Immunohistochemical expression of p53 and p21 in gliomas: a clinicopathological study

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Abstract

Background and objective: To evaluate p53 and p21 proteins over expression in gliomas and their relation to some clinico-pathological parameters.

Methods: From September 2009 to July 2010, a formalin fixed, paraffin embedded blocks of 60 gliomas cases were collected in addition to 44 astrocytomas, 5 oligodendrogliomas, 2 oligoastrocytomas, and 9 ependymomas. These cases were evaluated by immunohistochemistry using streptavidin-biotin method.

Results: Overall, 53% of gliomas were positive for p53; these cases formed 66% of astrocytomas, 20% of oligodendrogliomas and 100% of oligoastrocytomas. In contrast to astrocytomas, all ependymoma cases were negative for p53 protein. There was a significant association of p53 expression with patient's age and tumor site. On the other hand, p21 expression was positive in 25% of gliomas; they comprised 23% of astrocytomas, 40% of oligodendrogliomas, 50% of oligoastrocytomas and 22% of ependymomas. Both p53 and p21 expressions seemed to be raising from low to high grades astrocytoma but they did not reach level of significance.

Conclusion: The results of the present study suggest that p53 overexpression is common in gliomas and p21 expression is less common. There was a trend of both marker expressions increasing with higher grades.

Keywords: Glioma, p53, p21, Immunohistochemistry

Introduction

The term 'glioma' encompasses all tumors that are thought to be derived from one type of glial cells (astrocytes, oligodendrocytes or ependymal cells). It is the most common primary central nervous system neoplasm [1]. Four grades of gliomas are recognized by the WHO ranging from grade I tumors, the biologically least aggressive, to grade IV, the biologically most aggressive tumors. The histological criteria for malignancy grading are not uniform for all tumor types and thus all tumors must be classified before the malignancy grade can be determined [1-3]. p53 protein is a tumor suppressor gene that plays a key role in cellular response pathways for cell-cycle control apoptosis, genomic stability, senescence, differentiation and angiogenesis. Alterations of p53 function are the most changes in human malignancies [3]. Immunohistochemically, the wild-type p53 protein is slightly expressed in normal cells, having a short half-life (20-30minutes) because of a rapid degradation by intact mdm2 protein enzymes. On the other hand, its mutant version (inactive) is more stable, promoting its nuclear accumulation and thus making its detection immunohistochemically possible [4]. p21 is a protein that is directly induced by p53 through its regulation of cell cycle. It binds to and inactivates cyclin dependent kinases including those involved in G1 phase of cell cycle and thereby inhibits the cell cycle from progression [5]. This study was carried out to identify the immunohistochemical expression of p53 and p21 in different types of gliomas and to correlate the expression of both gene proteins with the patients' age

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and gender and with tumor site, type and grade.

Methods

From September 2009 to July 2010, a paraffin embedded blocks of 60 glioma cases were reviewed. The cases were registered at the department of histopathology at Rizgari Teaching Hospital as well as some other private laboratories in Erbil City. Clinical datas including age, sex and site of the tumors were collected from the histopathology reports. Four µm thick sections were taken and stained again with H&E and reviewed to confirm the histopathological typing & graded according to the WHO classification and grading system. Other sections were processed for immunohistochemical analysis. Sections were incubated at room temperature with monoclonal mouse anti-human p53 antibody (clone Do7, code M7001, Dako Denmark) and anti human p21 antibody (Clone SX118, code M7202, Dako Denmark). Positive controls (a strongly p53-positive breast carcinoma and a non proliferative compartment of gastrointestinal tract, for p21) and negative controls using parallel sections of tumor samples without the primary antibody were included in the analysis to ensure the specificity of the reactions. Results were considered positive when equal or more than 5% clear cut nuclear staining tumor cells detected under high power field (x 400). Statistical analysis was done by using statistical package for social sciences (SPSS) version 11 computer software. Cross tables and associations between different variables were measured using different tests. P≤0.05 was considered significant.

