningiomas expressed high p 63 immunoreactivity contrary to other studies. High grade meningiomas showed increased Ki67 labelling index. P63 immunoexpression was high with predictability of recurrence across all the grades.

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Introduction

Meningiomas constitute 24 to 30% of all the Central Nervous System tumors.[1]They are known to have a female preponderance and are more commonly seen in patients who are in the fourth decade of their life.[2] The current World Health Organization (WHO) 2016 classification has identified 15 histopathological subtypes of meningiomas ,which have been graded on the basis of their recurrence potential and net growth rate.[3] Morphological features such as increased mitoses, geographic necrosis, and invasion into the brain parenchyma are not always reliable, in representing the overall biologic nature of lesion.Inspite of total resection 7-20% of benign (Grade I), 29- 40% of atypical (Grade II), 50-78% of anaplastic (Grade III) meningiomas are known to recur.[4]

All radiologically and clinically suspected meningeal tumors may at times histopathologically prove to be rare non meningothelial tumors of meninges. Their histological recognition is a major concern as it has an effect on the clinical outcome and further treatment modality.

Several immunohistochemical (IHC) biomarkers such as Ki-67[1,7,8] P63,[9],Claudin[6] , and the absence of progesterone receptors have been proposed to assist conventional methods of tumour grading.[5] Use of one single IHC marker has often been found to be inadequate in definitively predicting the grades of these meningiomas. P63 is a structural homologue of the p53 tumor suppressor gene has been suggested to have a role in oncogenesis[7] . P63 is not only involved in maintaining epithelial stem-cell populations but also known to play an important role in cellular differentiation and neoplasia. So far p63 has been frequently used in the diagnosis of prostate and breast carcinoma.[8,9] Additionally the p63 protein has been found to have a strong association with squamous-cell carcinoma.[9]

In the available literature only few studies [10] have used p63 immunostaining along with Ki-67 LI expression in various grades of meningiomas. We thus report this study which was carried out to assess the correlation between p63 and Ki 67 immunoexpression across all grades of meningiomas.

Material and Methods

In this retrospective study squash cytosmears were prepared from the fresh biopsy samples of intracranial and intraspinal meningothelial and non meningothelial lesions sent to the Department of Neuropathology, Osmania Medical College for intraoperative pathological consultation. The squash smears prepared were stained using 1% aqueous toluidine blue and rapid H&E method. Paraffin block sections were prepared from the residual and subsequently resected tumor tissue and stained using routine H & E method. A total of 144 cases received over a five year period from Jan 2011 to May 2016 were analyzed A total of 144 cases were reported as meningiomas on squash cytosmears. 125/144 cases had cyto-histological correlation remaining 19 cases had varied diagnosis. A total of 130 cases were reported as meningiomas Available clinical data, including patient age, sex, clinical and radiological findings were retrieved from the records. Ki67 and P63 IHC was carried out on 125 cases of histologically proven meningiomas. Five cases were omitted from the present study for various technical reasons

Ki-67 and P63 Immunohistochemistry Technique: Sections of 4-micron thickness using Leica 2255 microtome collected on poly-L-lysine coated slides were subjected to IHC by routine indirect immunoperoxidase technique using primary antibody (Ki67, monoclonal antibody, DakoCytomation, Glostrup Denmark, dilution 1;50) and p63 (clone; Dak-p63 DakoCytomation, Glostrup Denmark dilution 1:50), followed by incubation in secondary antibody for 30 min (Polymer link detection kit, DakoCytomation, Glostrup, Denmark).At least 1000 tumor nuclei were counted at high magnification (40× objective) without recounting same areas and the average was expressed as percentage by two trained pathologist. Foci of hemorrhage and necrosis were excluded.

