ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

The Relationship of Clinicopathological Features, Ki-67 Proliferation Index, IDH1, EGFR, and p53 Mutations with Prognosis in Glioblastomas

Glioblastomlarda Klinikopatolojik Özelliklerin, Ki-67 Proliferasyon Endeksi, IDH1, EGFR ve p53 Mutasyonlarının Prognoz ile İlişkisinin Değerlendirilmesi

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ABSTRACT

Aim: *Glioblastoma is the most common malignant brain tumor. In the literature, few reports examine the relationship between morphologic findings of glioblastomas and patient prognosis. This study investigates the effects of morphological conclusions, IDH1, EGFR, p53 expressions, and Ki-67 proliferation index on patient prognosis in glioblastoma patients.*

Material and Method: *This study evaluated 166 patients diagnosed with glioblastoma between 2014 and 2017 in the Faculty of Medicine, Department of Pathology. Morphological findings (broad necrosis, focal necrosis, palisaded necrosis, microvascular proliferation, atypia, cellularity, lymphocyte infiltration, mitosis, cell type) were classified according to their presence/absence or intensity. IDH1, EGFR (Epidermal Growth Factor Receptor), p53 expressions, and Ki-67 proliferation indexes were grouped according to staining/non-staining conditions or staining percentages. The relationship between these findings and postoperative survival time was investigated.*

Results: *There was no statistically significant relationship with survival between morphologic findings, IDH1, EGFR, p53 expressions, and Ki-67 index.*

Conclusion: *Morphological and immunohistochemical features are insufficient to predict glioblastoma prognosis. Referring to molecular methods in estimating the prognosis may be more appropriate.*

Keywords: *glioblastoma; IDH1; p53; ki-67; EGFR*

ÖZET

Amaç: *Glioblastoma en sık görülen malign beyin tümörüdür. Literatürde glioblastomların morfolojik bulguları ile hasta prognozu arasındaki ilişkiyi inceleyen az sayıda yayın bulunmaktadır. Bu çalışmanın amacı, glioblastoma hastalarında morfolojik bulgular, IDH1, EGFR, p53 ekspresyonları ve ki-67 proliferasyon endeksinin hasta prognozu üzerindeki etkilerini araştırmaktır.*

Materyal ve Metot: *Bu çalışmada Tıp Fakültesi Patoloji Anabilim Dalı'nda 2014–2017 yılları arasında glioblastoma tanısı konulan toplam 166 hasta değerlendirildi. Morfolojik bulguların (geniş nekroz, fokal nekroz, palizadlanan nekroz, mikrovasküler proliferasyon, atipi, sellülarite, lenfosit infiltrasyonu, mitoz, hücre tipi) varlığı/yokluğu veya yoğunluğuna göre sınıflandırıldı. IDH1, EGFR, p53 ekspresyonları ve ki-67 proliferasyon endeksleri boyanma/boyanmama durumları veya boyanma yüzdelerine göre gruplandırıldı. Bu bulgular ile postoperatif sağkalım süresi arasındaki ilişki araştırıldı.*

Bulgular: *Morfolojik bulgular, IDH1, EGFR, p53 ekspresyonları, ki-67 endeksi ile hasta sağkalımı arasında istatistiksel olarak anlamlı bir ilişki bulunamadı.*

Sonuç: *Morfolojik ve immünohistokimyasal özellikler glioblastomaların prognozunu tahmin etmek için yeterli olmayabilir. Prognozu tahmin etmede moleküler yöntemlere başvurmak daha uygun olabilir.*

Anahtar kelimeler: *glioblastoma; IDH1; p53; ki-67; EGFR*

Introduction

Glioblastoma is a grade IV diffuse astrocytic tumor. It is the most common malignant brain tumor in adults, constituting 15% of all intracranial neoplasms and 45–50% of primary malignant brain tumors[1,2]. While diagnosing Central Nervous System (CNS) tumors was based solely on microscopic morphological features, the World Health Organization (WHO) suggested in 2016 that molecular parameters should be used in central nervous system tumor classification and morphological features[3]. As knowledge of the molecular basis of tumors

