Prognostic Value of IDH-1, PTEN and EGFR Expression in High Grade Gliomas

Meryem AKTAN[1], Sidika FINDIK[2], Gul KANYILMAZ[1], Berrin Benli YAVUZ[1], Mehmet KOC[1]

[1] Necmettin Erbakan University, Faculty of Medicine, Department of Radiation Oncology
[2] Necmettin Erbakan University, Faculty of Medicine, Department of Pathology, Konya, TURKEY

ABSTRACT

Gliomas are the most common primary brain tumors in adults. Despite advances in modern diagnostic procedures and therapies, the prognosis is still poor. To improve survival and the knowledge about the biological and clinical presentation of gliomas, more individualised and targeted treatments are needed. The aim of this study was to correlate the immunostaining patterns of isocitrate dehydrogenase-1 (IDH-1), phosphatase and tensin homolog (PTEN) and epidermal growth factor (EGFR) with progression-free survival (PFS) and overall survival (OS) in high grade gliomas patients. We analized 60 high grade gliomas who underwent surgery and standard chemoradiotherapy. Immunohistochemical methods were used to classify the IDH-1 gene mutation presence, staining patterns of PTEN and EGFR in tumor samples of the diagnosis. Median follow-up time was 18.9 months. There was significant relation between IDH-1 mutation and OS. Median OS was 37.9 months for patients with IDH-1 mutation, 12.4 months for patients with no mutation ($p< 0.001$). Median PFS was 29.8 months for patients with no IDH-1 mutation and 70.4 months for patients with IDH-1 mutation ($p= 0.03$). There was no significant relation between PTEN and EGFR immunopattern and OS or PFS in univariate analysis. However, there was significant relation between immunoreactivity of PTEN and OS ($p= 0.03$), immunointensity of PTEN and OS ($p= 0.02$) in multivariate analysis. In conclusion, the relationship between EGFR mutation and OS and PFS can also be demonstrated by studies with more patients.

Keywords: Glioma, IDH, EGFR, PTEN

ÖZET

Yüksek Dereceli Gliomlarda IDH-1, PTEN ve EGFR Ekspresyonunun Prognostik Önemi

Gliomalar erişkinlerde en sık görülen primer beyin tümörleridir. Modern teşhis prosedürleri ve tedavilerindeki ilerlemelere rağmen, prognoz hala zayıftır. Gliomaların biyolojik ve klinik seyri hakkında daha detaylı bilgi edinmek ve sağkalıma etkilerini belirleyebilmek için kişiye özel ve hedefe yönelik tedavilere ihtiyaç vardır. Bu çalışmanın amacı, isocitrate dehydrogenase-1 (IDH-1), fosfataz ve tensin homolog (PTEN) ve epidermal büyüme faktörünün (EGFR) immün boyama modellerini progresyonsuz sağkalım (PS) ve genel sağkalım (GS) ile ilişkilendirmektir. Standart cerrahi ve kemoradyoterapi uygulanan 60 yüksek dereceli glioma hastası analiz edildi. İmmünhistokimyasal yöntemler, tanıdaki tümör örneklerinde IDH-1 gen mutasyonu varlığını, PTEN ve EGFR'nin boyanma paternlerini sınıflandırmak için kullanıldı. Ortanca takip süresi 18.9 aydı. IDH-1 mutasyonu ve GS arasında anlamlı ilişki vardı. Ortanca GS, IDH-1 mutasyonu olan hastalar için 37.9 ay, mutasyon saptanmayan hastalar için 12.4 ay idi ($p< 0.001$). Ortanca PS, IDH-1 mutasyonu olmayan hastalar için 29.8 ay ve IDH-1 mutasyonu olan hastalar için 70.4 ay idi ($p= 0.03$). Tek değişkenli analizde PTEN ve EGFR immünopatterni ile GS yada PS arasında anlamlı ilişki bulunamadı. Bununla birlikte, çok değişkenli analizde PTEN'in immünoreaktivitesi ile GS ($p= 0.03$), PTEN'in immünointensivitesi ve GS ($p= 0.02$) arasında anlamlı bir ilişki vardı. Sonuç olarak, EGFR mutasyonu ile GS ve PS arasındaki ilişki de hasta sayısının daha fazla olduğu çalışmalarla gösterilebilir.

