RESEARCH ARTICLE

Editorial Process: Submission:12/17/2024 Acceptance:04/18/2025

Correlation between Isocitrate Dehydrogenase Mutation and Immunohistochemical Expression of DNA Mismatch Repair Proteins in the Prognosis of Gliomas

Nelly Mohamed Saeed[1], Hayam Rashed[1], Samia Hussein[2]*, Amira S Al-Karamany[2], Wael Elmesallamy[3], Ahmad Barakat Waley[4], Ahmed A Obay[5], Shimaa A Gharieb[5], Kareem Amgd[6], Sara Mohammed Ibrahim[7], Mohamed I Abdelhamid[8], Tarik Khamis[9], Asmaa Hussein Mohamed[1]

Abstract

Background: Gliomas comprise about a third of all brain tumors and 80% of malignant brain cancers. Isocitrate dehydrogenase (*IDH*) normally converts isocitrate to α-ketoglutarate (α-KG), but mutant *IDH* gives an oncometabolite, 2-hydroxyglutarate (2-HG) that inhibits α-KG-dependent dioxygenases and inhibits cellular differentiation. **Objectives:** This work aims to study the mutation in the *IDH1* gene and the expression of mismatch repair (*MMR*) proteins in gliomas and to study the relationship between *IDH1* mutation and *MMR* expression and the prognosis of gliomas. **Methods:** This study included 60 patients with gliomas. Brain tissues were used for DNA extraction with subsequent mutation analysis. Paraffin blocks of brain tissues were prepared for routine histopathological examination and immunohistochemical examination of *MMR* proteins. **Results:** *IDH1* mutation and MLH1 and MSH2 expressions were not statistically associated with any of the studied patient or tumor characteristics. No statistically significant association was observed between *MSH6* expression in the studied patients and tumor characteristics. No significant association was detected between *IHC* expression for *MLH1, MSH6, MSH2* expression, and *IDH*1 mutation. No significant association was determined between the expression of MSH6 and *IDH1* mutation, or MLH1 expression. A significant association was determined between *MSH2* and *MSH6* expression. There was a significant association between *IDH1* mutation, *MSH6*, and *MSH2* expressions and glioma progression-free survival (PFS). Log Rank test showed that mutant *IDH1* and *MSH6* expressions had a favorable prognosis. **Conclusion:** *IDH1* mutation and *MMR* proteins (*MLH1, MSH6,* and *MSH2*) could help predict glioma outcome.

Keywords: Mismatch repair proteins- isocitrate dehydrogenase mutation- gliomas

Asian Pac J Cancer Prev, **26 (4)**, 1447-1457

Introduction

Gliomas comprise about third of all brain tumors and 80% of malignant brain cancers. Glioblastoma is the most frequent type in adult and elderly patients, while it is rare in children [1]. The World Health Organization (WHO) classifies gliomas according to the histological grading into grade I gliomas (pilocytic astrocytoma), grade II gliomas including diffuse astrocytoma, oligodendroglioma, and oligoastrocytoma, grade III gliomas including anaplastic astrocytoma, anaplastic oligodendroglioma and anaplastic oligoastrocytoma, and grade IV (glioblastomas) [2]. In 2021, the WHO classification of adult gliomas was further updated to include histopathological and molecular criteria [3].

Isocitrate dehydrogenase (IDH) converts isocitrate into α-ketoglutarate (α-KG), but mutant IDH gives an oncometabolite, 2-hydroxyglutarate (2-HG) that inhibits α-KG-dependent dioxygenases and inhibits cellular differentiation [4]. Recurrent mutations in the *IDH1* gene were detected in glioblastomas, low-grade glioma, secondary glioblastoma [5], acute myeloid leukemias, cholangiocarcinoma [6], chondrosarcoma [7], and angioimmunoblastic T cell lymphoma [8]. Forty percent

*[1]Department of Pathology, Faculty of Medicine, Zagazig University, Egypt. [2] Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Zagazig University, Egypt. [3] Department of Neurosurgery, Faculty of Medicine, Zagazig University, Egypt. [4] Department of Medical Oncology, Faculty of Medicine, Zagazig University, Egypt. [5] Department of Clinical Oncology and Nuclear Medicine, Faculty of Medicine, Zagazig University, Egypt. [6]Faculty of Medicine, Zagazig University, Egypt. [7] Department of Clinical Pathology, Faculty of Medicine, Zagazig University, Egypt. [8] Department of Surgery, Faculty of Medicine, Zagazig University, Egypt. [9]Department of Pharmacology, Faculty of Veterinary Medicine, Zagazig University, Egypt. *For Correspondence: samiahussein82@hotmail.com*

of adult gliomas are associated with IDH mutation. Additionally, grade III gliomas can be categorized into 3 prognostic groups according to IDH and ATRX mutations [9].

Therapeutic modalities can be designed according to the molecular subtype and WHO grading. To identify this in a tumor sample, detection of the mutated DNA besides immunohistochemical evaluation of the directly affected proteins is required. Multiple markers are currently used in clinical practice (such as GFAP, EMA, MGMT, P53, NeuN, Oligo2, EGFR, VEGF, *IDH1*, Ki-67, 1p/19q). These markers are highly correlated with glioma prognosis [10]. IDH mutation is the main marker for subtype recognition in diffuse gliomas. Its role in gliomas has been well-established both biologically and clinically [11]. Importantly, IDH-mutant gliomas have a significantly better prognosis than IDH-wild gliomas [12].

Acquired mismatch repair (*MMR*) deficiency occurs in recurrent IDH-mutant gliomas as a resistance mechanism against alkylating chemotherapy [13]. DNA replication occurs during the cell cycle in the S-phase by DNA polymerases. However, this process is liable to mistakes. Point mutations and microsatellite instability are the hallmarks of replication repair deficiency. *MMR* system is responsible for replication fidelity [14]. Mutations in the *MMR* genes (*MSH6, PMS2, MSH2*, and *MLH1*) cause tumor syndromes including Lynch syndrome and Constitutional Mismatch Repair Deficiency with a higher liability for cancer [15].

Novel biomarkers have been paid great attention to enhance diagnostic and prognostic ability and to open a new era of more fruitful treatment plans. Thus, this study aims to investigate the mutation of *IDH1* and the expression of *MMR* proteins in gliomas and the relation between *IDH1* mutation and *MMR* expression and prognosis of gliomas.