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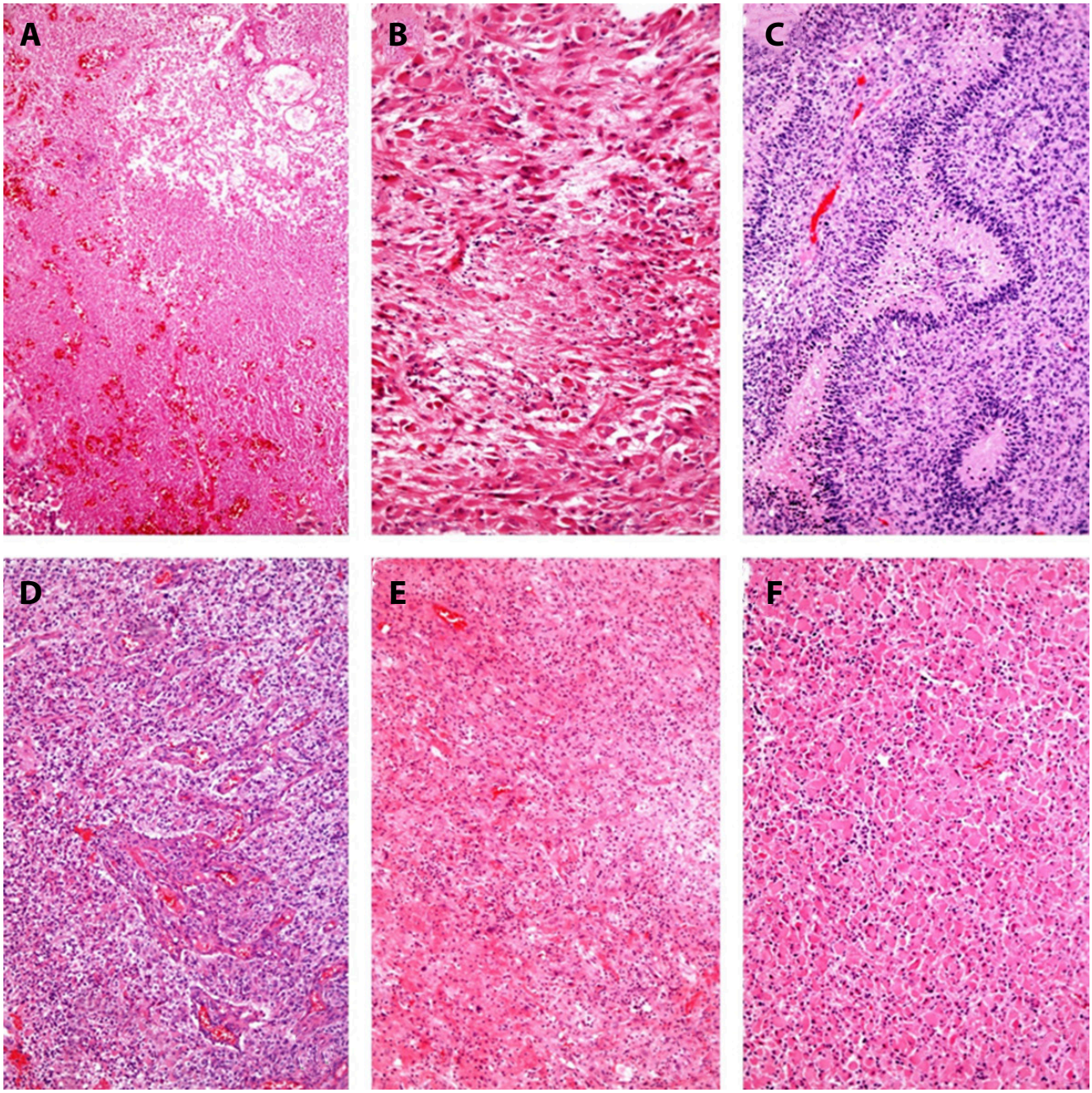

Figure 1. a–f. *Large necrosis (×200)(a). Focal necrosis (×200)(b). Palisaded necrosis (×200)(c). Microvascular proliferation (×200)(d). Lymphocyte infiltration (×400) (e). Gemystocytic cell presence (×200)(f).*

increases, the latest 2021 classification has provided a more precise classification of many CNS tumors[4]. In the previous classification, diffuse gliomas of adults were divided into 15 entities. In the latest classification, it is divided into only three groups: astrocytoma, IDH-mutant; oligodendroglioma, IDH-mutant and 1p/19q codeleted; and glioblastoma, IDH-wild type[4].

