Original Article

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Pathology Section

Differential Expression and Utilisation of CD34, EMA, PR, and Inhibin in Differentiating Meningioma, Haemangioblastoma, and Solitary Fibrous Tumour: A Cross-sectional Study

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ABSTRACT

Introduction: Mesenchymal tumours of the Central Nervous System (CNS) include meningioma, haemangioblastoma, and Solitary Fibrous Tumour (SFT). Although conventional histopathological examination remains the mainstay in diagnosis, Immunohistochemistry (IHC) plays an important role in CNS tumours, owing to its diagnostic significance and prognostic implications.

Aim: To evaluate whether combined use of inhibin, Epithelial Membrane Antigen (EMA,) Progesterone Receptor (PR) and CD34 improves diagnostic accuracy in meningeal and non meningeal mesenchymal tumours of CNS.

Materials and Methods: This was a cross-sectional retrospective observational study conducted in the Department of Pathology, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India, October 2022 to December 2022 in which all brain tumour specimens diagnosed as meningeal or non meningeal mesenchymal tumours from January 2022 to August 2022 were included in the present study. Sections were immunostained with an IHC panel of EMA, PR, inhibin, CD34, and Ki67. The immunohistochemical expression of EMA, PR, inhibin, and CD34 was expressed as frequencies and percentages, along with sensitivity and diagnostic accuracy evaluation. Ki67 was assessed in the hotspots and expressed as a percentage. The data were presented as frequencies and percentages.

Results: A total of 60 patients were included, with an age range from 12 to 73 years and a mean age of 48.2 years. Female predominance, constituted 40 (67.5%) of the study population. Out of the total cases, 49 (81.7%), 9 (15%), and 2 (3.3%) cases were of meningioma, haemangioblastoma, and SFTs, respectively. Among the 49 cases of meningioma, the majority 47 (96%) showed diffuse and strong staining for EMA with a cytoplasmic staining pattern±membranous accentuation. PR was expressed in 45 (92%) of the meningiomas. Inhibin was diffusely expressed in stromal cells of haemangioblastoma 9 (100%). A characteristic cytoplasmic positivity of CD34 was observed in both cases 2 (100%) of SFT. The Ki67 index of anaplastic and atypical meningiomas was 15%, while all other tumours had a very low proliferation index of 2%.

Conclusion: The IHC panel comprising CD34, EMA, and inhibin was useful in the differential diagnosis of both meningeal and non meningeal mesenchymal tumours in the CNS, even in cases with overlapping histomorphological features. Inhibin had a very high sensitivity and diagnostic accuracy for the diagnosis of haemangioblastomas. EMA and PR also had fairly good sensitivity and diagnostic accuracy for the diagnosis of meningiomas. CD34 had good sensitivity but very poor diagnostic accuracy for the diagnosis of SFT.

Keywords: Cluster of differentiation, Epithelial membrane antigen, Immunohistochemistry, Meningioma, Progesterone receptor

INTRODUCTION

Mesenchymal tumours of the CNS include meningioma, haemangioblastoma, and SFT. These tumours originate from mesodermally-derived precursor cells and are commonly seen arising from the dura or meninges [1]. Distinguishing these tumours from one another poses a diagnostic challenge when they show similar or overlapping morphology. Although conventional histopathological examination remains the mainstay in diagnosis, IHC plays an important role in CNS tumours, owing to its diagnostic importance, prognostic implications, and therapeutic response. In addition, these antibodies help to clarify the nature of cellular maturation, tissue differentiation, and tumour progression. IHC can also help in distinguishing tumours which have overlapping morphology, thus minimising diagnostic dilemma [2].