Anahtar Kelimeler: Glioma, IDH, EGFR, PTEN

ORCIDs: Meryem Aktan: 0000-0002-0447-7784 Sidika Findik: 0000002-3364-7498 Gul kanyilmaz: 0000-0003-2333-353x
Berrin Benli Yavuz: 0000-0001-7816-4687 Mehmet Koc: 0000-0002-1433-8403

INTRODUCTION

Glioma is a term used to describe tumors derived from glial cells and divided into subgroups based on the histological morphology and similarity of their differentiated glial cells.[1] In adults, gliomas are the most common primary brain tumors for about 70% of the primary central nervous system (CNS) neoplasms.[2] High grades gliomas (HGGs), defined by the World Health Organization (WHO) as grade III and IV gliomas, compose the majority of malignant primary brain tumors.[3] Patients with high grade gliomas have poor prognosis. Patients with glioblastoma (GBM, WHO Grade IV) who are treated with maximum safe tumor resection and standard radiochemotherapy with temozolomide achieve a median survival of 14.6 months.[4]

The high mortality rate of gliomas is attributable to their high recurrence rate and invasiveness.[5] Despite advances in modern diagnostic procedures, novel surgical techniques, and subsequent radiochemotherapy, the prognosis of patients suffered from HGGs remains dismal.[6] To improve the survival further and the knowledge about the biological and clinical presentation of gliomas, more individualised and targeted treatments are needed. As a result of this, complementary diagnostic and prognostic markers based on conventional pathology are directed towards working with potential individualized therapeutic targets and specific tumor-associated molecular markers.[7]

Particularly in recent years, molecular findings are identified as important biological markers of therapeutic response and/or the clinical outcome. Some of them are mutation of isocitrate dehydrogenase-1 (IDH-1), phosphatase and tensin homolog (PTEN) and overexpression of epidermal growth factor receptor (EGFR).

Mutations in the IDH-1 gene were identified in specifically low and high grade gliomas, including GBM. IDH-1 mutations occur predominantly in younger individuals, with the secondary form of cancer and longer survival. The most common IDH-1 mutations in the protein occur in the amino acid position 132 and determine the substitution of arginine for histidine (R132H).[8,9,10]

Phosphatase and tensin homolog is a tumor suppressor gene which is located on chromosome 10q23.3. PTEN plays important roles in the regulation of cell prolifera¬tion, invasion, adhesion, DNA damage, repair and apoptosis. PTEN is one of the most commonly lost or downregulated genes implicated in brain, prostate and breast cancers.[11,12,13] The mutations of PTEN have been involved with malignant progression of glioma and correlated with a shorter overall survival in glioma.[14,15] However, the prognostic significance of PTEN in glioma is still controversial. Some studies have suggested that PTEN gene mutations in glioma were associated with poor survival.[16] Conversely, some studies have reported that PTEN gene mutations have no correlation with survival.[17]

High protein levels of EGFR occur in about 90% of astrocytic tumors. Alterations in EGFR gene are more commonly found in primary GBM, and this gene may also participate in tumor angiogenesis.[2] Rearrangements and amplifications of EGFR are highly indicative of high-grade gliomas, with a worse prognosis estimated from histopathologic grading.[18]

The aim of this study was to correlate the immunostaining patterns of IDH-1, PTEN and EGFR with progression-free survival (PFS) as well as overall survival (OS) in high grade gliomas patients.

PATIENTS and METHODS

The design of the present study was approved by the Ethical Committee and Institutional Review Board of the university (2014/19) where the study was conducted. Informed consent was obtained from all of the patients before the treatments.

Patient Characteristics:

Between January 2010 and January 2016, we retrospectively analyzed 60 newly diagnosed patients with histologically confirmed HGG from surgical samples at our university. The patients enrolled in this study met the following inclusion criteria for this study: 1) pathological diagnosis of HGG; 2) adult patients (> 18 years of age at diagnosis); 3) only patients submitted to the standard protocol of treatment (surgery with total or subtotal gross re-

Table 1. Technical specifications of antibodies used for immunohistochemical analysis.