Appropriate positive and negative controls were used for both the antibodies. Myoepithelial cells of the breast and basal cells of prostate were taken as positive controls. The primary antibody was omitted in negative controls. Immunoreactivity of p63 was scored semi quantitatively based on nuclear positivity within neoplastic cells as, Grade 0 : no staining, Grade 1+: <10% cells, Grade 2+ : 10 -50 % cells, Grade 3+: >50% cells. Immunoreactivity for Ki-67 was assessed by counting at least 1000 tumor nuclei at high magnification (40x) without recounting the same area and the average was expressed as percentage. Foci of necrosis were excluded

Statistical Analysis: The relationship between P63 expression and the Histological grades were analyzed by using ANOVA, independent t-test and by Chi-square test using SPSS 14.1 version. The results were considered statistically significant if the P value was <0.05.

Results

This study encountered 19 discordant cases on squash cytology out of a total 144 CNS samples. They had varied histopathological diagnosis. 4 tumors were diagnosed as hemangiopericytoma, 3 as schwannoma, 2 as hemangioblastomas,1 case each of hemangioma, PNET,

mesenchymal chondrosarcoma and neuricysticercosis.5 cases of non meningeal tumors were histologically confirmed as meningiomas. In 125 histologically proven meningiomas the demographic distribution was found to be statistically non significant with a female preponderance 85 (68.1%) vs 40 (31.9%) males. There were 108 (86.4%) intracranial meningiomas as compared to 17(13.6%) intraspinal meningiomas. The age of the patients ranged from 4-75 years including 7 children below the age of 18years. The maximum number of cases were seen in the age group of 40-49 years (35.4%) followed by 30-39 years (18%).The least number of cases were seen in patients of age group 0-9 years (1%).

Geographically 62% meningiomas were located on brain convexities, 15% at skull base, 5%at Cerebello-pontine angle, 5%of cases at various locations and the remaining 13%cases at spinal location.

Histological grading of meningiomas was done according to WHO CNS tumor classification.112 out of 125 tumors were classified as WHO grade I of which meningotheliomatous subtype being the commonest accounting for 27%,transitional 26% ,fibroblastic 25%,psammomatous 10%,angiomatous 4%,microcystic 0.8%,secretory and metaplastic 1.6% each.

7(5.6%) tumors were classified as WHO grade II. Six cases in this grade were diagnosed as atypical meningiomas which infiltrated the brain parenchyma as depicted in figure G and figure H.A single case of clear cell variant of meningioma was also reported. Grade III tumors accounted for 6 (4.8%) cases contributed by papillary, rhabdoid and anaplastic meningiomas characterized by high degree of anaplasia, foci of necrosis and its infiltrative nature.

It was observed that mean P63 expression in the 6th decade was highest at 12.5 with total mean in all age groups at 8.13 similarly mean Ki67 LI was at 1.725 being highest in the 6th decade .The mean P63 expression in males was 6.82 and 8.74 in females .The mean Ki67 LI in males was 1.495 and 1.55 in females. Age and sex related correlation for test of significance could not be established.

Meningothelial meningiomas were the most commonly occurring grade I tumors with a mean Ki 67 LI of 3.212 whereas secretory, microcystic subtype showed 0.608 mean Ki67 LI. The mean P63 positivity index in the meningothelial meningiomas was highest at 23.06 amongst Grade I category and lowest at 0.234 in secretory micro cystic type respectively. The mean P 63 expression in WHO grade II subtypes of atypical and clear cell meningioma was observed as 92.8. The mean P63 expression in papillary meningioma with rhabdoid features was 22.33 and a lone case of anaplastic meningioma expressed mean of 97.16 being the highest in our series. Thus meningothelial meningiomas in WHO grade I category and tumors in WHO grade II and grade III categories showed higher P63 expression.

Discussion

A total number of 144 CNS tumor cases undergoing neurosurgical intervention were assessed using routine intraoperative cyto-pathological methods.19 cyto-histological discordant tumors were analyzed. Discordance resulted due to varied cytomorphology with increased cellularity, vascularity and technical limitations.

