Short Note

The Role of Glial Fibrillary Acidic Protein (GFAP) in the Diagnosis of Neuroepithelial Tumors

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Abstract

Objectives: The aims of this study are to identify the Immunohistochemical (IHC) expression of Glial Fibrillary Acidic Protein (GFAP) in different types of neuroepithelial tumors in Mosul city and to correlate the results with grade of tumor, with the results of other studies and to assess the diagnostic role of GFAP in the diagnonsis of neuroepithelial tumors and their differentiation from neuroglial tumors.

Patients and Methods: This study included 56 cases of neuroepithelial tumors. 22 cases were collected during the period extending from October 2007 to May 2008. (The rest of the cases were retrieved from a filing system extending back to 2004). In addition to two miscellaneous tumors, (one meningioma and the other secondary adenocarcinoma). All cases were obtained from Al- Jamhuri Teaching Hospital in the western side of Mosul City, Northern Iraq and some private laboratories. Typing and grading of the tumors were done according to World Health Organization (WHO) classification system. IHC procedure was done for GFAP using polyclonal antibodies and chromogen visualizing system. A semi-quantitative histochemical score was used to record the results of GFAP staining according to the system established by Catherine L. Nutt et al.

Results: Thirty seven cases were diagnosed as astrocytoma, while 8 cases out of ependymoma, 4 cases of oligodendroglioma, and three cases medulloblastoma were shown. In addition, this study revealed that one case for each of: oligoastrocytoma, Medulloepithelioma, atypical rhabdoid tumor and astroblastoma. Glial Fibrillary Acidic Protein (GFAP) was expressed in 85.7**%** of neuroepithelial tumors. Higher GFAP positivity was found in glioma than other types of neuroepithelial tumors (P value <0.05). On the other hand, GFAP was expressed in (36%) of astrocytoma. In oligodendroglioma, 3 cases out of 4 were positive while all cases of ependymoma were positive. In addition, oligoastrocytoma was positive while the remaining cases of neuroepithelial tumors were negative. In general, each type of glioma had special staining pattern of GFAP. GFAP status was found to be inversely related with the grade of glioma (P value <0.05).

Conclusions: GFAP is expressed more frequently in glioma than in other neuroepithelial tumors and this result is similar to many other studies done outside Iraq and it is correlated inversely with the grade of tumor. So, it is a valid supplementary diagnostic procedure for neuroepithelial tumors and a reliable marker to differentiate between glial from non-glial tumors on one hand and between different types of glial tumors on the other hand.

Keywords: Immunohistochemistry, Glial Fibrillary Acidic Protein**,** Neuroepithelial Tumors**.**

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Introduction

Neuroepithelial tumors account for about 56 % of all primary brain tumors. [1, 2] According to the results of world cancer report, the incidence rate for malignant tumors of the nervous system is 6-8 new cases per 100,000 population/year. [2] In Nineveh province/Iraq, the incidence rate of nervous system tumors was 1.5 per 100,000.This estimate is based on cancer rate during 2006. [3]

The diagnosis of neuroepithelial tumors of Central Nervous System (CNS) relies on a combination of clinical assessment, imaging studies and pathological tests. [4] The latter include cytological preparation, histopathological examination, using frozen section and paraffin embedded biopsy, immunohistochemical study, cytogenetic analysis and ultra structural study. [4-7]

The glial fibrillary acidic protein is a major constituent of glial cytoplasmic filaments. [8] It is one of the class III intermediate filaments that maps in human to chromosome 17q 21. It provides valuable tools to study cell determination and differentiation. [9]

Immunohistochemistry is used to illustrate the value of this marker in the diagnosis of CNS neoplasia and to highlight potential diagnostic pitfall. [9-13]

Patients and Methods

In a pro and retrospective study, 56 cases of neuroepithelial brain tumors have been collected from AL- Jumhuri Teaching Hospital in the western side of Mosul city in northern Iraq and some private laboratories. Twenty two cases were collected during the period extending from October 2007 to May 2008. The rest of the cases were requested from the filing system extending back to 2004.