Primary antibody	Clone	Dilution	Origin
IDH-1 R132H	ab212470	1/100	England
PTEN	ab31392	1/100	England
EGFR	ab231	1/100	England

IDH-1: isocitrate dehydrogenase-1; PTEN: phosphatase and tensin homolog; EGFR: epidermal growth factor receptor

section followed by initial radiotherapy (RT) with temozolomide (TMZ) (75 mg/m^2/day) chemotherapy); and than chemotherapy (200 mg/m^2 TMZ), 4) available and consistent data from the patients' charts and from the histopathological sections. Patients and/or surgical samples that did not fulfill all the listed criteria were excluded from the study. Using the patients charts and electronic medical records, the following data were obtained: sex, age, tumor pathology, tumor location, size and extent of surgery, Karnofsky performance status (KPS) before RT, RT technic, date of recurrence, neurological classification, last follow-up or death and survival time.

Histopathological Analysis:

The specimens had been fixed in 10% formaldehyde and processed for paraffin embedding, 4 μm thick sections mounted on to posi¬tively charged slides (Slides ISOTHERM Technical Laboratory Glass Materials, Germany) and were stained with hematoxylin and eosin (H&E). Histopathological examination, typing and grading were performed by an experienced neuropathologist, according to the last edition of the World Health Organization (WHO) classification of CNS tumors.[19] Diagnosis of GBM was confirmed by identifying at least three of the following features in astrocytic tumors: mitotic figures, cellular atypia, microvascular proliferation and/or necrosis.

Immunohistochemistry:

Immunocytochemistry was performed using an automated avidin biotin system (Ventana BenchMark XT; Ventana Medical Systems, Tucson, AZ, USA) by using the antibodies shown in Table 1. In all reactions, positive and negative controls of the immunohistochemical reaction were used. The immunostaining patterns for PTEN and EGFR were evaluated considering both cellular and tissue distribution. The number of immunopositive cells in ten high power (40x) areas was counted and the percentage of immunopositive cells was estimated. The ratio of positive cells/total number of cells was calculated for each field. The mean value of the ten fields obtained from a section was considered as the estimated percentage of immunoreactivity assigned to the tumor sample and percentage interval designated as class 1: 0-25%; class 2: 26-50%; class 3: 51-75%; and class 4: >75%.[20] Cytoplasmic positivity was noted with irregular distribution in the lesion, immunointensity ranging from weak to strong and categorized in 4 classes as following; 0 (-): negative; 1(+): light or focal; 2 (++): moderate; 3 (+++): strong positive cells.21 For the evaluation of IDH-1 mutation, IDH-1 R132H antibody has been used, according to the presence or absence of neoplastic cells with cytoplasmic and nuclear staining was interpreted as immunopositive. The results were accepted as positive if a focal or diffuse immunopositivity was detected and negative if no tumor cell was immunopositive.[8,21]

Statistical Analysis

The clinical endpoints were OS and PFS. OS was defined as the time between the diagnosis date and death or last follow up. PFS was defined as the time between the diagnosis date and recurrence. The OS and PFS rates were generated using Kaplan–Meier methods. The possible factors identified by univariate analyses were further entered into the Cox regression analysis to determine independent predictors of PFS and OS. The relation between IDH-1, PTEN, EGFR mutations and development of progressionon at primary tumor and death rates were analyzed using Fisher's exact test. Statistical analyses were performed with the SPSS for Windows software program version 18 (Chicago, USA) and a p value less than 0.05 was regarded as significant.

Table 2. Univariate and multivariate analysis for factors related to overall survival (OS)

Factor analyzed	Predictive factors	Median OS Months (Range)	p- value for multivariate analysis	p- value for univariate analysis
Gender	Male	21.9 (14.3-29.5)	0.4	0.05
	Female	16.8 (9.9-23.8)		
Age	< 50 years	38.7(10.8-66.6)	0.001	0.04
	> 50 years	15.9 (9.8-22)		
Tumor pathology	AA	17.6 (5.6-29.5)	0.05	0.8
	GBM	17.1 (10.4-23.8		
Tumor size	< 5 cm	17.6 (12.7-22.5)	0.1	0.01
	> 5 cm	21.9 (5.4-38.3)		
Type of surgery	Total	26.3 (16.7-35.9)	<0.001	0.03
	Subtotal	15.7 (10-21.3)		
RT technic	3D CRT	12.4 (7.5-17.2)	0.02	0.3
	IMRT	23 (10.8-35.3)		
KPS before RT	< 70	8.4 (3-37.9)	0.001	0.001
	> 70	28.5 (3-64)		
Neurological classification	Class 1	32.9 (24.8-41.0)	0.001	0.001
	Class 2	12.1 (10.1-14.1)		
	Class 3	10.2 (2.0-18.4)		