Isocitrate dehydrogenase 1 (IDH1) mutation status is one of the most important prognostic factors determining patient survival. Studies have shown that IDH-mutant type glioblastoma has a better prognosis than IDH-wild type glioblastoma and IDH-wild type anaplastic astrocytoma[5].

Glioblastomas progress rapidly despite surgical resection, radiotherapy, and treatment consisting of the chemotherapeutic agent temozolomide (TMZ), and the average survival time is 15 months [6–8]. This study investigates the effects of immunohistochemically detected p53, IDH1, EGFR mutations, Ki-67 expression, and clinicopathological features on patient prognosis.

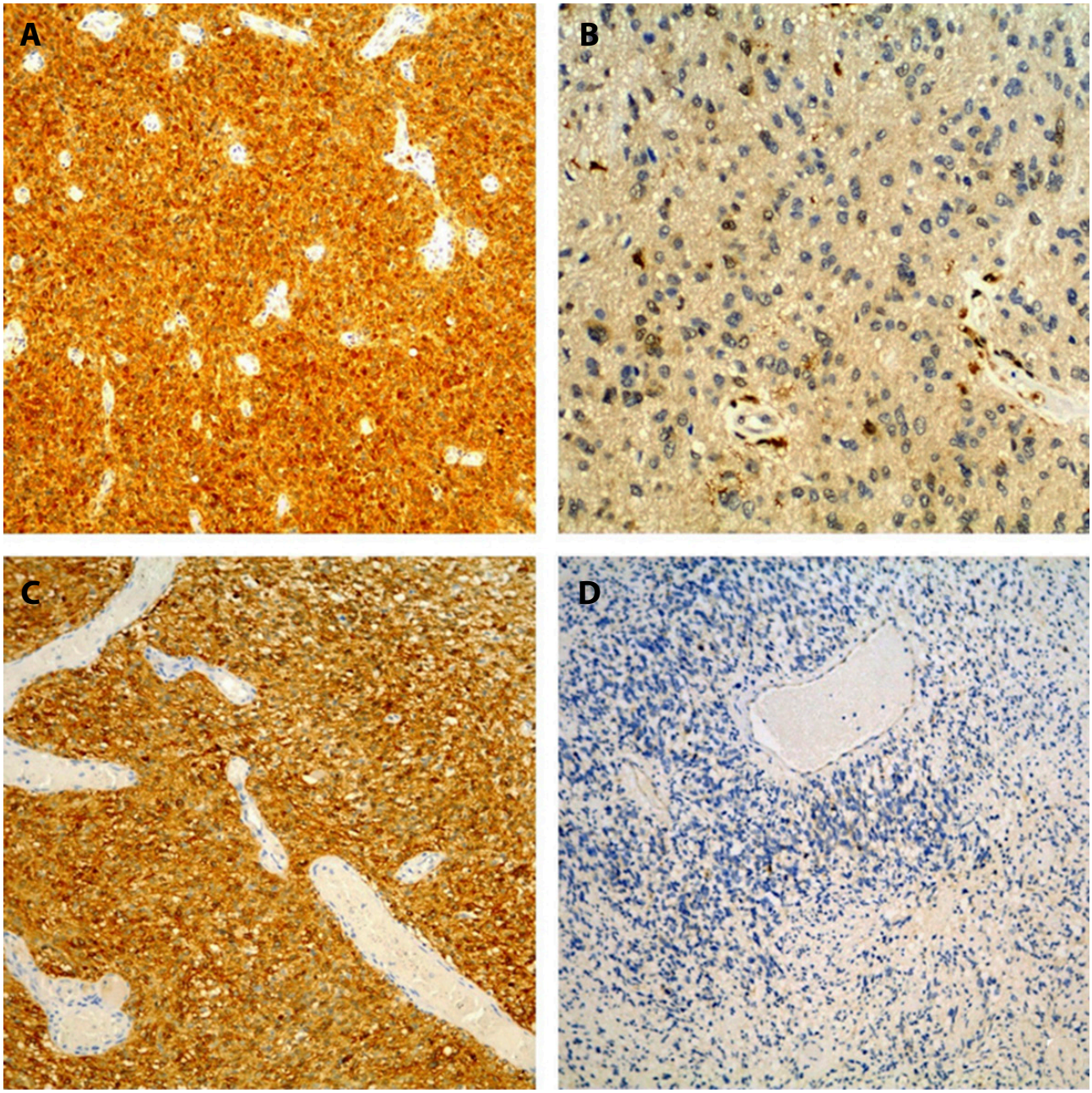

Figure 2. a–d. *Positive staining with IDH1 (×400)(a). Negative staining with IDH1 (×400)(b). Positive staining with EGFR (×400)(c). Negative staining with EGFR (×400)(d).*