Meningiomas are common in middle aged woman with only few cases being reported in males and pediatric age group. Age and Gender of the patient has no influence on the proliferative activity and recurrence potential of meningiomas. Our series noted no statistical significance of Ki67 and P63 expression with respect to age and gender. Recurrence was noted in only four cases, three of which were males and all four recurred meningioma cases were located in the skull base. Tumors occurring in other locations showed no statistical significance across various grades with respect to Ki67 LI and P63 immunoexpression. Other studies compared to in literature either used P63 or Ki67immunoexpression as an adjunct to conventional histopathological grading techniques. This study has used a combined grading system of P63 and Ki67 immunoexpression along with conventional grading method as per classification of CNS WHO tumor guidelines in all the 125 cases.

In the present study84% meningiomas in WHO grade I category did not show P63 immunoexpression, tumors in meningotheliomatous meningiomas subtype expressed more than 10-50% of nuclear positivity in the same group. All the meningiomas in WHO grade II and WHO grade III category showed strong P63 and Ki 67 nuclear expression. Value of significance between by using Independent t-test between grades I and II, grades II and III for P63 and Ki67 immunoexpression are 0.0001 and 0.001 respectively. Whereas comparison between grades I and III was 0.001 and 0.243 respectively, thus the p value is significant when meningiomas in WHO grade I category were compared with tumors in WHO grade II & WHO grade III categories.

In this present study four meningioma cases out of a total of one twenty five meningioma cases recurred clinically. A total of twenty meningothelial meningioma cases out of 112 WHO grade I meningiomas showed a high Ki-67 LI and P63immunoexpression which was also suggested by Guarnaschelli et al [14].Three cases were histologically

categorized to WHO grade I with minimal atypia and no evidence of brain invasion; however these meningioma cases showed focal hypercellularity. The remaining single case was histologically categorized into WHO grade II. The mean Ki-67 LI and P63immunoexpression scoring of recurrent meningiomas was 8.6% and 62.5% respectively. Period of recurrence ranged from 2.1 to 9.5 years with a mean of 6 years. Among the recurrent tumors the p value for

Table1: Correlation of P63 and Ki67 with respect to Age, Sex, Histological grade and morphological subtypes:

	Mean		SD		Standard Error		95% CI (Lower bound)		Statistical test	P value	
Age	P63	Ki67	P63	Ki67	P63	Ki67	P63	Ki67		P63	Ki67
1-10yrs	0.0	0.6	0.0	0.0	0.0		0.0				
11-20yrs	10.72	1.6	21.36	1.43	8.721		-11.70				
21-30yrs	04.76	1.37	11.79	1.42	3.151		-2.04				
31-40yrs	7.94	1.43	17.77	1.85	3.094		1.64		independent t-test		0.998
41-50yrs	7.66	1.59	16.99	1.8	2.505	NA	2.61	NA		NA	
51-60yrs	12.5	1.725	25.53	2.11	6.383		-1.10				
61-70yrs	8.88	1.65	16.53	1.64	5.848		-4.95				
71-80yrs	0.0	1.4	0.0	0.0	0.0		0.0				
Total	08.13	NA	17.85	NA	1.597		4.97				
Histological subtypes											
Grade 1											
Meningothelial	23.06	3.212	25.07	2.398	4.364	0.417	14.17	0.417			
Fibroblastic	0.36	0.569	0.903	0.447	0.177	0.093	-0.01	0.036			
Transitional	7.08	1.421	16.23	1.072	2.825	0.204	1.33	1.005			NA
Psammomatous	0.54	0.510	1.168	0.510	0.268	0.0	-0.03	0.575			
Angiomatous	0.32	0.63	0.921	0.436	0.212	0.212	-0.021	0.673		NA	
Secretory and Microcystic	0.234	0.608	0.781	0.483	0.197	0.139	1.253	0.301			
Grade 2											
Metaplastic	1.0		1.414		1.0		-11.71				
Atypical and Clear	92.8		4.62		21.36		4.219				
Grade 3											
Papillary with Rhabdoid	22.33		13.79		190.3		11.23				
Anaplastic	97.16		NA		NA		NA				
Total (125 cases)	8.13		17.86		1.597		4.97				
Sex											
Male (40 cases)	6.82	1.495	17.06	1.837	$2.698E_0$	0.290			independent t-test	0.56	0.85
Female (85 cases)	8.74	1.55	18.28	1.720	$1.983E_0$	0.188					

Table 2: Comparison of present study with other studies.