Materials and Methods

This prospective cohort study was conducted in the Pathology, Medical Biochemistry and Molecular Biology, Neurosurgery, and Oncology Departments, Faculty of Medicine, Zagazig University, in the period from July 2021 to July 2024 after receiving approval from the institutional review board (IRB) of the Faculty of Medicine, Zagazig University. The present study included 60 patients with gliomas. DNA extraction from glioma tissues was performed. Paraffin blocks of brain tissues were prepared for routine histopathological examination and immunohistochemical examination. All clinicopathological data were obtained from the patient's reports and his follow- up data. Patients with gliomas with complete clinical data and sufficient material were included in the study. The clinical data were reviewed from the patient's medical files regarding the age, sex, the site of lesion, and MRI radiology. Paraffin blocks of all studied cases were cut into 3-5 μm thickness sections and stained with hematoxylin and eosin (H&E) to evaluate the histopathological grade.

DNA extraction

DNA was extracted from glioma tissues using the G-spin Total Genomic DNA Extraction Kit (iNtron Biotechnology, Seongnam, Korea).

Detection of IDH-1 mutation

RotorGene Q (Qiagen, Germany) real-time PCR cycler was used to identify *IDH1* mutations. A primer surrounding the mutation site was created using the previously published protocol by Horbinski et al. [16]. The probes were *IDH1* wild-type complementarity. This approach is based on the difference in Tm between wild-type and mutant amplicons. It enables the detection of all potential mutation subtypes using a single set of primers and probes. The Tm of each mutation subtype reflects the thermodynamic stability of mismatched and complementary probe-target duplexes. The probes bind to the sample DNA flawlessly without mutations, dissociating at a higher Tm to display a single peak. On the other hand, probes will bind to the mutant amplicon inadequately and dissociate at a lower Tm if a heterozygous point mutation is present. As a result, two melting peaks are produced, one higher for the wild-type allele and one lower for the mutant allele. A 202-bp PCR product was obtained for detecting *IDH1* mutations using the following primers: forward (5′-ACGGTCTTCAGAGAAGC-3′), reverse (5′-GGTGTAGATACCAAAAGATAAGAAT-3′), and two probes (5′-LC640-ATGATAGGT TTTACCCATCCACTCACAAGC-3′ and 5′-ATCCCCATAAGCATGACGACCTA-FL-3′). All primers and probes were purchased from (ThermoFisher, USA).

Immunohistochemical procedure

Paraffin blocks were cut into 3–5 μm and deparaffinized using xylene, and then rehydrated using alcohol. The sections were immune-stained with an *MLH1* antibody (clone ES05), *MSH2* antibody (clone FE11), *MSH6* antibody (clone EP49), and PMS2 antibody (clone EP51). Staining was done on the Leica BONDMAX automated *IHC* platform, and antibody detection was done using a biotin-free bond polymer detection system (Leica Microsystems).

Evaluation of MMR immunostaining

A negative stain in tumor cells in the presence of retained internal control indicates deficient *MMR* [17].

Statistical analysis

The collected data were analyzed using SPSS 22.0 for Windows (IBM Inc., Chicago, IL, USA) and MedCalc 13 for Windows (MedCalc Software bvba, Ostend, Belgium). Continuous variables were expressed as the mean ± standard deviation and median (range), and categorical variables were expressed as percentages.

Results

Clinicopathological features of the studied glioma cases

Among our participants, 37 cases (61.7%) were males, and 23 cases (38.3%) were females. The mean age was

44.5 ± 15.3 years ranging from 12 to 80 years with the median 45 years. Most patients aged between 40 and 60 (51.7%), while 36.7% were less than 40 years and 11.7 % were more than 60 years. Fifty cases (83.3 %) were presented with gliomas in the brain, while 10 cases (16.7%) were in the spinal cord. Most cases were on the left side (43.3%), while 35 % were on the right side, 16.7% were non-lateralized, and only 5% were multicentric. The most common site was in the frontal lobe (23.3%). Other sites were the temporal lobe (15%), occipital lobe (10%), cerebellum (10%), cervical cord (10%), parietal lobe (8.3%), corpus callosum (5%), thoracic cord (5%), and 13.3% were in multiple lobes. The size of the most included gliomas was 2-4 cm (38.3%). Other gliomas' size was <2 cm, 4-6 cm, and >6 cm with (21.7%, 20%, and 20%, respectively) (Table 1).

Histopathological and immunohistochemical findings

Forty-six cases (76.7%) were diagnosed as astrocytoma (glioblastoma was the most common, 38.3%), 4 cases were oligodendrogliomas, 7 cases were ependymomas and 3 cases were diffuse astrocytic and oligodendrocytic tumors. Forty percent of the cases with glioma were grade II, 21.7% were grade III, and 38.3% were grade IV. Among grade IV, 12 cases were primary, and 11 cases were secondary (Table 2, Figure 1).

IDH1 mutation and MMR immune expression in the studied cases

Wild *IDH1* represented 51.7% of cases, while 48.3% were mutant (Table 1). One case showed negative *MLH1* expression (deficient expression). Three cases had negative *MSH6* expression. *MSH2* expression was negative in 2 cases. However, all cases were PMS2 positive (intact expression) (Table 2) (Figures 2-5).

Relation Between IDH1mutation and MMR proteins and clinicopathological criteria in glioma patients

IDH1 mutation was not statistically associated with any studied patient or tumor characteristics. Additionally, there was no significant association between *MLH1* expression and the studied variables except for tumor site (P=0.013) (Table 3). Moreover, no statistically significant association was observed between *MSH6* and *MSH2* expressions and any tumor characteristics (Table 4).

Relation Between IDH1mutation and the expression of MMR proteins

In addition, no significant association was detected between *MLH1*, *MSH6*, or *MSH2* expression and *IDH1* mutation (Table 5). Moreover, no significant association was determined between the expression of *MSH6* and *IDH1* mutation, or *MLH1* expressions. However, a significant association was determined between *MSH2* and *MSH6* expression (P=0.002) (Supplementary Table 1).

Relation between IDH1mutation and MMR protein expression and patient outcome

By following up with patients for 36 months, sixty percent of the participants (36 cases) showed progression while 40% (24 cases) did not. 93.3 % of the patients had free survival for 6 months, 65 % for 12 months, 52.3% for 18 months, 34.7% for 24 months and 34.7% for 30 months (Table 1, Figure 6A).

