ORIGINAL ARTICLE

UDC: 616.831/.832-006-037
DOI: https://doi.org/10.2298/VSP241008042H

Immunohistochemical analysis of IDH1, ATRX, p53, and Ki-67 in glioblastoma and diffuse infiltrative glioma: therapeutic and prognostic correlation

Imunohistohemijska analiza IDH1, ATRX, p53 i Ki-67 kod glioblastoma i difuznog infiltrativnog glioma: terapijska i prognostička korelacija

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Abstract

Background/Aim. The most common molecular alterations in high-grade astrocytoma include mutation of the isocitrate dehydrogenase (*IDH*) gene, loss of 1p19q, and *p53* mutation. The aim of the study was to determine the prevalence of high-grade astrocytoma and glioblastoma and to examine the immunohistochemical staining patterns of IDH1, alpha-thalassemia/mental retardation X-linked (ATRX), p53, and Ki-67, as well as neoplastic morphological findings, treatment response, and effects on prognosis. **Methods.** Patients with *IDH*-mutant or *IDH*-wild-type glial tumors diagnosed at our center between January 2016 and January 2022 were included in the study. Patients were divided into groups according to age as follows: 7–40, 41–55, 56–64, and ≥ 65 years. The impact of demographic and clinical features on survival was analyzed. The effects of IDH1, p53, ATRX, and Ki-67 parameters on treatment success and prognosis were investigated. The Chi-square test was used to compare independent categorical variables, while the McNemar test was used for dependent categorical variables between the groups. Kaplan-Meier method and Cox proportional regression model (forward model) were used to estimate the mean and median survival times, failure rates, and hazard ratios. **Results.** In the study, 115 (56.1%) patients were male and 90 (43.9%) were female. The patients ranged in age from 7 to 84 years. There was no significant relationship between gender and age groups on survival (p = 0.113). However, there was a significant association between the glioblastoma grade and survival (p = 0.024). There were 65 (31.7%) patients who died. The mean overall survival of all patients was 45.2 months (median: 24 months). While 45 (21.2%) patients were found to have *IDH1* mutation, the number of patients negative for the mutation was 160 (78.8%). Overall survival was significantly longer in *IDH1*-positive patients (mean: 65.8, median: 80) than in *IDH1*-negative patients (mean: 25.7, median: 22) (p = 0.019). **Conclusion**. It was found that mutations of *IDH1* and *ATRX* and overexpression of p53 alone significantly impacted the prognosis of glioblastoma patients. However, radiotherapy and chemotherapy had a positive effect on patient survival. Survival can be increased by adding additional treatments to patients with *ATRX* mutations.

Key words:
astrocytoma; glioblastoma; ımmunohistochemistry; mutation; neoplasm grading; survival.

Apstrakt

Uvod/Cilj. Najčešće molekularne promene kod astrocitoma visokog stepena uključuju mutaciju gena izocitrat dehidrogenaze (*IDH*), gubitak 1p19q i mutaciju *p53*. Cilj rada bio je da se utvrde prevalencija astrocitoma visokog stepena i glioblastoma, i da se ispitaju obrasci imunohistohemijskog bojenja IDH1, *alpha-thalassemia/mental retardation X-linked* (ATRX), p53 i Ki-67, kao i morfološki nalazi neoplazije, odgovor na lečenje i efekti na prognozu. **Metode.** Studijom su obuhvaćeni bolesnici sa *IDH* mutiranim ili *IDH-wild-type* glijalnim tumorima, dijagnostikovanim u našoj ustanovi od januara 2016. do januara 2022. godine. Bolesnici su bili podeljeni u grupe prema starosti: 7–40, 41–55, 56–64 i ≥ 65 godina. Analiziran je uticaj demografskih i kliničkih osobina bolesnika na njihovo preživljavanje. Ispitivani su efekti parametara IDH1, p53, ATRX i Ki-67 na uspešnost lečenja i prognozu. Za poređenje nezavisnih kategorijalnih varijabli korišćen je *Chi-square* test, dok je za zavisne kategorijalne

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varijable između grupa korišćen McNemar-ov test. Kaplan-Meier i Cox proporcionalni regresioni model (*forward* model) su korišćeni za procenu vremena preživljavanja [srednja vrednost (SV) i medijana (M)], stopa neuspeha i stepeni rizika (*hazard ratios*). **Rezultati**. U studiji je bilo 115 muškaraca (56,1%) i 90 žena (43,9%). Bolesnici su bili životnog doba od 7 do 84 godine. U pogledu preživljavanja, nije utvrđena značajna povezanost između grupa formiranih na osnovu pola i starosti bolesnika (p = 0,113). Međutim, postojala je značajna povezanost između stepena glioblastoma i preživljavanja (p = 0,024). Preminulo je 65 (31,7%) bolesnika. Srednje ukupno preživljavanje svih bolesnika iznosilo je 45,2 meseca (M: 24 meseca). Dok je 45 bolesnika (21,2%) imalo *IDH1* mutaciju, bez mutacije je bilo 160 bolesnika (78,8%). Ukupno preživljavanje bilo je značajno duže kod *IDH1* pozitivnih bolesnika (SV: 65,8 meseci; M: 80 meseci), nego kod *IDH1* negativnih bolesnika (SV: 25,7 meseci; M: 22 meseca) (p = 0,019). **Zaključak.** Otkriveno je da mutacije *IDH1* i *ATRX* i prekomerna ekspresija samog p53 značajno utiču na prognozu kod bolesnika sa glioblastomom. Međutim, radioterapija i hemoterapija imale su pozitivan efekat na preživljavanje bolesnika. Preživljavanje bolesnika sa *ATRX* mutacijama se može povećati dodavanjem dodatnih tretmana.