Grades	Rushing et al[11] (n=37)	Sharifi et al[12] (n=54)	Jain et al[13] (n=85)	Present study (n=125)
GRADE I	51%	35%	35%	16%
GRADE II	92%	71%	62%	100%
GRADE III	75%	100%	64%	100%

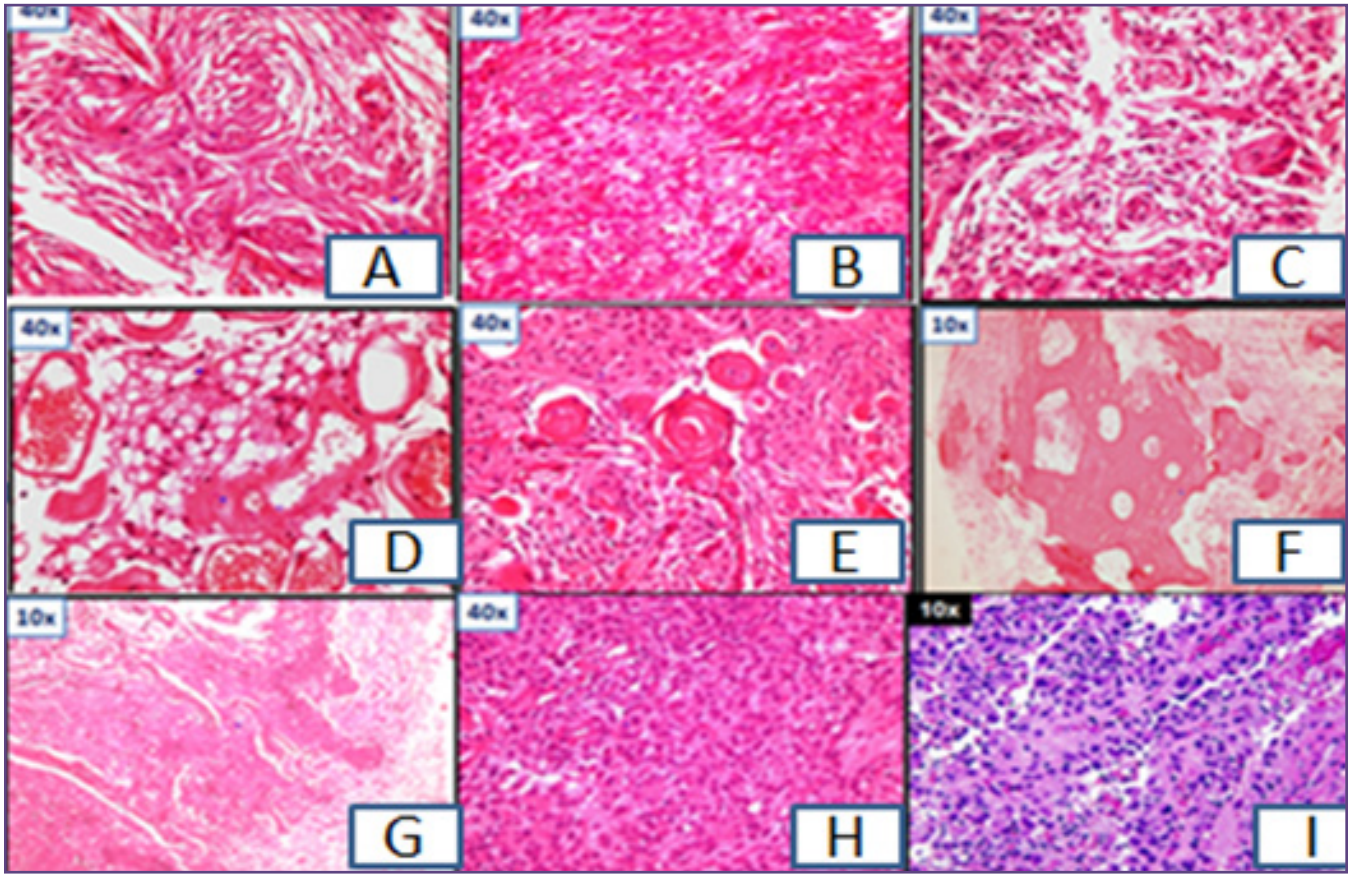
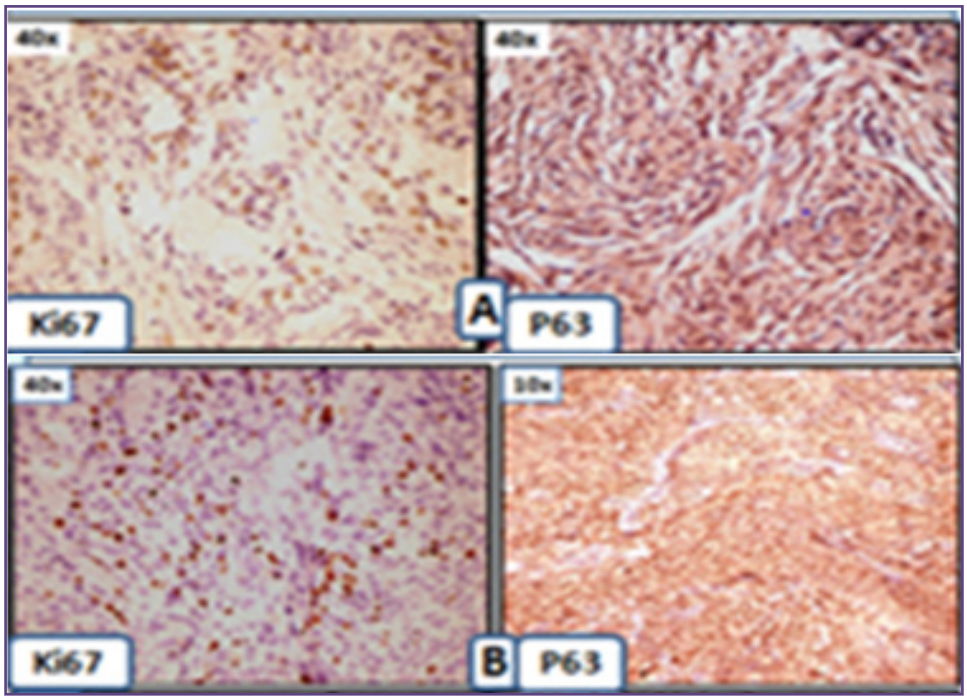


