GFAP Expression in Brain Tumors: An Immunohistochemical Study

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ABSTRACT

Background and Objectives: To identify the Immunohistochemical expression of glial fibrillary acidic protein (GFAP) in different types of brain tumors, and to correlate the results with patient's age and sex, and with tumor type, site and grade.

Patients and Methods: During the two year period, from July 2007 to June 2009, 52 cases of neuroepithelial tumors were collected from Rizgari Teaching Hospital, and private labs in Hawler city. Typing and grading of tumors were done according to the World Health Organization (WHO) classification system. Immunohistochemical staining was done for GFAP using polyclonal antibodies and chromogen visualizing system. A semi quantitative histochemical score was applied to the GFAP staining.

Results: Of the 52 collected cases, 36 were astrocytoma, 6 medulloblastoma, 5 oligodendroglioma, 3 ependymoma, 1 oligoastrocytoma, and 1 ganglioglioma. GFAP was expressed in 86.5% of neuroepithelial tumors. Higher positivity was found in gliomas than in other neuroepithelial tumors ($p < 0.001$). A significant correlation between GFAP expression with the patient's age and site of the tumor was found. It was inversely correlated with the grade of glioma.

Conclusions: GFAP expression is helpful in identification of glial from non-glial neuroepithelial tumors. It is of a great use to highlight the tumor grade particularly if the scoring system is applied.

Key words: Brain tumors, GFAP, Immunohistochemistry .

INTRODUCTION:

Tumors of central nervous system (CNS) rank the 7th malignancy in Iraq and they are the most frequent solid tumors among children[1,2] According to WHO classification, CNS tumors comprise more than 50 clinicopathological entities; they vary in their biologic behavior and response to therapy[3, 4]. GFAP is an intracytoplasmic intermediate filament protein, it has a molecular weight of approximately of 50 KD, and it's specific for cytoskeleton of glial cells such as astrocytes[5]. It was first described by Amico Bignami in the white matter plaques from patients with a longstanding multiple sclerosis[6]. Highly specific polyclonal antibodies to human GFAP are available since 1975 and their applications in immunohistochemical .(IHC) studies are extremely helpful in determining GFAP is strongly expressed in astrocytes and other glial cells mainly in the cytoplasm than cellular processes while the reverse is true for astrocytoma in which the expression is more intense over the cellular processes than the cytoplasm [8].**Aims of the study:**

1.To identify the Immunohistochemical expression of GFAP in different types of neuroglial tumors.

2.To correlate the results with patients age, sex, and with type, site and grade of the tumors.

PATIENTS AND METHODS:

A total of 52 cases of neuroglial brain tumors were collected from Rizgari Teaching Hospital and private labs in Hawler city during a two year period, extending from July 2007 to June 2009. Data including patient's age and sex and

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was taken from the histopathological reports. For each case, Hematoxylin and eosin (H&E) stain was done using 4 µm thick sections. Typing and grading of the tumors were done according to WHO classification system[4]. The IHC technique used was carried out by the labelled streptavidin biotin method on paraffin sections using polyclonal rabbit GFAP antibody and kits manufactured by DAKO Corporation (Dako Denmark A/S), 3-3'-diaminobenzidine tetrahydrochloride (DAB) was used as chromogen, and the antigen retrieval was done by microwave heat. Three µm sections 'were mounted on silanized slides and allowed to dry overnight at 56°C, deparaffinized with xylene and blocked for endogenous peroxidase with 3% H_2O_2. The buffer used was Tris-buffered saline (TBS, 0.05 M) and the counterstain was Harris hematoxyllin. Appropriate positive and negative controls were run in every case[9, 10, 11]. Scoring of GFAP expression was done semiquantitavely for both cell number and staining intensity. The number of positive cells was given as: 0 for none, 1 for< 5%, 2 for 5-25%, 3 for 25-75%, and 4 for 75-100% of positive cells. While of 0–3+. A total score of 0-2 was regarded as negative, while ≥ 3 regarded as positive[12]. Statistical analysis was done by using statistical package for social sciences (SPSS) version 15 using the chi-square (X^2) test. P <0.05 was considered as the level of significance.