Haematoxylin and Eosin (H and E) stain of sections which were prepared from paraffin block after sectioning of 4 micro thicknesses. Typing and grading of the tumors were done according to WHO classification and the grading system took into consideration the cellularity, mitosis, cellular and nuclear atypia, vascular proliferation and necrosis. [1]

GFAP status was assessed immunohisto-chemically on Formalin Fixed Paraffin Embedded (FFPE) tissue of the tumor, using Rabbit polyclonal antibody code (N1056) (Dako, Carpintera) and Envision system G/2 code (K5355) visualizing system with chromogen. Positive and negative control slides were included in each run of staining. For the evaluation of GFAP staining, a semi quantitative histochemical scoring was applied for both cell number (proportional score) and staining intensity (intensity score). The total score was calculated from the summation of both proportional score and intensity score according to Catherine L. Nutt et al. [14]

Statistical Analysis

The relationship between GFAP expression and the clinicopathologic variables were analyzed by the X^2 – test or Fisher Freeman Holton's and Fisher's exact test, when necessary. The results were considered statistically significant if the P value was <0.05. [15]

Results

During a period of 8 months, 56 cases with neuroepithelial brain tumors have been studied concerning GFAP expression and its relation to type and grade of tumor with assessment of its role in the diagnosis of the collected cases. Thirty seven (66 %) out of 56 cases were diagnosed as astrocytoma, 8 (14.3%) cases showed ependymoma, while 4 (7.1%) of oligodendroglioma, and 3 (5.4%) of medulloblastoma were found. Only 1 (1.8%) case of oligoastrocytoma, astroblastoma, medulloepithelioma and atypical rhabdoid tumor was found.

The frequencies of different types of neuroepithelial brain tumors are shown in Table (1).

Table (1): Percentage of GFAP expression in different types of neuroepithelial tumors.

Types	Total		GFAP +ve		GFAP -ve		p-value
	No.	%	No.	%	No.	%	
Astrocytoma	*37*	*66.0*	*36*	*75.0*	*1*	*12.5*	
Ependymoma	*8*	*14.3*	*8*	*16.7*	*0*	*0.0*	
Oligodendroglioma	*4*	*7.1*	*3*	*6.3*	*1*	*12.5*	*
Medulloblastoma	*3*	*5.4*	*0*	*0.0*	*3*	*37.5*	*<0.001*
Oligoastrocytoma	*1*	*1.8*	*1*	*2.0*	*0*	*0.0*	
Astroblastoma	*1*	*1.8*	*0*	*0.0*	*1*	*12.5*	
Medulloepithelioma	*1*	*1.8*	*0*	*0.0*	*1*	*12.5*	
Rhabdoid tumor	*1*	*1.8*	*0*	*0.0*	*1*	*12.5*	
Total	*56*	*100*	*48*	*100*	*8*	*100*	

****According to Fisher Freeman Halton test.***

In addition, 2 miscellaneous cases (meningioma and secondary adenocarcinoma) were enrolled in this study.

One case of neuroepithelial tumors was diagnosed as glioblastoma, and the other one as a clear cell ependymoma. They were stained with GFAP and restained one year after the recurrence of the same tumor.

Regarding astrocytoma, 3 (8.1%) cases were of WHO grade I (Pilocytic astrocytoma), while 11 (30.5%) cases were of WHO grade II (Fibrillary astrocytoma) (Figure 1). These cases included one case of gemistocytic variant, while the other 1 (2.7%) case was of WHO grade III (anaplastic astrocytoma) (Figure 2).

On the other hand, 22 (59.5%) cases were of the WHO grade IV (glioblastoma), (Figure 3), including 2 cases of giant cell variant and 1 case of gliosarcoma variant (Figure 4).

Regarding ependymoma, all cases were the WHO grade 2 (Figure 5). Three cases (75%) out of 4 cases of oligodendroglioma were of WHO grade II (Figure 6). The remaining 1 (25%) case of WHO grade III. This study revealed that GFAP expression was positive in 48 (85.7%) cases with neuroepithelial tumors and negative in 8 (14.3%) of cases.

The frequency of GFAP expression was the highest in glioma cases (P value < 0.05) Table (1). Most of these cases of astrocytoma which were 36 (97.3%) cases showed positive GFAP staining.

In addition, 3 cases of oligodendroglioma showed positive GFAP expression.

This study revealed that all cases of ependymoma 8 (100%) cases and oligoastrocytoma were positive.

