OPEN ACCESS

Research Paper

The Role of Glial Fibrillary Acidic Protein and Epithelial Membrane Antigen in the Differentiation and Diagnosis of Glial Tumors

Summer Osam Ahmed[1], Khitam Razzaq Kadhim[2]

ABSTRACT:

BACKGROUND:

CNS tumors diagnosis represents a challenge in histopathological examination. EMA and GFAP are widely used markers in neurosurgical pathology that help in the diagnosis of many commonly occurring tumors and differentiating them from other mimickers.

OBJECTIVE:

Evaluation of the value of EMA and GFAP in the diagnosis of ependymal tumors compared to other glial tumors. Evaluate the pattern of expression of these markers. The value of glial fibrillary acidic protein positivity in the diagnosis of ependymoma and diffuse fibrillary astrocytoma.

MATERIALS AND METHOD:

The present study is a retrospective study over a period of one year, the paraffin blocks were collected from the archive of the department of pathology, Ghazi Al –Hariri specialized surgical hospital, Baghdad medical city, over the period of 4 years, from March 2015 to October 2019, they included 15 cases of ependymoma and 15 cases of diffuse fibrillary astrocytoma. Total number of cases was 30 cases. The markers used for this study were EMA, and GFAP. The manual method of staining was used in this study.

RESULTS:

Both markers showed positivity in studied cases, in EMA the presence of 5 EMA dots/ high-power field were associated with a 86% sensitivity and 80% specificity. The ring-like EMA positive structures show less sensitivity (33%), but it is highly specific (100%). GFAP was useful in highlighting the growth pattern of these tumors, and in ependymoma it was mostly expressed in cells and fibrillary processes around vessels (pseudorosettes) and in true rosettes. In asttrocytom it showed staining in most tumor cells.

CONCLUSION:

The use of EMA and GFAP together in the diagnosis of CNS tumor, has a good role in the distinction of these tumors and in reaching more specific diagnosis. Although not expressed in all ependymomas, distinct punctate and ring like EMA staining considered a sensitive and specific markers of ependymal differentiation in glial tumors. As for GFAP marker, although it was expressed in both ependymoma and glial tumors, it can provide significant value in highlighting the growth pattern of these tumors, and in excluding other mimickers like metastatic tumors. Together, EMA and GFAP can aid in patient diagnosis and management.

KEYWORDS: GFAP, EMA, Ependymoma, Diffuse fibrillary astrocytoma, Brain tumors.

1Al-Amal National Hospital for Cancer management
2Collage of Medicin/ Baghdad University

Iraqi Postgraduate Medical Journal, 2024; Vol. 23(4): 472- 477
DOI: 10.52573/ipmj.2024.136783 *Received: September 3, 2023,* *Accepted: September 17, 2023*

INTRODUCTION:

Brain tumors often represents a diagnostic challenge, clinicl data, radiologicl findings and other ancillary studies can help provide differential diagnosis, but histopathological examination is the final step to reach more definit result. however, brain tumors histological diagnosis is not always straightforward due to similarties in morphological charechtarisics within different types of tumors and many subtypes within the same tumor. In adition, non-neoplastic (reactive and inflammatory) lesions can mimic tumors, like reactive gliosis. therfore, immunohistochemical stains and molecular studies has become vital for an definit diagnosis and subtyping. [(1)]

Recent data from Iraq's national cancer registry in the 9th of December 2019, showed that betwee 2017–2018 there are over 31,500 cancer and tumor-related cases in Iraq, and consier cancer is as one of the leading causes of mortality in the country (11% of total deaths). Never the less,

there was no accurate studies or statistics regarding CNS tumors or its types. [2]

Accurate diagnosis of central nervous system tumors can be quite challenging. The use of immunohistochemistry has a significant role in differential diagnosis and in enhancing diagnostic accuracy. utiization of an adequate panel of selected immunohistochemical stains is not helpful in only reachin final diagnosis, but IHC is also of great help in predicting the prognosis for certain brain tumors, which has an impact on patient management plan.[3]

In addition, the recent use of intraoperative rapid immunohistochemical analysis of CNS tumors in addition to conventional rapid intraoperative diagnosis, contributes to deciding appropriate treatment strategies and facilitating early initiation of chemotherapy if indicated.[4]

MATERIALS AND METHOD:

The present study is a retrospective study over a period of one year, the samples were collected from the period of March 2015 to October 2019 in Department of Pathology, Ghazi Al –Hariri specialized surgical hospital, medical city in Baghdad. in all the cases Relevant clinical data including imaging details and peroperative investigations were documented The specimens were comprised of total 30 cases of CNS and spinal cord tumors including Diffuse Fibrillary Astrocytoma and Ependymoma (convevtional, myxopapillary and anaplastic variants).