Meningiomas are commonly benign, mesenchymal tumours representing 13%-30% of all primary intracranial tumours [3]. They are derived from the meningothelial cells of the arachnoid mater. Meningiomas exhibit a wide range of histological appearances and have been classified into World Health Organisation (WHO) subtypes. The common differential diagnosis of meningiomas is that of SFT, particularly with the fibrous variant. The other is haemangioblastoma, wherein their morphological features may overlap with the microcystic or clear cell variant of meningioma. In such challenging cases, IHC helps establish a definitive diagnosis. The most commonly used IHC panel for meningiomas includes EMA, PR, CD34, Vimentin, Glial fibrillary acidic protein, and Ki67 [4].

Haemangioblastoma is a highly vascular, slow-growing, lipid-rich tumour constituting about 2% of all intracranial tumours. Commonly, it involves the cerebellum and spinal cord, occurring sporadically or in association with von Hippel-Lindau syndrome [5]. Being a vascular neoplasm, differentiating haemangioblastoma from vascular meningioma or SFT is challenging. It has been reported that

the recurrence rate of sporadic haemangioblastoma is between 6.25%-20% following subtotal resection of lesions [5]. Therefore, a sensitive marker is needed that would help establish a definitive diagnosis to initiate appropriate treatment in time. Inhibin is a 32 kDa dimeric glycoprotein (with alpha and beta subunits) that belongs to the transforming growth factor beta-family, which is normally produced by ovarian granulosa cells and testicular Sertoli cells. Inhibin has recently been shown to be a sensitive marker for haemangioblastoma [6].

The primary aim of the present study was to evaluate whether the combined use of inhibin, EMA, PR, and CD34 improves diagnostic accuracy in meningeal and non meningeal mesenchymal tumours in the CNS. The objectives of the present study were to evaluate the sensitivity of EMA, inhibin, PR, and CD34 in the above tumours and establish the role of inhibin as a specific IHC marker for haemangioblastomas.

MATERIALS AND METHODS

This was a cross-sectional retrospective observational study conducted in the Department of Pathology, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India, from October 2022 to December 2022.

Inclusion criteria: All brain tumour specimens (complete or partial resections) which were on microscopy diagnosed as meningeal or non meningeal mesenchymal tumours in the period from January 2022 to August 2022 were included in the study.

Exclusion criteria: Cases that received any neoadjuvant therapy were excluded from the study.

Study Procedure

The diagnostic categories were meningioma, haemangioblastoma, and SFT based on the 5th edition of the WHO classification of brain tumours [7]. Samples were fixed with 10% buffered formalin, routinely processed, and embedded. Serial sections 4 µm thick were cut and stained with Haematoxylin and Eosin (H&E) for routine histologic examination. Histologic diagnosis was made according to the World Health Organisation (WHO) classification (2021) [7].

For IHC, 4 µm thick sections from the paraffin blocks were cut on precoated poly-l-lysine slides. Sections were immunostained using a polymer-based detection method and augmented by heat-induced antigen retrieval (using a pressure cooker). An IHC panel of EMA, PR, inhibin, CD34, and Ki67 was performed on all cases. Primary antibodies for differentiation in this study included EMA (Monoclonal Mouse antibody, Mc-5 Clone, Biocare Medical), inhibin (Monoclonal Mouse Antibody, R1 clone, Biocare Medical), CD34 (Monoclonal Mouse Antibody, Qbend/1 clone, Biocare Medical), and PR (Rabbit monoclonal, SP2 clone, Biocare Medical). The immunoreactivity was interpreted based on the staining pattern (cytoplasmic, membranous, or nuclear). Ki-67 (Mouse monoclonal, E3 Ubiquitin Protein Ligase 1 (MIB-1), Biocare Medical) positivity was quantified in the hotspots based on nuclear staining and expressed as a percentage.

