Original Paper

The importance of IDH1, ATRX and WT-1 mutations in glioblastoma

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Numerous genetic pathways associated with glioblastoma development have been identified. In this study, we investigated the prognostic significance of IDH1 and ATRX mutations and WT-1 and p53 expression in glioblastomas and that of surgical methods, radiotherapy and chemotherapy. 83 patients with glioblastomas were retrospectively evaluated. Immunohistochemical analysis was performed for IDH1, ATRX and WT-1 expression. Tumour cells were positive for IDH1 in 9.6% of the patients. In 4.8% of the patients, loss of ATRX expression was observed in tumour cells; 86.7% of the patients were WT-1 positive, and 12.05% of the patients were p53 positive. No statistically significant difference was found in the progression-free and overall survival according to IDH1, ATRX, WT-1 and p53 expression. There was a statistically significant difference in the progression-free and overall survival according to the radiotherapy status. There was a statistically significant difference in the overall survival according to the chemotherapy status. There was no statistically significant difference in the progression-free and overall survival according to the surgical method. IDH1 and ATRX mutations, p53 overexpression and WT-1 expression alone did not have a significant effect on the prognosis of patients with glioblastoma; however, radiotherapy and chemotherapy had a positive effect on survival.

Key words: glioblastoma, immunohistochemistry, IDH1, ATRX, WT-1.

Introduction

Glioblastomas are the most malignant brain tumours, constituting 45-50% of primary malignant brain tumours [1]. In glioblastoma, the mean survival is 15 months, with a combination of maximal safe surgical resection, adjuvant radiotherapy and concurrent adjuvant temozolomide treatment [2]. Usually, patients with tumours respond poorly to radiotherapy and chemotherapy [3]. Therefore, there is a need for developing new therapeutic approaches for glioblastomas. The most promising treatment approach is the identification of genetic pathways leading to the development of glioblastomas [4]. In study conducted in 2008, it was found that IDH1/2 mutations played a role in the genetic pathways leading to glioblastoma formation [5]. IDH mutations occurred in the early stages of tumourigenesis, affected glial precursor cells and were acquired before TP53 mutations and 1p/19q co-deletion [6]. In the 2016 WHO classification, glioblastomas have been classified according to molecular markers such as IDH mutations

or 1p/19q deletion [7]. IDH1/2 mutation states play a role in the classification of diffuse gliomas [5]. Mutations of IDH2 are less frequent than those of IDH1. The frequency of IDH1 mutations is low in primary glioblastomas; however, it is seen in 60-80% of secondary glioblastomas developing from astrocytomas or oligodendroglial tumours [8]. IDH-mutant gliomas have a better prognosis than IDH-wild type gliomas [7]. ATRX encodes a protein involved in the chromatin rearrangement pathway, allowing the histone H3.3 to be incorporated into heterochromatin [9]. ATRX mutations occur in approximately 57% of secondary glioblastomas, but they is rare in primary glioblastomas. In glioblastomas, ATRX mutations are often accompanied by IDH1 and TP53 mutations [10]. ATRX mutations are a good prognostic factor [11, 12]. Wilm's tumour (WT-1) gene is a tumour suppressor gene encoding the protein that acts as a transcription factor involved in cell growth and differentiation [13]. WT-1 plays a role in gliomagenesis and it is overexpressed in astrocytic tumours; it is correlated with the grade and Ki-67 proliferation index. High WT-1 levels may be caused by cellular proliferation [14, 15]. In a study by Cancer Genome Atlas, genetic changes in the p53 pathway have been reported in 90% of glioblastomas [16]. TP53 mutation is an early genetic alteration leading to secondary glioblastoma formation [16, 17]. The aim of this study was to investigate the prognostic significance of IDH1, ATRX, WT-1 and p53 expressions and that of surgical methods, radiotherapy and chemotherapy in patients with glioblastomas using immunohistochemical methods.

Material and methods

Overall, 83 patients investigated in the Pathology Department of Pamukkale University, Faculty of Medicine, between 2010 and 2016 and diagnosed with glioblastomas were included in the study and evaluated retrospectively. Haematoxylin-eosin-stained preparations for all subjects were prepared from formalin-fixed paraffin-embedded tissue samples, and all immunohistochemical preparations previously applied and kept in the archives were re-evaluated. The block that best reflected the tumour morphology was selected for each case, and immunohistochemical staining for WT-1, ATRX and IDH1 was performed on this block. P53 (Ventana, DO-7 clone, pre-diluted) immunohistochemistry preparations in the archives were re-evaluated. Dianova polyclonal antibody (clone H09, 1/20) was used for IDH1, Sigma-Aldrich polyclonal antibody (clone HPA001906, 1/100) was used for ATRX and polyclonal antibody (Ventana, clone 6F-H2, pre-diluted) was used for WT-1; 3-μm thick sections were obtained from formalin-fixed, paraffin-embedded tissue

samples selected for immunohistochemical staining, placed on electrostatically charged slides and dried in an incubator at 60°C for at least 2 hours. The entire staining process, including deparaffinization and antigen release, was performed using the Ventana BenchMark LT fully automated machine.

