Original Article

Expression of Ki 67 and P53 immunohistochemical markers in central nervous system astrocytoma

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Abstract:

Background: Astrocytic tumors are the most common primary tumors of the central nervous system. Several grading systems are used to grade astrocytomas. The most widely used system is the World Health Organization (WHO) classification (1979, 1993, 2000, and 2007) that grades astrocytomas (I-IV) based on cytological atypia, mitotic activity, vascular proliferation, and necrosis: pilocytic astrocytoma (grade I), diffuse astrocytoma (grade II), anaplastic astrocytoma (grade III), and glioblastoma (grade IV).

Objectives: The aim of this study is to evaluate p53 over expression , Ki-67 expression in astrocytomas and Correlate these two markers with histologic grade of astrocytomas.

Methods: Formalin fixed, paraffin-embedded blocks from 40 patients with brain astrocytoma included in this retrospective study. LSAB (Labeled Strept-Avidin, Biotin) method was employed for immunohistochemical detection of Ki – 67 and P53.

Results: P53 was detected in (25%) of the cases and was significantly positively correlated with grade IV. Ki-67 labeling index was (>5%) in (50%) of the cases. Both biomarkers were positively correlated with each other, and the grade of astrocytoma; however, Ki67 is a better marker for differentiating (diagnostic marker) between the grades of astrocytoma than p53.

Conclusion: P53 overexpression and ki-67 expression plays an important role in pathogenesis of astrocytoma evolution, as they positively associated with higher tumor grade.

Key words: Ki – 67, P53, Astrocytoma.

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Introduction:

Astrocytic tumors are the most common primary tumors of the central nervous system (1). The annual incidence of CNS tumors ranges from 10 to 17 per 100,000 persons for intracranial tumors. In Iraq, CNS tumors are the fifth most common tumor in adults and the second most common in children(2). Several grading systems are used to grade astrocytomas. The most widely used system is the World Health Organization (WHO) classification (1979, 1993, 2000, and 2007) that grades astrocytomas (I-IV) based on cytological atypia, mitotic activity, vascular proliferation, and necrosis: pilocytic astrocytoma (grade I), diffuse astrocytoma (grade II), anaplastic astrocytoma (grade III), and glioblastoma (grade IV)(3).

The monoclonal antibody Ki-67 is commonly used as a diagnostic power in astrocytic tumors(4). Ki-67 is a nuclear antigen expressed in the G1, S, G2, and M phases of the cell cycle. The monoclonal antibody MIB-1 detects this nuclear antigen expressed by proliferating cells during the entire cell cycle. The percentage of immunopositive cells are referred to as the Ki-67 labeling index (LI)(5). Most of the studies demonstrate that Ki-67 LI differentiates well between diffuse astrocytomas WHO grade II (AII) and anaplastic astrocytomas (AA) and between (AII), as well as glioblastomas muthi formi (GM), but not between AA and GM. P53 has been described as "the guardian of the genome", referring to its role in conserving stability by preventing genome mutation (6). Loss of function in the p53 tumor suppressor gene due to mutation occurs early in astrocytoma tumorigenesis in about 30–40% of cases(7). Genetic instability due to the impaired ability of p53 to mediate DNA damage repair further facilitates a new genetic abnormalities, leading to malignant progression of an astrocytoma into anaplastic astrocytoma(8). This is reflected by a high rate of p53 mutation (60–70%) in anaplastic astrocytomas(8). Additional genetic abnormalities leading to progression of astrocytoma to glioblastoma multiforme(9).

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Methods

In this retrospective study, a total of 51 formalin-fixed, paraffin-embedded brain excisional biopsies from Iraqi patients covering the period from January 2006 to December 2013. The study included 10 cases of pilocytic astrocytomas ,12 cases of diffuse fibrillary astrocytomas, 8 cases of anaplastic astrocytomas , and 10 c1ases of glioblastomas, were retrieved from the archival materials of a neurosurgical hospital in Baghdad. All the clinicopathological parameters (such as age, gender and tumor grade) were obtained from the available histopathological reports. From each block, 4 sections of 4 µm thickness were taken. One section was stained with hematoxylin and eosin (Fig. 1) for revision of the histopathological diagnosis, and the other 2 sections were stained immunohistochemically for Monoclonal Mouse Anti-

Human Ki-67 Antigen, clone M 7240 and Monoclonal Mouse Anti- Human p53, Clone DO – 7.Technical negative controls were obtained by omitting the primary antibody for the two markers under identical test condition, respectively. Sections from a squamous cell carcinoma known to be immunoreactive for Ki-67 and p53 were used as a positive control.