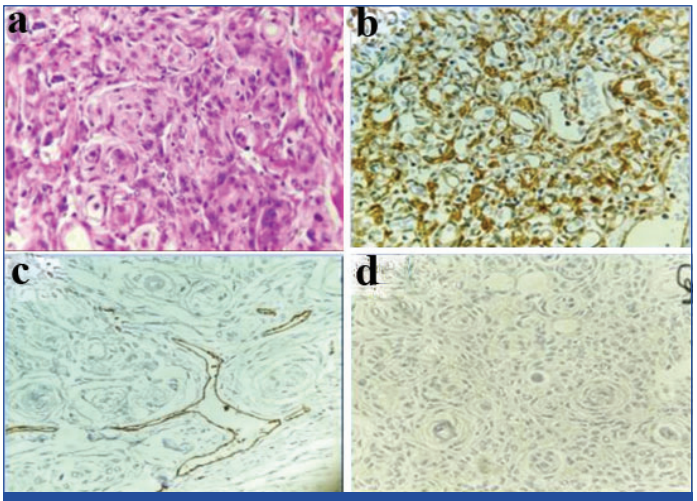
All tumours in which the tumour cells completely lacked immunostaining or showed faint staining in a minority (<5%) of tumour cells were scored as negative. IHC findings were rated as positive when at least 5% of the tumour cells were unequivocally stained in the cytoplasm (inhibin), nucleus (PR), or cell membrane (EMA, CD34) [2].

STATISTICAL ANALYSIS

The data were expressed as frequencies and percentages, along with an assessment of the sensitivity and diagnostic accuracy of the immunohistochemical markers.

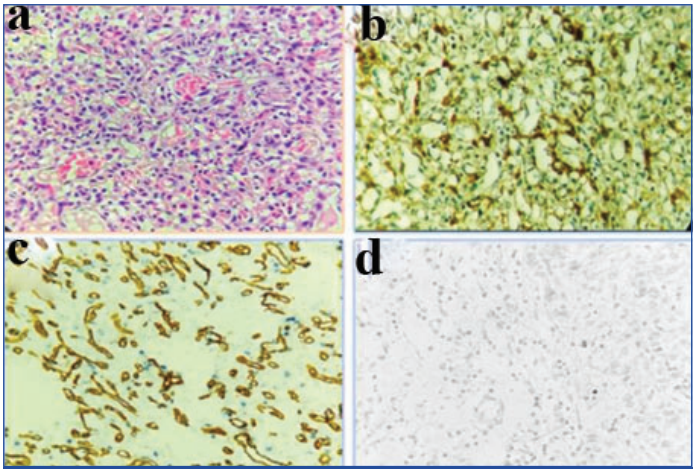
RESULTS

A total of 60 patients were included in the present study, of which 49 (81.7%) cases were meningioma and its subtypes, 9 (15%) were haemangioblastoma, and 2 (3.3%) were SFT. The age of the patients varied from 12 to 73 years, with a mean age of 48.2 years. Female predominance, constituted 67.5% of the study population. Histological findings revealed that the most common subtype of meningioma was transitional (n=16, 32.6%), followed by meningothelial (n=11, 22.4%), fibroblastic (n=9, 18.3%), angiomatous (n=5, 10.2%), psammomatous (n=3, 6.1%), atypical (n=3, 6.1%), papillary (n=1, 2.1%), and anaplastic (n=1, 2.1%). Histological features included syncytial arrangement and whorls of meningothelial cells with abundant eosinophilic cytoplasm, oval nuclei with delicate chromatin. Intranuclear pseudo inclusions, psammoma bodies, prominent vascular networks, and increased mitotic activity were observed depending on the subtype [Table/Fig-1a-d].



[Table/Fig-1]: Meningioma. A case of meningothelial meningioma exhibiting prominent whorled architecture: (a) with positive expression of EMA; (b) and negative expression of CD34; (c) and inhibin; (d) on Immunohistochemistry (IHC, X400).

All nine cases of haemangioblastoma demonstrated a rich vascular network of numerous thin-walled vessels with neoplastic stromal cells interspersed in between. The stromal cells demonstrated minimal pleomorphism with clear, foamy, lipid-laden cytoplasm [Table/Fig-2a-d].