There was a highly significant association between wild *IDH1* and glioma progression (P<0.001), as 96.8% of wild *IDH1* showed progression and mutant *IDH1* showed a mean of about 30 months survival compared with about 13 months for those with wild *IDH1*. By Log Rank test, there was a significant difference and mutant *IDH1* showed a favorable prognosis (P<0.001) (Supplementary Table 2, Figure 6B).

Concerning *MLH1* expression, a highly significant association was detected between its expression and each of the PFS and the occurrence of progression (P<0.001 for each) (Supplementary Table 2, Figure 6C).

In addition, a highly significant association between

Table 1. Patient Characteristics of the Studied Patients (N=60)

Patient characteristics	All studied patients (N=60)	
	No.	
Gender		
Male	37	61.70
Female	23	38.30
Age (years)		
Mean± SD	44.50±15.33	
Median (Range)	45 (12 – 80)	
Age group		
≤40 years	22	36.70
>40-60 years	31	51.70
>60 years	7	11.70
Location of tumor		
Brain	50	83.30
Spinal cord	10	16.70
Side of tumor		
Left side	26	43.30
Right side	21	35
Non-lateralized	10	16.70
Multicentricity	3	5
Site of tumor		
Frontal lobe	14	23.30
Temporal lobe	9	15
Parietal lobe	5	8.30
Occipital lobe	6	10
Multiple lobes	8	13.30
Corpus callosum	3	5
Cerebellum	6	10
Cervical cord	6	10
Thoracic cord	3	5
Size of tumor		
<2 cm	13	21.70
2-4 cm	23	38.30
4-6 cm	12	20
>6 cm	12	20

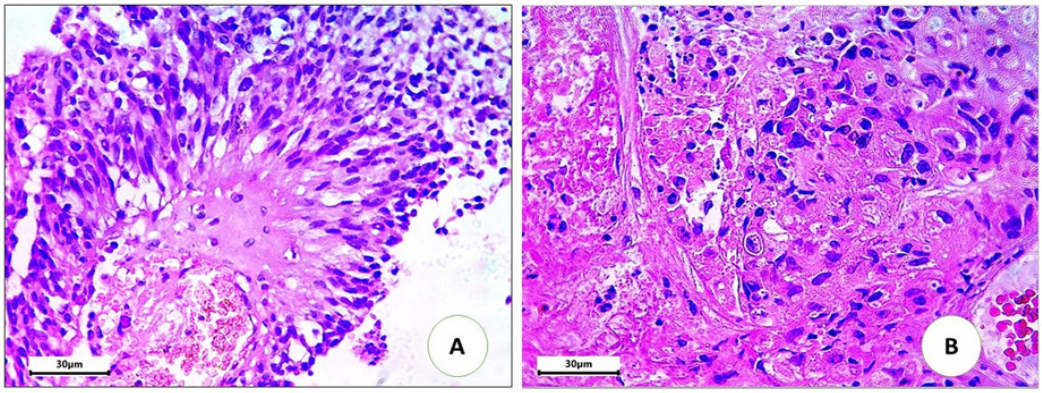

Figure 1. A: Ependymoma (grade II): showing ependymal cells arranged around vascular spaces (Perivascular pseudo-rosettes) (H&E stain, x400 original magnification); B: Anaplastic astrocytoma (grade III): showing marked nuclear atypia, increased cellularity, significant proliferative activity (H&E stain, x400 original magnification).

Table 2. Pathological and Immunohistochemical Results of the Studied Patients (N=60)

	All studied patients (N=60)	
	Number	%
Diagnosis		
Astrocytoma	46	76.70
Diffuse astrocytoma	2	3.30
Gemistocytic astrocytoma	8	13.40
Anaplastic astrocytoma	13	21.70
GBM	23	38.30
1ry GBM	12	
2ry GBM	11	
Oligodendroglioma	4	6.60
Ependymoma	7	11.70
Diffuse astrocytic and Oligodendrocytic tumors	3	5
Grade		
Grade II	24	40
Grade III	13	21.70
Grade IV	23	38.30
Grade IV (1ry)	12	20
Grade IV (2ry)	11	18.30
MLH1 IHC		
Negative	1	1.70
Positive	59	98.30
MSH6 IHC		
Negative	3	5
Positive	57	95
MSH2 IHC		
Negative	2	3.30
Positive	58	96.70
PMS2 IHC		
Negative	0	0
Positive	60	100

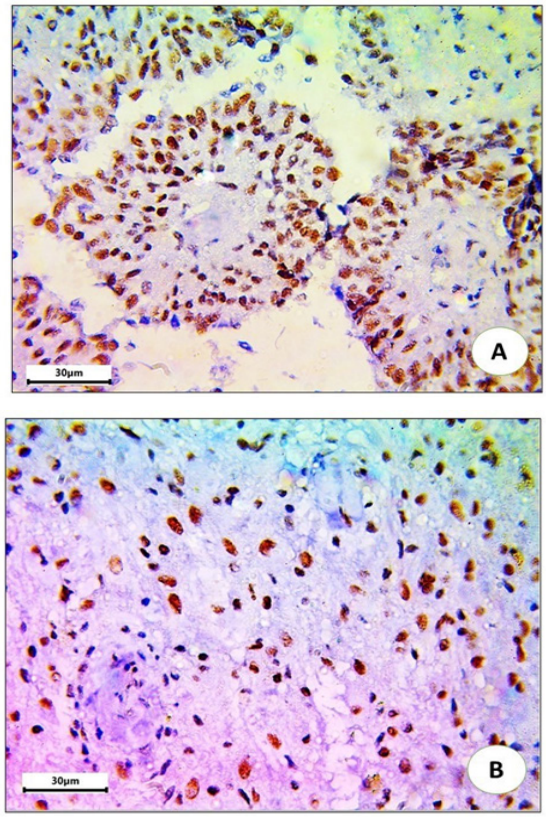

Figure 2. A: Ependymoma (grade II): showing diffuse positive nuclear MLH1 staining of tumor cells (intact expression) (MLH1 IHC stain, x400 original magnification); B: Anaplastic Astrocytoma (grade III): showing diffuse positive nuclear MLH1 staining of tumor cells. (MLH1 IHC stain, x400 original magnification).

MSH6 expression and PFS was found, but no association with the occurrence of progression (P=0.268). Intact expression showed about 22 months of survival compared with only about 6 months for those with loss of *MSH6* expression. By Log Rank test, there was a significant difference by *MSH6* and its positive expression showed a favorable prognosis (P<0.001) (Supplementary Table 3, Figure 6D).