Cytoplasmic staining was performed, and staining intensity in tumour cells was semiquantitatively evaluated for IDH1. Cases with widespread intense cytoplasmic staining in tumour cells were considered as "IDH1 positive" for its mutation [18], and those with no tumour cell staining were considered as 'IDH1 negative'. For ATRX evaluation, staining results of vascular endothelial cells and normal glial cells were considered as the internal positive control. Nuclear staining in tumour cells was evaluated and calculated as a percentage. The presence of nuclear ATRX staining in < 10% of tumour cells showed expression loss for ATRX and was considered as 'ATRX positive' for its mutation [19]. For WT-1evaluation, staining results of vascular endothelial cells were considered as the internal positive control. Cytoplasmic staining was evaluated in tumour cells. The cell percentage that was positive on staining was calculated. Staining in > 50% of tumour cells was considered as WT-1 positive, and staining in ≤ 50% of tumour cells was considered as WT-1 negative. Strong nuclear staining in ≥ 80% tumour cells was considered as p53 overexpression and "p53 positive" mutation.

Information on surgical treatment was obtained from the Department of Neurosurgery. Stereotactic biopsy as well as gross total and subtotal resections were performed surgically for patients depending on tumour localization. Information on postoperative radiotherapy treatment was obtained from Radiation Oncology Department records and that on chemotherapy treatment, progression-free survival (PFS) and overall survival (OS) data was obtained from Medical Oncology Department records. Survival data from the day of first diagnosis until December 2017 were used to calculate prognosis.

Statistical analysis

All analyses were performed using the SPSS software (version 21.0, SPSS Inc., Chicago, IL, USA). Descriptive statistics were presented as number, percentage, mean and standard deviation, median and minimum and maximum values. OS and PFS were used to predict the prognosis of IDH1 and ATRX mutations and WT-1 and p53 expression and to calculate the effect of treatment on prognosis. OS was defined as the time between the diagnosis and patient death or final follow-up. PFS was defined as the time between the diagnosis and relapse or final follow-up. Kaplan-Meier and log-rank tests were used for survival analysis. A p-value of < 0.05 was considered statistically significant.

Ethical approval was obtained for this study from Pamukkale University Non-invasive Clinical Research Ethics Committee (Dated 06/28/2016, No. 13).

Results

This study was conducted with 83 patients with glioblastomas who were selected from those diagnosed in the Pathology Department of Pamukkale University, Faculty of Medicine, between 2010 and 2016. The mean age of the 83 patients was 57.95 ±12.70 years, and the median age was 62 years; 49.4% (n = 41) of the patients were female and 50.6% (n = 42) were male. The male/female ratio was 1.02/1. In terms of histopathological classification 96.4% (n = 80) of the patients were diagnosed with classic glioblastoma, 1.2% (n = 1) of the patients were diagnosed with giant cell glioblastomas and 2.4% (n = 2) of the patients were diagnosed with gliosarcomas. According to clinical history, 95.2% (n = 79) of the patients had primary glioblastomas and 4.8% (n = 4) had secondary glioblastomas. Three of the patients with secondary glioblastomas were previously diagnosed with diffuse astrocytoma, and 1 was diagnosed with gemistocytic astrocytoma registered in our pathology laboratory. The tumours were excised by gross total resection in 44.6% (n = 37) of the patients and by subtotal resection in 53% (n = 44) of the patients. Owing to tumour localization, diagnostic stereotactic biopsy was performed in 2.4% (n = 2) of the patients; 83.1% (n = 69) of the patients received radiotherapy (60 Gray dose) five days a week for 6 weeks after surgery, and 16.9% (n = 14) of the patients could not receive radiotherapy owing to poor general condition. Temozolomide (75 mg/m²/day) was concurrently administered to the patients receiving radiotherapy. After radiotherapy, 67.5% (n = 56) of the patients received 6 courses of 150-200 mg/m² of temozolomide treatment for 5 days once every 28 days in the Oncology Clinic; 32.5% (n = 56) of the patients could not receive treatment owing to poor general condition. Based on available data until December 2017, 7.2% (n = 6) of the patients survived, and 92% (n = 77) of the patients died. The distribution of patients according to clinical findings is shown in Table I.

Immunohistochemical findings in glioblastoma

Immunohistochemical results for IDH-1, ATRX, WT-1 and p53 staining are shown in Table II. 90.4% (n = 75) of 83 patients were IDH1 negative. Cytoplasmic IDH1 staining in tumour cells was observed in 9.6% (n = 8) of the patients (Figs. 1A, B), and 95.2% (n = 79) of the patients had nuclear ATRX staining in ≥ 10% of tumour cells and were considered as "no ATRX mutation". More than 90%

nuclear expression loss in tumour cells was observed in 4.8% (n = 4) of the patients, and these were considered as "ATRX mutation" (Figs. 1C, D). WT-1 staining ranged from 5% to 98%, with a mean staining percentage of 68.37% ±20.071% and a median of 70%. The percentage of WT-1 staining was > 50% in 86.7% (n = 72) of the patients and were evaluated as WT-1 positive. The percentage of WT-1 staining was ≤ 50% in 13.3% (n = 11) of the patients and were evaluated as WT-1 negative (Figs. 2A, B). The mean p53 staining percentage was 28.63 ±28.921%, and the median value was 15%. p53 staining percentage was ≥ 80% in 12.05% (n = 10) of the patients and were evaluated as positive. p53 staining percentage was < 80% in 87.95% (n = 73) of the patients and were evaluated as p53 negative (Figs. 2C, D).

