Original Article

Epidermal growth factor receptor, vascular endothelial growth factor, mouse double minute 2 homolog, Ki 67, and p53 expression in glioblastoma: A survival analysis including the prognostic value of clinical, histopathological, and immunohistochemical parameters

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Abstract

Objective: The objective of the study was to describe the clinical, histopathological, and immunohistochemical profile of glioblastoma in patients and to correlate these findings with patient survival.

Materials and Methods: Thirty cases of histopathologically diagnosed glioblastomas were included in this study. These cases were analyzed in detail for certain clinical and histopathological parameters. Immunohistochemical staining for p53, epidermal growth factor receptor, vascular endothelial growth factor, mouse double minute 2 homolog (MDM2), and Ki67 was done, and scores were calculated. Results of these findings were correlated with patient survival.

Results: A retrospective analysis of the histopathology records and clinical case files was done in thirty cases of glioblastoma (World Health Organization Grade IV). The mean age of presentation was 50.6 years with a male predilection. The most common involved site was the frontal lobe. Among the clinical parameters, age of the patient and extent of surgical resection showed a significant correlation with the patient survival. Histopathological parameters showed no significant correlation with the patient survival, while among the immunohistochemical parameters, expression of MDM2 showed a significant correlation with the patient survival.

Conclusion: In this study incorporating clinical, histopathological, and basic panel of immunohistochemistry, age of the patient, extent of the surgical resection, and expression of MDM2 showed a significant correlation with the patient survival.

Keywords: Clinical, glioblastoma, histopathology, immunohistochemistry, survival

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INTRODUCTION

Glioblastoma is the most common primary brain tumor in adults, accounting for 15% of all the primary brain tumors and 60%–75% of all astrocytic tumors, with a median survival range of 1.5–2 years.[1] Glioblastoma and its variants correspond histologically to the World Health Organization (WHO) Grade IV. In the WHO Classification

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of Tumours of the Central Nervous System (2016), glioblastoma has been classified into three categories: glioblastoma, IDH-wild-type; glioblastoma, IDH-mutant; and glioblastoma, not otherwise specified (NOS). Among the three, IDH-wild-type glioblastoma synonymous with primary glioblastoma is the most common and most malignant astrocytic glioma, accounting for approximately 90% of all glioblastomas; typically affecting adults (mean age at diagnosis of 62 years).[2] IDH-mutant glioblastomas synonymous with secondary glioblastoma account for approximately 10% of all glioblastomas, manifest in younger patients (mean age at diagnosis of 45 years), and carry a significantly better prognosis.[3] Glioblastoma, NOS encompasses the cases in which IDH mutation status has not been fully assessed.

Overall, glioblastomas do exhibit significant clinical, pathological, and molecular heterogeneity.[4] In this purview, this study was undertaken to analyze the clinical and histopathological spectrum of thirty glioblastomas received in our department over the past 5 years. The aim was to describe the clinical, histopathological, and immunohistochemical profile (Ki 67, p53, epidermal growth factor receptor [EGFR], vascular endothelial growth factor [VEGF], and mouse double minute 2 homolog [MDM2]) of glioblastomas in patients and to correlate these findings with patient survival.

MATERIALS AND METHODS

A retrospective analysis of the histopathology records and clinical case files was done in thirty cases of histopathologically diagnosed cases of glioblastoma (WHO Grade IV) received over a period of 5 years in our department. These cases were analyzed for defined clinical and histopathological parameters with some relevant immunohistochemical parameters. Clinical parameters included age, gender, clinical features such as signs and symptoms at onset, duration of symptoms, localization of tumor, treatment modalities, and role of adjuvant therapy. Histopathological parameters included the extent of necrosis, pattern of microvascular proliferation, mitotic activity, and presence of other components or variants. Immunohistochemical parameters included expression of p53, EGFR, VEGF, Ki67, and MDM2. The score was calculated as a percentage of positively labeled cells. Overall 1000 tumor cells were counted in systematically randomized fields throughout the section. Nuclear expression of markers was scored for p53, Ki67, and MDM2, cytoplasmic expression was scored for VEGF, whereas for EGFR, both membranous and cytoplasmic expression was scored. The expression for all immunohistochemical markers was evaluated quantitatively as the percentage of positive tumor cells over total tumor cells (%). A cutoff of ≥20% positivity was labeled as positive, whereas <20% was labeled as negative [Table 1].