Materials and Methods

The records of patients diagnosed with glioblastoma in 2014–2017 in the Pathology Department of the Faculty of Medicine were accessed by examining the electronic hospital database. Age, gender, clinical and radiological information of the patients were taken from the hospital database and recorded.

While patients diagnosed with glioblastoma and whose clinical and radiological data can be accessed regardless of age and gender were included in the study, patients without clinical and radiological data, tissues with poor fixation-follow-up quality, and tissues for which Hematoxylin-Eosin (HE) stained preparations were not available were excluded from the study. Survival data between the days of the operation and September 2019 were used to calculate the prognosis. The survival information of the patients was accessed through the Death Notification System.

Paraffin blocks and preparations were obtained from the archive. Hematoxylin-Eosin stained preparations

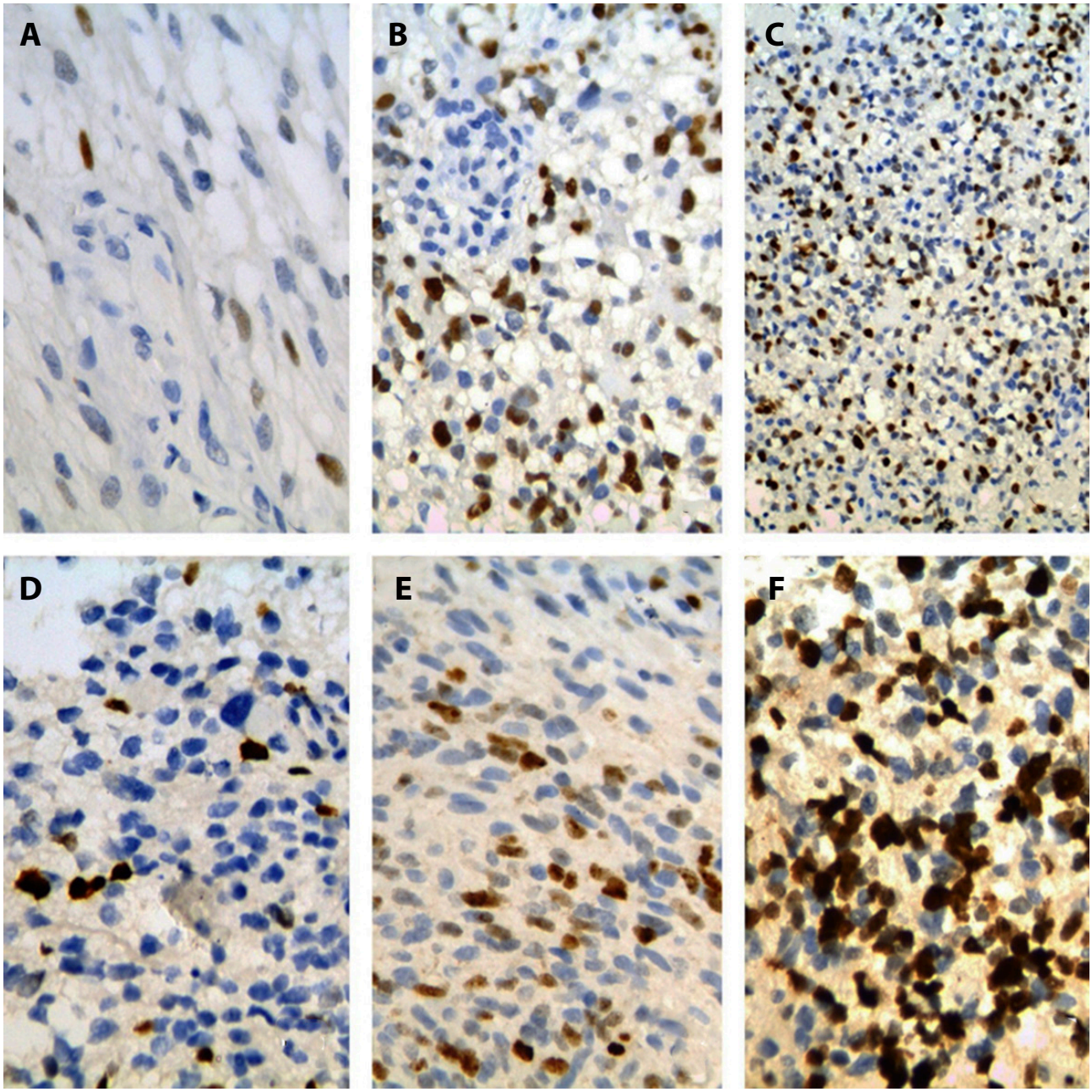

Figure 3. a–f. *Score 1 staining with p53 (×400)(a). Score 2 staining with p53 (×400)(b). Score 3 staining with p53 (×400)(c). Score 1 staining with Ki-67 (×400)(d). Score 2 staining with Ki-67 (×400)(e). Score 3 staining with Ki-67 (×400)(f).*

belonging to the patients were classified according to the presence/absence or density of morphological findings (large necrosis, focal necrosis, palisaded necrosis, microvascular proliferation, atypia, cellularity, lymphocyte infiltration, mitosis, cell type) (Fig. 1). The most suitable preparations for immunohistochemical staining were selected, and for immunohistochemical staining, 4 micrometer thick sections were cut from these blocks. The properties of the immunohistochemical marker used in the study are given in Table 1. For IDH1 evaluation, cases with significant cytoplasmic staining in tumor cells were positive, whereas cases without staining or weak staining in tumor cells were considered negative. For EGFR evaluation, cases with cytoplasmic and membraneous staining of tumor cells were evaluated as positive, while cases without staining were considered negative (Fig. 2). P53 and Ki-67 scores were classified as follows: score 1: <10% positivity of tumor cells, score 2:10–30% positivity of tumor cells, and score 3: >30% positive tumor cells (Fig. 3).