AA: Anaplastic astrositoma; GBM: Glioblastome multiforme; 3D CRT: Three dimensional conformal radiotherapy; IMRT: Intensity modulated radiotherapy; KPS: Karnofsky performance status

RESULTS

A total of 60 patients with newly diagnosed and histologically confirmed high grade gliomas were included in this study. Of all patients, 17 (28%) were female, 43 (72%) were male and the median age was 58 (range: 19-83 years). Eighteen (30%) tumors were diagnosed as anaplastic astrositoma (AA) (WHO Grade III) and 42 (70%) tumors were GBM (WHO Grade IV). Five of the GBM patients were diagnosed as secondary GBM. Twenty two (37%) tumors were found in the temporal lobe, 20 (33%) in the frontal lobe, 14 (23%) in the parietal lobe and 4 (7%) in the occipital lobe. The median tumor size at the time of diagnosis was 5 cm (range: 2-9 cm). Twenty five (42%) patients were treated with gross total resection, 35 (58%) were treated with subtotal resection. Before the RT, median KPS was 80 (50-100). All patients were treated with standard therapy (surgery and then RT with concurrent TMZ chemotherapy, also known as the Stupp protocol22). Twenty four (40%) patients were treated with three- dimensional conformal radiotherapy (3DCRT), 36 (60%) patients were treated with intensity modulated radiotherapy (IMRT). Patients were classified according to neurological status. Patients who did not need care were defined as class 1(45%, n= 27), patients requiring home care were defined as class 2 (38.3%, n= 23) and patients who have hospitalized were class 3 (16.7%, n= 10).

Median follow-up time from diagnosis was 18.9 months (range: 3-70.4 months). Clinical and radiologic progression has developed in 18 patients (30%). Median time to recurrence or progression was 16.2 months (range 5.7-27.7 months). Median overall survival was 17.6 months (range 5.6-29.5 months) for AA, 17.1 months for GBM (range 10.4-23.8 months) (p= 0.05). The 1 and 3-year survival rates were 72% and 38%, respectively for AA and 66% and 9%, respectively for GBM.

Results of univariate and multivariate analysis for factors related to OS are shown in Table 2.

Immunopositivity for the mutated form of IDH-1 R132H was observed in 16 (25%) of 60 malign glioma patient tumor samples. We identified the IDH-1 mutation in 6 of AA, 8 of primary GBM and 2 of secondary GBM patients. The seperation of pri-

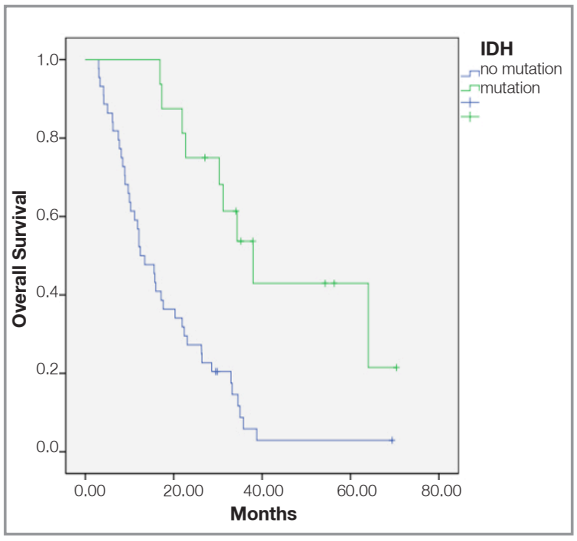

Figure 1. Overall survival of patients with or without IDH-1 R132H mutation

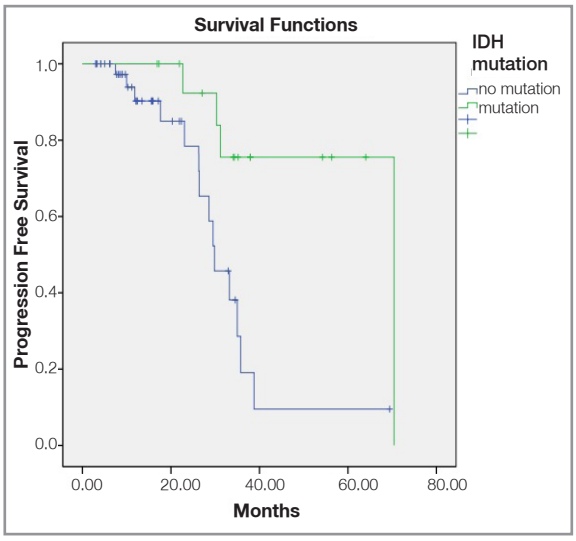

Figure 2. Progression- free survival of patients with or without IDH-1 R132H mutation