Statistical analysis

SPSS software (17.0 version, IBM, South Asia Pvt Ltd, Bengaluru, India) was used for the statistical analyses. Chi-square test was applied to the sets of categorical data to evaluate the association between the variables. $P \leq 0.01$ was considered statistically significant.

RESULTS

Clinical data

The mean age of the presentation was 50.63 years (range: 20–78 years). There was a male preponderance with 19 male (63.3%) and 11 female patients (36.7%). There was a wide range in the duration of symptoms, varying from 7 days to 1 year. Nine (30%) patients presented with the clinical features indicating focal neurological deficits and raised intracranial tension (ICT). Seven (23.3%) patients presented with clinical features indicating raised ICT. Six (20%) patients presented with the clinical features, suggesting focal neurological deficits, raised ICT, and behavioral/neurocognitive changes. Three (10%) patients presented with the clinical features indicating raised ICT and behavioral/neurocognitive changes, whereas three (10%) patients presented with the clinical features, indicating focal neurological deficits and behavioral/neurocognitive changes. Two (6.7%) patients presented with the clinical features indicating focal neurological deficits. Overall, the most common location was basifrontal in 19 (63.3%) cases. All the patients underwent surgery, a near-total/gross total resection was achieved in 17 (56.7%) cases. Remaining 13 (43.3%) cases had a subtotal resection.

All the patients were referred for the adjuvant therapy. In the follow-up, it was found that 28 (93.3%) patients went for radiotherapy/chemotherapy. Two (6.7%) patients did not opt for any sort of adjuvant therapy.

Histopathology data

Of the 30 cases, large areas of necrosis (>50%) were seen in 7 (23.3%) cases, whereas remaining 23 (76.7%) cases showed relatively less necrosis (<50%). Microvascular proliferation showing endothelial cell proliferation and glomeruloid tufts both were seen in 19 (63.3%) cases, while in 11 (36.7%) cases, only endothelial cell proliferation was observed. Twenty-four (80%) cases had mitotic count ≤5/HPF, while six (20%) cases had mitotic count ≥5/HPF [Figure 1]. In this study, three (10%) cases of giant cell glioblastoma

Table 1: Table showing the clinical, histopathological and immunohistochemical parameters

Clinical parameters	Histopathological parameters	Immunohistochemical parameters
Age (years) ≤50 >50	Extent of necrosis ≤50%; >50%	p53 (nuclear) Positive (≥20%); negative (<20%)
Gender (male; female)	Microvascular proliferation ECP; ECP and GT both	EGFR (membranous/cytoplasmic) Positive (≥20%); negative (<20%)
Clinical features Signs and symptoms at onset (FNDs; hemiparesis, aphasia; raised ICT; seizure, NV, headache; BNCs)	Mitosis ≤5/HPF; >5/HPF	VEGF (cytoplasmic) Positive (≥20%); negative (<20%)
Duration of symptoms (days) ≤30 31-90 >90	Additional HP features Absent; present	Ki-67 (nuclear) Positive (≥20%); negative (<20%)
Localization Basifrontal/temporal; any other; all		MDM2 (nuclear) Positive (≥20%); negative (<20%)
Treatment Incomplete excision; complete excision		
Adjuvant therapy Taken; not taken		
Survival		