Table 1. *The properties of the immunohistochemical marker*

Immunohistochemical marker	Brand	Clone	Dilution	Positive control
IDH1	Histonova	H09	1/20	Glial tumor
p53	Novocastra	DO-7	1/800	Colon adenocarcinoma
EGFR	Novocastra	EGFR.113	1/10–1/20	Placenta
Ki-67	Novocastra	SP 6	1/100–1/500	Lymph node

Table 2. *The clinical findings of the cases*

Age	
The mean age	59.88
Distribution range	19–85
Gender	
Male	96 (57.8%)
Female	70 (42.2%)
Most common localizations	
Frontal lobe	53 (31.9%)
Temporal lobe	52 (31.3%)
Parietal lobe	31 (18.7%)
Multifocality	
Present	28 (16.9%)
Absent	125 (75.3%)
Radiotherapy	
Received treatment	104 (62.7%)
Not received treatment	49 (29.5%)
Chemotherapy	
Received treatment	86 (51.8%)
Not received treatment	67 (40.4%)
Survival	
Alive	14 (8.4%)
Dead	152 (91.6%)

Table 3. *The histopathological findings of the cases*

Cellular composition	
Presence of gemystocyte	78 (47%)
Presence of giant cells	30 (38.1%)
Presence of oligodendroglial cells	77 (46.4%)
Presence of sarcomatous component	6 (3.6%)
Presence of small cell component	42 (25.3%)
Classification according to Primary and Secondary	
Primary glioblastoma	56 (33.7%)
Secondary glioblastoma	70 (42.2%)
Broad necrosis	
Present	135 (81.3%)
Absent	31 (18.7%)
Focal necrosis	
Present	91 (54.8%)
Absent	75 (45.2%)
Palisading necrosis	
Present	81 (48.8%)
Absent	85 (51.2%)
Microvascular proliferation	
Present	126 (75.9%)
Absent	40 (24.1%
Cellularity increase	
Present	94 (56.6%)
Absent	72 (43.4%)
Marked atypia	
Present	23 (13.9%)
Absent	143 (86.1%)
Mitotic count	
<10	107 (64.5%)
10–25	37 (22.3%)
>25	22 (13.3%)
Lymphocytic infiltration	
Present	67 (40.4%)
Absent	99 (59.6%)

Statistical Analysis

The statistical analyses of the results were performed using IBM Statistical Package for Social Sciences (SPSS) statistical software package. Continuous variables were expressed as median (minimum-maximum) and mean ± standard deviation, while categorical variables were expressed as n (%). Pearson's chi-square test was used to compare categorical variables. The log-Rank test was used to determine the difference between survival times, and average survival times were given by the Kaplan-Meier method. P values less than 0.05 were considered statistically significant.