Each type of glioma showed different intensity of GFAP stain and different staining pattern.

Gliosarcoma showed positive staining of glial element and negative for sarcomatous element (Figure 4).

The expression of GFAP was negative in all cases of astroblastoma, medulloblastoma, medulloepithelioma, and rhabdoid tumor.

Both secondary adenocarcinoma and meningioma were also negative.

The pattern of GFAP stain was characteristic in each type of gliomas.

There was a significant inverse relation between both total score and proportional score of GFAP and the grade of glioma with P value < 0.05 (Table 2 and 3). While no statistical significance was found between intensity score of GFAP and grade of glioma (Table 4).

Table (2): Relationship between GFAP Total Score (TS) and the grade of glioma.

Glioma score / Grade	No.	0-2		3-4		5		6-7		p-value
		No.	%	No.	%	No.	%	No.	%	
I	3	0	0.0	0	0.0	0	0.0	3	11.5	
II	22	0	0.0	2	33.3	5	31.3	15	57.7	*
III	2	0	0.0	2	33.3	0	0.0	0	0.0	<0.001
IV	22	1	50.0	2	33.3	11	68.7	8	30.8	
Oligoastrocytoma	1	1	50.0	0	0.0	0	0.0	0	0.0	
Total	50	2	100	6	100	16	100	26	100	

Using Fisher Freeman Halton test

Table (3): Relationship between GFAP Proportional Score (PS) and grade of glioma.

Grade	No.	Positivity <5%		5-25%		25-75%		75-100%		p-value
		No.	%	No.	%	No.	%	No.	%	
I	3	0	0.0	0	0.0	0	0.0	3	12.0	
II	22	1	50.0	1	20.0	6	35.3	14	56.0	*
III	2	0	0.0	1	20.0	0	0.0	1	4.0	<0.001
IV	22	1	50.0	3	60.0	11	64.7	7	28.0	
Total	49	2	100	5	100	17	100	25	100	

Using Fisher Freeman Halton test.

Table (4): Relationship between GFAP Intensity Score(IS) and the grade of glioma.

Intensity / Grade	Weak		Strong		p-value
	No.	%	No.	%	
I (n=3)	0	0.0	3	6.8	
II (n=22)	2	40.0	20	45.5	*
III (n=2)	1	20.0	1	2.2	0.056 (NS)
IV (n=22)	2	40.0	20	45.5	
Total	5	100	44	100	

NS = Not significant according to Chi-square test.

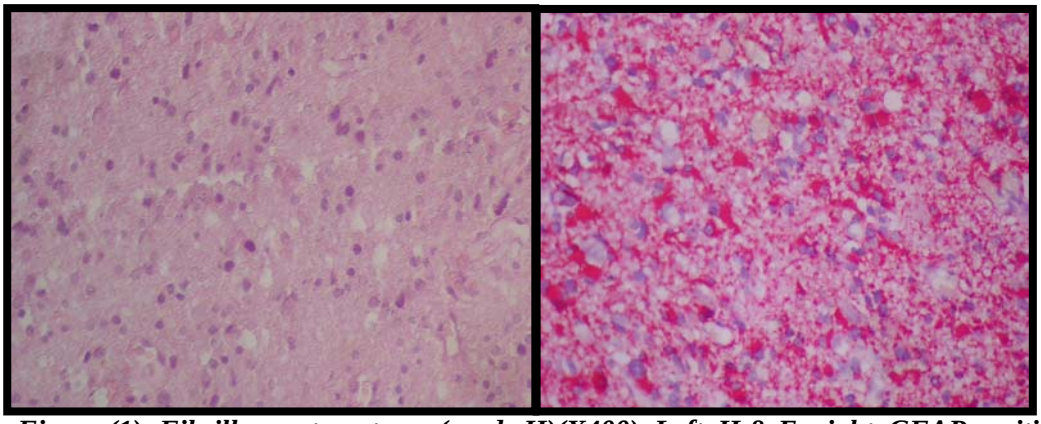

Figure (1): Fibrillary astrocytoma (grade II)(X400). Left, H & E; right, GFAP positive.