The paraffin blocks were collected from the archived cases, 3 sections of 5 µm thickness were taken from paraffin tissue blocks. Histologic examination of hematoxylin and eosin (H and E) stained sections (Fig. 3.1b) and immunohistochemical studies done to diagnose and classify various brain tumors according to the latest classification of central nervous system (CNS) tumors by World Health Organization (WHO).[5] The expression pattern of GFAP and EMA was studied in these cases.

The immunohistochemical stain evaluation for GFAP and EMA was done according to intensity of markers expression and the percentage of tissue expressing the markers.[6] As there was no negative result in the current study, the scoring system was determined as follow:

- Score 1: focal expression of light intensity staining.
- Score 2: partial tissue expression of moderate intensity.
- Score 3: diffuse tissue expression of stronge intensity.

RESULT:

In the total of 15 cases of ependymoma tumors in this study as represented in Table 1, 9/15 ependymomas (60%) located at spinal cord, 3/15 (20%) in the posterior fossa and 3/15 (20%) supratentorially.

The criteria of diagnosis for anaplastic ependymoma were met by 3 tumors (20%). Myxopapillary ependymomas (3 cases) accounted for 20% of spinal tumors. The age of the patients varied from 1.5 to 60 years. The gender of patient with ependymoma was found to be 6 female (40%) and 9 male (60%) as shown in (Table1)

Table 1: Localization, tumor grade and patient age of 15 ependymomas.

Localization	Grade I	Grade II	Grade III	Age in years
Supratentorial	0	2	1	14-60
Posterior fossa	0	1	2	1.5-18
Spinal	3	6	0	6-66
Total	3	9	3	1.5-66

All cases of ependymal tumors showed positive reaction for EMA immunostaining (100%). The antigen expression pattern was found in the cytoplasm and overlapping the nucleus and showed accentuation around the blood vessels in some areas of tumor (Fig.3.1a). The distinct dot-like intracytoplasmic EMA immunoreactivity expression pattern was notted in most of tumors 13/15 (86%) (Fig. 1b), the 2 remaining cases did not show this pattern of expression, it showed mainly cytoplasmic and membranous staining. Additionally, intracytoplasmic or luminal ring-like EMA staining pattern was observed in 5/15 tumors (30%) (Fig. 3.1c), the remaining positive cases showed mainly cytoplasmic and membranous staining without ring-like structure. Although there was slight increase in the score in children and young age group, there was no significant correlation in score with the gender or the type of tumor. (Table 2)

In diffuse fibrillary astrocytic tumors all found to be positive with EMA staining. The expression pattern of the stain was found to be cytoplasmic, membranous and overlying the nucleus (Fig 1 d). There was no ring like intracytoplasmic structure pattern of expression found in astrocytoma, and the dote like pattern of expression was found in 3 of the astrocytoma cases.

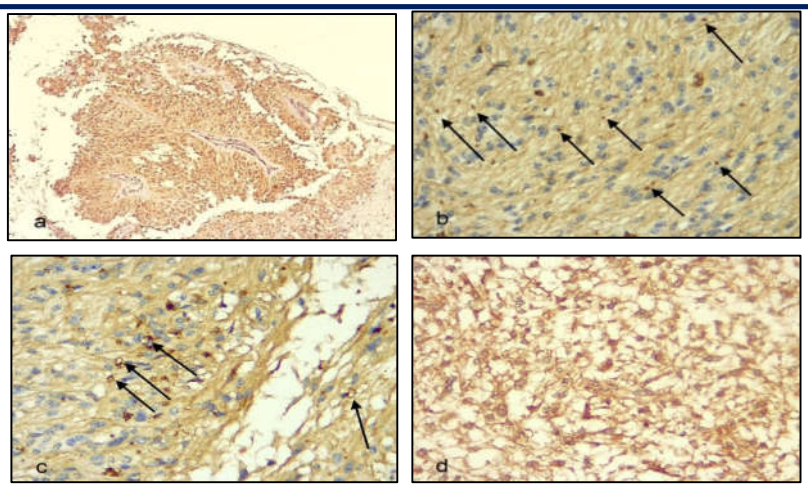

Figure 1: A. epithelial membrane antigen cytoplasmic and membranous expression in ependymomas, 10x power field. B. dote like pattern of EMA in ependymomas, 40x power field. C. ring like intracytoplasmic structure of EMA in ependymomas, 40x power field. D. diffuse fibrillary astrocytoma expression of epithelial membrane antigen, 10x power field.