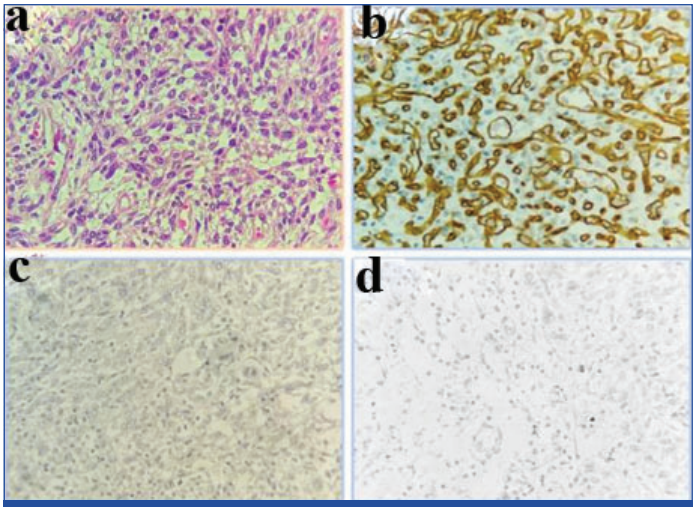


[Table/Fig-2]: Haemangioblastoma. A case of hemangioblastoma exhibiting stromal cells with clear foamy cytoplasm in vascular networks: (a) with positive immunoexpression of inhibin; (b) and negative expression of CD34; (c) and EMA; (d) in the stromal cells (IHC. 400x).

Both cases of SFT exhibited a "patternless pattern" of short fascicles with alternating hyper and hypocellular areas, thick collagen bands, and dilated branching vasculature [Table/Fig-3a-d].

Immunohistochemical expression: [Table/Fig-4] summarises the results of immunohistochemical expression.

- The majority (47 cases, 96%) of meningioma showed diffuse and strong staining for EMA with a cytoplasmic staining



[Table/Fig-3]: Solitary Fibrous Tumour (SFT). A case of SFT exhibiting spindle cells in patternless architecture: (a) with positive immunoexpression of CD34; (b) and negative expression of EMA; (c) and inhibin (d) (IHC, 400x).

pattern±membranous accentuation. However, two cases (4%) of atypical meningioma were immunonegative for EMA. Haemangioblastoma and SFT did not show any expression for EMA.

- Inhibin was diffusely expressed in stromal cells of haemangioblastoma (9 cases, 100%). None of the cases of meningioma and SFT showed inhibin expression.
- A characteristic cytoplasmic positivity of CD34 was observed in all cases (2 cases, 100%) of SFT. Focal cytoplasmic positivity was also seen in fibroblastic and papillary subtypes of meningioma (10 cases, 20%). The rest of the meningioma subtypes and haemangioblastoma were negative for CD34.
- The majority of meningiomas (45 cases, 92%) showed strong nuclear staining for PR. However, three cases (6%) of fibroblastic and one case (2%) of papillary subtype were immunonegative for PR.
- The Ki67 index of anaplastic and atypical meningiomas was 15%, while all other tumours had a very low proliferation index of 2%.

IHC markers	Meningioma; n=49	Haemangioblastoma; n=9	Solitary Fibrous Tumour (SFT); n=2
EMA	Positive except in 2 cases of atypical meningioma	Negative	Negative
Inhibin	Negative	Positive	Negative
CD34	Very focal weak positive in fibroblastic and papillary subtypes	Negative	Positive
PR	Positive except in 4 cases (3 fibroblastic and 1 papillary subtype)	Negative	Negative
Ki67	Proliferation index of 2% except in atypical and anaplastic meningioma (15%)	Proliferation index of 1%	Proliferation index of 1%

[Table/Fig-4]: Immunohistochemical expression of the different tumours.

The sensitivity of EMA and PR for the diagnosis of meningiomas was 95.9% and 91.8%, respectively. The diagnostic accuracy of EMA and PR for the diagnosis of meningiomas was 95.9% and 91.8%, respectively. However, inhibin had 100% sensitivity and diagnostic accuracy for the diagnosis of haemangioblastomas. CD34 had 100% sensitivity but a very low diagnostic accuracy (16.7%) for the diagnosis of SFT [Table/Fig-5-7].