Table I. The distribution of patients according to clinical findings

PARAMETER	
Age	
Median age	57.95 ±12.70 (62)
Range	21-83
Sex	
Male	42 (50.6%)
Female	41 (49.4%)
Male/female	1.02/1
Histopathological classification	
Classical glioblastoma	80 (96.4%)
Giant cell glioblastoma	1 (1.2%)
Gliosarcoma	2 (2.4%)
According to the clinical history	
Primary glioblastoma	79 (95.2%)
Secondary glioblastoma	4 (4.8%)
Surgery	
Gross total resection	37 (44.6%)
Subtotal resection	44 (53%)
Stereotactic biopsy	2 (2.4%)
Radiotherapy	
Receive	69 (83.1%)
Not receive	14 (16.9%)
Chemotherapy	
Receive	56 (67.5%)
Not receive	27 (32.5%)
Survival	
Exitus	77 (92%)
Alive	6 (7.2%)

Table II. IDH1, ATRX, WT-1 and p53 immunohistochemical results and survival analysis

GLIOBLASTOM	N	%	MEDIAN PFS, MONTHS	P-VALUE	MEDIAN OS, MONTHS	P-VALUE
IDH1						
Positive	8	9.6	5	0.217	11	0.297
Negative	75	90.4	4		8	
ATRX						
Positive	79	95.2	15	0.214	15	0.342
Negative	4	4.8	4		8	
WT1						
Positive	72	86.7	10	0.800	15	0.454
Negative	11	13.3	2		11	
P53						
Positive	10	12.05	4	0.697	6	0.798
Negative	73	87.95	5		8	