Results

Our study evaluated one hundred sixty-six cases diagnosed with glioblastoma between 2014 and 2017. The mean age of 166 cases was 59.88, and the median age was 61 years. The ages of the cases ranged from 19 to 85 years. 42.2% (n=70) of the cases were women; 57.8% (n=96) were male. The male/female ratio was found to be 1.37/1. The cases are most frequently localized in the frontal lobe, temporal lobe, and parietal lobe, and their incidence rates are 31.9% (n=53), 31.3% (n=52), and 18.7% (n=31), respectively. 42.2% (n=70) of 126 cases (75.9%) were IDH1 mutant glioblastoma; 33.7% (n=56) was IDH1 wild glioblastoma; IDH1 staining could not be applied to the remaining 40 cases (24.1%) because it was exhausted in our department or nonspecific results were obtained

Table 4. *The staining rates with immunohistochemical markers*

IDH1	Positive	42.2% (n=70)
	Negative	33.7% (n=56)
p53	Score 1	61.4% (n=102)
	Score 2	15.1% (n=25)
	Score 3	18.7% (n=31)
Ki-67	Score 1	10.2% (n=17)
	Score 2	56% (n=93)
	Score 3	30.1% (n=50)
EGFR	Positive	54.2% (n=90)
	Negative	3% (n=5)

from the staining. The mean age of IDH1 mutant cases was 60.26, while the mean age of IDH1 wild cases was 59.44. According to the data until September 2019, 8.4% (n=14) of 166 cases were still alive, and 91.6% (n=152) had died. The clinical findings of the cases are given in Table 2, histopathological findings in Table 3, and the staining rates with immunohistochemical markers are given in Table 4.

The patients' mean overall survival (OS) was 15.52±1.22 months, and the median was 11 months. No significant difference was found between overall survival times according to cellular components. According to the other histopathological characteristics of the cases (large necrosis, focal necrosis, palisaded necrosis, microvascular proliferation, pronounced cellularity, significant atypia, number of mitoses, lymphocytic infiltration), no significant difference was detected between overall survival times. P values were p=0.123, p=0.951, p=0.112, p=0.668, p=0.765, p=0.845, p=0.097, p=0.875, respectively.

There was a statistically significant difference between the overall survival times of the patients according to their focality, radiotherapy status, and chemotherapy status ($p < 0.05$).

There was no statistically significant difference between the overall survival times of the cases according to the expression of IDH1 and EGFR. P values were p=0.896 and p=0.268, respectively. No statistically significant difference was found between the groups regarding overall survival according to p53 expression and the Ki-67 index. P values were p=0.110 and p=0.241, respectively.

Discussion

Glioblastoma is the most common malignant brain tumor in adults, constituting approximately 45–50% of primary malignant brain tumors[1,2]. In the study conducted by Bouvier et al.[9] on 63 glioblastomas, the mean age of the cases was 56±13 years, and in the study by Popova et al.[10], the mean age was 48 years. Our study's average age of 166 glioblastoma cases was 59.88 years.

Isocitrate dehydrogenase mutations were first described in 2008 and reported by Parsons[11]. In this study, the authors said that patients with IDH1 mutation were mostly secondary glioblastoma, the patients were young, and their overall survival rate was higher. IDH1 mutation is observed in less than 10% of primary glioblastoma; and seen in about 70% of secondary glioblastoma. IDH1 antibody results were available in 126 of 166 glioblastoma cases in our study, and we found IDH1 expression in 70 (42.2%) of 126 cases. IDH1 staining could not be applied to the remaining 40 cases (24.1%) because it was exhausted in our department, or nonspecific results were obtained from the staining. The presence of IDH1 and IDH2 mutations in glioblastomas is a good prognostic factor. Hartmann et al.[5], in their study of 382 cases, showed that the prognosis was better in tumors with IDH1 mutations than in tumors without IDH mutations. Our study did not observe a statistically significant difference in mean overall survival between patients with IDH1 mutation and patients without IDH mutation.