Fig. 1: Variants of Meningioma, (H & E Stain). A:Meningothelial,B: Fibroblastic, C:Transitional, D:Angiomatous, E: Psammomatous, F: Metaplastic, G&H :Atypical with brain parenchymal invasion, I:Papillary.



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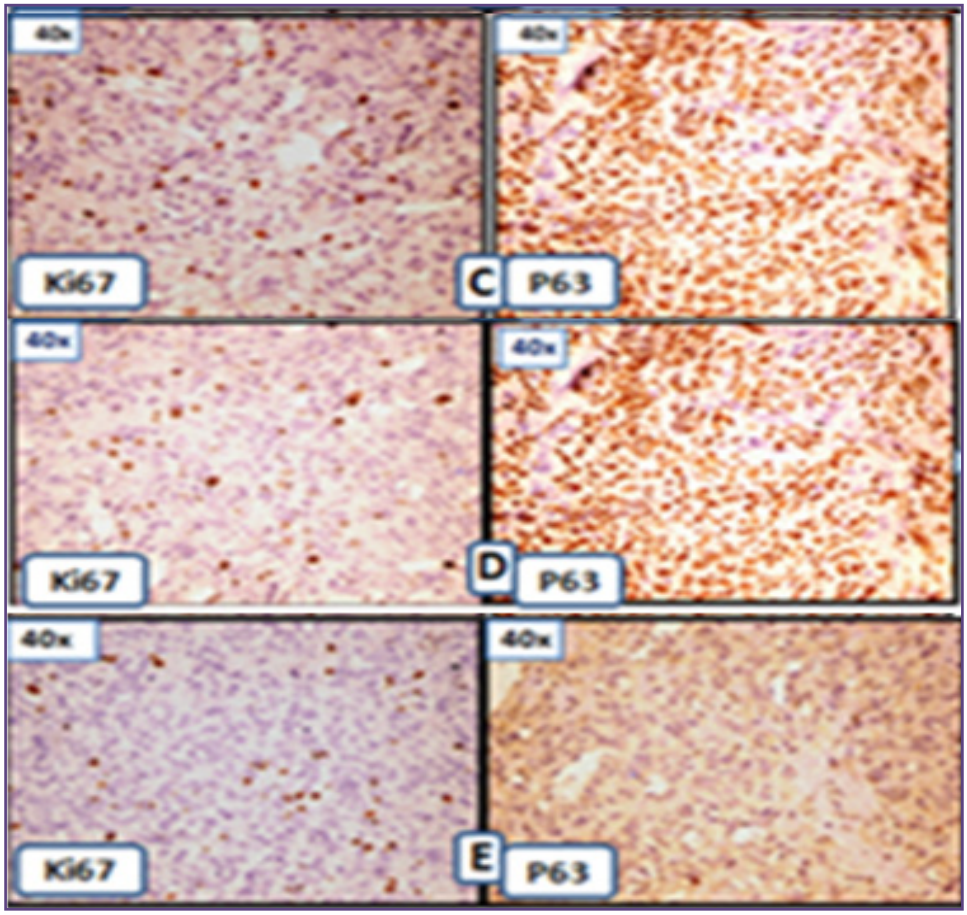

Fig. 2:A Grade I Meningothelial Meningioma low Ki67 LI(3%),P63<10%,B: Grade I High Ki67 8% LI and P63(3+) Positivity,C: Grade 2 Atypical Meningioma,Raised Ki67LI(8%), P63(3+),D:Grade 2 Atypical Meningioma Low Ki 67 LI (1%) with high P63(3+),E: Papillary Meningioma,High Ki67 LI(9%) and P63(3+).

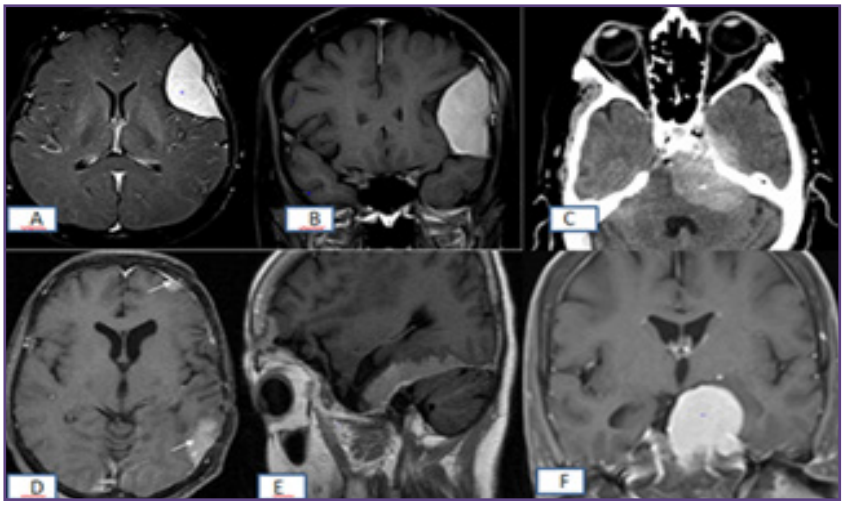

Fig. 3: Radiologic images depicting various sites of Meningiomas. A: T2W MRI Saggital section showing fronto-temporal lesion with dural enhancement. B:T1W MRI Coronal section showing parieto-temporal lesion with dural enhancement, C: CT image showing basally located dural enhancement. D:T1W MRI Sagittal section showing parieto –occipital dural lesion. E:T1W MRI Coronal section showing tentorial enhancement. F: T1W MRI Coronal section showing falx cerebri dural enhancement.