PFS – progression-free survival; OS – overall survival

P-value was obtained by log rank test of Kaplan Meier survival analysis
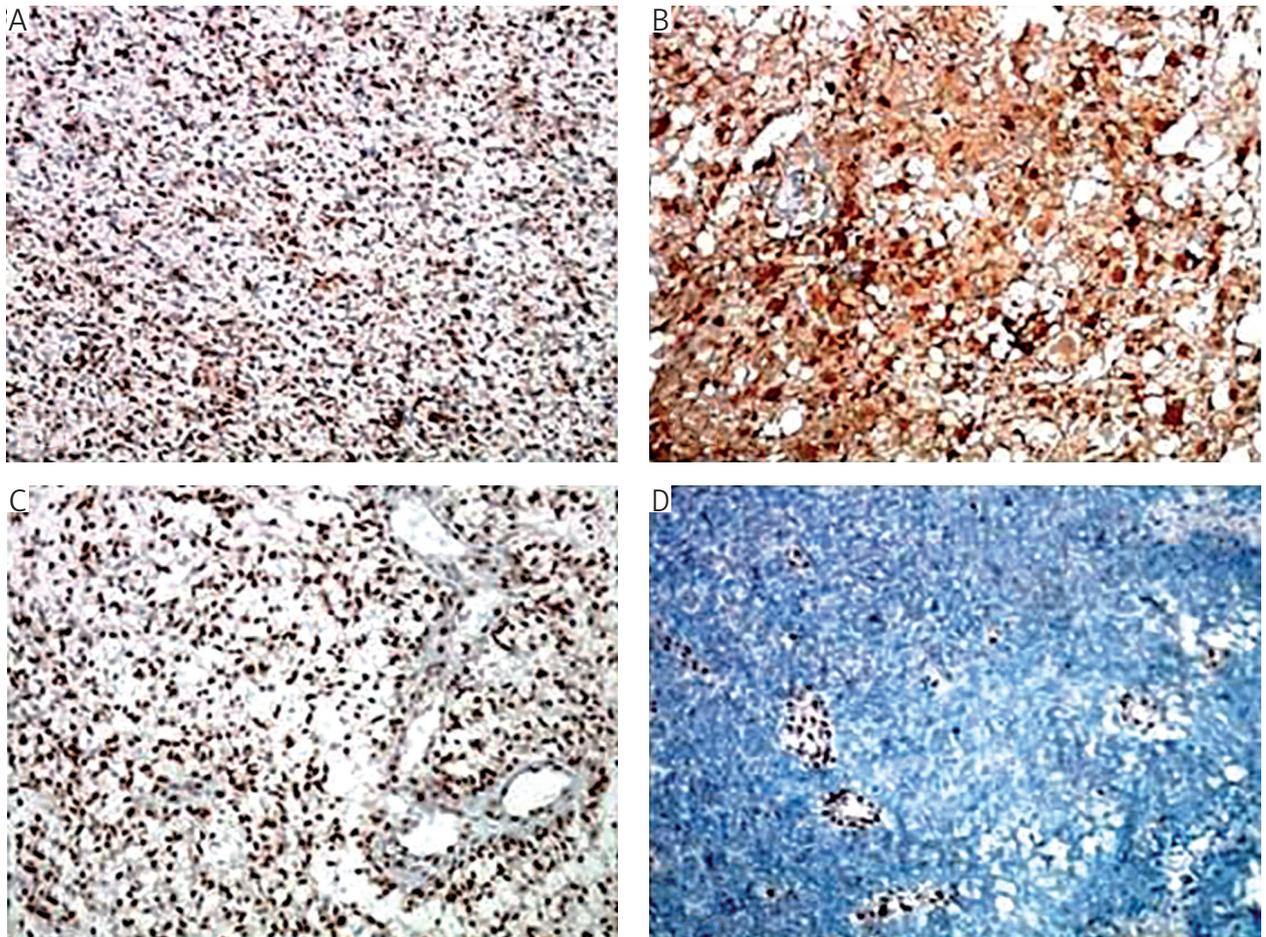
Fig. 1. Glioblastoma cases. A, B) Strong cytoplasmic IDH1 positivity in glioblastoma (IDH1, 200×). C) ATRX positivity in glioblastoma (ATRX, 200×). D) ATRX negativity in glioblastoma, endothelial cells positive as internal control (ATRX, 200×)
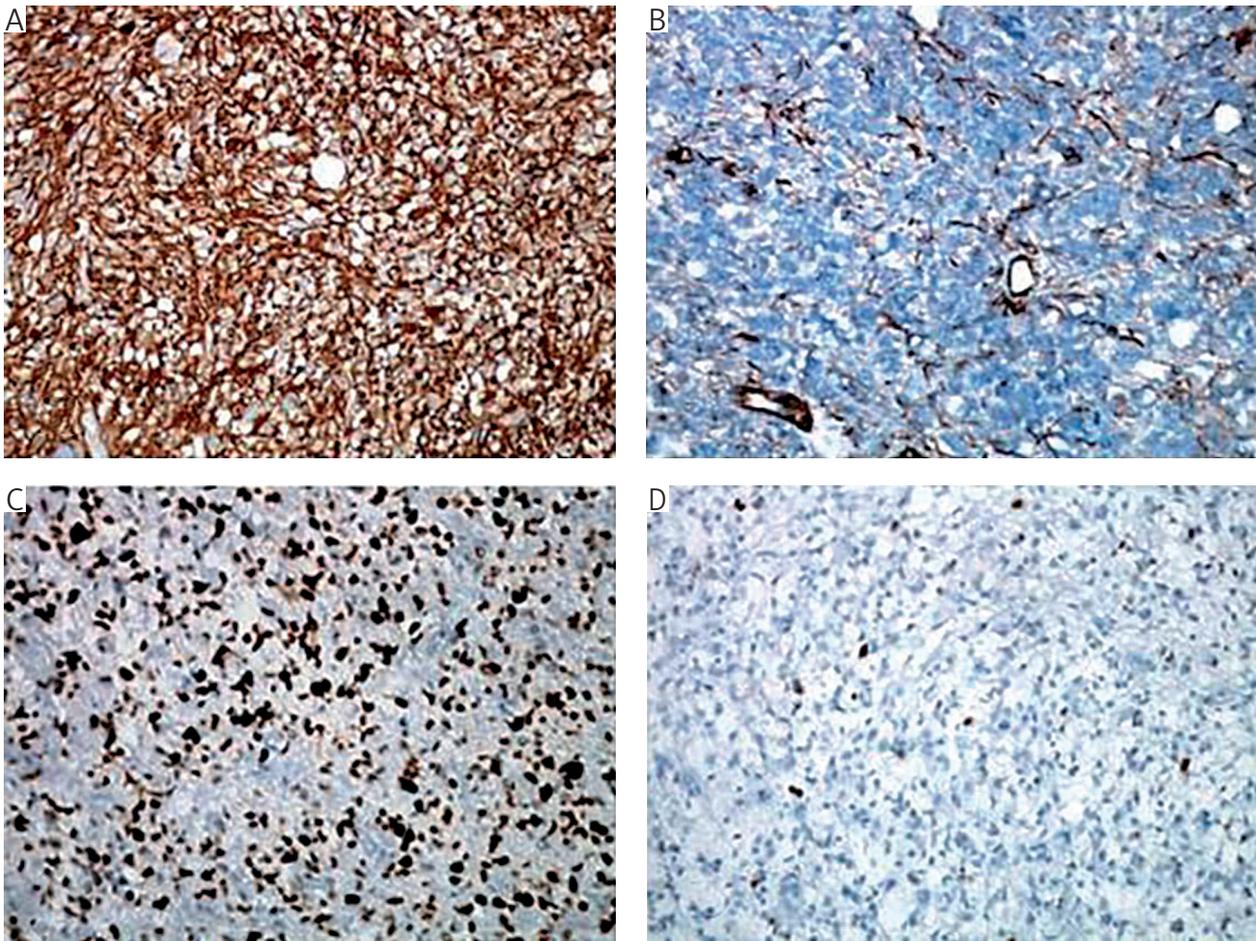
Fig. 2. Glioblastoma cases. A) WT-1 positivity in glioblastoma (WT-1, 200×). B) WT-1 negativity in glioblastoma (WT-1, 200×). C) P53 positivity in glioblastoma (P53, 200×). D) P53 negativity in glioblastoma (P53, 200×)

Survival findings

Overall, 7.2% (n = 6) of 83 patients were alive as of December 2017; and 92.8% (n = 77) of the patients died. The mean PFS was 8.994 ±1.321 months, and the median PFS was 5 months. The mean OS was 11.878 ±1.364, and the median OS was 8 months. Survival analysis according to the expression states of IDH-1, ATRX, WT-1 and p53 is shown in Table II. Survival analysis based on radiotherapy, chemotherapy and surgery is shown in Table III.