RESULT:

Majority of tumors were supratentorial in location 32 (61.5%). The remainders were infratentorials 20 (38.5%). Histopathologically, astrocytoma was the most frequent CNS tumor 36 (69.2%), followed by medulloblastoma 6 (11.6%), oligodendroglioma 5 (9.6%), and ependymoma 3 (5.8%). There was one case of oligoastrocytoma and another ganglioglioma. Most cases of glioma fell within grade 2 (Table 1). Immunohistochemically, GFAP was expressed in 35 (97.2%) cases of astrocytoma (Figure 1) and all cases of ependymoma (Figure 2), oligodendroglioma (Figure 3), oligoastrocytoma and ganglioglioma. No expression was identified in medulloblastoma (Table 2). There was a significant relationship between GFAP

Table1: Distribution of gliomas by grade

Grade	Astrocytoma	Oligodendro-glioma	Ependymoma	Oligoastrocytoma
1	2	0	0	0
2	15	3	3	0
3	6	2	0	1
4	13	0	0	0
Total	36	5	3	1

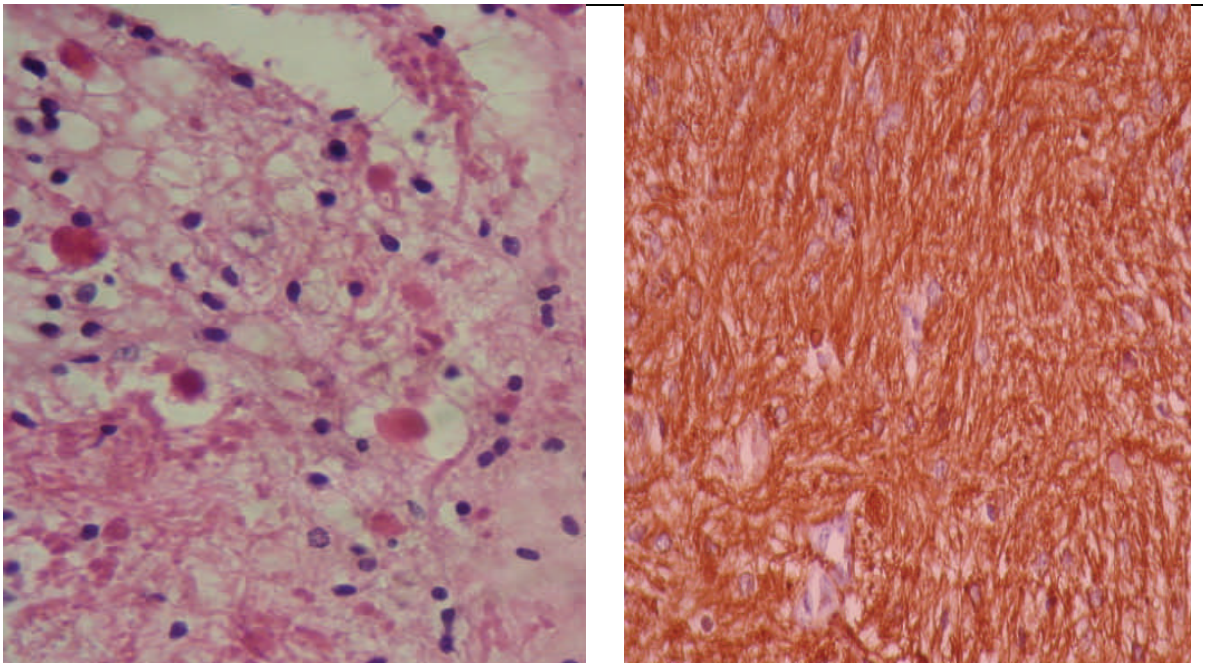

Figure 1: Grade I (pilocytic) astrocytoma H&E (A) and GFAP expression (B) (X 400)