Data were analyzed using SPSS version 16 and Microsoft Office Excel 2010. Data were presented as Mean ± SD, and as number and percentage. One way ANOVA was used to compare mean age among different grades. Chi-Square test and Fisher Exact test were used to compare frequency. The P - value would be considered significant if it was less than 0.05.

Results:

Table (1) showed significant differences (($r = 0.421$; $P \leq 0.02$) between p53 LI and grade of tumor, low p53 LI was more frequent in grade I, II and III than high p53 LI, while the reverse was found in grade IV.

Table (1) P53 labeling index in regard to grade of the tumor

Grade	NO.	(LOW) P53 < 20%		(HIGH) P53 > 20%	
		NO.	%	NO.	%
I	10	9	90	1	10
II	12	10	83.3	2	16.7
III	8	6	87.5	2	12.5
IV	10	4	40	6	60
P value		≤ 0.02			

Strong Ki-67 LI was more frequent in tumors of grade III and IV in comparison with tumors of grade I and II. In addition, there was a significant positive correlation ($r = 0.724$, $p \leq 0.01$) between grade of tumor and Ki-67 LI as shown in table (2).

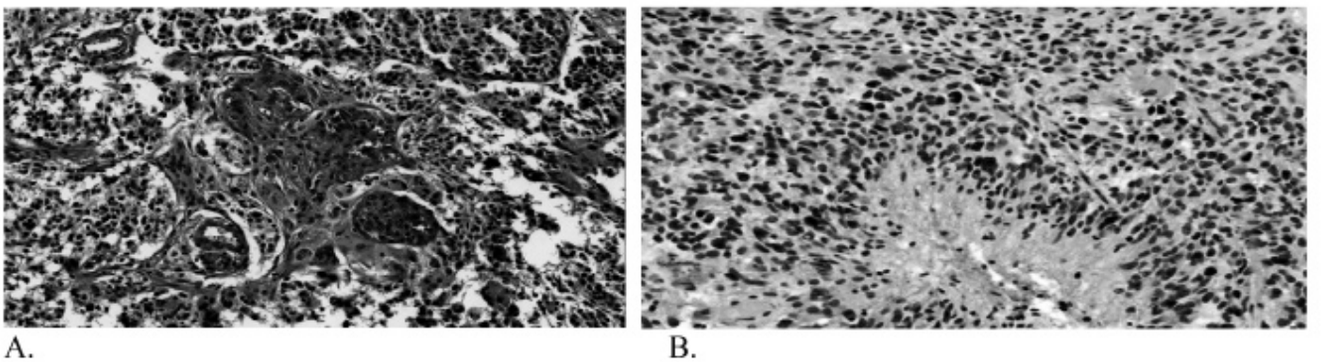
Table (2) Ki-67 labeling index (cut-off point)according to grade of tumor

Grade	NO.	Ki 67 < 5%		Ki - 67 > 5%	
		NO.	%	NO.	%
I	10	10	100	0	0
II	12	8	67	4	33
III	8	0	0	8	100
IV	10	0	0	10	100
P value		≤ 0.01			

Table (3) shows 52.5% of patients revealed low p53 expression < 20% and low Ki-67 <5% indices, but 22.5% of them showed low p53 expression < 20% and high Ki-67 > 5% indices , on the other hand 10% patients possess high p53 expression >20% and low Ki-67 < 5% indices, meanwhile 15% have high P53 > 20% and high Ki-67 > 5% indices. The total percentage of these crossing labeling indices showed significant differences.

Table (3) Correlation between p53 and Ki- 67 labeling indices (($r = 0.373$; $p \leq 0.03$)

	Ki - 67 < 5%		Ki - 67 > 5%		Total		P value
	NO.	%	NO.	%	NO.	%	
P53<20%	21	52.5	9	22.5	30	75	≤ 0.03
P53>20%	4	10	6	15	10	25	
Total	25	62.5	15	37.5	40	100	



A. B.

Figure 1: Glioblastoma multiforme (grade IV) stained with H& E is demonstrating the marked vascular proliferation and glomeruloid vascular structure (A),hypercellularity with marked hyperchromatism , pleomorphism. and area of necrosis (B). (×40).