In cases of ependymal tumors, Glial fibrillary acidic protein was seen in all the cases studied (100%). It was present in some ependymomal cells and absent from others. The protein was in the cytoplasm of the cell body and in the processes, but not in the nuclei. (Fig 2A).

In diffuse fibrillary astrocytoma, all cases showed positivity for GFAP (100%), the staining was positive in the intermediate filaments of astrocytes and negative in the nucleus (Fig 2B).

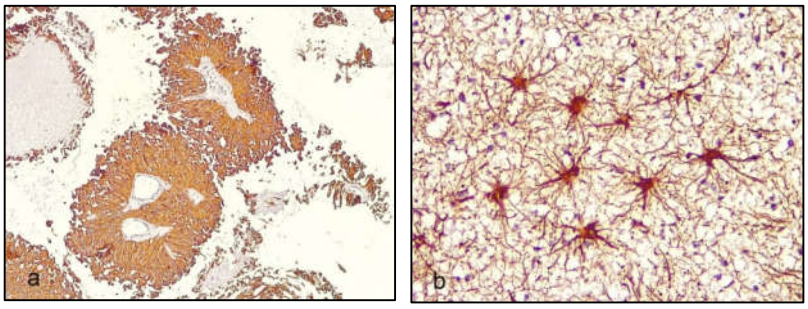

Figure 2: Expression of glial fibrillary acidic protein. A. in ependymoma, cytoplasmic and membranous pattern of staining, 10x power field. B. in diffuse fibrillary astrocytoma, cytoplasmic pattern of staining and negative in the nucleus, 40x power field.

Correlation of the certain characteristics like the age, the gender and type of tumor was studied in relation to the score of EMA and GFAP expression. Other than slight tendency for more intense EMA expression was found in age group less than 25 years (P value < 0.05) and a small increase in intensity of GFAP expression in males compared with females (P value < 0.05), there was no other significant correlation in these markers compared with characteristic features as shown in (Table 2) and (Table 3)

Table 2: Association between epithelial membrane antigen and certain characteristics.

variable	EMA Score 1	EMA Score 2	EMA Score 3	Total	P value
Age in years					
0-25	3	6	5	14	0.0001
>25	3	13	0	16	
Gender					
Male	5	10	3	18	**0.372**
Female	1	9	2	12	
Tumor type					
Ependymoma	3	9	3	15	**0.28**
Diffuse Fibrillary Astrocytoma	3	10	2	15	

Table 3: Association of glial fibrillary acidic protein and certain characteristics.

Variable	GFAP Score 1	GFAP Score 2	GFAP Score 3	Total	P value
Age in years					
0-25	3	6	5	14	0.223
>25	4	8	4	16	
Gender					
Male	5	7	6	18	0.011
Female	2	7	3	12	
Tumor type					
Ependymoma	5	6	4	15	0.099
Diffuse Fibrillary Astrocytoma	2	8	5	15	

DISCUSSION:

In a previous study done in 2003 by Hasselblatt M and Paulus W. [7], the extent and pattern of epithelial membrane antigen (EMA) immunostaining in ependymomas studied as compared to other glial tumors. The result helped to determine sensitivity as well as specificity of EMA staining, the cases used were 54 cases of ependymomas were in comparison to 54 cases of glioblastomas, 43 cases of fibrillary astrocytomas and 21 cases of oligodendrogliomas. The slides showed ring-like EMA staining in 17/54 ependymomas (31%) where as distinct punctate intracytoplasmic EMA immunoreactivity oserved in 48/54 ependymomas (89%). less frequency of Dot-like EMA immunoreactivity showed with Glioblastoma [32/54 (59%), $P<0.05$ vs ependymomas], oligodendrogliomas [2/21 (10%), $P<0.001$ vs ependymomas] and fibrillary astrocytomas [10/43 (23%), $P<0.001$ vs ependymomas] , and the lack of obvious ring-like EMA staining. Determination of Sensitivity and specificity of punctate EMA staining for ependymoma diagnosis compared to diffrent glial tumors was done: the finding of 5 EMA dots/ HPF (high-power field) was correlated with a sensitivity of 72% and a specificity of 81%. less sensitivity (32%), and higher specificity (100%) was determined withh ring-like EMA positive structures.