ICT: Intracranial tension, ECP: Endothelial cell proliferation, GT: Glomeruloid tuft, HPF: High-power field, EGFR: Epidermal growth factor receptor, VEGF: Vascular endothelial growth factor, MDM2: Mouse double minute 2 homolog, FNDs: Focal neurological deficits, NV: Nausea and vomiting, BNCs: Behavioral and neurocognitive changes, HP: Histopathological

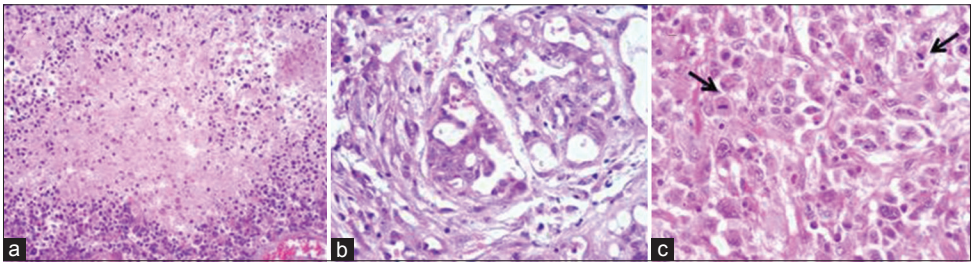

Figure 1: Histopathology showing (a) Large areas of necrosis (H and E, ×200). (b) Microvascular proliferation forming glomeruloid tufts (H and E, × 400). (c) Brisk mitotic activity (H and E, ×400)

were reported [Figure 2]. Additional features (including oligodendroglial and gemistocytic component) were present in 12 (40%) cases [Figure 3].

Immunohistochemistry data

Of the 30 cases, 14 (46.7%) cases showed immunopositivity for p53 and 16 (53.3%) cases showed immunonegativity. Sixteen (53.3%) cases showed overexpression of EGFR, whereas 14 (46.7%) cases showed immunonegativity. Twenty-seven (90%) cases showed overexpression of VEGF and three (10%) cases showed immunonegativity. Ki 67 showed overexpression in 17 (56.7%) cases, while 13 (43.3%) cases showed immunonegativity. MDM2 was expressed in 22 (73.3%) cases, while 8 (26.7%) cases showed immunonegativity [Figure 4].

Survival analysis

Multivariate analysis was done by applying Chi-square test to evaluate the correlation of survival with the clinical, histopathological, and immunohistochemical parameters. Age of the patient and expression of MDM2 were the most significant predictor of survival in this study ($P < 0.001$) [Table 2].

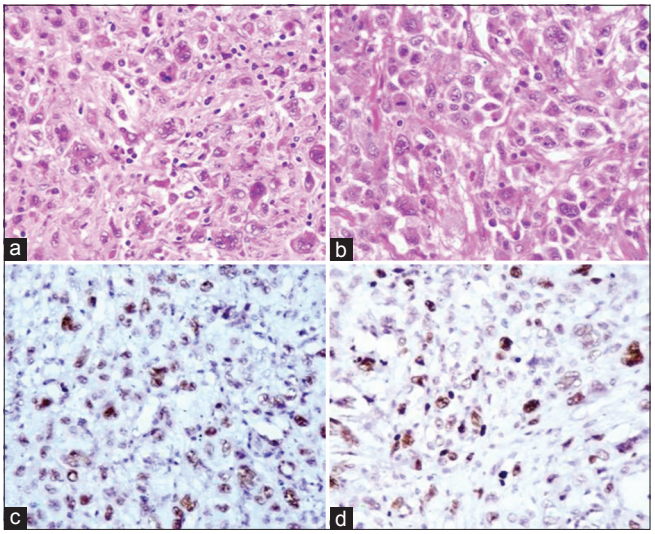

Figure 2: Histopathology showing (a) Predominant giant cells in glioblastoma (H and E, ×400). (b) Brisk mitosis (H and E, ×400). (c) Glial fibrillary acidic protein-positive giant cells (×400). (d) High Ki 67 proliferation index (×400)