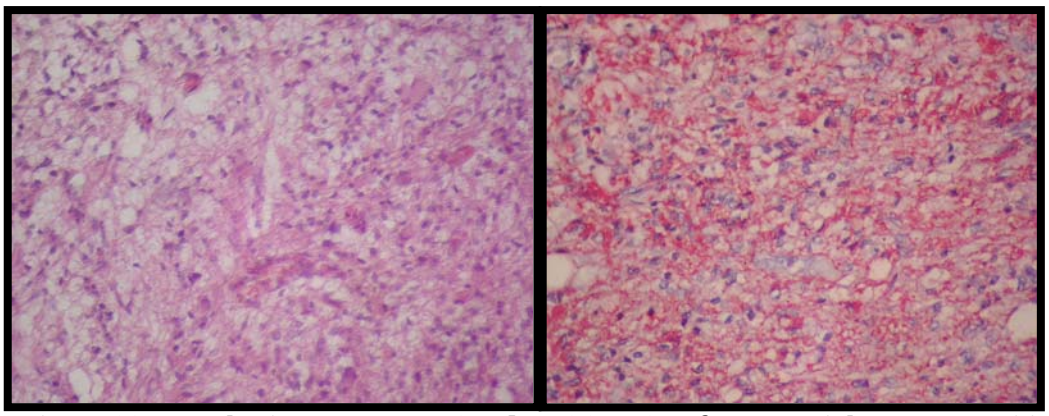

Figure (2): Anaplastic astrocytoma (grade III)(x200).Left, H&E; right, GFAP positive.

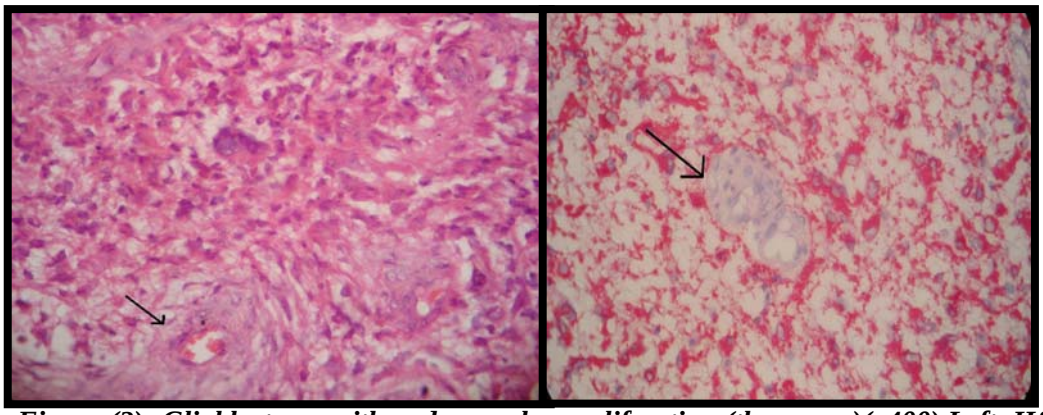

Figure (3): Glioblastoma with endovascular proliferation (the arrow)(x400).Left, H& E; right GFAP positive.

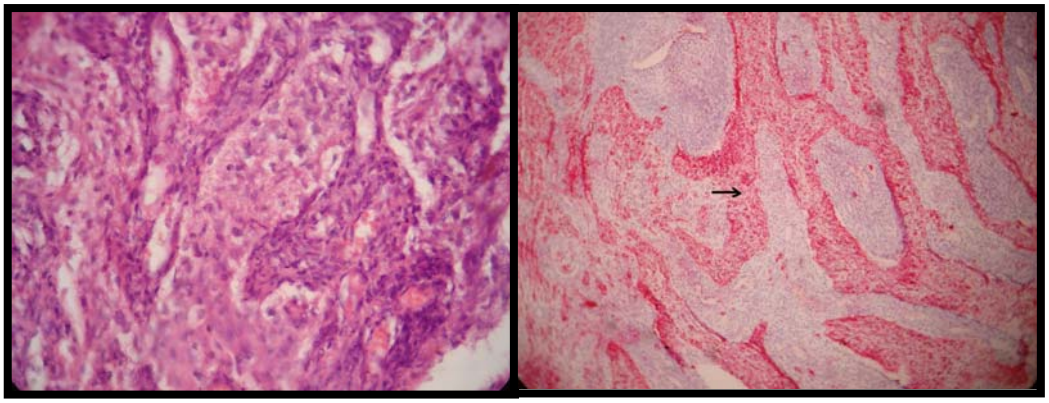

Figure (4): Gliosarcoma (x400).Left, H&E; right, GFAP positive glial element(the arrow) & negative sarcomatous element.