Similarly, *MSH2* expression showed a highly significant association with PFS (P<0.001), but not

Table 3. Relationship between Patient Characteristics and *IDH1* Mutation and MLH1 IHC Staining.

Patient characteristics	N	*IDH1* mutation		P	MLH1 IHC		P
		Wild *IDH1* (N=31) N (%)	Mutant *IHD1* (N=29) N (%)		Negative MLH1 (N=1) N (%)	Positive MLH1 (N=59) N (%)	
Gender							
Male	37	20 (54.1)	17 (45.9)	0.639 [a]	1 (2.7)	36 (97.3)	1.000[a]
Female	23	11 (47.8)	12 (52.2)		0 (0)	23 (100)	
Age (years)							
Mean± SD		41.61±16.07	47.58±14.13	0.193 [b]	43	44.52±15.46	0.817[b]
Median (Range)		42 (12 – 70)	48 (15 – 80)			45 (12 – 80)	
Age group							
≤40 years	22	14 (63.6)	8 (36.4)	0.199 [c]	0 (0)	22 (100)	0.700[c]
>40-60 years	31	14 (45.2)	17 (54.8)		1 (3.2)	30 (96.8)	
>60 years	7	3 (42.9)	4 (57.1)		0 (0)	7 (100)	
Grade							
Grade II	24	13 (54.2)	11(45.8)	0.067 [c]	0 (0)	24 (100)	0.884[c]
Grade III	13	8 (61.5)	5 (38.5)		1 (7.7)	12 (92.3)	
Grade IV (1ry)	12	10 (83.3)	2 (16.7)		0 (0)	12 (100)	
Grade IV (2ry)	11	0 (0)	11 (100)		0 (0)	11 (100	
Location of tumor							
Brain	50	26 (52)	24 (48)	1.000[a]	0(0)	50 (100)	0.167 [a]
Spinal cord	10	5 (50)	5 (50)		1(10)	9 (90)	
Side of tumor							
Left side	26	15 (57.7)	11 (42.3)	0.726 [a]	0(0)	26 (100)	0.166 [a]
Right side	211	9 (42.9)	12 (57.1)		0 (0)	21(100)	
Non-lateralized	10	5 (50)	5 (50)		1 (10)	9 (90)	
Multicentricity	3	2 (66.7)	1 (33.3)		0 (0)	3(100)	
Site of tumor							
Frontal lobe	14	5 (35.7)	9 (64.3)	0.585 [a]	0(0)	14 (100)	0.013 [a] *
Temporal lobe	9	7 (77.8)	2 (22.2)		0(0)	9 (100)	
Parietal lobe	5	3 (60)	2 (40)		0(0)	5 (100)	
Occipital lobe	6	2 (33.3)	4 (66.7)		0(0)	6 (100)	
Multiple lobes	8	3 (37.5)	5 (62.5)		0(0)	8 (100)	
Corpus callosum	3	2 (66.7)	1 (33.3)		0(0)	3 (100)	
Cerebellum	6	4 (66.7)	2 (33.3)		0(0)	6 (100)	
Cervical cord	6	3 (50)	3 (50)		0(0)	6 (100)	
Thoracic cord	3	2 (66.7)	1 (33.3)		1(33.3)	2 (66.7)	
Size of tumor							
<2 cm	13	6 (46.2)	7 (53.8)	0.641 [c]	1 (7.7)	12 (92.3)	0.181[c]
2-4 cm	23	14 (60.9)	9 (39.1)		0(0)	23 (100)	
4-6 cm	12	6 (50)	6 (50)		0(0)	12 (100)	
>6 cm	12	5 (41.7)	7 (58.3)		0(0)	12 (100)	

Categorical variables were expressed as number (percentage), [a], Chi-square test; [b], Mann Whitney U test; [c], Chi-square test for trend; P-value<0.05 is significant.

with progression occurrence (P=0.512). *MSH2* intact expression showed about 21 months survival compared with 5.5 months for those with lost *MSH2* expression. By the Log Rank test, there was a significant difference by *MSH2* as positive expression showed a favorable prognosis (P<0.001) (Supplementary Table 3, Figure 6E).

Relation between IDH1mutation and MMR protein expression and patient management

No significant association was detected between *IDH1* mutation and *MLH1* expression and each of the extent of surgical resection (P=0.602 and 0.780, respectively), the size of residual tumor (P=0.411 and

Table 4. Relationship between Patient Characteristics and MSH6 IHC and MSH2 IHC Staining

Patient characteristics		MSH6 IHC			MSH2 IHC		
	N	Negative MSH6 (N=3) N (%)	Positive MSH6 (N=57) N (%)	P	Negative MSH2 (N=2) N (%)	Positive MSH2 (N=58) N (%)	P
Gender							
Male	37	3 (8.1)	34 (91.9)	0.297[a]	2 (5.4)	35 (94.6)	0.519[a]
Female	23	0 (0)	23 (100)		0 (0)	23 (100)	
Age (years)							
Mean± SD		41.66±16.07	44.64±15.43	0.623[b]	47.50±17.67	44.39±15.41	0.805[b]
Median (Range)		35 (30 – 60)	45 (12 – 80)		47.50 (35 – 60)	45 (12 – 80)	
Age group							
≤40 years	22	2 (9.1)	20 (90.9)	0.258[c]	1 (4.5)	21 (95.5)	0.583[c]
>40-60 years	31	1 (3.2)	30 (96.8)		1 (3.2)	30 (96.8)	
>60 years	7	0 (0)	7 (100)		0 (0)	7 (100)	
Grade							
Grade II	24	0 (0)	24 (100)	0.441[c]	0 (0)	24 (100)	0.298[c]
Grade III	13	2 (15.4)	11 (84.6)		1 (7.7)	12 (92.3)	
Grade IV (1ry)	12	0 (0)	12 (100)		0 (0)	12 (100)	
Grade IV (2ry)	11	1 (9.1)	10 (90.9)		1 (9.1)	10 (90.9)	
Location of tumor							
Brain	50	2 (4)	48 (96)	0.427[a]	1 (2)	49 (98)	0.308[a]
Spinal cord	10	10 (10)	9 (90)		1 (10)	9 (90)	
Side of tumor							
Left side	26	2 (7.7)	24 (92.3)	0.535[a]	1 (3.8)	25 (96.2)	0.526[a]
Right side	21110	0 (0)	21 (100)		0 (0)	21 (100)	
Non-lateralized	3	1 (10)	9 (90)		1 (10)	9 (90)	
Multicentricity		0 (0)	3 (100)		0 (0)	3 (100)	
Site of tumor							
Frontal lobe	14	0 (0)	14 (100)	0.516[a]	0 (0)	14	0.538[a]
Temporal lobe	9	0 (0)	9 (100)		0 (0)	9	
Parietal lobe	5	1 (20)	4 (80)		0 (0)	5	
Occipital lobe	6	0 (0)	6 (100)		0 (0)	6	
Multiple lobes	8	1 (12.5)	7 (87.5)		1 (12.5)	7 (87.5)	
Corpus callosum	3	0 (0)	3 (100)		0 (0)	3	
Cerebellum	6	0 (0)	6 (100)		0 (0)	6	
Cervical cord	6	1 (16.7)	5 (83.3)		1 (16.7)	5 (83.5)	
Thoracic cord	3	0 (0)	3 (100)		0 (0)	3	
Size of tumor							
<2 cm	13	1 (7.7)	12 (92.3)	0.932[c]	1 (7.7)	12 (92.3)	0.872
2-4 cm	23	1 (4.3)	22 (95.7)		0 (0)	23 (100)	
4-6 cm	12	0 (0)	12 (100)		0 (0)	12 (100)	
>6 cm	12	1 (8.3)	11 (91.7)		1 (8.3%)	11 (91.7)	