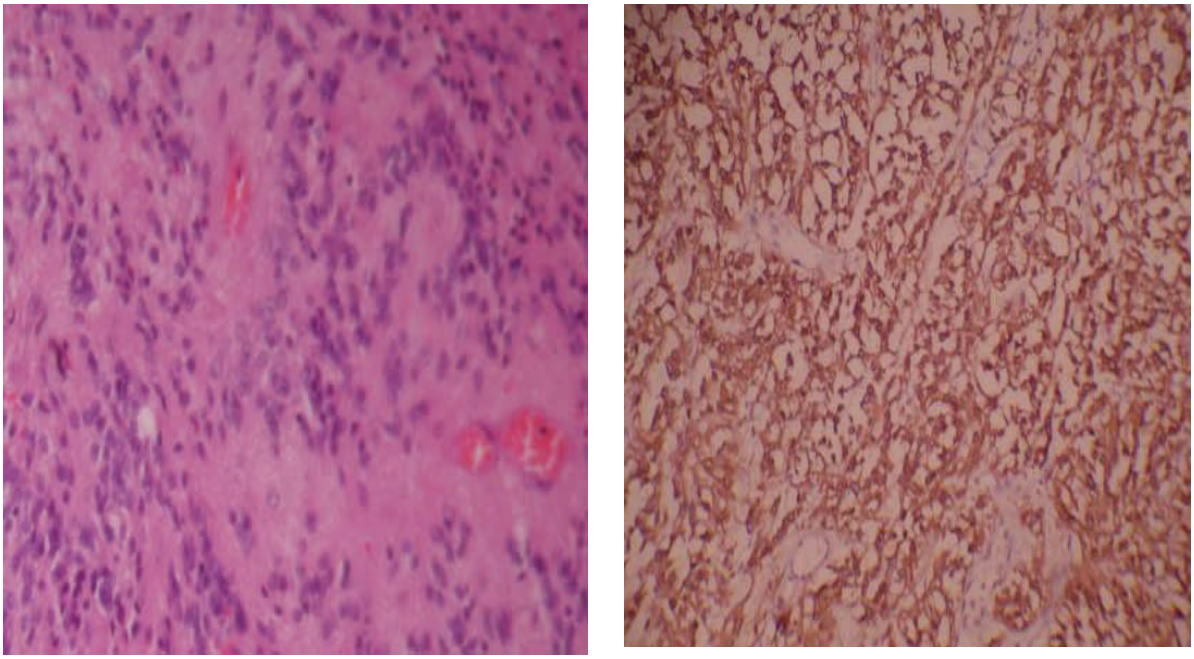

Figure 2: Ependymoma H&E (A) and GFAP expression (B) (X 400)

Life. No significant correlation was noted between GFAP expression and sex (Table 4). The supratentorial tumors more significantly expressed GFAP than (Table5). Regarding GFAP scoring, there was a highly significant inverse correlation between the total score of GFAP and grade of glioma (Table 6).

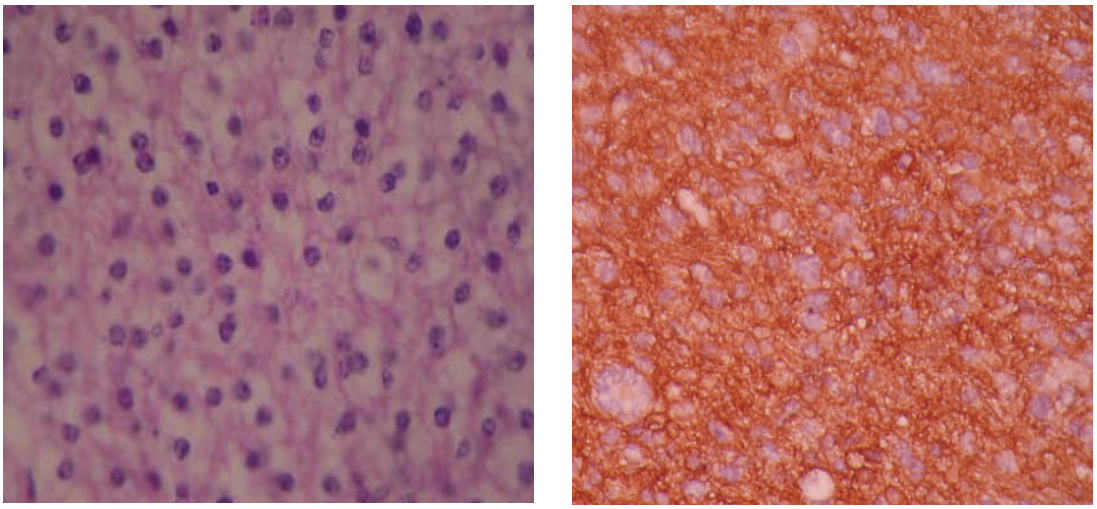

Figure 3: Oligodendroglioma H&E (A) and GFAP expression (B) (X 400)