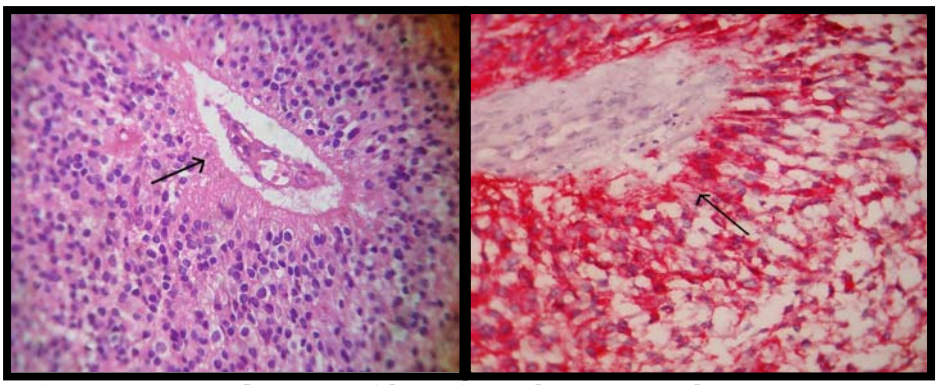

Figure (5): Ependymoma with perivascular rosette (the arrow)(x400). Left H&E & right, GFAP positive.

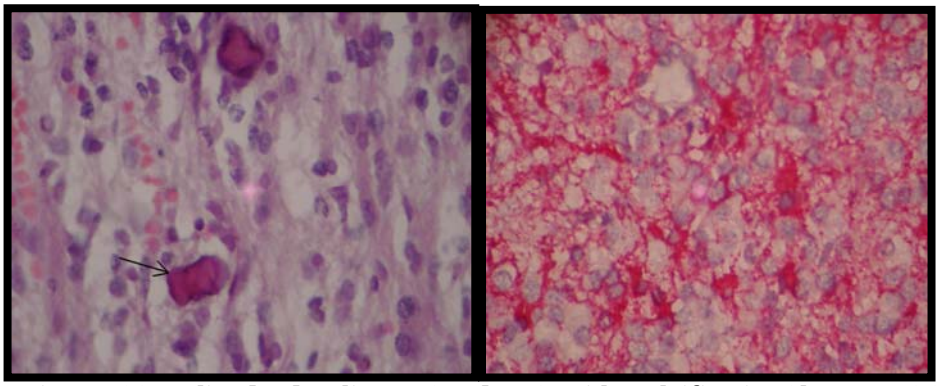

Figure (6): Oligodendroglioma (grade II) with calcification(the arrow)(x400). Left, H&E; right, GFAP positive.

Discussion

Immunohistochemical Assessment of GFAP status is an essential component of the evaluation of neuroepithelial tumors of CNS. Although GFAP status provides prognostic information, currently the major clinical value of determining GFAP status is to assess the likelihood of the patient's response to chemotherapy. [1, 5, 8]

In the current study, the GFAP expression was found to be positive in 85.7% of neuroepithelial tumors & this result agrees with that reported by Jones et al. [15] who revealed positive GFAP expression in 88.3%.

This study revealed that 96% of glial tumors were positive for GFAP, this is similar to that found by Trevor Jones R in 1982, [22] Gullotta et al. in 1985. [23] This result clarifies the importance of GFAP in differentiating glial from non-glial tumors.

Generally, astrocytoma reveals intense GFAP staining when compared to other gliomas especially at the fibrillary processes. These characteristics of astrocytic neoplasm were high lightened by the GFAP stain. High expression of GFAP was shown in astrocytic tumors with P value < 0.05.

Giant cells were positive in this study and this agrees with that found by Reyaz et al. in 2005.[8]

Regarding ependymoma, all cases showed positive GFAP expression, this agrees with that reported by Miettinen et al. in 1986, [24] Maruno in 1987. [25] However, these findings were different from that found by Reyz et al. in 2005 [8] who showed that 2 out of 4 cases were positive for GFAP and this may be due to his small sample size.