Results

Out of the total 60 glioma cases, 44 were astrocytomas; the remaining cases included 5 oligodendrogliomas; 2 oligoastrocytomas; and 9 cases of ependymomas. The age of patients ranged from 1 to 70 years with the mean age of 35±18.7 years; 61.7% were males and 38.3% were females with male to female ratio equals 1.6:1. Table 1, shows age and gender distribution as well as types and histological grading of the studied cases. More than half of glioma cases were located in the anterior fossa (38 cases, 63.3%); the remaining 22 cases (36.7%) were posterior fossa tumors.

Table 1: Histological grades of the studied glioma types

Type n (%)	Grade	No.	%	Age (years) Mean±SD	M:F
Astrocytoma 44 (73.3%)	I	7	11.67	21 ±13.3	5.2:1
	II	13	21.67	30 ±18	1.67:1
	III	12	20.00	43 ±16.4	2:1
	IV	12	20.00	45 ±14.6	1.4:1
Oligodendroglioma 5 (8.3%)	II	3	5.00	33 ±12.3	4:1
	III	2	3.33		
Oligoastrocytoma 2 (3.3%)	III	2	3.33	33 ±7.7	1:1
Ependymoma 9 (15%)	II	8	13.33	18 ±18.9	1:0.8
	III	1	1.67		
Total	**-**	**60**	**100.00**	**35±18.7**	**1.6:1**

Nuclear p53 immunoreactivity was observed in 32/60 of cases (53%). The expression was more frequent among patients older than 30 years with a significantly highest positivity in the seventh decade (p value= 0.0052) (Table 2). The gender had no influence; however, anterior fossa tumors expressed p53 significantly more than tumors in posterior fossa (p value = 0.005). Regarding the relation of p53 expression to the tumor type, it was found that 29 of 44 (65.9%) cases of astrocytomas and both cases of oligoastrocytomas showed p53 expression (figure 1). On the other hand, all ependymoma cases and 4/5 of oligodendrogliomas showed negative p53 expression. There was no significant relation between p53 expression and type of gliomas. Results revealed that high grade astrocytomas showed more p53 expression when compared with grade I astocytomas; however, this difference did not reach the significance level (Table 3). Only 15 out of 60 (25%) cases of gliomas showed positive nuclear expression for p21. More than half of positive cases were between 41-60 years (p= 0.0219) (table 2). There was no difference in p21 expression among males and females. Tumors in the anterior and posterior fossa did not show any difference either (p=0.36). When types of gliomas were analyzed, 22.7% of astrocytomas, 40% of oligodendrogliomas, 50% of oligoastrocytomas and 22% of ependymomas showed nuclear p21 expression with no significant variations, p= 0.067 (Figure 2). Likewise, although there was a trend for more p21 expression among Grade II, III and IV than grade I astrocytomas, the difference did not reach a significant level (p value > 0.05) (Table 3).

Table 2: Age distribution and p53/p21 expression

Age groups (years) (n)	p53 (+ve) Number (%) *	p21 positive Number (%) **
1-10 (11)	4 (36.4)	1 (9.1)
11-20 (4)	1 (25)	1 (25)
21-30 (12)	5 (41.7)	3 (25)
31-40 (12)	9 (75)	1 (8.3)
41-50 (11)	6 (54.5)	4 (36.4)
51-60 (6)	3 (50)	4 (66.7)
61-70 (4)	4 (100)	1 (25)