Ključne reči:
astrocitom; glioblastoma; imunohistohemija; mutacija; neoplazme, određivanje stadijuma; preživljavanje.

Introduction

Diffuse glial tumors account for 15–20% of all central nervous system (CNS) tumors and 80% of high-grade CNS tumors [1–3]. High-grade astrocytoma (HGA), particularly glioblastoma (GB), classified as World Health Organization (WHO) grade 4, is one of the most aggressive and poorly prognostic primary brain tumors. GB is the most common malignant glial tumor in adults [4–6]. Although morphology is important based on the new classification system, classification is based on molecular features in the case of incompatibility between histological and molecular features [7]. The most common molecular alterations in gliomas include mutations in the isocitrate dehydrogenase (IDH) gene, loss of 1p19q, and *p53* mutations. According to the 2021 WHO classification, all *IDH*-wild-type gliomas are classified as GB, while *IDH*-mutated high-grade gliomas are classified as grade 4 astrocytoma. With this classification, GB accounts for 15% of all brain tumors and 45% of malignant brain tumors [8].

Three different IDH enzymes convert isocitrate to alpha-ketoglutarate by oxidative decarboxylation. More than 90% of IDH mutations in gliomas affect the *IDH1* gene, with the remainder affecting the *IDH2* gene [9]. Glioma formation begins with an *IDH* mutation at an early stage and continues with a *p53* mutation in astrocytomas and loss of 1p19q in oligodendrogliomas [10, 11].

IDH1 and *IDH2* mutations have emerged as early mutations in diffuse astrocytic tumors, and determination of IDH status by immunohistochemical (IHC) staining or sequencing has become a standard in diagnosing these tumors [12]. Approximately 80% of adult grade 2/3 gliomas carry *IDH1* or *IDH2* mutations [13]. The presence of an *IDH* mutation is more important than histologic grade and other molecular features in determining prognosis in high-grade gliomas (grade 3/4) [14]. In studies, *IDH* mutation is observed in 54–100% of diffuse astrocytomas (WHO grade 2), 66.1% of anaplastic astrocytomas (WHO grade 3), and 64–93% of oligodendrogliomas. In addition, mutations are present in 50–88% of secondary GB and only 5% of primary GB [4, 11, 15].

IDH mutation is the strongest prognostic factor in gliomas, and studies show that tumors with *IDH* mutation have a better prognosis than those without *IDH* mutation (wild-type) [4, 11, 12, 16]. Moreover, another observation is that morphologically low-grade gliomas with *IDH*-wild-type behave like GB [11]. Indeed, studies have shown that an *IDH* mutation is a stronger predictor of average survival than a histologic grade [14, 15]. Based on these studies, *IDH*-negative tumors are classified as GB regardless of their histologic features. Positive tumors are classified as oligodendrogliomas or astrocytomas with *IDH* mutations (grades 2–4) according to the detection of 1p19q [8].

The tumor suppressor gene *p53* is located on the short arm of chromosome 17, and its mutations cause the continuous production of the p53 protein and accumulation in cells [17]. Although widespread and strong staining of gliomas with IHC p53 staining is indicative of a *p53* mutation, the sensitivity of this method varies among studies. In studies in which the threshold was set at 10% of tumor cells, the sensitivity of the p53 IHC method ranged from 77% to 91%, and the specificity ranged from 78% to 92% [18].

Alpha-thalassemia/mental retardation syndrome X-linked (ATRX) gene mutations were reported in 70–75% of grade 2 and 3 astrocytomas, 68% of oligoastrocytomas, and 57% of secondary GB, while it is rarely observed in primary GB [4, 11, 19]. *ATRX* mutations were first described in pediatric and adult GB in 2011 [11, 20]. While the *ATRX* mutation is observed in 90% of *IDH*-mutated astrocytomas, it is rare in *IDH*-wild-type GB [15, 21, 22].

A high Ki-67 labeling index in GB is an important indicator of malignancy. Levels of Ki-67 ≥ 20% are associated with more rapid tumor growth and poorer prognosis [23].

IHC staining is frequently used in neuropathology to indirectly demonstrate molecular changes because they are generally cheaper, more widely available, and provides faster results than molecular tests. IHC staining can reveal the absence of protein synthesis due to nonsense gene mutations or homozygous gene losses. Tumors with concurrent *IDH1* mutation and *ATRX* loss are typically associated with a more favorable prognosis and longer overall survival (OS) compared to GB patients with *IDH*-wild-type status.