Ki-67 and p63 were highly significant as 0.0043 and 0.0009 respectively. Remaining 16 meningioma cases of WHO grade I category with high Ki-67 LI and correspondingly high P63 immunoexpression are under follow-up for the past two years and have showed no recurrence till the time of this report.

Conclusion

The present study has made an effort to grade meningiomas by using conventional as well as adjunct immunohistochemical markers P63 and Ki67.The expression of P63 is variable in different grades of meningiomas.p63 alone cannot make a distinction between low and higher grades of meningiomas. High grade meningiomas showed increased Ki67 labeling index and high P63 immunoexpression is a predictor of recurrence.There was statistically significant increase in P63 protein immunoexpression and Ki67LI between the grades hence further molecular characterization and gene profiling studies may define the biological behaviour of meningiomas.

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Competing Interests

None declared

References

1. Ostrom QT, Gittleman H, Farah P, Ondracek A, Chen Y, Wolinsky Y, et al. CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States 2006-2010. Neuro-Oncol. 2013; 15(2):ii1-ii56.
2. Kasuya H, Kubo O, Tanaka M, Amano K, Kato K, Hori T. Clinical and radiological features related to the growth potential of meningioma. Neurosurg Rev 2006; 29:293-7.
3. Lloyd RV, Kovacs K, Young Jr WF, Farrel WE, Asa SL, Trouillas J, Kontogeorgos G, Sano T, Scheithauer B, Horvath E. Tumours of the pituitary gland. In: DeLellis RA, Lloyd RV, Heitz PU, Eng C, editors. World Hearth Organization Classification of Tumours. Pathology and genetics of tumours of endocrine organs. Lyon: IARC Press; 2004. 9-47.
4. Karamitopoulou E, Perentes E, Melachrinou M, et al Proliferating cell nuclear antigen immunoreactivity in human central nervous system neoplasms. Acta Neuropathol 85:316-322, 1993
5. Nakasu S, Nakasu Y, Nakajima M, Matsuda M, Handa J. Preoperative identification of meningiomas that are highly likely to recur. J Neurosurg 1999; 90:455-62
6. Hahn HP, Bundock EA, Hornick JL Immunohistochemical staining for claudin-1 can help distinguish meningiomas from histologic mimics. Am J Clin Pathol. 2006 Feb; 125(2):203-8
7. Hopf NJ, Bremm J, Bohl J, Perneczky A. Image analysis of proliferating cells in tumors of the human nervous system: an immunohistological study with monoclonal Antibody Ki67. Neurosurg 1994; 35:917-23
8. LoMuzio L, Campisi G, Farina A. Effect of p63 expression on survival in oral squamous cell carcinoma. Cancer Invest. 2007; 25:466-469.
9. Sharifi N, Katebi M. An immunohistochemical study of p63 protein expression in meningioma. Iranian J Pathol 2008; 3: 146-150.
10. Devaprasath A, Chack G. Diagnostic validity of the Ki-67 labeling index using the MIB-1 monoclonal antibody in the grading of meningiomasNeurology India2003;51:336-340
11. Rushing EJ. Correlation of P63 immunoreactivity with tumor grade in meningiomas.Inter J Surg Pathol 2008; 16(1):38-42
12. Sharifi N, Katebi M. An immunohistochemical study of p63 protein expression in meningioma. Iranian J Pathol 2008; 3:146-150.
13. Jain D. Correlation of P63 protein expression with histological grade of meningiomas: An immunohistochemical study. Inter J Surg Pathol 2012; 20(4):349-354.
14. Guarnaschelli JJ, Stawicki SP. Brief communication: Recurrent brain meningiomas. OPUS 12 Scientist 2008; 2:32-4.