Survival analysis based on IDH1

The mean PFS in IDH1-positive patients was 13.375 ±4.950 months (median: 5 months) and that in IDH1-negative patients was 8.255 ±1.257 months (median: 4 months). The mean OS in IDH1-positive patients was 15.250 ±4.616 months (median: 11 months) and that in IDH1-negative patients was 11.298 ±1.345 months (median: 8 months). There was no statistically significant difference in PFS and OS according to IDH1 expression (p = 0.217 and p = 0.297, respectively; Figs. 3A, B).

Survival analysis based on ATRX

In patients with loss of ATRX expression, the mean PFS was 15.5 ±3.279 months (median: 15 months), and the mean OS was 17.25 ±2.955 months (median: 15 months). In patients without any loss of ATRX expression, the mean PFS was 8.752 ±1.397 months (median: 4 months), and the mean OS was 11.662 ±1.434 months (median: 8 months). There was no statistically significant difference in PFS and OS according to ATRX expression (p = 0.214 and p = 0.342, respectively; Figs. 3C, D).

Survival analysis based on WT-1

The mean PFS in WT-1-positive patients was 8.627 ±1.287 months (median: 5 months) and that in WT-1-negative patients was 9.182 ±3.396 months (median: 3 months). The mean OS in WT-1-positive patients was 11.407 ±1.389 months (median: 7 months) and that in WT-1-negative patients was 13.091 ±3.174 months (median: 12 months). There was no statistically significant difference in PFS and OS according to WT-1 expression (p = 0.800 and p = 0.454, respectively; Figs. 3E, F).

Table III. Survival analysis based on radiotherapy, chemotherapy and surgery

Glioblastoma	N	%	Median PFS, months	P-value	Median OS, months	P-value
RT						
Receive	69	83.1	6	0.000	11	0.000
Not receive	14	16.9	1		1	
KT						
Receive	56	67.5	6	0.079	12	0.006
Not receive	27	32.5	2		2	
Surgery						
Gross total resection	37	44.6	4	0.983	12	0.516
Subtotal resection	44	53	5		6	

RT – radiotherapy; KT – chemotherapy; PFS – progression-free survival; OS – overall survival

P-value was obtained by log rank test of Kaplan Meier survival analysis

Survival analysis based on P53

The mean PFS in patients with p53 overexpression was 11.000 ± 4.297 months (median: 4 months). The mean PFS in patients without p53 overexpression was 8.666 ± 1.394 months (median: 5 months). The mean OS in patients with p53 overexpression was 13.000 ± 4.142 months (median: 6 months). The mean OS in patients without p53 overexpression was 11.583 ± 1.409 months (median: 8 months). There was no statistically significant difference in PFS and OS according to P53 overexpression ($p = 0.697$ and $p = 0.798$, respectively; Figs. 3G, H).

Survival analysis based on radiotherapy

The mean PFS was 1.429 ± 0.173 months (median: 1 month) in patients who did not receive radiotherapy and 10.529 ± 1.524 months (median: 6 months) in patients who received radiotherapy. There was a statistically significant difference in the PFS based on radiotherapy status ($p = 0.000$; Fig. 4A). The mean OS was 1.429 ± 0.173 months (median: 1 month) in patients who did not receive radiotherapy and 13.998 ± 1.518 months (median: 11 months) in patients who received radiotherapy. There was a statistically significant difference in OS based on their radiotherapy status ($p = 0.000$; Fig. 4B).

Survival analysis based on chemotherapy

There was no statistically significant difference in the PFS based on chemotherapy status ($p = 0.079$; Fig. 4C). The mean OS was 8.963 ± 3.091 months (median: 2 months) in patients who did not receive chemotherapy and 13.317 ± 1.286 months (median: 12 months) in patients who received chemotherapy. There was a statistically significant difference in OS based on chemotherapy status ($p = 0.006$; Fig. 4D).

Survival analysis based on surgical method

Two patients who underwent stereotactic biopsy were excluded from the evaluation owing to the small sample size. Of the 37 patients who underwent gross total resection, 91.9% (n = 34) died. Of the 44 patients who underwent subtotal resection, 93.2% (n = 41) died. There was no statistically significant difference in PFS and OS based on the surgical method ($p = 0.983$, $p = 0.516$, respectively; Figs. 4E, F).

Discussion

Owing to recently developed molecular techniques, important biomarkers have been found for the diagnosis and prognosis of glioblastomas. These markers provide valuable information about the pathogenesis of gliomas and have become the target for new therapeutic approaches [20]. In this study, we evaluated the expression and prognostic significance of IDH1, ATRX, WT-1 and p53 in patients with glioblastomas and the prognostic significance of surgical methods, radiotherapy and chemotherapy on their survival.