The aim of this study was to determine the prevalence of HGA and GB diagnosed at our hospital, as well as to

analyze the IHC patterns of IDH1, ATRX, p53, and Ki-67, alongside neoplastic morphological findings, treatment response, and impact on prognosis.

Methods

This study was approved by the Baskent University Institutional Review Board (Project No. KA22/157, from March 29, 2022) and supported by the Baskent University Research Fund.

Parameters IDH1, p53, ATRX, and Ki-67 have been routinely used for the diagnosis of glial tumors in our center since 2016. Our study enrolled patients diagnosed with *IDH*-mutated or *IDH*-wild-type glial tumors between January 2016 and January 2022. Demographic and clinical features of the patients were analyzed. All variables were recorded at the time of enrollment in the study.

Sections from formalin-fixed paraffin-embedded tissue blocks were subjected to IHC analysis using a Leica/Bond-Max (Australia) automated immunostainer. Following our daily diagnostic and research practices, we used 4 μm-thick sections. IHC reactions were performed using the streptavidin-biotin-peroxidase complex method with antibodies listed in Table 1. Positive and negative IHC controls were used in all cases. Tumors exhibiting strong nuclear staining in more than 10% of cells were considered positive for p53 [18]. The Ki-67 proliferation index was determined by counting positively stained cells in 10 high-power fields (400× magnification) within the area of highest proliferation and expressed as a percentage. IHC evaluations were performed by two pathologists. According to the 2021 WHO classification, 160 cases were GB *IDH1*-wild-type, and 45 cases were HGA *IDH1* mutant.

All patients were divided into groups according to age as follows: 7–40 years, 41–55 years, 56–64 years, and ≥ 65 years.

Statistical analysis

The Chi-square test was used to compare independent categorical variables, while the McNemar test was applied for dependent categorical variables between the groups. Kappa and accuracy values were also calculated. OS was calculated from the time of diagnosis to the time of death. The Kaplan-Meier method and Cox proportional regression model (forward model) were used to estimate the mean and median survival times, failure rates, and hazard ratios (HR). Log-rank test was used to compare the survival distributions between groups. The prognostic ability of parameters was evaluated for OS in both univariate and multivariable Cox regression models. Categorical variables were expressed as numbers and percentages. The value of $p < 0.05$ was considered significant. The analyses were performed using the statistical package SPSS version 22.0.

Results

A total of 205 patients were evaluated in the study. The impact of IDH1, p53, ATRX, and Ki-67 parameters, along with demographic and clinical features, on patient survival was investigated (Tables 2 and 3).

Table 1

Technical specifications of antibodies used for immunohistochemical analyses

Primary antibody	Clone	Dilution	Origin
IDH1	H09	1/60	Dianova/Germany
ATRX	H-300	1/50	Santa Cruz-Biotechnology
p53	D0-7	1/200	Dako/Denmark
Ki-67	MIB-1	1/200	Dako/Denmark

IDH1 – isocitrate dehydrogenase 1; ARTX – alpha-thalassemia/mental retardation syndrome X-linked; Ki-67 – Kicl 67.

Table 2

Demographic and clinical features of the patients according to prognosis

Parameter	Alive (n = 140)	Dead (n = 65)	Total (n = 205)	*p*
Gender				
female	64 (71.1)	26 (28.9)	90 (43.9)	
male	76 (66.1)	39 (33.9)	115 (56.1)	0.443
Age groups				
7–40	21 (63.6)	12 (36.4)	33 (16.1)	
41–55	49 (80.3)	12 (19.7)	61 (29.8)	
56–64	35 (64.8)	19 (35.2)	54 (26.3)	
≥ 65	35 (61.4)	22 (38.6)	57 (27.8)	0.113
Diagnosis				
astrocytoma	22 (88.0)	3 (12.0)	25 (12.2)	
glioblastoma	118 (65.6)	62 (34.4)	180 (87.8)	0.024

Table 2 (continued)

Parameter	Alive (n = 140)	Dead (n = 65)	Total (n = 205)	*p*
Comorbidity				
no	135 (70.3)	57 (29.7)	192 (93.7)	
yes	5 (38.5)	8 (61.5)	13 (6.3)	0.017
Treatment				
other	67 (80.7)	16 (19.3)	83 (40.5)	
RT	6 (42.9)	8 (57.1)	14 (6.8)	
TMZ+RT	67 (62.0)	41 (38.0)	108 (52.7)	0.002
Recurrence				
no	133 (70.0)	57 (30.0)	190 (92.7)	
yes	7 (46.7)	8 (53.3)	15 (7.3)	0.062
Prognosis				
alive	133 (100.0)	0 (0.0)	133 (64.9)	
recurrence/alive	7 (100.0)	0 (0.0)	7 (3.4)	
dead	0 (0.0)	57 (100.0)	57 (27.8)	
recurrence/dead	0 (0.0)	8 (100.0)	8 (3.9)	0.001

RT – radiotherapy; TMZ – temozolamide; n – number.