mary and secondary GBM was done on the basis of pathological confirmation. There was significant relation between IDH-1 mutation and OS. Median OS was 12.4 months (range 8.1-16.6 months) for patients with no mutation, and 37.9 months (range 27.7-48.8 months) for patients with IDH-1 mutation ($p< 0.001$) (Figure 1). Progression was detected in 18 patients. There was no IDH-1 mutation in 14 patients with progression, while IDH-1 mutation was detected in 4 patients with progression. Median PFS was 29.8 months (range 24.4-35.2 months) for patients with no mutation, on the other hand 70.4 months for patients with IDH-1 mutation ($p= 0.03$) (Figure 2).

Regarding the immune pattern, immunoreactivity and immunointensity results of PTEN and EGFR, results of IDH-1 mutation status are shown in Table 3 for OS and PFS. In both univariate and multivariate analysis, the significant effect of IDH1 mutation on OS and PFS was shown statistically. There was no significant relation between PTEN and EGFR immunopattern and OS in univariate analysis. However, there was significant relation between immunoreactivity of PTEN and OS ($p= 0.03$), immunointensity of PTEN and OS ($p= 0.02$) in multivariate analysis.

DISCUSSION

Today, despite the advances in treatment modalities including surgery, radiotherapy and chemotherapy, malign glioma patients have poor prognosis and overall survival time is even lower for GBM. Several studies have identified clinical and tumor characteristics and treatment-related prognostic factors like age, race, KPS, tumor localization, extent of surgical resection and post-operative chemoradiotherapy.[23,24] Alterations in cancer cell metabolism are now well accepted as one of the principal hallmarks of the carcinogenesis and tumor progression. Therefore, markers in the cellular dimension are important for understanding the biology and behavior of the cancer cell and developing new strategies accordingly. Methylation of O-6-methylguanine-DNA methyltransferase (MGMT) promoter has been found as a predictive molecular marker for GBMs' response to treatment.10 The IDH-1 gene is an important inducer that causes the WHO classification of brain glial tumors to change in 2016.[19] Several studies reported a favorable impact of the IDH mutation in malign glioma patients.

The IDH-1 mutation can be seen in 32% of central nervous system tumors, 23% of bone tumors, 8% of biliary tract tumors, 6% of thyroid cancer, and many other tumor types. IDH-1 mutations are pre-

Table 3. Results for overall survival and progression-free survival differences in relation to IDH-1, PTEN and GFR status in univariate and multivariate analysis

	Patients n (%)	Median OS Months (range)	p-value in univariate analysis	p-value in multivariate analysis	Median PFS Months (range)	p-value in univariate analysis	p-value in multivariate analysis
IDH-1 mutation							
No mutation		12.4 (8.1-16.6)	<0.001	<0.001	29.8 (24.4-35.2)	0.03	0.05
Mutation (+)		37.9 (27.7-48.1)		70.4			
PTEN- immunoreactivity							
0-25%	14 (23.3%)	15.5 (0-33.2)			34.9 (18.7-51)		
26-50%	16 (26.7%)	12.4 (4.7-20.0)	0.2	0.03	30.2 (25.9-34.6)	0.9	0.2
51-75%	16 (26.7%)	17.1 (13.8-20.4)			35.7 (31-40.4)		
>75%	14 (23.3%)	22.7 (8.2-37.2)			31.1 (27.7-34.5)		
PTEN-immunointensity							
Negative	6 (10%)	15.5 (2.4-28.7)			34.9 (15.9-54)		
+ (light/ fokal)	10 (16.7%)	17.6 (1.4-33.8)	0.3	0.02	70.7	0.3	0.1
++ (moderate)	30 (50%)	21.9 (5.4-38.4)			55.5 (29.8-41.6)		
+++ (strong)	14 (23.3%)	15.7 (9.4-22.0)			31.6 (20.4-32.1)		
EGFR-immunoreactivity							
0-25%	19 (31.7%)	12.4 (10.7-14.1)			35.7 (19.2-52.2)		
26-50%	14 (23.3%)	26.2 (12.3-40.1)	0.7	0.2	70.4	0.8	0.1
51-75%	18 (30%)	21.9 (12.4-31.4)			34.9 (27.4-42.5)		
>75%	9 (15%)	16.8 (0-30.9)			31.1(28.4-33.8)		
EGFR- immunointensity							
Negative	11 (18.3%)	12.1 (8.0-16.1)			28.5 (6.3-50.7)		
+ (light/ fokal)	11 (18.3%)	21.9 (10.4-33.3)	0.7	0.8	34.9 (21.8-48)	0.6	0.5
++ (moderate)	31 (51.7%)	22.3 (12.5-32.1)			35.7 (27.5-43)		
+++ (strong)	7 (11.7%)	15.7 (0-33.0)			34.9 (28.9-41)		