In the literature, the results of studies that investigated the effect of the Ki-67 index on the clinical course of GBM patients were variable. In our study, we scored Ki-67 values in three groups, as in the study performed by Popova et al.[10] on 219 glial tumors, and we did not detect a statistically significant difference between Ki-67 values and overall survival. It could be because the sampled tissue did not reflect the entire tumor, the tumors had heterogeneous characteristics, and Ki-67 evaluations differed between the observers.

Epidermal growth factor receptor amplification is seen in approximately 40% of primary glioblastomas. The results in the literature investigating the effect of EGFR on the clinical course of GBM patients were variable. In the study conducted by Bouvier-Labit et al.[9] on 63 glioblastoma cases, no significant relationship was found between EGFR and survival. When we looked at the relationship between EGFR expression status and overall survival, no statistically significant difference was found between them. This was thought to be due to the poor compatibility of EGFR immunohistochemistry results with EGFR amplification.

P53 and IDH mutations occur in the early stage of gliomagenesis. The results in the literature investigating the effect of p53 mutation status on the clinical course of GBM patients were variable. In our study, we divided the p53 score into three groups, as in the study performed by Popova et al.[10]. When we examined the relationship between p53 staining rate and overall survival, we did not find a statistically significant difference.

The diagnosis of glioblastoma is based on tissue pattern rather than cell type. There are few studies examining the effects of morphological findings on prognosis. Two comprehensive malignant glioma studies show that necrosis results in a significantly worse prognosis in anaplastic glioma with both oligodendroglial and astrocytic components; patients with tumor necrosis were found to have considerably shorter mean survival than patients without tumor necrosis[12,13]. Bigner et al.[14] examined the relationship of histopathological features with EGFR amplification status and found no significant relationship between necrosis, palisaded necrosis, multinuclear giant cells, and microvascular proliferation. It was found to be borderline significant with lymphocytic infiltration. In the study of Palma et al.[15], consisting of 42 cases, it was reported that lymphocytic infiltration in the tumor positively affected survival. In our study, we classified the cases according to the presence/absence of morphological features such as large necrosis, focal necrosis, palisaded necrosis, microvascular proliferation, cellularity, atypia, infiltration, and cell type, and we examined their overall survival. We detected that none of the morphological findings had any effect on survival.

Ahmadipour et al.[16] investigated the effect of proliferation markers and multifocality on survival in their study of 565 cases. They found the overall survival to be 13.5 months in single lobe involvement, 11.4 months in multifocal involvement of the same hemisphere, and 9.3 months in contralateral hemisphere involvement. As a result of their studies, they mentioned that multifocality can be used as an independent prognostic factor. Our study found that the mean overall survival time in multifocal cases was 9.857±1.444 months, and the mean overall survival time was 16.896±1.521 months in unifocal cases. We observed a statistically significant difference between the overall survival times of the cases according to their focality.

In the phase 3 study conducted by Perry et al.[17], adding TMZ to short-term radiotherapy was associated with significantly longer survival. In our study, we found the mean overall survival time in patients who received radiotherapy was 18.294±1.565 months, and it was 9.347±1.592 months in patients who did not receive radiotherapy; the average overall survival time in patients who received chemotherapy was 20.374±1.786 months, and it was 9.164±1.246 months in patients who did not receive chemotherapy. We observed a statistically significant difference between the overall survival times of the patients according to their radiotherapy and chemotherapy status.

Conclusion

Our study investigated the effects of clinical and morphological features, IDH1, EGFR, Ki-67, and p53 expression states on patient prognosis in glioblastoma cases. As a result, the data showed that the morphological features, IDH1, p53, EGFR expressions, and Ki-67 proliferation index did not significantly affect glioblastoma survival.

The survival of patients who received radiotherapy and TMZ chemotherapy was statistically significantly longer than those who did not receive treatment. It had been observed that multifocality had a negative effect on patient survival. Morphological and immunohistochemical features are not sufficient to predict the prognosis of glioblastomas. Referring to molecular methods in estimating the prognosis may be more appropriate.

Statement of Ethics

The approval for this study was obtained from Uludag University, Faculty of Medicine, Ethics Committee of Medical Research, dated April 09, 2019, and numbered 2019–7/28.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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