Values are given as numbers (percentages). $p < 0.05$ was considered significant.

Table 3

Immunohistochemical characteristics of tumors according to patient prognosis

Parameter	Alive (n = 140)	Dead (n = 65)	Total (n = 205)	*p*
IDH1				
negative	111 (69.4)	49 (30.6)	160 (78.8)	
positive	29 (64.4)	16 (35.6)	45 (21.2)	0.530
p53				
negative	60 (66.7)	30 (33.3)	90 (43.9)	
positive	80 (69.6)	35 (30.4)	115 (56.1)	0.658
ATRX				
negative	13 (54.2)	11 (45.8)	24 (11.7)	
positive	127 (70.2)	54 (29.8)	181 (88.3)	0.113
Ki-67				
negative (< 20)	95 (72.5)	36 (27.5)	131 (63.9)	
positive (≥ 20)	45 (60.8)	29 (39.2)	74 (36.1)	0.084
IDH1 and p53				
no risk for both	21 (67.7)	10 (32.3)	31 (15.1)	
at least one risk	67 (68.4)	31 (31.6)	98 (47.8)	
risk for both	52 (68.4)	24 (31.6)	76 (37.1)	0.997
IDH1 and ATRX				
no risk for both	5 (55.6)	4 (44.4)	9 (4.4)	
at least one risk	32 (62.7)	19 (37.3)	51 (24.9)	
risk for both	103 (71.0)	42 (29.0)	145 (70.7)	0.386
IDH1 and Ki-67				
no risk for both	21 (77.8)	6 (22.2)	27 (13.2)	
at least one risk	82 (67.2)	40 (32.8)	122 (59.5)	
risk for both	37 (66.1)	19 (33.9)	56 (27.3)	0.518
ATRX and p53				
no risk for both	9 (56.3)	7 (43.8)	16 (7.8)	
at least one risk	75 (70.1)	32 (29.9)	107 (52.2)	
risk for both	56 (68.3)	26 (31.7)	82 (40.0)	0.540
ATRX and Ki-67				
no risk for both	9 (52.9)	8 (47.1)	17 (8.3)	
at least one risk	90 (74.4)	31 (25.6)	121 (59.0)	
risk for both	41 (61.2)	26 (38.8)	67 (32.7)	0.065
p53 and Ki-67				
no risk for both	51 (78.5)	14 (21.5)	65 (31.7)	
at least one risk	73 (62.9)	43 (37.1)	116 (56.6)	
risk for both	16 (66.7)	8 (33.3)	24 (11.7)	0.097

Values are given as numbers (percentages).

For abbreviations, see Table 1.

Among the patients, 115 (56.1%) were male and 90 (43.9%) were female, with ages spanning from 7 to 84 years. There was no significant association between gender and age groups (7–40, 41–55, 56–64, ≥ 65) in terms of survival ($p = 0.113$). However, there was a significant association between grade and survival ($p = 0.024$). A significant association was observed between GB and HGA concerning survival ($p = 0.024$). A total of 65 (31.7%) cases resulted in death. The mean OS in all cases was 45.2 months (median: 24 months).

While 45 (21.2%) cases were found to have the *IDH1* mutation, the number of cases without the mutation was 160 (78.8%). According to the 2021 WHO classification, 160 patients were diagnosed with GB. Among the *IDH1*-mutated cases, 14 were grade 2–3 gliomas, and 31 were classified as grade 4 astrocytomas. Of these 31 *IDH1*-mutated grade 4 astrocytomas, 19 (61.3%) were primary and 12 (38.7%) secondary.

There was a high concordance of 75% between *IDH1* and other markers, particularly between *ATRX* (positive/non-mutated) and *IDH1* (negative-wild-type) (Table 4). Loss of *ATRX* was observed in 21 (10.2%) cases, and 16 were GB patients.

OS was found to be longer in *ATRX* mutant negative ($p = 0.004$) (Figure 1A), *p53* positive ($p = 0.023$) (Figure 1B), and Ki-67 < 20% ($p = 0.060$) (Figure 1C) cases. In addition, OS was sifmeangnificantly longer in *IDH1* positive cases

Table 4

Compatibility of IDH1 with other markers (agreement)

Parameter	IDH1		
	no risk – positive (n)	risk – negative (n)	agreement (%)
p53			
positive	31	84	
negative	14	76	52
ATRX			
negative	9	15	
positive	36	145	75
Ki-67			
negative (< 20)	27	104	
positive (≥ 20)	18	56	40

n – number; % – percentage. For other abbreviations, see Table 1.