[a], Chi-square test; [b], Mann Whitney U test; [c], Chi-square test for trend; P<0.05 is significant.

0.210, respectively), radiotherapy (P=0.140 and 1.000, respectively), or concurrent chemotherapy (P=0.752 and1.000, respectively) (Supplementary Table 4).

In addition, *MSH6* and *MSH2* expressions were not statistically associated with the extent of surgical resection (P=0.682 and 0.662, respectively), the size of residual tumor (P=0.430 and 0.840, respectively), radiotherapy (P=1.000 and 1.000, respectively), or concurrent chemotherapy (P=0.268 and 0.512, respectively) (Supplementary Table 5).

Discussion

The WHO categorization scheme for adult gliomas was considerably modified to integrate crucial new genetic results on diffuse gliomas. Brain tumor classification was

Table 5. Relationship between IHC Staining for MLH1/MSH6/MSH2 and *IDH1* Mutation

IHC staining for MLH1/MSH6/ MSH2	N	Wild *IDH1* (N=31)		Mutant *IHD1* (N=29)		Test	P
		No.	%	No.	%		
MLH1 IHC							
Negative	1	1	100%	0	0%	0.951[a]	1
Positive	59	30	50.80%	29	49.20%		
MSH6 IHC							
Negative	3	1	33.30%	2	66.70%	0.425[a]	0.606
Positive	57	30	52.60%	27	47.40%		
MSH2 IHC							
Negative	2	0	0%	2	100%	2.212[a]	0.229
Positive	58	31	53.40%	27	46.60%		

Categorical variables were expressed as number (percentage); [a], Chi-square test: P<0.05 is significant

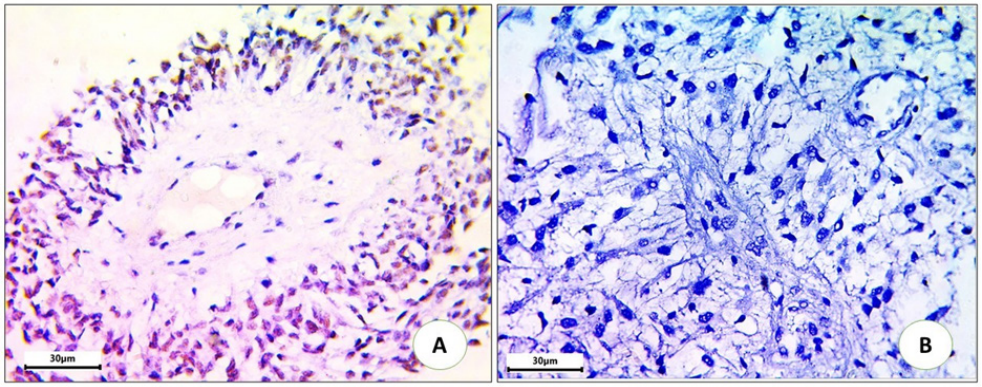

Figure 3. A: Ependymoma (grade II): showing diffuse positive nuclear MSH6 staining of tumor cells (intact expression) (MSH6 IHC stain, x400 original magnification); B: Anaplastic astrocytoma (grade III): showing negative nuclear MSH6 staining of tumor cells (lost expression) (MSH6 IHC stain, x400 original magnification).

expanded to encompass immunohistopathological and genetic criteria [18].

In our study, the age of the included patients ranged from 12 to 80 years with a mean age of 44.5 years and the commonest age group was 40-60 years. This agreed with Larjavaara et al. [19] who reported that the age of glioma patients ranged from 20 to 69 years old with a mean age of 49.2 years. Also, Roohani et al. [20] reported that the mean age was 43.57 years. However, they found the commonest age group was 30-40 years old. However, Lin et al. [10] found that the median age at diagnosis for all primary gliomas was 37.7 years old. This discrepancy can be explained by the wider age range in their study where it ranged from 1 to 82 years old.

In this study, the occurrence of gliomas in male patients was more common than in females with a ratio of 1.6:1. Similarly, the ratio of males to females was 1.4:1 in the study performed by Lin et al. [10]. In addition, Roohani et al. [20] reported that male to female ratio was 1.8:1 in cerebral glioma [20]. These results can be explained by the neuroprotective effects of estrogen where late menarche and early menopause (shorter exposure to

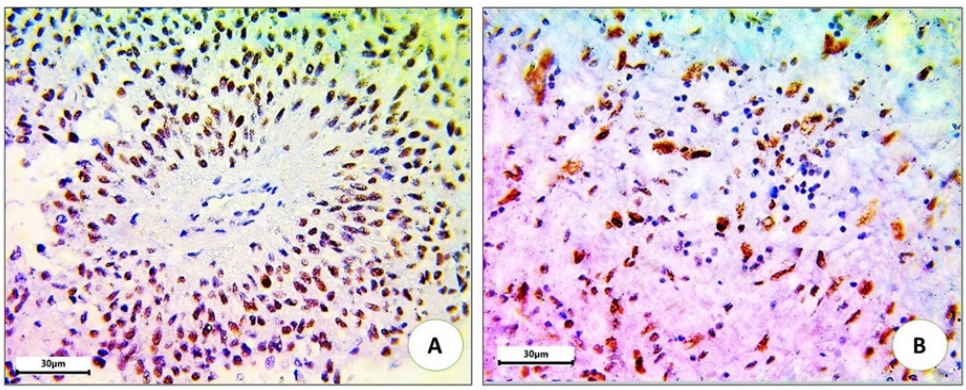

Figure 4. A: Ependymoma (grade II): showing diffuse positive nuclear MSH2 staining of tumor cells (intact expression) (MSH2 IHC stain, x400 original magnification); B: Anaplastic astrocytoma (grade III): showing diffuse positive nuclear MSH2 staining of tumor cells (MSH2 IHC stain, x400 original magnification).