* P value: 0.0052, ** P value: 0.0219

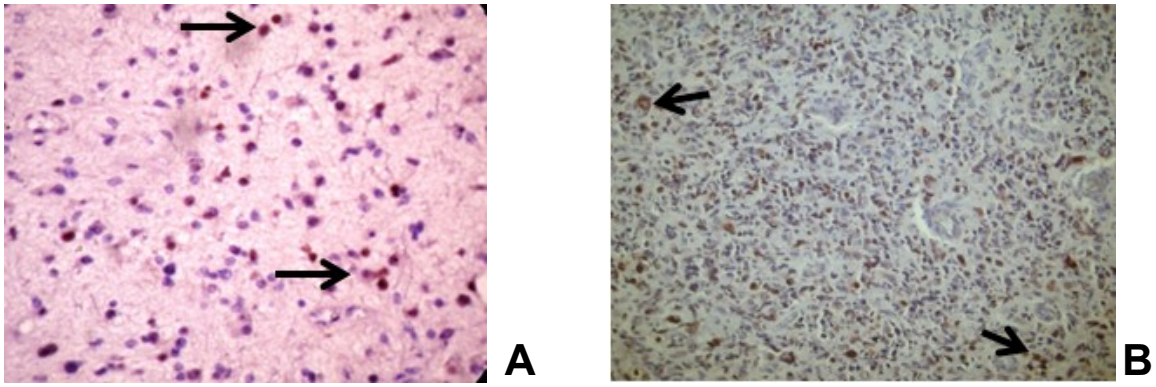

Figure 1: Nuclear expression of p53 (arrows) in grade II (A) and grade IV astrocytomas (B) (x200)

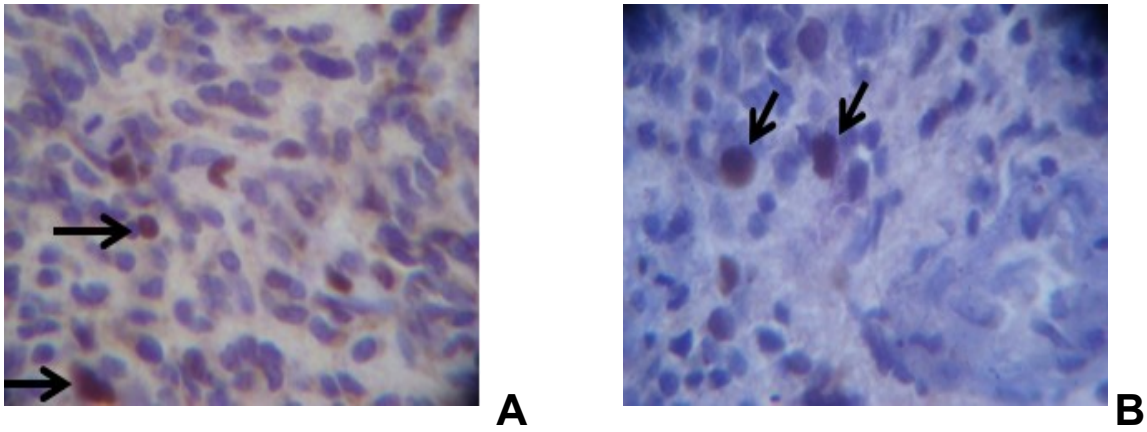

Figure 2: p21 nuclear expression (arrows) in grade IV astrocytoma (A) and grade II ependymoma (B) (x400)

Table 3: p53 and p21 expression in astrocytomas

Astrocytoma	Positive p53 Number (%)	Positive p21 Number (%)
Grade I	2 (29)	0 (0)
Grade II	9 (69)	2 (15)
Grade III	9 (75)	5 (42)
Grade IV	8 (67)	3 (25)
Total	28 (63.6)*	10(22.7) **

* P value > 0.05, ** P value > 0.05

Combined p53 and p21 expression: Four phenotypic patterns of combined p53/p21 expression were observed among the gliomas: (A), p53 expression without p21 (p53 +/p21-) identified in 25 (42%) cases; (B), p21 expression without p53 (p53-/p21+) seen 8 (13%) cases; (C), absence of both proteins (p53-/p21-) observed in 20 (33%) cases; and (D), simultaneous expression of both markers (p53+/p21+) found in 7 (12%) cases. The expression of p53 alone (Pattern A) was significantly higher than all other patterns (p value = 0.04), Figure 3. Results showed that pattern-A was observed significantly higher in astrocytomas than other glioma types (P-value = 0.017), Table 4. However, combined expression patterns did not differ in the different astrocytoma grades (p-value > 0.05), Table 5.