OS: Overall survival; PFS: progression-free survival; PTEN: phosphatase and tensin homolog; EGFR: epidermal growth factor receptor; IDH: isocitrate dehydrogenase

sented mostly in diffuse astrocytomas (64%), AA (49%), GBM (9%) and oligodendrogliomas (2%) in primary brain tumors group.[26] Since the IDH mutation has been shown for the first time in GBM, it has opened new paths to understand GBM and cancer biology.[27] IDH-1 mutations are also important for their clinical consequences. A recent meta-analysis confirmed the prognostic role of IDH-1/2 mutations in gliomas.[28] Recent studies revealed the important role of mutated IDH-1 in the assessment of astrocytoma and GBM patients' prognosis. The most common form of IDH1 mutations is R132H amino acid substitution with the prevalence of 90% among IDH1-mutant tumors.[29] Polivka et al. showed that IDH1 mutation R132H was a strong prognostic factor for patients with GBM.[30]

The result of our study supports that the IDH-1 R132H mutation was a strong prognostic factor for patients with malign glioma. In both univariate and multivariate analyzes, the presence of IDH1 mutation was found to be statistically significant for both OS and PFS. The proportion of IDH1- mutated tumors was higher in our study for primary GBM than in other similar studies. Six patients were AA, 8 were primary GBM and only 2 were secondary GBM. It was not possible to clinically distinguish between primary and secondary GBM, so we used the pathologic data for primary and

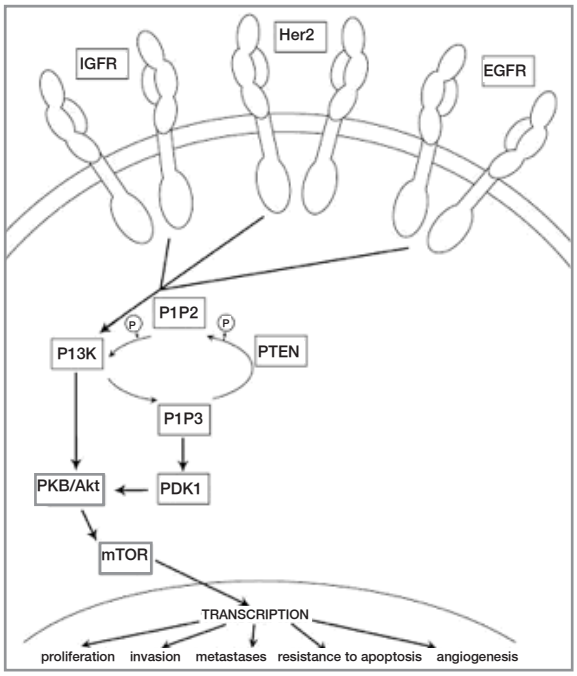

Figure 3. Role of EGFR and PTEN in the PI3K-AKT signaling pathway.

secondary GBM. The rate of progression in IDH-1 mutant patients was also lower in this study.

Several studies have addressed whether PTEN mutations could be regarded as a potential candidate in predicting clinical outcome of malign gliomas, whereas for GBM, studies reported inconsistent results. Some studies found strong correlation between PTEN mutation and survival.[31,32] On the other hand, some of them were not related.[33,34] Smith et al. showed that PTEN mutation was an important prognostic factor in patients with AA and in older patients with GBM, respectively.[32] In a meta-analys, Han et al. suggested that PTEN mutation was associated with poor prognosis in glioma patients. However, that finding was derived from data in observational studies and subgroup analysis indicated an association with only some group patients, and a strong correlation between PTEN mutation and poor prognosis was observed in AA, but in GBM, this relationship disappeared.[31] In the study conducted by Yang et al, a total of 11 patients with AA and 11 patients with GBM were enrolled. The median OS of patients with or without PTEN mutations was 67 and 99 weeks, respectively. However, the difference of the median survival time between the two groups was not statistically significant.[17]