The expression of GFAP was strong mainly in ependymal rosettes and perivascular. This fact has been established by others. [1, 10, 19] The increase of GFAP expression and intensity in both low grade astrocytoma and ependymoma appears to be related to the development of fibrillary processes and GFAP may have a similar function in these two types of cells [19] since several studies found that astrocytes lacking GFAP do not form the extensions usually present with neurons. [1, 10, 19] Another explanation of increased GFAP expression in low grade astrocytoma is the presence of Rosenthal fibers which contain heavy inclusions of GFAP&B-crystalline. [8.]

Regarding oligodendroglioma, all cases were positive except one and this agrees with that reported by Vyberg et al. in 2006. [26]

The expression of GFAP was less intense and the pattern was ring like around the nucleus only with occasional short cytoplasmic processes and this is because the cells of this tumor usually contain a low level of GFAP and most cells which stain positively in oligodendroglioma are reactive astrocytes. [26]

In oligoastrocytoma (mixed glioma), the astrocytic element was stronger than oligodendroglial element. The tendency of astroblastoma to be GFAP positive for variable levels was not demonstrated in this study because only one case was included. On the other hand, in medulloblastoma, all of the cases were negative and this differs from a study done by Marsden et al. [27] and from that reported by McLendon et al.[28] which may be due to reactive astrocytosis rather than neoplastic cells, while one case of meningioma was GFAP negative as expected. In addition, the secondary adenocarcinoma was negative too.

GFAP has a major role in differentiating adenocarcinoma from aggressive and very malignant forms of gliosarcoma.

Regarding the two recurrent tumors in this study, ependymoma showed the same intensity but less positivity, while glioblastoma stained less intensely and with less positivity after recurrence. This may be attributed to recurrent tumors that are more malignant and less differentiated, so lower levels of GFAP are expected to be found. The importanc of GFAP as a glial marker was obvious in this study as being positive in nearly all cases of glioma. Furthermore, the pattern of staining was characteristically different in each type of glial tumors which confirms the diagnosis by H & E and solves the problem of trouble cases.

Controversial data have been reported with regard to the relationship of GFAP expression and the grade of glioma. In this study, there was a significant inverse relationship between the grade of glioma and the total GFAP score on one hand and the proportional score on the other hand with P value< 0.05. This result is consistent with those reported by others. [12, 25, 29] On the contrary, Hannah C Cheung et al. [30] Jossef Zamecnuk et al. [31] and Tajika et al. [32] failed to show any significant relation.

In this study, a statistical significant relation was found between the intensity score of GFAP and the tumor grade but with P value near significant (P value < 0.05).

Low grade astrocytoma (I & II) showed increased expression of GFAP staining especially at the fibrillary processes. Grade III astrocytoma showed diffuse cytoplasmic pattern. In glioblastoma, the majority of cases (68.7%) stained with less positivity and showed lower score. These results indicate that GFAP level decrease with the increase of the astrocytomas grade.

A considerable number of cases of glioblastoma (30.8%) showed high GFAP scores, this may be due to several factors including aberrant regulation associated with neoplastic transformation in glioma which may occur sometimes [19] or due to astrogliosis reaction around the tumor which may give false impression for strong GFAP staining of the tumor. [27] Low level of GFAP was associated with signs of malignancy such as pleomorphism, mitosis, necrosis and vascular proliferation.

Conclusion

GFAP is expressed more frequently in glioma than in other neuroepithelial tumors and this result is similar to many other studies done outside Iraq and it is correlated inversely with the grade of tumor. So it is a valid supplementary diagnostic procedure for neuroepithelial tumors and a reliable marker to differentiate between glial from non-glial tumors on one hand and between the different types of glial tumors on the other hand.

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دور البروتين الدبقي الليفي الحامضي في تشخيص أورام الظهارة العصبية

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الملخص

الهدف: تهدف هذه الدراسة إلى التحري عن بروتين الدبقي الليفي الحامضي (GFAP) بالطريقة المناعية–النسيجية–الكيميائية في مختلف أنواع أورام الظهارة العصبية في مدينة الموصل، كما تهدف إلى إيجاد العلاقة بين النتائج وعمر المريض، والجنس، وموقع الورم ومرتبة الورم ومقارنتها مع نتائج دراسات أخرى، وتهدف أيضاً إلى تعرّف دورها في تشخيص أورام الظهارة العصبية وتمييزها عن بعضها.