DISCUSSION

Glioblastomas are the most common and the most malignant of all brain tumors, characterized by genetic

instability, heterogeneous histology, and unpredictable clinical behavior with a dismal prognosis.[4] Survival of patients with glioblastoma depends on certain clinical, histological, immunohistochemical, and molecular variables. Over the years, some studies have highlighted the effect of clinical factors such as age, Karnofsky performance score (KPS), extent of surgical resection, and radiotherapy on survival. A study by Umesh *et al*. has analyzed clinical and immunohistochemical prognostic factors in adult glioblastoma patients.[5] Few studies have incorporated molecular markers along with the clinical parameters to correlate them with survival. In the current study, the key clinical features, the major histopathological and relevant immunohistochemical parameters were analyzed and correlated with survival of the patients.

The clinical variables in predicting survival in glioblastomas have been well defined in the past few years. Among the clinical variables, younger age has been unequivocally associated with a prolonged survival in many studies.[6-8] Near total/gross total resection was another clinical variable associated with better survival in glioblastoma patients in many studies.[9-11] In one study, clinical variables inclusive of age, KPS, and extent of surgical resection were the most significant prognostic factors influencing survival.[12] In our study, among the clinical parameters, age of the patient emerged as significant prognostic factor as it showed a significant correlation with the patient survival ($P < 0.05$). A marginal correlation was found between the extent of resection of the tumor and patient survival; however, it did not show any statistical significance.

Among the histopathological parameters, some studies have suggested that certain histopathological features in glioblastomas are associated with patients' clinical outcome, like the presence of necrosis has been considered a predictive factor for poor survival of patients with glioblastoma.[13] In their study, Pierallini *et al*. reported that patients of glioblastoma showing necrosis in >35% of tumor had a significantly shorter survival time.[14] A large population-based study by Homma *et al*. has shown no correlation between microvascular proliferation and patient survival in glioblastomas.[13] Several studies have shown longer survival of patients with glioblastoma containing an oligodendroglial component.[15-17] However, in contrast, He *et al*. reported that the survival of patients with glioblastoma containing an oligodendroglial component did not differ from those of patients with ordinary glioblastoma.[18] Our study in discordance to the existing literature did not show any correlation with any of these features.

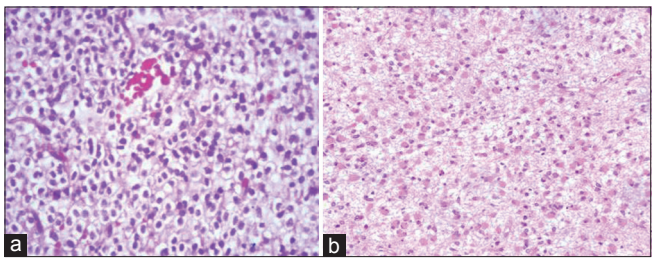

Figure 3: Histopathology showing (a) Oligodendroglial component (H and E, ×400). (b) Gemistocytic component (H and E, ×200)

Among the immunohistochemical markers, with regard to the significance of EGFR overexpression as a prognostic factor, several studies have shown variable results. Studies conducted by Heimberger *et al*., Cobbers *et al*., Rainov *et al*., and Waha *et al*. overall did not show any correlation between EGFR overexpression and survival,[19-22] while several other studies concluded that overexpression of EGFR is a negative prognostic indicator with respect to survival.[23-28] In a study by Montgomery *et al*., the authors had studied the prognostic correlation of p53 and MDM2 in 36 cases of glioblastomas and had found poor survival in cases that showed overexpression of p53 and MDM2.[29] In contrast, in the present study, we found a positive correlation between MDM2 overexpression and the patient survival with no significant correlation between p53 overexpression and survival. There is a paucity of literature correlating the expression of Ki67 and VEGF with survival in glioblastomas. In our study, we did not find any correlation between Ki67, VEGF expression, and patient survival.