Abdullah et al. analyzed 37 glioma patients and observed an insignificant relation between PTEN mutations and survival time. In that study, only seven patients showed PTEN mutations and the authors did not report the median survival time for each group. They analyzed the PTEN mutation in multivariate Cox regression analysis and showed that PTEN mutations did not have an impact on the survival time of patients with gliomas (p= 0.074). However, the PTEN mutations were only found in 7 of 37 cases (18.9%) and the authors defined that a significant association might be found if the sample size was larger than the sample in that study.35 On the other hand, Cario et al. showed that PTEN loss was not associated with worse OS in newly diagnosed GBM patients.[33] They examined the data of 73 newly diagnosed GBM patients treated in temozolomide era. Temozolomide is an alkylating chemotherapeutic agent that can penetrate the blood brain barrier and make it more effective in treating brain tumors than previously used chemotherapies.[36] There is evidence that temozolomide may be more effective in eradicating GBM cancer cells with PTEN loss. McEllin, et al. reported that human glioma cell lines with PTEN loss were more sensitive to temozolamide. They found that PTEN deficient glioma cell lines were inadequate for the repair of double-strand breaks induced by temozolomide, which caused more apoptosis.[37] This can explain why PTEN loss does not predict poor survival in GBM patients treated with temozolomide.

PTEN is a tumor suppressor gene that codes for a phosphatase that acts on PIP3 and regulates the PI3k/Akt pathway.[31] The PI3k/Akt pathway can be up-regulated due to mutation such as deletion and translocation in one or more of its downstream effectors (Figure 3). In addition to studies that demonstrate the independent prognostic significance of loss of PTEN in glioblastoma, there are studies that demonstrate the importance of EGFR amplification and Akt pathway activation in tumorigenesis.[30,38,39] The overexpression of EGFR contributes to the proliferation, differentiation, migration, survival, and increases tumor angiogenesis and invasiveness of cancer cells.[2] In support of this information Carico et al. found a correlation between PTEN loss and EGFR amplification. In that study, they found that PTEN status alone was not an independent prog-

nostic factor for OS. This finding simply implied a complex molecular relationship between PTEN and its regulators and effectors such as EGFR in the tumorigenesis of glioblastoma.[33] This suggests that there may be a complex molecular relationship between glioblastoma tumorigenesis and PTEN, its regulators and effectors like as EGFR. Xu et al. analyzed the effects of different PTEN mutations on disease-free survival (DFS) of patients with GBM, which reflects the effectiveness of treatment and the tendency for cancer recurrence. 586 GBM patients were selected for analysis in this study. They described four PTEN mutations; wild type, missense, nonsense and frameshift. They analyzed the effect of PTEN mutation on DFS. As a result, nonsense PTEN mutations were associated with significantly shorter DFS (median 3.8 months) compared to other mutations or wild-type genotype (median 7.2 months). On the contrary, missense or frameshift mutations showed no significant association with DFS. Moreover, overexpression of PTEN protein was also associated with shorter DFS (median 6.0 months) than other cases (median 7.0 months).[34]

As a conclusion; in this study, we analyzed the IDH1, PTEN and EGFR mutation of AA and GBM groups with immunohistochemical evaluation. According to statistical analyses, IDH-1 mutation was a positive prognostic factor for OS and PFS in malign gliomas. There was a significant relation between PTEN immunoreactivity, PTEN immunointensity and OS in multivariate analysis. However, there was no significant relation between immunoreactivity or immunointensity of EGFR and OS or PFS. Thus, the relationship between EGFR mutation and OS and PFS can also be demonstrated by studies with more patients.

There were some limitations of this study, including the semiquantitative assessment method by immunohistochemical staining and relatively small sample size. Futher research, using quantitative reverse transcription polymerase chain reaction with immunostaining may yield more sensitive results.

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Correspondence:
Dr. Meryem AKTAN
Necmettin Erbakan Üniversitesi
Meram Tip Fakültesi
Radyasyon Onkolojisi Anabilim Dali
42080 Meram, KONYA / TURKEY

Tel: (+90-332) 223 68 52
e-mail: meryemaktan@gmail.com