DNA sequencing methods, fluorescence *in situ* hybridisation and pyrosequencing methods have been used to detect IDH mutations in patients with glioblastomas [21]. Capper et al. compared the DNA sequencing method and immunohistochemical method for detecting IDH1 mutations in 186 patients with gliomas. Using R132H-mutation specific antibodies, they determined the sensitivity and specificity of the immunohistochemical method to be 94% and 100%, respectively. They reported that immunohistochemical methods could be used as a standard procedure owing to the difficulty associated with genetic analysis methods such as DNA sequencing [22]. In studies in which immunohistochemical methods were performed, Popova *et al.* detected IDH1 mutations in 11% patients with glioblastomas [23], and Chaurasia
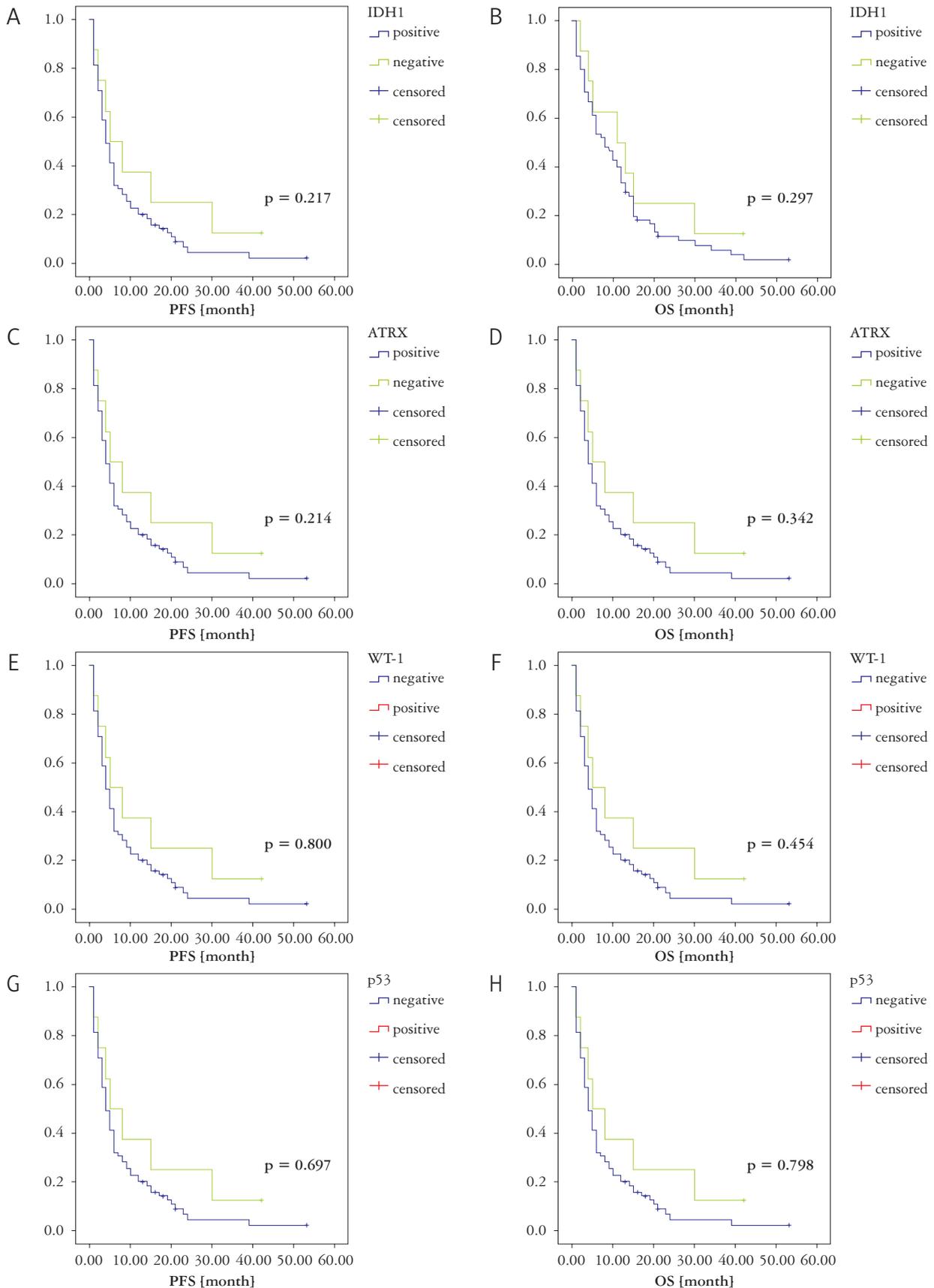
Fig. 3. Kaplan-Meier curves. Progression-free survival (PFS) and overall survival rate (OS) for patients with glioblastoma. **A)** PFS according to IDH1 expression. **B)** OS according to IDH1 expression. **C)** PFS according to ATRX expression. **D)** OS according to ATRX expression. **E)** PFS according to WT-1 expression. **F)** OS according to WT-1 expression. **G)** PFS according to p53 expression. **H)** OS according to p53 expression. P-values were calculated by the log-rank test
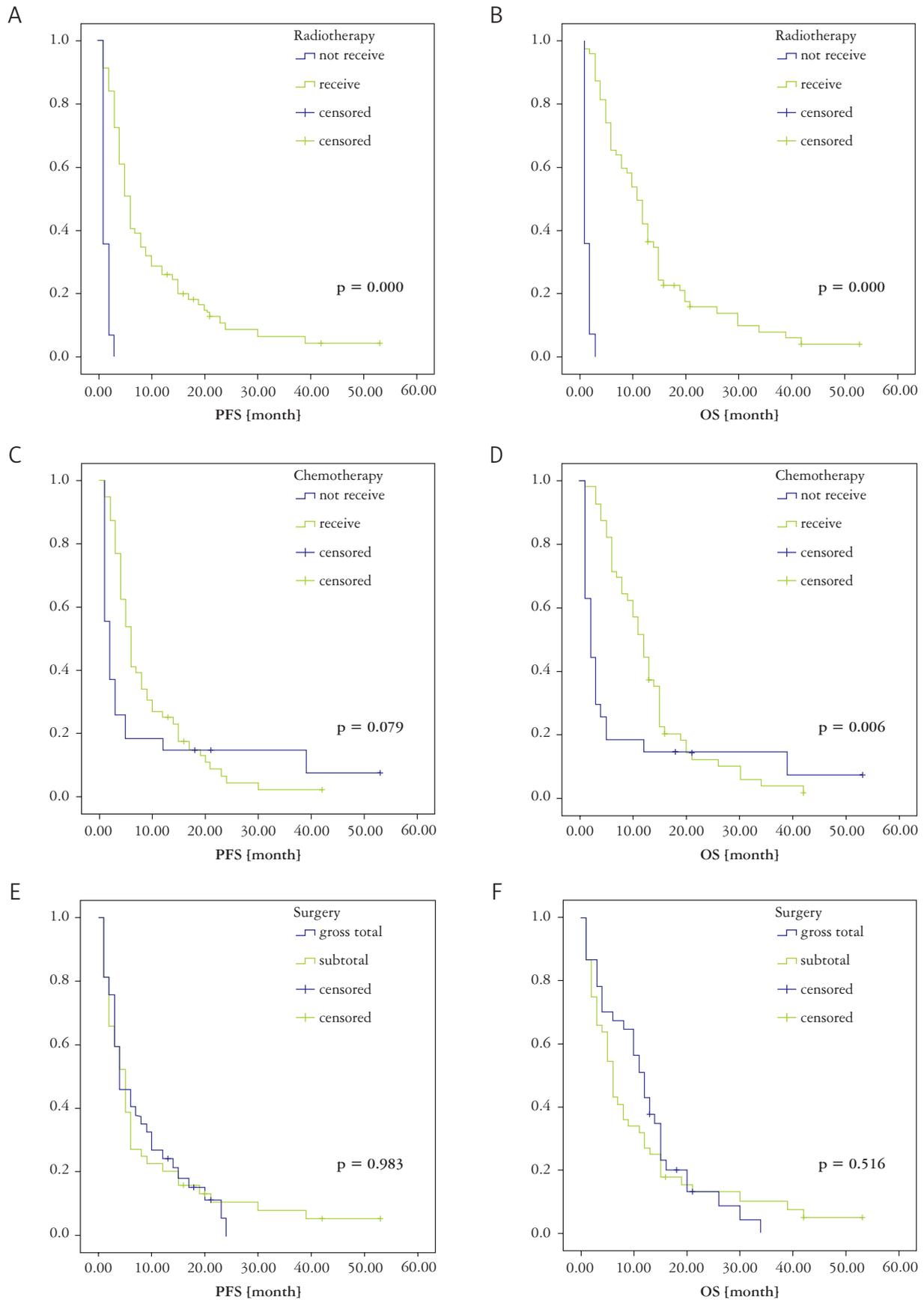
Fig. 4. Kaplan-Meier curves. Progression-free survival (PFS) and overall survival rate (OS) for patients with glioblastoma. **A)** PFS according to radiotherapy. **B)** OS according to radiotherapy. **C)** PFS according to chemotherapy. **D)** OS according to chemotherapy. **E)** PFS according to surgery. **F)** OS according to surgery. P-values were calculated by the log-rank test

