RESEARCH COMMUNICATION

Gliomas: Correlation of Histologic Grade, Ki67 and p53 Expression with Patient Survival

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Abstract

Background: Gliomas are grouped into grades 1 to 4 on the basis of morphologic criteria. Grade is the most significant prognostic factor determining survival, but various proliferation markers are being increasingly employed by histopathologists as adjuncts to conventional morphologic variables to determine prognostic behavior of brain tumors. The most widely used and useful of these are MIB1 (Ki67) and p53. Objective: To correlate World Health Organization (WHO) grades of glial neoplasms and expression of MIB1 and P53 by these tumors with patient survival at the end of one year. Material and Methods: 50 consecutive cases with confirmed diagnosis of various histologic types of glial neoplasms were included. Grading was done according to the WHO grading system for CNS neoplasms. Immunohistochemical staining of p53 and MIB1 (Ki67) was performed and scores were calculated. Results: A significant correlation was shown between WHO histologic grade and patient survival (p value:0.004) and a marginal correlation was seen between MIB1 score and patient survival (p value: 0.233). Conclusion: Histologic grade is the most important prognostic factor with respect to patient survival in glial neoplasms. Immunohistochemical staining with MIB1 and p53 may serve as an additional useful toolin determining the clinical course in combination with and as an adjunct to tumor grade. However, the fact that follow-up was available in only twenty out of the fifty cases is a limitation of the present study.

Keywords: Gliomas - histologic grade - MIB1 - p53

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Introduction

Gliomas, which include astrocytomas, oligodendrogliomas and ependymomas, are the commonest primary brain neoplasms. They range in histological appearance and clinical behavior from well differentiated, indolent tumors to anaplastic, rapidly growing tumors (Kleinhues et al., 1995). According to the WHO classification of CNS neoplasms, gliomas are grouped into various grades (1 to 4) on the basis of cellularity, nuclear atypia, mitotic activity, pseudo-palisading necrosis and or microvascular proliferation (Kleinhues and Cavence, 2000).

Various studies have shown that tumor grade is the most statistically significant prognostic factor determining patient survival, and good correlation is found between increasing tumor grade and adverse prognosis i.e. higher grade corresponds to decreasing patient survival. (Kleinhues et al., 1995; Kleinhues and Cavence, 2000; Brat et al., 2008)

Nowadays, various proliferation markers are being increasingly employed by histopathologists as adjuncts to conventional morphologic variables (outlined above) to determine the prognostic behavior of brain tumors. The most widely used and useful of these proliferation markers are MIB1 (Ki67) and p53 (Cunningham et al., 1997; Johannssen and Torp, 2006). Immunohistochemical methods are used to demonstrate the expression of these markers by CNS neoplasms, and various studies have found excellent correlation between quantitative increases in the expression of MIB1 and p53, and poor prognosis with reduced interval to recurrence and reduced patient survival (Jaros et al., 1992; Cunningham et al., 1997; Johannssen and Torp, 2006).

The aim of the present study was to correlate the WHO grades of glial tumors and expression of proliferation markers (MIB1 and p53) by these tumors with patient survival at the end of one year. And to see whether any correlation exists between increasing tumor grade and MIB1 and p53 scores.

Materials and Methods

This was a descriptive study 50 consecutive cases with confirmed diagnosis of glioma were retrieved from the files. Only cases which had been definitively graded according to the WHO classification (2) were included in the study. Cases with inconclusive diagnosis, and cases with diagnosis of glioma but without definite grading were excluded. All cases were reviewed by one of the authors (SHH), and for each case, a representative block was selected and immunohistochemical stains MIB1

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(Ki67) and p53 were performed. Nuclear staining was considered positive.

Immunohistochemical analysis was performed using p53 and Ki-67 antibodies (DAKO, Denmark). Each assay included positive and negative controls. Paraffin sections of 3-micrometer thickness were placed on glass slides coated with Poly-L-Lysine (Sigma chemical Co, USA) to promote adhesion. Slides were kept overnight at 37°C. The sections were dried at 60°C for 45 to 60 minutes. These sections were then deparaffinized in xylene, rehydrated through a graded alcohol series and rinsed with water. For Antigen retrieval the slides were treated in Tris EDTA buffer ph 9.0 at high temperature in microwave 3 times for 10 minutes each and the slides were then allowed to cool for 10-15 minutes at room temperature. The slides were washed well in distilled water. Endogenous peroxidase was blocked with blocking solution (peroxidase) for five minutes. The slides were rinsed well in distilled water and then with Tris buffer at pH7.6 and were treated with p53 and MIB1 (Ki-67) antibodies for 30 minutes at room temperature. The sections were washed with Tris buffer and treated with Polymer (Envision system K 5007). The slides were treated in DAB solution for 5-7 minutes, washed well in tap water, and were then counter stained with hematoxylin, dehydrated with graded alcohol, cleared in xylene phenol and xylene. Slides were finally mounted in DPX. Brown nuclear staining was considered positive both for p53 and Ki-67 antibodies.