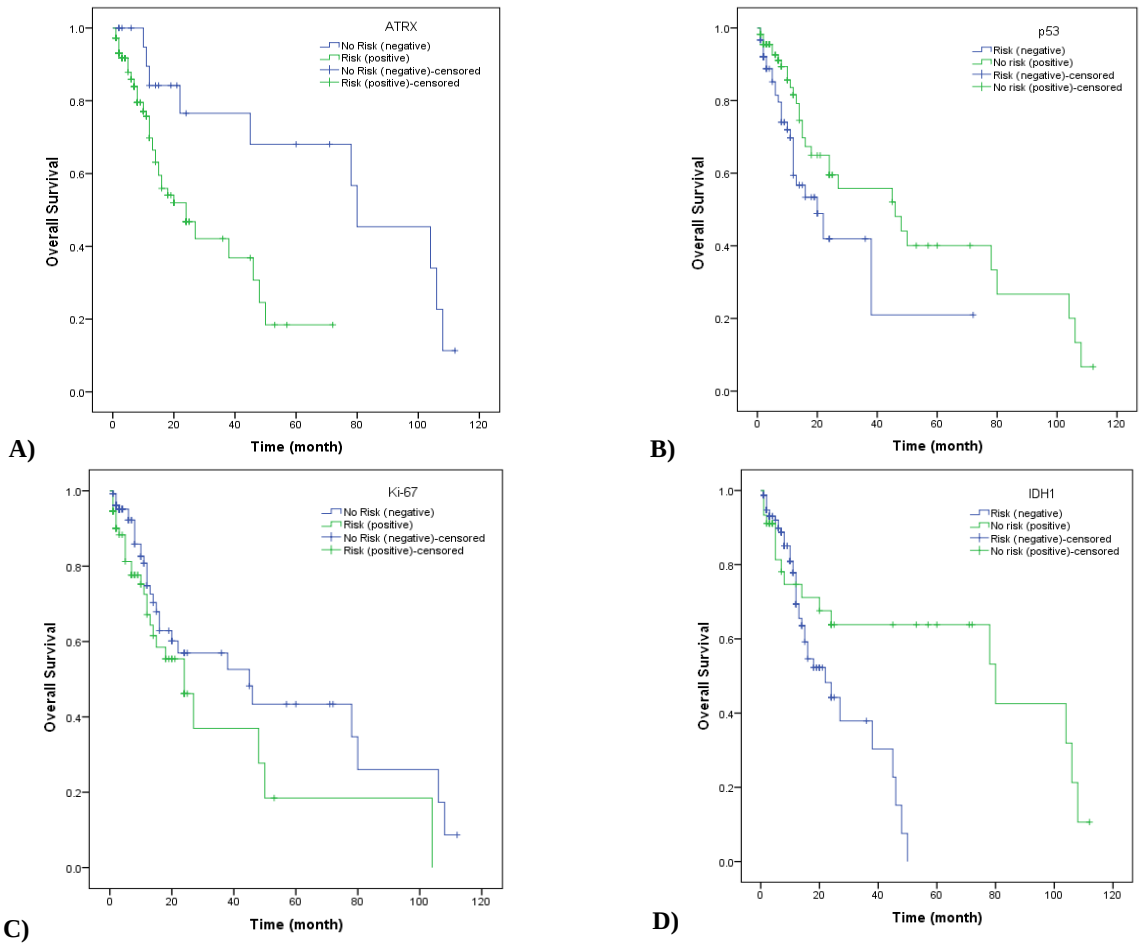

Fig. 1 – Overall survival curves following stratification by ATRX mutations (A), p53 mutations (B), Ki-67 < 20% positivity (C) and IDH1 mutations (D). IDH – isocitrate dehydrogenase; ATRX – alpha-thalassemia/mental retardion syndrome X-linked.

(mean OS: 65.8, median: 80) than in negative cases (mean OS: 25.7, median: 22) ($p = 0.019$) (Figure 1D).

When examining the prognostic ability of the combined markers (summarized in Table 5), patients with an *IDH1* mutation and *ATRX* mutation had the longest OS (mean: 98, median: 106 months; $p = 0.001$) (Figure 2A), while Ki-67 ≥ 20% and p53 negative patients had the shortest OS (mean: 13 months; $p = 0.003$) (Figure 2B).