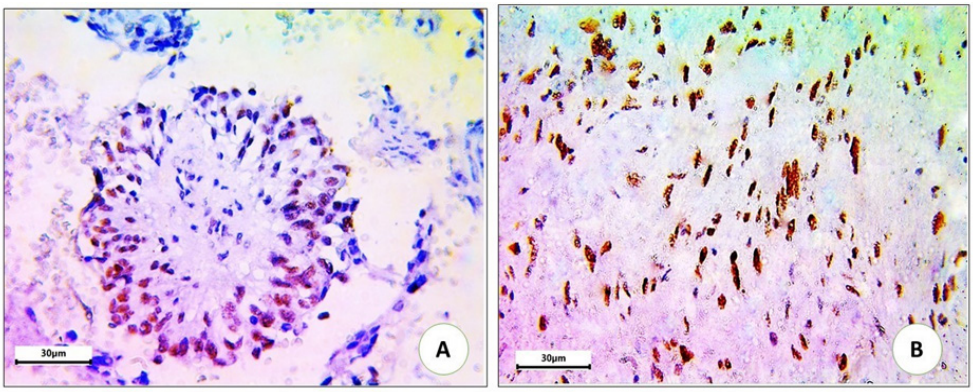

Figure 5. A: Ependymoma (grade II): showing diffuse positive nuclear PMS2 staining of tumor cells (intact expression) (PMS2 IHC stain, x400 original magnification); B: Anaplastic astrocytoma (grade III): showing diffuse positive nuclear PMS2 staining of tumor cells (PMS2 IHC stain, x400 original magnification).

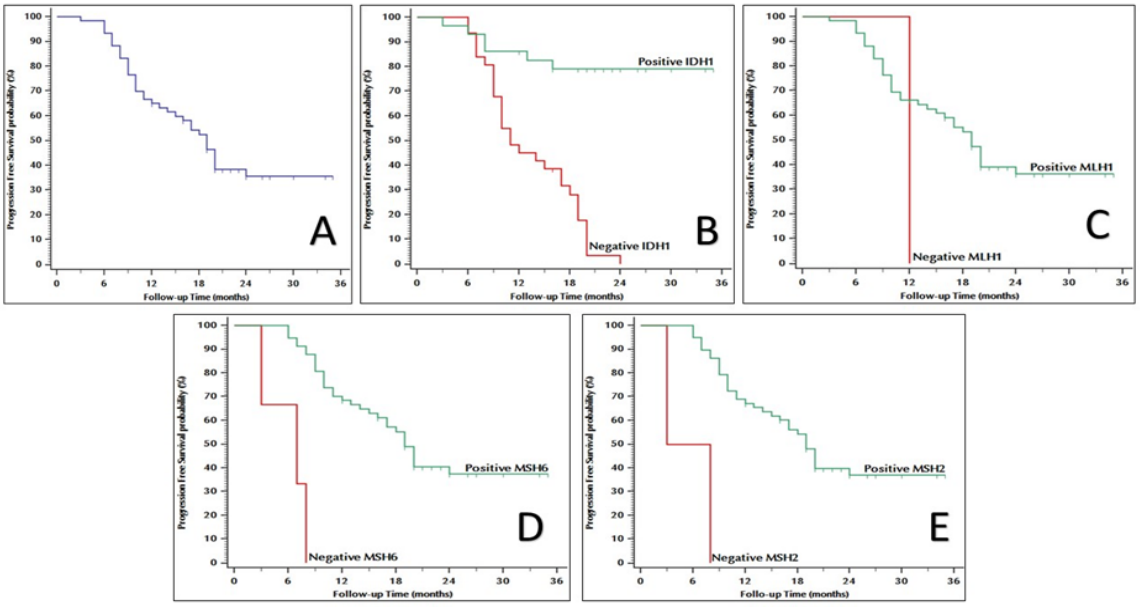

Figure 6. Kaplan Meier Plot for Progression Free Survival (PFS) among the Studied Patients A: for all patients, B: stratified by IDH1 IHC staining, C: stratified by MLH1 IHC staining, D: stratified by MSH6 IHC staining, E: stratified by MSH2 IHC staining.

female hormones) were associated with increased glioma risk [21]. Moreover, an increase in testosterone levels has been reported in glioblastoma, which suggests that a testosterone-activated AR signaling pathway has a key role in its proliferation, migration, and invasion [22].

In our study, the majority of cases were brain gliomas. This result was also confirmed by Lin et al. [10] where 92.9% of cases occurred in the brain and Roohani et al. [20] where brain gliomas represented 85% of cases. Also, the unilateral tumor location was a majority that was more common on the left side. These findings were very near to the results of Roohani et al. [20] where the unilateral location was 93.1%. However, almost equal right and left-side distributions were detected. Also, Larjavaara et al. [19] detected the bilateral location was found in 4.9% of cases.

In our study, the frequency of gliomas in different brain lobes showed that the frontal lobe was the most recorded followed by the temporal lobe, the occipital lobe, and the parietal lobe. Our results were similar to Lin et al. [10]where gliomas mostly occurred in the frontal lobe (35.8%) and temporal lobe (17.4%) . In addition, Larjavaara et al. [19] and Roohani et al. [20] found near results where higher frequencies of gliomas were in the frontal lobe, the temporal lobe, the parietal lobe, and the occipital lobe in order.

Our work showed that the tumor maximal dimension was ≤6 cm in 80% of patients. Similarly, Lin et al. [10] found that the average diameter of glioma was 4.9 cm . This was far from Magrini et al. [23] and Edward et al. [24]. They stated that the maximal dimension of gliomas was ≤6 cm in only 48% and 57.3% of the patients, respectively.

Reifenberger et al. [25] stated that astrocytoma was the most common glioma type comprising about 70% of glial tumors . This was not far from our results as 76.7% of cases were astrocytoma.