et al. detected IDH1 mutations in 10.4% patients with glioblastomas [11]. Pekmezci *et al.* conducted DNA sequencing and immunohistochemistry methods and detected IDH mutations in 14% of 360 patients with glioblastomas [24]. In the present study, we detected IDH1 mutations in 9.6% of 83 patients with glioblastomas by immunohistochemical methods. Our results are consistent with those of other studies in which immunohistochemical methods were performed.

IDH1 mutations are a good prognostic marker [5, 8, 25]. Glioblastomas with a IDH1 mutation had a better prognosis than anaplastic astrocytoma without any IDH1 mutation [25]. Kim *et al.* reported that IDH1/2 mutations had no prognostic value in low-grade gliomas [26]. In a meta-analysis, Chen *et al.* evaluated the prognostic value of IDH1/2 mutations and examined 15 studies for OS and 10 studies for PFS. They found that IDH1/2 mutations were associated with longer OS and PFS in patients with glioblastomas [27]. Combs *et al.* investigated patients with primary glioblastomas and found that OS was significantly longer in patients with IDH1 mutations and that there was a significant difference in the OS; however, they did not observe a significant difference in the PFS between patients with IDH mutants and IDH-wild type primary glioblastomas [28]. Paldor *et al.* compared 21 patients with IDH-mutant glioblastomas and 21 with IDH-wild type glioblastomas in terms of OS and PFS and found no statistically significant difference. They reported that IDH mutations did not provide a better prognosis of glioblastomas [29]. In the present study, although the mean as well as median PFS and OS were longer in patients with IDH mutations than those with wild type IDH, there was no statistically significant difference.