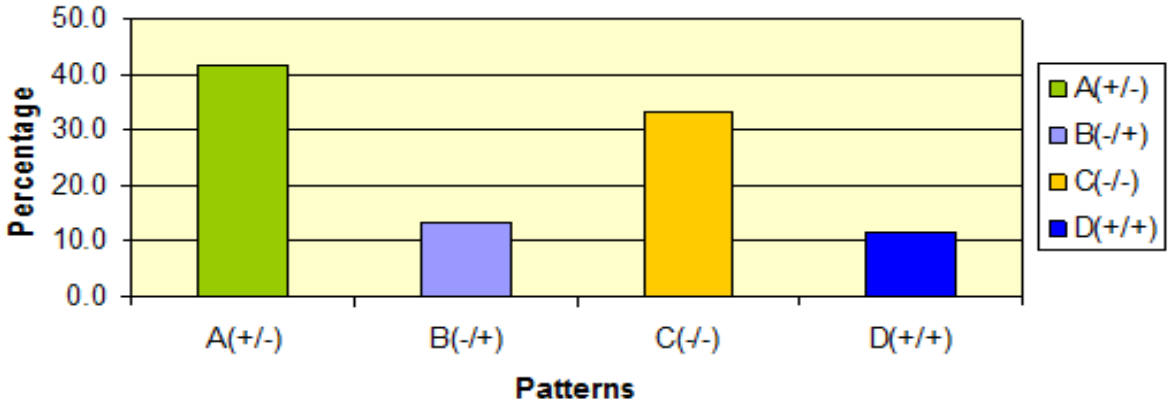

Figure 3: Combined p53/p21expression patterns.

Table 4: Combined p53/p21 expression in the different types of Gliomas

Glioma type	A (+/-) No. (%)	B (-/+) No. (%)	C (-/-) No. (%)	D (+/+) No. (%)
Astrocytoma	23.0 (38.3)	4.0 (6.7)	11.0 (18.3)	6.0 (10)
Oligodendendroglioma	1.0 (1.7)	2.0 (3.3)	2.0 (3.3)	0.0 (0)
Oligoastrocytoma	1.0 (1.7)	0.0 (0)	0.0 (0)	1.0 (1.7)
Ependymoma	0.0 (0)	2.0 (3.3)	7.0 (11.7)	0.0 (0)

Table 5: Combined p53/p21 expression among different grades of astrocytomas

Pattern	Low grade No. (%)	High grade No. (%)	p value
A	10 (22.7)	13 (29.5)	
B	1 (2.3)	3 (6.8)	>0.05
C	8 (18.2)	3 (6.8)	
D	1 (2.3)	5 (11.4)	

Discussion

Investigating the differences in the immunohistochemically detectable expression of cell cycle proteins between different types and grades of gliomas is an attempt to understand the genetic alterations associated with the cell cycle deranged regulation [6]. It has been shown that evaluation of p53 nuclear expression alone may fail to provide significant clinical information but such evaluation if combined with other gene proteins like p21 phenotype could be more informative in spite of the fact that both are linked to the p53 status [7]. Loss or mutation of p53 is especially common in gliomas and is reported to be the earliest detectable event in their development [8]. In the present study, p53 was detected in 53.3% of gliomas, a finding that is comparable with that reported by other authors from Brazil and USA who found positive expression in about 54% of their cases [5, 9]. In our series, p53 expression was observed to be more frequent among patients older than 30 years age, a finding which was also observed in other studies, [10, 11] which probably related to the higher glioma grade occurring with increasing age [12]. Our results are in agreement with two other studies who demonstrated more p53 expression in gliomas lying in the anterior fossa than the posterior fossa [13, 14]. In the current series, p53 expression was variable among different glioma types with a trend toward more expression in astrocytomas and oligoastrocytoma although this difference did not reach a statistically significant level ($p > 0.05$). To our knowledge, p53 expression in gliomas varies widely among different studies ranging from 27% to 70%, Table 6. The sample size, the immunostaining method, the technique used for antigen retrieval, the antibodies applied and the subjectivity in scoring vary greatly in different studies, in addition to the absence of a uniform cut off value for definition of positive tumors; these are contributory factors which may change the real value of the marker used [15].