Matching with previous studies, glioblastoma

multiforme was the most common type of astrocytoma in our study [26, 27]. Moreover, GBM represented the majority of gliomas (29.7%) in the study performed by Lin et al. [10]. However, the differences between the incidence rates of different types of gliomas in the present study and other studies may be explained by ethnic and geographical variations. The genetic polymorphisms, the number of available cases, and exclusion criteria may also contribute.

In the current study, there were about 40% in grade II and grade IV. These results were near to those of Roohani et al. [20]. Grade II tumors represented 30% and grade IV represented 46.5% of adult cerebral gliomas . However, in a large study by Magrini et al. [23] in 12 Italian radiation oncology centers, adult cerebral gliomas were 72% of grade IV, and 7% of grade II. The variation may be attributed to the variation in sample size.

Miller et al. [28] cleared that *IDH* mutation is one of the numerous molecular criteria important for the subtype diagnosis of diffuse gliomas. Importantly, all *IDH*-mutant gliomas had a much better outcome than malignant diffuse *IDH*-wild gliomas such as glioblastoma.

IDH1 results showed that 48.3% of gliomas were mutant. About 46% and 40% were positive in grade II and III gliomas, respectively. We reported that all cases of secondary GBM were positive for *IDH1* mutation, while only 16.7% of primary cases were positive. This was similar to the results of Cambruzzi [29] who stated that *IDH* mutations are rare in primary GBM. Primary and secondary GBM are genetically and clinically different despite their similarities on a histological basis. Primary GBM arises with no previous history of a lower-grade lesion while secondary GBM evolves from low-grade astrocytoma .

In our study, we found a non-significant association between *IDH1* mutation and other variables. However, a significant association was found between *IDH1* mutation and the absence of glioma progression and improved PFS. Similar to our findings, Zeng et Al.[30] established that patients with *IDH1/2* mutation survived significantly longer than patients with *IDH1/2*-wildtype gliomas. In addition, Xia et al. [31] found that *IDH1/2* mutation had significant advantages in PFS and OS in diffuse gliomas. Moreover, Miller et al. [28] demonstrated that *IDH1* mutation was a strong prognostic factor in gliomas, whatever the grade where multivariate analysis confirmed it as an independent favorable predictor of outcome. Similarly, adult glioblastoma studies showed that *IDH* mutations predict prolonged PFS as well as increased overall survival. De Quintana-Schmidt et al. [32]found that the median survival in patients with *IDH1* mutation was 23.6 months compared with 11.9 months in the cases with *IDH1*-wildtype.

Regarding response to treatment, our study didn't find any significant associations between *IDH1* mutation and patient management modalities. On the other side, Juratli et al. [33] found that radiotherapy and chemotherapy (mostly procarbazine, 1-(2-chloroethyl)-3-cyclohexyl-l-nitrosourea, and vincristine (PCV)) significantly improved PFS and OS in *IDH*-mutant secondary high-grade astrocytoma patients. In addition, Kurdi et al. [34] determined that concomitant radiotherapy with Temozolomide chemotherapy significantly increased than isolated radiotherapy in patients with *IDH*-wildtype glioblastoma .

The presence of mutated *IDH* can affect epigenetic alterations with a higher probability of alteration of *MMR* protein expression in gliomas. DNA mismatch repair (*MMR*) is a system that identifies and repairs mismatched nucleotides to guarantee genomic stability and integrity. It depends on four key genes: *MSH2*, *MSH6*, *MLH1*, and *PMS2* [35].

In this study, *MSH6* and *MSH2* expression were lost in 5% and 3.3% of cases, respectively. *MLH1* showed the least deficient expression with 1.7%. Whilst PMS2 was intact in all cases. So, PMS2 was excluded from statistical analysis. Caccese et al. [36] identified immunohistochemical loss of *MMR* protein expression (partial or complete) in 12.1% of the patients. Among them, 4.2% showed a complete immunohistochemical loss of at least one *MMR* protein. Both *MSH2* and *MSH6* were lost in 33% of patients, while both PMS2 and *MLH1* were lost in 23% of patients. In addition, a significantly lower *MMR* protein expression was detected in recurrent GBM compared to primary glioblastoma .

We found no statistically significant association between *MSH6* or *MSH2* and any of the studied criteria. Similarly, Caccese et al. [36] found no statistically significant association with gender while they found a higher probability of immunohistochemical loss of *MMR* proteins in grade III than in grade IV gliomas.

In our study, there was a highly significant association between *MSH6*, *MSH2*, and *MLH1* expressions and PFS. However, Caccese et al. [36] denied that. In addition, Kawaguchi et al. [37] found no difference between OS and the *MMR* mutation irrespective of *IDH* mutation.

On correlating *IDH1* mutation and *MMR* protein expressions, no significant association was found, but only *MSH2* expression was statistically associated with *MSH6* expression. On the other side, immunohistochemical loss of *MMR* proteins can detect hypermutated gliomas with high sensitivity and specificity as demonstrated by McCord et al. [38]. Also, Caccese et al. [37] showed a significant correlation between *MMR* protein loss in high-grade gliomas and *IDH* mutation .

In our study, there was no significant association between any of the *MMR* proteins and patient management. However, Caccese et al. [36] found that tissues obtained after temozolomide therapy showed a higher probability of immunohistochemical loss of *MMR* protein expression than the cases analyzed before treatment.

Knowledge of the predictors of immunotherapy efficacy is important to identify the subgroup of patients responding to this treatment. Hence, the immunohistochemical loss of *MMR* protein expression could be a valid predictor of checkpoint inhibitor efficacy in gliomas [39].

In conclusion, *IDH1* mutation and *MMR* proteins (*MLH1*, *MSH6*, and *MSH2*) could help predict glioma outcomes.

Author Contribution Statement

WE, ABW, AO, SG, SMI, ASE, KA, TK, AHM interpreted the data. NMS, HR, MIA and SH wrote the main manuscript text. All authors reviewed the manuscript.

Acknowledgements

Not applicable.

Ethical approval and consent to participate

This study got approval from the Institution Review Board of the Faculty of Medicine, Zagazig University (ZU-IRB#11075-17-9-2023).

Availability of data and material

Available upon a reasonable request from the author.

Competing interests

The authors declare that there is no conflict of interest.