طريقة العمل: شملت هذه الدراسة 56 حالة خلال الفترة الممتدة من تشرين الأول 2007 إلى أيار 2008، إذ تم تجميع 56 حالة من أورام الظهارة العصبية بالإضافة إلى حالتي ورم متشتتة (ورم سحائي وسرطانة غدية ثانوية). جمعت الحالات من المستشفى الجمهوري التعليمي والمختبرات الخاصة في دراسة مستقبلية واسترجاعية.(هذه الدراسة شملت حالات ضمن الفترة ما بين 2004 إلى 2007). تم تصنيف الأورام ومرتبتها وفقا لنظام التصنيف لمنظمة الصحة العالمية. لقد تم التحري عن بروتين الدبقي الليفي الحامضي بالطريقة المناعية–النسيجية–الكيميائية باستخدام المضادات متعددة النسل ونظام إظهار نوع المعلم ب(الكروموجين). تم اعتماد مقياس نسيجي كيميائي شبه كمي لتسجيل نتائج بروتين الدبقي الليفي الحامضي وفقا للنظام المستخدم من قبل كاثرين.

النتائج: تراوحت أعمار المرضى بين 3 أشهر و63 سنة (بمعدل 8, 28 سنة).كانت غالبية الحالات (66,1%) في الفص الأمامي للجمجمة في حين باقي الحالات (33.9%) كانت في الفص الخلفي. من ضمن الحالات المجمعة كانت 37 حالة ورم الخلايا النجمية، و8 حالات ورم البطانة النخاعية، و4 حالات دبقوم قليلات التغصن، و3 حالات ورم اورمة النخاع، وحالة واحدة من كل من: ورم دبقي مختلط، وورم ظهاري نخاعيني، وورم عضلي مخطط لانموذجي، وورم الأرومة النجمية. لقد وجد بروتين الدبقي الليفي الحامضي في 85,7% من حالات أورام الظهار العصبي. ووجدت نسبة عالية من بروتين الدبقي الليفي الحامضي في الأورام الدبقية مقارنة مع الأورام الظهارية العصبية الأخرى

(P<0,001). كانت جميع حالات أورام الخلايا النجمية موجبة لبروتين الدبقي الليفي الحامضي باستثناء حالة واحدة. كانت النتيجة مشابهة بالنسبة لحالات دبقوم قليلات التغصن، بينما كانت جميع حالات أورام البطانة النخاعية موجبة. كان ورم الدبقي المختلط موجبا أيضا بينما الحالات المتبقية كانت كلها سالبة للبروتين المذكور. لكل نوع من الأورام الدبقية كان لصبغة بروتين الدبقي الليفي الحامضي طابع خاص بذلك النوع. لقد تناسب بروتين الدبقي الليفي الحامضي عكسيا مع مرتبة الورم (P=0,001) ووجد بنسبة عالية ضمن الأعمار التي تتراوح بين 21 و30 سنة (P=0,001). كما وجد بنسبة عالية في الأورام الواقعة ضمن الفص الأمامي للجمجمة (P=0,008)، في حين لم تظهر أية علاقة بين البروتين المذكور وجنس المريض(P=0.07) .

الاستنتاجات: إن بروتين الدبقي الليفي الحامضي يوجد بنسبة عالية في الأورام الدبقية بالمقارنة مع أورام الظهار العصبي الأخرى، وهذه النتيجة مشابهة لكثير من الدراسات الأخرى التي أجريت خارج العراق.

إن بروتين الدبقي الليفي الحامضي يتناسب عكسيا مع مرتبة المرض.

إن للبروتين الدبقي الليفي الحامضي دوراً تشخيصياً مساعداً وفعالاً لأورام الظهارة العصبية، وهو مؤشر يعول عليه لتمييز الأورام الدبقية من غير الدبقية من ناحية وللتمييز بين مختلف أنواع الأورام الدبقية من ناحية أخرى.

الكلمات الدالة: الطريقة المناعية–النسيجية–الكيميائية، بروتين الدبقي الليفي الحامضي، أورام الظهارة العصبية.