Table 5

Mean and median overall survival according to related risk factors

Parameter	Overall survival				*p*
	total (n)	number of events	mean	median	
Gender					
female	90	24	31.3	38.0	
male	115	36	50.2	24.0	0.967
Age groups (years)					
7–40	33	10	72.1	80.0	
41–55	61	12	57.6	50.0	
56–64	54	19	14.6	15.0	
65+	57	19	13.8	13.0	< 0 .001
Grade					
2 + 3	25	2	95.3		
4	180	58	40.8	24.0	0.004
Diagnosis					
other	25	2	95.3		
glioblastoma	180	58	40.8	24.0	0.004
Comorbidity					
no	192	52	49.7	45.0	
yes	13	8	13.3	11.0	0.006
Treatment					
other	83	14	49.9	45.0	
RT	14	6	61.8	104.0	
TMZ+RT	108	40	46.1	38.0	0.328
IDH1					
negative	160	43	25.7	22.0	
positive	45	17	65.8	80.0	0.019
p53					
negative	90	28	29.3	20.0	
positive	115	32	52.8	46.0	0.023
ATRX					
negative	24	10	74.0	80.0	
positive	181	50	31.4	24.0	0.004
Ki-67					
negative (< 20)	131	32	52.9	45.0	
positive (≥ 20)	74	28	36.7	24.0	0.060
IDH1 and p53					
no risk for both	31	11	75.6	80.0	
at least one risk	98	27	28.3	20.0	
risk for both	76	22	22.5	22.0	0.004
IDH1 and ATRX					
no risk for both	9	5	98.0	106.0	
at least one risk	51	17	37.8	24.0	
risk for both	145	38	25.6	18.0	0.001
IDH1 and Ki-67					
no risk for both	27	7	82.2	80.0	
at least one risk	122	35	36.7	22.0	
risk for both	56	18	25.4	24.0	0.002
ATRX and p53					
no risk for both	16	7	85.8	104.0	
at least one risk	107	28	29.1	24.0	
risk for both	82	25	30.8	20.0	0.002
ATRX and Ki-67					
no risk for both	17	8	72.6	80.0	
at least one risk	121	26	49.1	38.0	
risk for both	67	26	25.2	24.0	0.010
p53 and Ki-67					
no risk for both	65	12	65.5	78.0	
at least one risk	116	40	37.6	24.0	
risk for both	24	8	13.7	-	0.003

For abbreviations, see Tables 1 and 2.

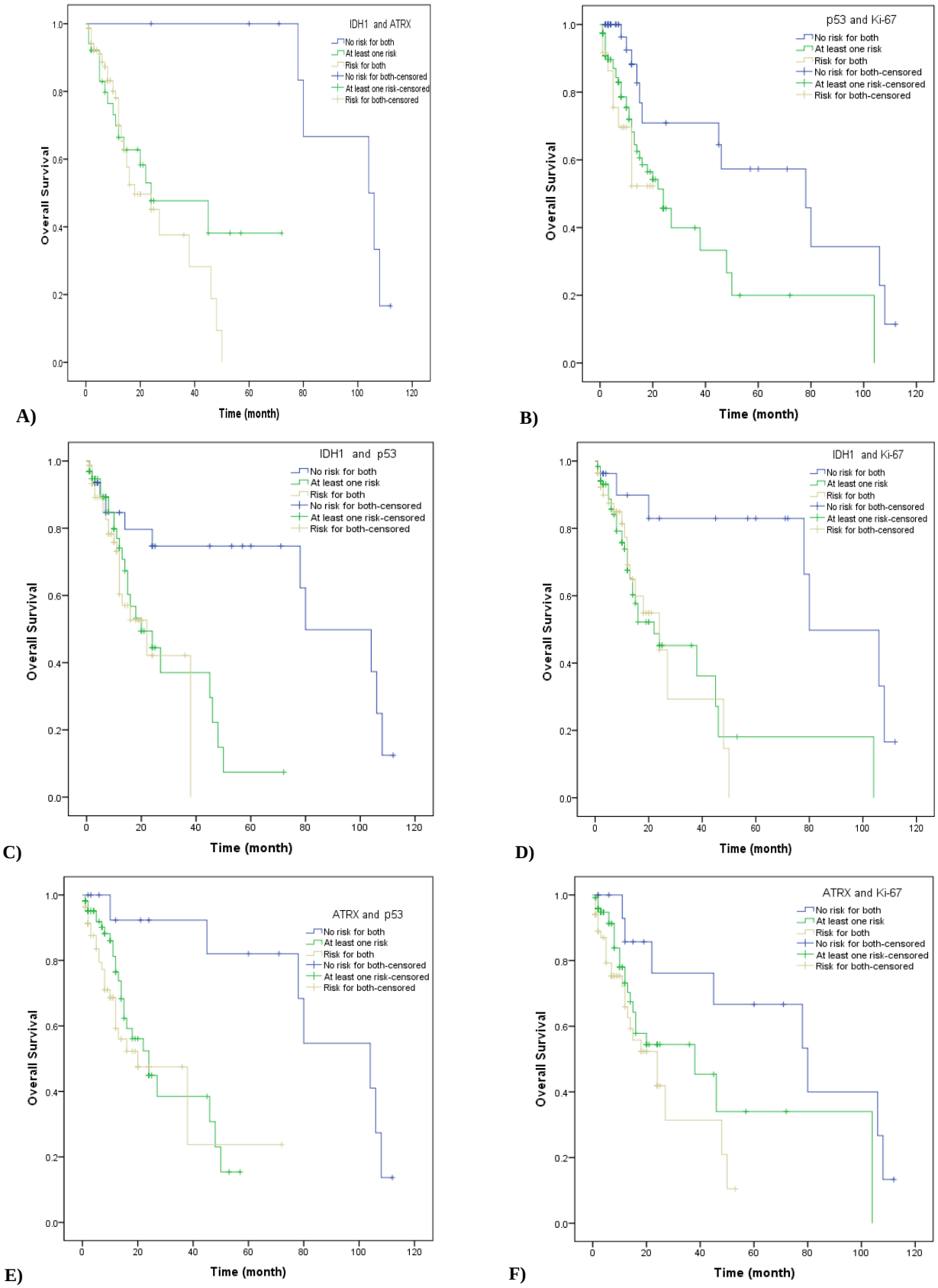

Fig. 2 – Overall survival curves following stratification according to IDH1, ATRX, p53 mutation status and Ki-67 positivity: A) IDH1 and ATRX; B) p53 and Ki-67; C) IDH1 and p53; D) IDH1 and Ki-67; E) ATRX and p53; F) ATRX and Ki-67. For abbreviations, see Figure 1.