In the present study, all of 15 ependymomas (100%) showed EMA immunostaining, which is due to dot-like punctate intracytoplasmic staining presence. This result of EMA positivity percent is higher than previously reported by Cruz-Sanchez FF, Figarella-Branger D, Uematsu Y, et al. in the years from 1992 to 2000 [8-11]. the monoclonal antibody in all of these studies E29 has been used (as well as in this study). Therefore, dot-like EMA immunoreactivity extent might be underestimated even though in methodology differences cannot be ruled out. In contrast to previous findings in 1990 found by Kaneko Y, Takeshita I, Matsushima T, et al, of increased [12] or found in 1988,1989 and 2000 by Uematsu Y, Rojas-Corona RR, Llena JF, et al, of decreased [10, 11,13] EMA staining in anaplastic ependymoma, no correlayion between the presence or extent of EMA immunostaining and grade of malignancy was observed in the present study.

specific dot-like EMA immunoreactivity presence occasionaly in high-grade astrocytomas in addition to diffuse staining with granular accentuation observed by Cruz-Sanchez FF, Garcia-Bachs M, Rossi ML, et al [8,14] has been previously reported in a study by Hasselblatt M, Paulus W. [7] and it might reflect ependymal differentiation of tumor cells. however the diagnosis of ependymoma compared to

diffuse astrocytoma in this study concetrats on the sensitivity and specificity of dot-like EMA stainin, one must keep in mind that with varous types of other tumors, EMA staining has been described, e.g., chordoid glioma of the third ventricle, meningioma, rhabdoid tumor or chordoma, albeit as membranous or diffuse staining patterns, as was found in previous studies by Ho DM, Hsu CY, Wong TT, et al. [15-17]

The sensitivity of intracytoplasmic ring like structure expression of EMA in Ependymal tumors is (33%) in the current study, as it was positive in only 5 of 15 cases of ependymoma. while it was found to be very specific (100%) as it showed no such pattern in astrocytic tumors stained with EMA.

The sensitivity of finding cytoplasmic dot like structure pattern of EMA in ependymal tumors is found to be high (86%), as it was observed in 13/15 of the ependymoma cases, and it was assessed by finding 5 dots or more/HPF, the calculated specificity was (80%).

There was no correlation of the extent of EMA expression and the site of tumors, the grade or the gender of the patients (P value more than 0.05). As for the age of patients with ependymal tumors, the stain showed tendency for more intensity of staining in age group less than 25 years (mainly children and adolescent age group) in comparison with older adults (P value less than 0.05).

Although all the tumors of ependymoma and diffuse fibrillary astrocytoma showed positive staining for GFAP, the stain accentuated the pattern of growth of these tumors. In ependymoma, it was mostly expressed in cells and fibrillary processes around vessels (pseudorosettes) and in true rosettes, making them easier to detect in case it was missed in H&E alone. In astrocytoma cases it highlighted the fibrillary processes and the astrocytic cells in cytoplasmic and membranous pattern. Giving this high expression percent of GFAP in ependymal tumors, this adds a great value when combined with EMA positivity in differentiation of ependymoma from other CNS tumors that may have similar radiologic or morphologic features. Several studies done by Madabhushi V, Venkata RI, Garikaparthi S, et al, showed the value of GFAP in the diagnosis of astrocytic tumors and in exclusion of other tumors [18,19].

CONCLUSION:

1. EMA was positive in all cases in this study, showing mainly cytoplasmic and membranous pattern of expression.
2. The dote like pattern of EMA expression was 86% sensitive and 80% specific for ependymal tumors.
3. The ring like intracytoplasmic structure of EMA expression was 33% sensitive and 100% specific in ependymal tumors.
4. EMA provides a great value in the diagnosis of ependymoma especially in combination with GFAP that will exclude other non-glial tumors.
5. GFAP is expressed in both ependymoma and glial tumors, showing mainly cytoplasmic pattern of expression.