CONCLUSION

In this study, among the clinical parameters, age of the patient, and among the immunohistochemical parameters, expression of MDM2 emerged as significant prognostic

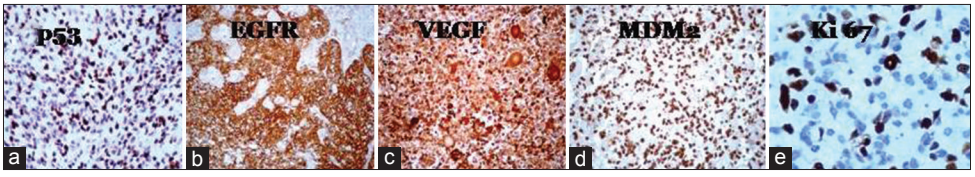

Figure 4: (a) Nuclear immunopositivity for p53 (x200). (b) Membranous and cytoplasmic immunopositivity for EGFR (x 200). (c) Cytoplasmic immunopositivity for VEGF (x 200). (d) Nuclear immunopositivity for MDM2 (x200). (e) Nuclear immunopositivity for Ki 67 (x200)

Table 2: Table showing the results of the statistical analysis

Variable	Survival duration		χ^2	*P*
	≤90 days (*n*=15) (%)	≥90 days (*n*=15) (%)		
Clinical parameters				
Age (years)				
≤50	20	73.3	8.57	0.003
>50	80	26.7		
Clinical features				
FND, hemiparesis, aphasia	6.7	6.7	0.921	0.969
Raised ICT, seizure, NV, headache	20.0	26.7		
FND, hemiparesis, aphasia, raised ICT, seizure, NV, headache	33.3	26.7		
Raised ICT, seizure, NV, headache, BNC	6.7	13.3		
FND, hemiparesis, aphasia, BNC	13.3	6.7		
All	20.0	20.0		
Duration of symptoms (days)				
≤30	26.7	33.3	2.540	0.281
31-90	60.0	33.3		
>90	13.3	33.3		
Localization				
Basifrontal/temporal	13.3	6.7	0.886	0.642
Any other	66.7	60.0		
All	20.0	33.3		
Treatment				
Incomplete excision	60.0	26.7	3.394	0.065
Complete excision	40.0	73.3		
Adjuvant therapy				
Not taken	13.3	0.0	2.143	0.143
Taken	86.7	100.0		
Histopathological parameters				
Extent of necrosis				
≤50%	66.7	86.7	1.677	0.195
>50%	33.3	13.3		
Microvascular proliferation				
ECP	26.7	46.7	1.292	0.256
ECP and GT both	73.3	53.3		
Mitosis				
≤5/HPF	86.7	73.3	0.833	0.361
>5/HPF	13.3	26.7		
Additional HP features				
Absent	66.7	33.3	3.333	0.068
Present	33.3	66.7		
Immunohistochemical parameters				
p53				
Negative	66.7	40.0	2.143	0.143
Positive	33.3	60.0		
EGFR				
Negative	46.7	46.7	0.000	1.000
Positive	53.3	53.3		
VEGF				
Negative	20.0	0.0	3.333	0.068
Positive	80.0	100.0		
Ki-67				
Negative	40.0	46.7	0.136	0.713
Positive	60.0	53.3		
MDM2				
Negative	6.7	46.7	6.136	0.013
Positive	93.3	53.3		

ICT: Intracranial tension, ECP: Endothelial cell proliferation, GT: Glomeruloid tuft, HPF: High-power field, EGFR: Epidermal growth factor receptor, VEGF: Vascular endothelial growth factor, MDM2: Mouse double minute 2 homolog, FND: Focal neurological deficits, NV: Nausea and vomiting, BNC: Behavioral and neurocognitive change, HP: Histopathological

factors as both showed a significant correlation with the patient survival. A marginal correlation was found between the extent of resection of the tumor and patient survival. All other clinical, histopathological, and immunohistochemical parameters included in this study did not show any significant correlation with the patient survival. However, the small sample size remains a limitation of this study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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