DISCUSSION

Meningiomas, haemangioblastomas, and SFTs are important mesenchymal neoplasms in the CNS. Although these tumours

	Meningioma	Mesenchymal tumours other than meningioma (11)	Sensitivity {True positive/(true positive+false negative) x100}	Diagnostic accuracy {(true positive+true negative)/(true positive+true negative+false positive+false negative)}
EMA+	47	0	95.9%	95.9%
EMA-	2	0		
PR+	45	0	91.8%	91.8%
PR-	4	0		

[Table/Fig-5]: Sensitivity analysis of Epithelial Membrane Antigen (EMA) and Progesterone Receptor (PR) in diagnosis of meningioma.

	Haemangio-blastoma	Mesenchymal tumours other than haemangio-blastoma (51)	Sensitivity {true positive/(true positive+false negative) x100}	Diagnostic accuracy {(true positive+true negative)/(true positive+true negative+false positive+false negative)}
Inhibin+	9	0	100%	100%
Inhibin-	0	0		

[Table/Fig-6]: Sensitivity analysis of Inhibin in diagnosis of haemangioblastoma.

	Solitary Fibrous Tumour (SFT)	Mesenchymal tumours other than Solitary Fibrous Tumour (SFT) (58)	Sensitivity {true positive/(true positive+false negative) x100}	Diagnostic accuracy {(true positive+true negative)/(true positive+true negative+false positive+false negative)}
CD34+	2	10	100%	16.7%
CD34-	0	0		

[Table/Fig-7]: Sensitivity analysis of CD34 in diagnosis of Solitary Fibrous Tumour (SFT).

have defined morphological features, they may cause diagnostic dilemmas due to overlapping morphology or when small biopsies are received for histopathological examination. Meningiomas may show morphological overlap with haemangioblastoma, especially when there is prominent microcystic change.

Meningiomas represent approximately one third of all primary intracranial tumours. In the present study, meningiomas were found to be the most common tumours, with varied histological appearances. Among the different WHO meningioma subtypes, the most commonly encountered were the meningothelial and fibroblastic subtypes, followed by the transitional subtype, which is similar to the results of Telugu RB et al., [8]. They also found meningothelial (26.3%) meningioma to be the most common subtype, followed by psammomatous (25.4%) and transitional (19.2%) types. In the present study, 47 (96%) cases of meningioma were positive for EMA, which was in concordance with the study done by Boulagnon Rombi C et al., where EMA was seen to be expressed by 90% of all meningiomas [2].

The authors observed a similar percentage positivity for PR, which was positive in 45 (92%) cases. PR-negative meningiomas may or may not be CD34 positive. However, a consistent expression of EMA was seen in the present study. Hence, the utility of EMA expression in identifying PR-negative meningiomas is highlighted. Although many studies report a consistent expression of CD34, the percentage positivity in the present study was only 6 (12%), which was restricted to cases of fibroblastic and papillary meningiomas. None of the meningiomas expressed inhibin, which was in concordance with the study done by Boulagnon Rombi C et al., [2]. In the present study, the combined use of PR, CD34, and EMA improved the diagnostic accuracy in meningiomas.

All cases of haemangioblastoma in the present study expressed diffuse cytoplasmic staining of inhibin exclusively in the stromal cells (100%). Similar results were noted by Jung SM and Kuo TT, and Sancheti S et al., [6,9]. Jung SM and Kuo TT, in their study, observed 91% (20/22) of cases of haemangioblastoma to be positive for inhibin [6]. Although the other studies have used inhibin alpha (inhibin A), the present study observed similar results with inhibin. CD34 was positive in the vessels; however, it was negative in stromal cells. None of the cases expressed EMA. This helped differentiate it from meningioma, where EMA was consistently positive. These findings demonstrate inhibin as a useful and differentiating marker for haemangioblastoma.