Positive immunohistochemical staining for Ki-67 is brown nuclear with a diffuse pattern, (Fig. 2). Ki-67 expression was evaluated semi-quantitatively. It was scored by counting at least 1000 cells in 10 high-power fields. Every brown stained nucleus was considered positive, irrespective of intensity. The percentage of positive stained cells were recorded as Ki-67 labeling index (Ki-67 LI)(10). For P53, cells labeled by the antibody displayed a staining almost entirely confined to the

nucleus and with a diffuse pattern, the color of the stain is brown, (Fig. 3). P53 expression was scored by counting 1000 cells in 10 high-power fields. Every brown stained nucleus was considered positive, irrespective of intensity. The percentage of positive stained cells were recorded as a p53 labeling index (P53 LI)(11).

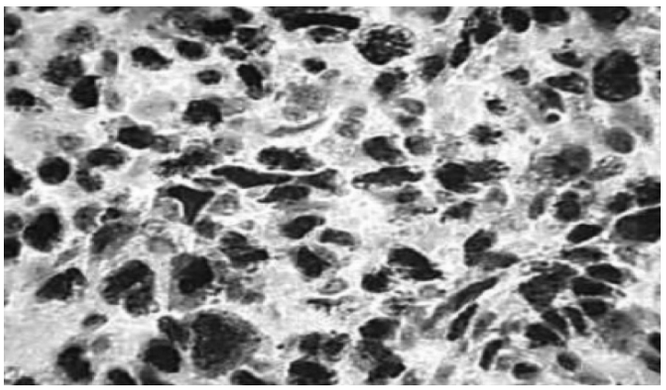


Figure 2: Glioblastoma multiforme (grade IV) stained immunohistochemically with anti-Ki-67 showing positive brown nuclear expression of Ki-67 with large number of stained nuclei reflecting active cellular proliferation (×40).

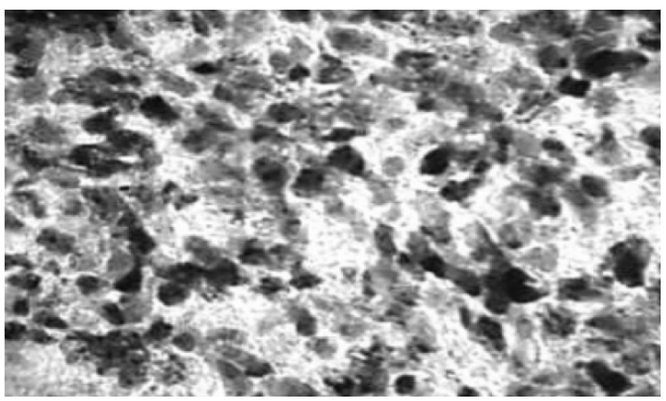


Figure 3: Glioblastoma multiforme (grade IV) stained immunohistochemically with anti-P53 showing positive brown nuclear expression of p53 (diffuse stained nuclei) with a large number of stained nuclei reflecting increased cellularity (×40).

Discussion

One of the best and well known immunohistochemical methods for evaluating the proliferation rate is quantitation of Ki-67, which was developed and introduced by Gerdes in 1993. A technique which shows all phases of the cell cycle except cells in G0 phase and because the use of Ki-67 antigen is restricted to frozen material, a new antibody (MIB-1) to an epitope of the Ki-67 antigen is used on paraffin-embedded tissue. Although this marker is a helpful marker for evaluating cellular proliferation, previous studies that used it in grading gliomas have shown conflicting results(1).

There was a highly significant correlation between the histopathological grade of astrocytomas and Ki-67 LI ($p \leq 0.01$). This result agrees with the studies done by Chaloob et al(12) and Abdelaziz et al(13)

The p53 gene is one of the major factors controlling cell proliferation, suppressing both growth and cell transformation. The loss of this tumor suppressor function can actively promote proliferation.

In regard to p53 labeling index, significant differences ($P \leq 0.01$) between low and high p53 labeling index in regard to grade of the tumor, low P53 labeling index was more frequent in patient of grade I, II, and III than high p53 labeling index. This mean that p53 overexpression is significantly associated with grade IV (glioblastoma multiforme), this result agrees with AL-Munem (14) study and Isolan et al(15) study.

Conclusion:

A significant correlation has been found between P53 protein overexpression with grade IV and the majority of grade I, II and III cases were negative. Ki-67 LI was significantly positively correlated with increasing grade of astrocytoma. Both p53 and ki-67 labeling indices are significantly positively correlated with the grade of astrocytoma and to each other.

Author contributions:

Mahmood Sh. Mahmood: collection of cases, immunohistochemical staining technique and interpretation of results with discussion.

Khitam R.Kadhim: supervisor of the research

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