Estimation of P53 and MIB-1 labelling index

The MIB-1 labelling index and p53 were calculated as a percentage of labelled nuclei per 1000 cells. One thousand tumor cells were counted in several areas of tissue where positively stained nuclei were evenly distributed. But in those cases with uneven distribution of positive nuclei, the tumor cells were counted in the areas with highest density of positive nuclei by visual analysis (Ralte et al., 2001).

Data Anaysis

Statistical data was analyzed by SPSS version 13. For all categorical variables like sex, morphology of tumor, frequencies and percentages were calculated. Median, Mean and Standard deviation were estimated for quantitative variables like age, MIB-1 and p53 score with 95% confidence interval. Survival analysis was used to calculate survival rate. P value of ≤ 0.05 was considered significant.

Results

Fifty glial neoplasms were included in the study. Thirty five cases (70%) were in males, while fifteen (30%) were in females. Ages of patients ranged from five to sixty seven years with a mean age of thirty five years. Eleven patients (22%) were under nineteen years of age. Glioblastoma multiforme (GBM) was the most common histologic type followed by oligodendroglioma. The histologic types of tumors included in the study along with the respective grades and immunohistochemical scores are shown in Table 1. Forty four percent cases were grade IV (grade IV astrocytomas or GBM). It was observed that the MIB 1(Figure 1) and p53 (Figure 2) scores were higher in tumors of increasing grades. Spearman's rank correlation coefficient was used to determine the relationship between the histologic grade and the proliferation markers.

Table 1. Immunohistochemistry Scores in Glial Neoplasms of Various Types

WHO Grade	No.	MIB1	P53
		Mean (Range)	Mean (Range)
Pilocytic astrocytoma, I	3	0.26 (0.2-0.4)	0.86 (0.4-1.5)
Myxopapillary ependymoma, I	2	0.65 (0.2-1.1)	1.4 (0.3-2.5)
Oligodendroglioma, II	11	0.61 (0.1-1.5)	3.5 (0.5-10)
Ependymoma, II	2	0.5 (?-?)	0.2 (0.2-0.4)
Diffuse Astrocytoma, II	1	0.3	2.0
SEGA, II	1	0.8	2.0
Anaplastic oligo -dendroglioma, III	6	2.91 (1.5-4.0)	10 (0.3-??)
Anaplastic astrocytoma, III	2	3.10 (2.2-4.0)	2.0 (1.5-2.5)
Astrocytoma, IV	22	7.95 (0.2-15)	7.77 (0.1-25)

A strong correlation was seen between grade and MIB1 scores, while a weak correlation was seen between grade and p53 scores, that is 0.78 for MIB1, and 0.37 for p53. The ranges for Spearman's rank correlation are 0.2-0.39 (weak correlation), 0.4-0.59 (moderate correlation), and 0.6-1.0 (strong correlation).

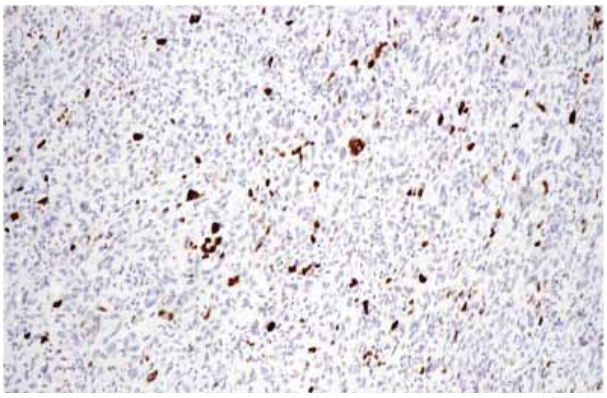

Figure 1. Anti MIB Antibody Positive Glioblastoma Multiforme (x20)

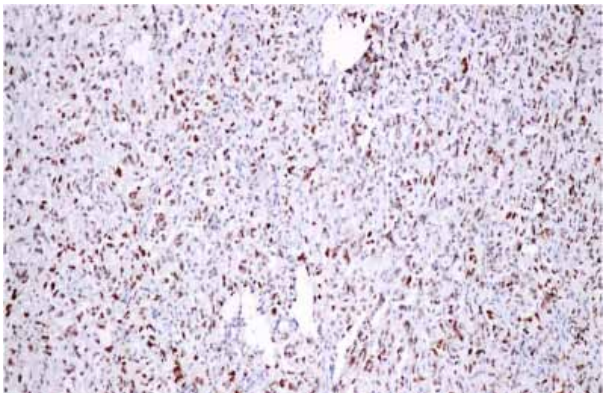

Figure 2. Strong Expression of p53 in Glioblastoma Multiforme (x20)

The patients were followed up for a period of one year. Followup was available, however, in twenty out of fifty cases. Among grade 1 tumors, follow up was available in all three cases of pilocytic astrocytomas, one out of the two myxopapillary ependymomas. Among the grade 2 tumors, follow up was available in three out of eleven oligodendrogliomas, and both cases of ependymoma. No follow up was available in the cases of diffuse astrocytoma and subependymal giant cell astrocytoma. Among grade 3 tumors, follow up was available in three out of six cases of anaplastic oligodendrogliomas and one out of two cases of anaplastic astrocytomas. Follow up was available in eight out of twenty two cases of grade 4 astrocytomas (GBM).