Table2: GFAP expression in the studied cases

Diagnosis	GFAP		Total (%)
	+VE	-VE	
Astrocytoma	35	1	36 (69.2%)
Oligodendroglioma	5	0	5 (9.6%)
Ependymoma	3	0	3 (5.8%)
Oligoastrocytoma	1	0	1(1.9%)
Ganglioglioma	1	0	1(1.9%)
Medulloblastoma	0	6	6 (11.6%)
Total	45	7	52 (100%)

Table3: GFAP expression by age

Age	GFAP		Total
	+VE	-VE	
1-10	6	3	9
11-20	4	2	6
21-30	8	0	8
31-40	7	1	8
41-50	12	0	12
51-60	3	1	4
61-70	5	0	5
Total	45	7	52

P value 0.043 (significant)

Table4: GFAP expression by sex

Sex	GFAP		Total
	+VE	-VE	
Male	27	5	32
Female	18	2	20
Total	45	7	52

P value 0.563 (non significant)

Table5: GFAP expression by site

Site	Diagnosis	GFAP		Total
		+VE	-VE	
Supratentorial	**Astrocytoma**	**26**	**1**	**27**
	Oligodendroglioma	**4**	**0**	**4**
	Oligoastrocytoma	**1**	**0**	**1**
	Total	**31**	**1**	**32**
Infratentorial	**Astrocytoma**	**9**	**0**	**9**
	Oligodendroglioma	**1**	**0**	**1**
	Ependymoma	**3**	**0**	**3**
	Ganglioglioma	**1**	**0**	**1**
	Medulloblastoma	**0**	**6**	**6**
	Total	**14**	**6**	**20**

P value 0.005 (significant)

Table6: GFAP scoring and grade of glioma

Grade	total score groups				Total
	0-2	3-4	5	6-7	
1	0	0	0	2	2
2	0	0	0	21	21
3	0	0	1	8	9
4	1	4	2	6	13
Total	1	4	3	37	45

Pvalue <0.001 (highly significant)

DISCUSSION:

Assessment of GFAP status is an essential component for confirmation of the diagnosis of neuroepithelial tumors and probably for assessment of the degree of tumor differentiation[13]. Although immunohistochemical expression of GFAP nowadays is a widely used marker for astrocytoma, it's not restricted exclusively to astrocytic lesions. Even other types of gliomas show GFAP positivity[14]. In the current study, GFAP expression was positive only in glial neuroepithelial tumors. This result clarifies the importance of differentiating glial from non-glial tumors. It was found to be positive in 86.5% of neuroepithelial tumors (P < 0.001), a the fact that almost all GFAP positive tumors were of low grades. This was strengthened by the observation of an inverse correlation between GFAP intensity and the grade of glioma (P < 0.001). A part from Pakistan, this finding is documented by many other studies (Table 7). Absence of GFAP expression in one case of astrocytoma may be explained by the fact that this case was of a high grade which may lose some of the differentiation features. The predominance of GFAP expression in the forth decade of life is explained by the predominance of astrocytoma among this age group. The same thing is applied for the predominance of GFAP expression in the supratentorial tumors (P value: 0.005).

Table7: GFAP expression in relation to grade in different studies

Author	Year	No. of cases	Country	GFAP relation to grade
Tascos N A etal[15]	1982	131	USA	Inverse
Tajika T etal[16]	1986	91	Japan	No relation
Bian X W[17]	1992	243	China	Inverse
Reyaz N etal[8]	2005	50	Pakistan	Direct
Layla G S[18]	2008	56	Iraq	Inverse
Current study	2009	52	Kurdistan	Inverse

CONCLUSION:

1.GFAP is a very reliable marker to differentiate between glial and non-glial tumors and can solve the problematic cases.

2.GFAP expression is inversely correlated with the grade of glioma particularly after application of the scoring system supporting the claim that it may be

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