The SFTs are rare mesenchymal neoplasms that account for less than 1% of all CNS tumours. CD34 remains an important and consistently positive immunohistochemical marker that is useful for the first-line diagnosis of SFT. In the present study, CD34 was expressed diffusely in both SFT cases, which is in concordance with the existing literature. They observed CD34 to be expressed in 73% (16/26) of SFT cases and in 8% (10/126) of meningiomas [2]. Another differential diagnosis is fibroblastic meningioma, where EMA is consistently negative in SFT, unlike meningioma (fibroblastic subtype), which consistently shows reactivity for EMA, as noted by Jaiswal S [10]. One of the cases showed focal expression of EMA; however, it was negative for inhibin.

Limitation(s)

The small sample size is a limitation of the study. However, larger studies with a bigger sample size are needed to evaluate the performance analysis of immunohistochemical biomarkers in the diagnosis of mesenchymal CNS tumours.

CONCLUSION(S)

Hence, it can be concluded that an IHC panel comprising CD34, EMA, and inhibin is useful in the differential diagnosis of both meningeal and non meningeal mesenchymal tumours in the CNS, even in cases where there are overlapping histomorphological features. Inhibin has a very high sensitivity and diagnostic accuracy for the diagnosis of haemangioblastomas. EMA and PR also have fairly good sensitivity and diagnostic accuracy for the diagnosis of meningiomas. CD34 has good sensitivity but very poor diagnostic accuracy for the diagnosis of SFT. Thus, inhibin has been shown to be a valuable and specific diagnostic marker for haemangioblastoma.

REFERENCES

[1] Bruner E, Welsh CA, Smith T. Can we reliably distinguish between supratentorial hemangioblastomas and the variant of meningioma that resembles them? American Journal of Clinical Pathology. 2012;138:A262.
[2] Boulagnon Rombi C, Fleury C, Fichel C, Lefour S, MarchalBressenot A, Gauchotte G. Immunohistochemical approach to the differential diagnosis of meningiomas and their mimics. J Neuropathol Exp Neurol. 2017;76:289-98.
[3] Hoang MP, Amirkhan RH. Inhibin alpha distinguishes hemangioblastoma from clear cell renal cell carcinoma. Am J Surg Pathol. 2003;27:1152-56.
[4] Madabhushi V, Venkata RI, Garikaparthi S, Kakarala SV, Duttaluru SS. Role of immunohistochemistry in diagnosis of brain tumours: A single institutional experience. Journal of Dr. NTR University of Health Sciences. 2015;4:103-11.
[5] Sun Hİ, Ösduman K, Usseli Mİ, Ösgen S, Pamir MN. Sporadic spinal hemangioblastomas can be effectively treated by microsurgery alone. World Neurosurg. 2014;82:836-47.
[6] Jung SM, Kuo TT. Immunoreactivity of CD10 and inhibin alpha in differentiating hemangioblastoma of central nervous system from metastatic clear cell renal cell carcinoma. Mod Pathol. 2005;18:788-94.
[7] Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D, et al. The 2021 WHO Classification of tumours of the central nervous system: A summary. Neuro Oncol. 2021;23:1231-51.
[8] Telugu RB, Chowhan AK, Rukmangadha N, Patnayak R, Phaneendra BV, Prasad BC, et al. Histopathological and immunohistochemical evaluation of meningiomas with reference to proliferative markers p53 and Ki-67. J Clin Diagn Res. 2016;10:EC15-19.
[9] Sancheti S, Menon S, Mukherjee S, Arun I. Clear cell renal cell carcinoma with hemangioblastoma-like features: A recently described pattern with unusual immunohistochemical profile. Indian J Pathol Microbiol. 2015;58:354-55.
[10] Jaiswal S. Role of immunohistochemistry in the diagnosis of central nervous system tumours. Neurol India. 2016;64:502-12.

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