Table 6

Results of Cox regression analyses

Parameter	β	SE	HR	95% CI for HR		Sig.
				lower	upper	
Age	0.034	0.011	1.0	1.0	1.1	0.002
Comorbidity	0.966	0.392	2.6	1.2	5.7	0.014
IDH1	0.731	0.372	2.1	1.0	4.3	0.049
ATRX	0.361	0.436	1.4	0.6	3.4	0.408
p53	0.470	0.287	1.6	0.9	2.8	0.101
Ki-67	0.388	0.256	1.5	0.9	2.4	0.130

For other abbreviations, see Table 1.

SE – standard error; HR – hazard ratio; CI – confidence interval.

Analysis of *IDH1* and *p53* expression patterns revealed that patients with *IDH1+*/*p53–* tumors had the most favorable outcomes, with the longest median OS of 75.6 months and progression-free survival (PFS) of 80 months. In contrast, the *IDH1–*/*p53+* group demonstrated the poorest prognosis, with the shortest median OS and PFS of 22.5 and 22 months, respectively. We identified that specific changes in protein profiles were associated with differences in patient survival times. Statistically significant differences in survival ($p < 0.05$) were observed across all combinations, including *IDH1*/*p53* ($p = 0.004$) (Figure 2C), *IDH1*/Ki-67 ($p = 0.002$) (Figure 2D), *ATRX*/*p53* ($p = 0.002$) (Figure 2E), *ATRX*/Ki-67 ($p = 0.010$) (Figure 2F), and *p53*/Ki-67. OS times according to a combination of indicators are illustrated in Figure 2 and summarized in Table 5.

After being standardized for age and comorbidities in the Cox regression model, only *IDH1* emerged as an independent and statistically significant predictor of OS (Table 6).

Radiotherapy (RT) and temozolomide (TMZ) were used in 108 (52.7%) cases, whereas RT only was used in 14 (6.8%) cases. The other 83 patients either did not start treatment at our hospital or were treated but could not receive regular treatment afterward.

Discussion

HGA and GB are the most common and aggressive primary brain tumors [1]. Although GB is frequently seen *de novo*, a few of them occur secondary to the transformation from low-grade astrocytoma (WHO grade 2) or anaplastic astrocytoma (WHO grade 3) [2]. In our series, 18 patients (5 stereotaxic, 13 resection biopsies) were previously diagnosed with low-grade glial tumors and transformed into grade 4 gliomas in the following years (1–11 years).

New molecular techniques and important biomarkers related to the prognosis and diagnosis of HGA/GB were discovered. These markers have emerged as targets for novel therapeutic strategies and offer important insights into the pathogenesis of gliomas [4]. While *IDH* gene mutations were observed with a frequency of 5% in primary GB, they were at a rate of 70–75% in grade 2–3 gliomas and secondary GB [24]. In our series, mutations were present in 11% of primary GB cases, 56% of grade 2–3 gliomas, and 67% of secondary GB. Mutations were more commonly observed in younger patients and were linked to a more favorable prognosis [25]. One of the most compelling clinical observations is the theory that *IDH* mutant gliomas follow a distinct biological course, with patients harboring these mutations demonstrating significantly longer survival compared to those with *IDH*-wild-type tumors [26].

The deoxyribonucleic acid (DNA) sequencing method and the IHC method were compared to detect *IDH1* mutations in 186 patients with glioma. Using antibodies specific for the *R132H* mutation, the sensitivity and specificity of the IHC method were determined to be 94% and 100%, respectively. The authors reported that IHC methods could be used as a standard procedure due to the difficulty of genetic analysis methods, such as DNA sequencing [27]. In subsequent studies, *IDH* mutation was between 10% and 14% in GB [4, 28]. Our study detected an *IDH1* mutation in 21.2 % of 205 patients with IHC methods.

IDH1 mutation is a good prognostic marker for OS and PFS in patients with GB.[13, 14]. Some meta-analyses have shown that *IDH1*/*IDH2* mutations are associated with longer OS and PFS in patients with GB [29]. However, some studies reported no statistically significant difference [4, 30]. Parsons et al. [31] reported that patients with *IDH*-mutated GB survived an average of 31 months, and patients with *IDH*-wild-type survived 15 months [31]. Similarly, Parsons et al. and other researchers found that the median OS of patients with stage III *IDH* mutant glioma was 20 months, whereas it was 65 months in patients with *IDH*-wild-type glioma [31, 32]. In our study, the mean and median OS were longer in patients with *IDH* mutations than in wild-type patients, and there was a statistically significant difference.