Although ATRX mutations are common in diffuse astrocytoma, they are rarely seen in oligoastrocytomas, oligodendrogliomas or glioblastomas. Immunohistochemical assessment of the loss of ATRX expression captures the majority of ATRX mutations and that the use of immunohistochemical tests for gliomas is highly reliable [10, 30]. Loss of ATRX expression has been examined in various studies by immunohistochemical methods. Reuss *et al.* reported a loss of ATRX expression in 18%, Liu *et al.* in 26% and Chaurasia *et al.* in 15.3% of patients with glioblastomas [11, 31, 32]. In the present study, loss of ATRX expression was detected in only 4 of 83 patients (4.8%) and was evaluated as ATRX mutation.

Chaurasia *et al.* and Cai *et al.* found that ATRX mutation in glioblastomas had a statistically significant effect on survival. They found that ATRX mutation was a good prognostic factor [11, 12]. Pekmezci *et al.* did not observe a significant difference in survival with respect to ATRX mutation status in IDH-mutant glioblastomas; however, the presence of ATRX mutation in IDH-wild type glioblastomas was associated with better survival [24]. Uppar *et al.* reported that ATRX, IDH and p53 biomarkers did not affect prognosis in paediatric patients with glioblastomas [33]. In the present study, we found that the mean PFS and OS were longer in patients with ATRX mutation than in those without ATRX mutation. However, we did not find any statistically significant difference in PFS and OS in our patients.

WT-1 inhibits p53-mediated apoptosis, stimulates tumour cell proliferation and increases cellular longevity [34]. WT-1 plays a role in gliomagenesis and is expressed in astrocytic tumours. WT-1 expression is correlated with the tumour grade [14, 15, 35, 36]. Studies have demonstrated high WT-1 expression in glioblastomas [15, 37]. Bourne *et al.* reported WT-1 expression in all cases of glioblastomas and found ≥ 20% WT1 expression in 36 out of 38 cases [38]. Consistent with the literature, we observed WT-1 expression in all of our patients with glioblastoma and found ≥ 20% WT-1 staining in 96.4% of our patients.

Rauscher *et al.* and Schwab *et al.* showed that WT-1 expression was associated with poor prognosis in patients with astrocytic tumours. Schwab *et al.* showed that WT-1 expression decreased significantly in the presence of IDH1 mutation and loss of ATRX expression [39, 40]. There are few studies investigating survival with respect to the WT-1 expression status in patients with glioblastomas. Camacho-Urkaray *et al.* found a significantly decreased survival in patients with glioblastomas with decreased WT-1 levels [41]. Rauscher *et al.* compared survival according to WT-1 expression in patients with glioblastomas and found no significant difference. They reported that WT-1 expression was not a prognostic factor in patients with glioblastomas [42]. Here, we investigated WT-1 expression only in patients with glioblastomas and did not find a significant difference in PFS and OS.

TP53 mutation is a genetic alteration that occurs early in patients with gliomas and is detected in majority of patients with low-grade diffuse astrocytomas. Its prevalence in anaplastic astrocytoma developing from diffuse astrocytomas and secondary glioblastomas is similar to that of diffuse astrocytoma [43]. There are conflicting reports on the effect of TP53 mutation on the prognosis in patients with glioblastomas. TP53 mutations are not associated with the prognosis [44, 45]. Conversely, Schmidt *et al.* and Ohgaki *et al.* found that TP53 mutations were a good prognostic factor in patients with glioblastomas [37, 46].

Chaurasia *et al.* examined 163 patients with glioblastomas by immunohistochemical methods and found better PFS in p53-negative patients; however, there was no significant difference in the OS [11].

Montgomery *et al.* observed a shorter life expectancy at high p53 levels and reported that p53 was a poor prognostic factor [47]. Ogura *et al.* reported that no significant difference was found in the survival with respect to p53 expression in patients with glioblastomas [18]. In the present study, we did not find a significant difference in PFS and OS with respect to p53 overexpression.

Stupp *et al.* compared survival in patients with glioblastomas who received only radiotherapy and those who received radiotherapy and subsequent adjuvant temozolomide treatment after surgery. They found the median survival time to be 14.6 months in the latter compared with a median survival time of 12.1 months in the former [2]. Ohgaki *et al.* found that patients undergoing surgery or patients receiving radiotherapy had a longer life expectancy [17]. Here, we compared the gross total and subtotal surgery in terms of PFS and OS in the patients included in our study but could not obtain statistically significant results. We found a statistically significant difference in PFS and OS with respect to the radiotherapy status. Consistent with the literature, we found that the survival of patients who received radiotherapy survived was longer. In the patients included in our study, the median OS was 2 months in those who did not receive adjuvant temozolomide treatment compared with patients who received adjuvant temozolomide treatment, for whom the median OS was 12 months. We observed a statistically significant difference in the OS with respect to chemotherapy status; however, we did not obtain significant results in PFS.

In conclusion, we found that IDH1 and ATRX mutations, p53 overexpression and WT-1 expression alone did not have a significant effect on the prognosis in patients with glioblastoma; however, radiotherapy and chemotherapy had a positive effect on their survival. These findings should be supported by future studies conducted on larger series of patients by molecular methods.

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