In our series, p53 expression was more among higher grades than grade I astrocytomas but not to a statistically significant level (p value >0.05). In two separate studies; Nieder et al, who made a systematic review on 28 studies, and Sarkar et al, they observed that p53 expression reached approximately 50% or even more of cases but without reaching a significant difference between the different grades [15,16]. In contrast, other studies found positive p53 immunostaining to be more encountered in more clinically aggressive tumors especially among adult grade II astrocytomas [10,17] and childhood grade III gliomas [12]. Likewise, Ono et al., Jaros et al. and Faria et al reported significant correlation between p53 expression and high grade

Table 6. Comparison of current study of p53 expression with other studies [15].

Reference	No. of cases	Cut-off level	P53 expression
Hilton *et al.* (1998)	96	>10%	46%
Kraus *et al.* (1994)	37	Any staining	27%
Baxendin-Jones (1997)	62	≥3%	50%
Bouvier-Labit (1998)	62	≥1%	43%
Kyritsis (1995)	48	Any staining	73%
Perry (1999)	85	>10%	54%
Louis *et al.* (1993)	24	NA	50%
Kirla et al. (2000)	77	NA	45%
Kordek *et al.* (1996)	56	NA	41%
Pardo *et al.* (2004)	74	NA	48%
Ono *et al.* (1997)	48	NA	39.5%
Jaros *et al.* (1992)	43	NA	44%
Lang *et al.* (1994)	31	NA	64%
The current study	60	5%	53%

gliomas [18,19,5]. In the present study, the positive index for p21 was 25%. Faria et al, reported 34% while Korkolopoulou et al, noted 50% p21 protein expressions [5,7]. Two of 9 cases of ependymomas were immunopositive for p21. Although a sufficient immunohistochemical study for p21 has not been performed yet, it has been mentioned in a study by Kamiya and Nakazato that p21 expression is a feature of ependymomas [20]. Like p53, p21 protein expression did not correlate with the tumor grade, a finding which is in agreement with that observed by Kamiya and Nazakato and Jung et al. [20,21] who claimed that p21 overexpression appears to be an early event in the development of glial neoplasms. Faria et al, [5] reported a tendency of reducing p21 expression with histological grade in contrast to Korkolopoulou et al, [7] who observed a raised percentage of p21 positive cases in the higher grade tumors. The addition of p21 expression can help in interpretation of some of the data concerning p53 expression since the antibody used (Do-7) detects both the wild-type and mutated p53 in addition to the fact that p53 overexpression is not always associated with mutation which make it difficult to draw conclusions [13]. Pattern A (p53+/p21-) immunophenotyping was the commonest immunophenotype (41%) and was significantly higher than the other phenotypes (B, p53-/p21+; C, p53-/p21- and D, p53+/p21+) ($p = 0.04$). Moreover, this pattern encountered significantly more in astrocytomas than other types of gliomas ($p= 0.017$). Similar finding has been reported by Jung et al. [21] who postulated that p53-dependent and p53-independent mechanisms account for p21 elevation in astrocytomas especially grade III and IV.

Conclusion

The results of the present study suggest that p53 and p21 over expression is not uncommon in gliomas. Immunopositivity of both markers increased proportionately with the histological grades of gliomas, in spite of this finding not reaching a statistically significant level.

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