In GB, *IDH1* and *p53* mutations are frequently accompanied by *ATRX* mutations [4, 21]. Although *ATRX* mutations are more common in diffuse astrocytomas, they are rarely observed in oligoastrocytomas and GB [4]. Studies have reported *ATRX* loss of 15.3%, 18%, and 26% in GB [19, 33, 34]. In our study, loss of *ATRX* was observed in 21 (10.2%) cases, and 16 were GB patients. There were studies indicating that *ATRX* mutation was statistically significant in terms of survival in GB and that *ATRX* mutation was a good prognostic factor [33, 35]. Additionally, some studies have shown that there is no significant difference in survival in GB/HGA with *IDH* mutation, regardless of the *ATRX* mutation status. However, they associated the presence of the *ATRX* mutation in *IDH*-wild-type GB with better survival [3].

Our study found OS to be longer in patients with an *ATRX* mutation (negative), which was statistically significant. In addition, the longest OS was observed in patients with *IDH1*-mutant/*ATRX*-wild-type tumors (98 months), while the shortest OS was seen in those with *IDH1*-wild-type/*ATRX*-wild-type tumors (25.6 months).

Cells with impaired *p53* function can develop genetic abnormalities and lead to the development of malignancies. Alterations in the *p53* gene are more common in secondary GB than in primary GB, and *p53* may be mutated in more than 65% of cases [2]. There are conflicting reports on the impact of *p53* mutation on prognosis in patients with GB. While some studies have found no association between *p53* mutations and prognosis [35], some other studies have identified a significant correlation between *p53* positivity and clinical survival [36, 37]. Additionally, studies have observed shorter life expectancy with high p53 levels and reported that p53 is a poor prognostic factor [2]. In our study, *p53* was positive in 115 cases and negative in 90 cases. The OS was longer in *p53* positive cases (mean: 46 months) and shorter in negative cases (mean: 20 months), which proved to be statistically significant.

Surgery is the first step in treating all patients with intracranial gliomas, usually with tissue diagnosis and tumor resection [15]. Standard treatment for newly diagnosed patients with HGA/GB includes maximal resection of the tumor, 60 Gray RT for 6 weeks, concurrent administration of TMZ, and six cycles of adjuvant therapy [15, 38]. One study found that survival was 12.1 months in patients who received RT alone, compared with 14.6 months in GB patients who received RT after surgery, followed by adjuvant TMZ therapy [39]. In retrospective studies, the five-year survival probability for GB was reported to be approximately 5%, whereas the one-year survival rate was reported to be 36% [5]. Cancer progresses within one year despite standard treatment in 70% of cases. In our study, the number of patients receiving only RT was 14, and OS was 61.8 months, whereas 108 patients receiving TMZ+RT survived 46.1 months. Consistent with the literature, patients who received RT had longer life expectancy, but no statistically significant results were obtained. We can explain this by the small number of patients who received RT. Targeted therapies are available for *IDH1* mutant tumors, and research is ongoing for *ATRX* mutations. Losing *ATRX* leads to DNA repair errors and may increase sensitivity to poly-adenosine diphosphate ribose polymerase inhibitors. Agents that inhibit telomerase in cells with *ATRX* loss are being investigated. For instance, by demonstrating *ATRX* loss in the tumor, we can provide patients with access to new treatment options.

Recent studies have been conducted to determine molecular characteristics and specific genetic features of gliomas to improve prognosis [40]. There are studies on loss of 1p19q heterozygosity, *p53*, *EGFR* and *PTEN* mutation, *MDM2* amplification, *MGMT* promoter methylation, and *IDH1* mutation [11, 13, 41–43]. Given that more than 80% of low-grade gliomas carry an *IDH* mutation, this survival benefit strongly depends on the presence of an *IDH* mutation [15].

In summary, we found that *IDH1* and *ATRX* mutations significantly impacted prognosis in GB patients with *p53* overexpression alone. In addition, RT and chemotherapy had a positive effect on their survival.

Limitations of the study

Although our university hospital serves as a regional center and draws a diverse patient population from neighboring provinces, the relatively small sample size and single-center design may limit the generalizability of our findings. The identification of more cases may change with the availability of comprehensive clinical, morphological, and molecular profiles. There are inconsistent findings in the current literature regarding certain prognostic markers, which may complicate the interpretation of the results. Future studies employing molecular methods are needed to confirm the findings and deepen our understanding. In addition to IHC staining in our study, further prospective clinical and molecular studies are highly desirable.

Conclusion

IDH1 mutant patients have been shown to have a better prognosis, and now only those with the *IDH1*-wild-type are referred to as glioblastoma. Not only *IDH1* mutation status but also the combination of other protein expressions may subdivide glioblastoma from a diagnostic point of view in the future. These proteins may be used as prognostic markers. Our results should be supported by future studies in larger patient series using molecular methods.

Conflict of interest

The authors of this paper declare no conflict of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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Received on October 8, 2024
Revised on February 16, 2025
Revised on April 14, 2025
Accepted on April 29, 2025
Online First June 2025