REFERENCES:

1. Munshi A, Jalali R. Therapy for glioma: Indian perspective. Indian J Cancer 2009;46:127-31.
2. World Health Organization Eastern Mediterranean Regional Office. Iraqi Tumor Registry Cancer Data for 2017-2018. Available from: http://www.emro.who.int/irq/iraq-news/cancer-data-for-20172018-announced-in-iraq.html. [Last accessed 2021 Mar 26].
3. Takei H, Bhattacharjee MB, Rivera A, Dancer Y, Powell SZ. New immunohistochemical markers in the evaluation of central nervous system tumors: A review of 7 selected adult and pediatric brain tumors. Arch Pathol Lab Med 2007;131:234-41.
4. Inoue, Akihiro, et al. "Usefulness of intraoperative rapid immunohistochemistry in the surgical treatment of brain tumors." *Neuropathology* 43.3 ,2023:209-20.
5. David NL, Arie P, Guido R, Andreas VD, Dominique FB, Webster KC. The 2016 World Health Organization Classification of Tumors of the Central Nervous System. Acta Neuropathol. 2016; 131: 803-20.
6. Cizkova, Katerina, et al. "Comparative analysis of immunohistochemical staining intensity determined by light microscopy, ImageJ and QuPath in placental Hofbauer cells." *Acta histochemica et cytochemica* 54.1 .2021:21-29.
7. Hasselblatt M, Paulus W. Sensitivity and specificity of epithelial membrane antigen staining patterns in ependymomas. Acta neuropathologica. 2003;106:385-88.
8. Cruz-Sanchez FF, Garcia-Bachs M, Rossi ML, Rodriguez-Prados S, Ferrer I, Coakham HB, Ferreres JC, Figols J, Palacin A Epithelial differentiation in gliomas, meningiomas and choroid plexus papillomas. Virchows Arch [B] .1992; 62:25–34.

9. Figarella-Branger D, Gambarelli D, Dollo C, Devictor B, Perez-Castillo AM, Genitori L, Lena G, Choux M, Pellissier JF Infratentorial ependymomas of childhood. Correlation between histological features, immunohistological phenotype, silver nucleolar organizer region staining values and post-operative survival in 16 cases. Acta Neuropathol .1991;82:208-16.
10. Uematsu Y, Rojas-Corona RR, Llena JF, Hirano A Distribution of epithelial membrane antigen in normal and neoplastic human ependyma. Acta Neuropathol .1989;78:325-28.
11. Vege KD, Giannini C, Scheithauer BW The immunophenotype of ependymomas. Appl Immunohistochem Mol Morphol .2000; 8:25-31.
12. Kaneko Y, Takeshita I, Matsushima T, Iwaki T, Tashima T, Fukui M Immunohistochemical study of ependymal neoplasms: histological subtypes and glial and epithelial characteristics. Virchows Arch [A] .1990; 417:97-103.
13. Cruz-Sanchez FF, Rossi ML, Esiri MM, Reading M Epithelial membrane antigen expression in ependymomas. Neuropathol Appl Neurobiol .1988;14:197-205.
14. Hitchcock E, Morris CS Cross reactivity of anti-epithelial membrane antigen monoclonal for reactive and neoplastic glial cells. J Neurooncol .1987;4:34-52.
15. Ho DM, Hsu CY, Wong TT, Ting LT, Chiang H Atypical teratoid/rhabdoid tumor of the central nervous system: a comparative study with primitive neuroectodermal tumor/medulloblastoma. Acta Neuropathol .2000;99:482-88.
16. Miettinen M Chordoma. Antibodies to epithelial membrane antigen and carcinoembryonic antigen in differential diagnosis. Arch Pathol Lab Med 1984;108:891-92.
17. Reifenberger G, Weber T, Weber RG, Wolter M, Brandis A, Kuchelmeister K, Pilz P, Reusche E, Lichter P, Wiestler OD Chordoid glioma of the third ventricle: immunohistochemical and molecular genetic characterization of a novel tumor entity. Brain Pathol 1999;9:617-26.
18. Madabhushi V, Venkata RI, Garikaparthi S, Kakarala SV, Duttaluru SS. Role of immunohistochemistry in diagnosis of brain tumors: A single institutional experience. J NTR Univ Health Sci. 2015;4:103-11.
19. Brehar FM, Arsene D, Brinduse LA, Gorgan MR. Immunohistochemical analysis of GFAP-δ and nestin in cerebral astrocytomas. Brain tumor pathology. 2015;32:90-98.