Only eight of the twenty patients (forty percent) were alive at the end of one year. These included all three patients with pilocytic astrocytoma and the single patent with myxopapillary ependymoma in whom follow up was available, two out of three patients with oligodendroglima in whom follow up was available and both patients with ependymoma in whom follow up was available. All patients with grade three and four tumors in which follow up was available which included three patients with anaplastic oligodendroglioma, one patient with anaplastic astrocytoma and eight patients with GBM were dead of the disease at the end of one year.

Of the twenty patients in whom follow up was available, sixteen had MIB1 score of less than five percent. Among these, eight patients were alive (sixty percent) and eight were dead of disease, four patients had MIB1 score of greater than five percent. All four had died of the disease within two years. These results show a marginal correlation between MIB1 score and patient survival (p=0.089).

Of the twenty patients in whom followup was available, twelve had p53 score of less than two percent. Among these, six patients were alive (fifty percent), and 6 were dead of disease. Eight patients had p53 score of greater than two percent, six out of these eight (seventy five percent) had died of the disease within one year. These results show that no significant correlation exists between p53 score and patient survival (p=0.233).

Discussion

A number of studies have shown that the histologic grade of a glial neoplasm remains the most statistically significant prognostic factor and there is a good correlation between increasing tumor grade (based on cellularity, nuclear atypia, mitotic activity, and pseudopalisading necrosis and /or microvascular proliferation) and adverse prognosis, with higher grade corresponding to decreased survival (Kleinhues et al., 1995; Kleinhues and Cavence, 2000; Brat et al., 2008). Our results also showed significant correlation between, increasing histologic grade and patient survival with a p value of 0.004 (see results). This corresponds to international published data. However, whereas several studies have found a good correlation between quantitative increases in the expression of proliferation markers MIB1 and p53 by immunohistochemical methods, and poor prognosis and reduced patient survival, (Jaros et al., 1992; Wakimoto et al., 1996; Cunningham et al., 1997; Johannssen and Torp, 2006) our study showed only a marginal correlation between MIB1 score and patient survival with a p value of 0.089; and no significant correlation between p53 score (determined by immunohistochemistry) and patient survival with a p value of 0.233 (see Results). These results are somewhat in contrast to other studies. However, western studies also differ in their conclusion regarding the usefulness of p53 as a prognsostic factor in glial neoplasms with some demonstrating its significant role, and others failing to demonstrate its usefulness (Pardo et al., 2004). Another study by Hilton et al., (1998) infact concludes that overexpression of neither p53 nor MIB1 has any significant role in predicting survival in patients with astrocytomas. However, we are aware of the fact that on the one hand, our patient size was small (due to financial constraints), and on the other hand, only twenty out of the fifty patients could be followed up. This is definitely a limitation of the present study. Since we receive cases from all over Pakistan, including places where a full fledged armed insurgency is going on; and even from war torn Afghanistan, it is not surprising that followup is available in very few cases. This lack of clinical information and lack of followup is not confined to CNS cases, rather it encompasses the entire spectrum of surgical pathology specimens and is a challenge that histopathologists face on a daily basis while practicing in a developing country like Pakistan. Our study also found a strong correlation between increasing histologic grade and MIB1 score; and a weak correlation between increasing grade and p53 score. (see results) Western studies have also shown that an increased MIB1 score is associated with higher histologic grade of glial neoplasms (Labit et al., 1998; Johannssen and Torp, 2006). However, it needs to be remembered that p53, a tumor suppression gene is very susceptible to mutations, and as a result p53 levels are often depleted. Tumors that retain p53 are more likely to respond to chemo and radiation therapy. Hence, studies are also looking at therapeutic strategies aimed at increasing p53 activity in tumor cells (Kumar et al., 2005).

In conclusion, our study demonstrates that WHO histologic grade remains the most important prognostic factor with respect to patient survival in glial neoplasms. Immunohistochemical staining with proliferation markers such as MIB 1 and p53 many serve as additional useful tools in determining the clinical course but cannot be used alone as prognostic factors and must be used in combination with and as adjuncts to the established histologic criteria, that is tumor grade. p53 expression can be helpful in tailoring the therapeutic modalitites in individual cases.

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