References

1. Schaff LR, Mellinghoff IK. Glioblastoma and other primary brain malignancies in adults: A review. Jama. 2023;329(7):574-87. https://doi.org/10.1001/jama.2023.0023.
2. Antonelli M, Poliani PL. Adult type diffuse gliomas in the new 2021 who classification. Pathologica. 2022;114(6):397-409. https://doi.org/10.32074/1591-951x-823.
3. Whitfield BT, Huse JT. Classification of adult-type diffuse gliomas: Impact of the world health organization 2021 update. Brain Pathol. 2022;32(4):e13062. https://doi.org/10.1111/bpa.13062.
4. Bhavya B, Anand CR, Madhusoodanan UK, Rajalakshmi P, Krishnakumar K, Easwer HV, et al. To be wild or mutant: Role of isocitrate dehydrogenase 1 (idh1) and 2-hydroxy glutarate (2-hg) in gliomagenesis and treatment outcome in glioma. Cell Mol Neurobiol. 2020;40(1):53-63. https://doi.org/10.1007/s10571-019-00730-3.
5. Tommasini-Ghelfi S, Murnan K, Kouri FM, Mahajan AS, May JL, Stegh AH. Cancer-associated mutation and beyond: The emerging biology of isocitrate dehydrogenases in human disease. Sci Adv. 2019;5(5):eaaw4543. https://doi.org/10.1126/sciadv.aaw4543.
6. Deshmukh S, Kelly C, Tinoco G. Idh1/2 mutations in cancer: Unifying insights and unlocking therapeutic potential for chondrosarcoma. Target Oncol. 2025;20(1):13-25. https://doi.org/10.1007/s11523-024-01115-3.
7. Liu Y, Xu W, Li M, Yang Y, Sun D, Chen L, et al. The regulatory mechanisms and inhibitors of isocitrate dehydrogenase 1 in cancer. Acta Pharm Sin B. 2023;13(4):1438-66. https://doi.org/10.1016/j.apsb.2022.12.019.
8. Internò V, Melaccio A, Vitale P, Spedaliere R, Buonfantino M, Messina R, et al. Therapeutic inhibition of isocitrate dehydrogenase mutations in glioma and cholangiocarcinoma: New insights and promises-a narrative review. Chin Clin Oncol. 2024;13(5):69. https://doi.org/10.21037/cco-24-17.
9. Wood MD, Halfpenny AM, Moore SR. Applications of molecular neuro-oncology - a review of diffuse glioma integrated diagnosis and emerging molecular entities. Diagn Pathol. 2019;14(1):29. https://doi.org/10.1186/s13000-019-0802-8.
10. Lin Z, Yang R, Li K, Yi G, Li Z, Guo J, et al. Establishment of age group classification for risk stratification in glioma patients. BMC Neurol. 2020;20(1):310. https://doi.org/10.1186/s12883-020-01888-w.
11. Berzero G, Di Stefano AL, Ronchi S, Bielle F, Villa C, Guillerm E, et al. Idh-wildtype lower-grade diffuse gliomas: The importance of histological grade and molecular assessment for prognostic stratification. Neuro Oncol. 2021;23(6):955-66. https://doi.org/10.1093/neuonc/noaa258.
12. Villanueva-Meyer JE, Wood MD, Choi BS, Mabray MC, Butowski NA, Tihan T, et al. Mri features and idh mutational status of grade ii diffuse gliomas: Impact on diagnosis and prognosis. AJR Am J Roentgenol. 2018;210(3):621-8. https://doi.org/10.2214/ajr.17.18457.
13. Suwala AK, Stichel D, Schrimpf D, Kloor M, Wefers AK, Reinhardt A, et al. Primary mismatch repair deficient idh-mutant astrocytoma (pmmrdia) is a distinct type with a poor prognosis. Acta Neuropathol. 2021;141(1):85-100. https://doi.org/10.1007/s00401-020-02243-6.
14. Tabori U, Hansford JR, Achatz MI, Kratz CP, Plon SE, Frebourg T, et al. Clinical management and tumor surveillance recommendations of inherited mismatch repair deficiency in childhood. Clin Cancer Res. 2017;23(11):e32-e7. https://doi.org/10.1158/1078-0432.Ccr-17-0574.
15. Kim B, Tabori U, Hawkins C. An update on the cns manifestations of brain tumor polyposis syndromes. Acta Neuropathol. 2020;139(4):703-15. https://doi.org/10.1007/s00401-020-02124-y.
16. Horbinski C. What do we know about idh1/2 mutations so far, and how do we use it? Acta Neuropathol. 2013;125(5):621-36. https://doi.org/10.1007/s00401-013-1106-9.
17. Alphones S, Chatterjee U, Singh A, Das A, Zameer L, Achari R, et al. Immunohistochemical screening for mismatch repair protein deficiency in paediatric high-grade gliomas - institutional experience and review of literature. Childs Nerv Syst. 2021;37(8):2521-30. https://doi.org/10.1007/s00381-021-05229-1.
18. Komori T. Grading of adult diffuse gliomas according to the 2021 who classification of tumors of the central nervous system. Lab Invest. 2022;102(2):126-33. https://doi.org/10.1038/s41374-021-00667-6.
19. Larjavaara S, Mäntylä R, Salminen T, Haapasalo H, Raitanen J, Jääskeläinen J, et al. Incidence of gliomas by anatomic location. Neuro Oncol. 2007;9(3):319-25. https://doi.org/10.1215/15228517-2007-016.
20. Roohani y, khalatbari m, sadeghipoor a, zaeri f, esmail ak, hosseinzadeh mr. The clinical trend and prognosis of patients with brain glioma. Iran J Pathol. 2010;77-82.
21. Altinoz MA, Ozpinar A, Elmaci I. Reproductive epidemiology of glial tumors may reveal novel treatments: High-dose progestins or progesterone antagonists as endocrino-immune modifiers against glioma. Neurosurg Rev. 2019;42(2):351-69. https://doi.org/10.1007/s10143-018-0953-1.
22. Rodríguez-Lozano DC, Piña-Medina AG, Hansberg-Pastor V, Bello-Alvarez C, Camacho-Arroyo I. Testosterone promotes glioblastoma cell proliferation, migration, and invasion through androgen receptor activation. Front Endocrinol (Lausanne). 2019;10:16. https://doi.org/10.3389/fendo.2019.00016.
23. Magrini SM, Ricardi U, Santoni R, Krengli M, Lupattelli M, Cafaro I, et al. Patterns of practice and survival in a retrospective analysis of 1722 adult astrocytoma patients treated between 1985 and 2001 in 12 italian radiation oncology centers. Int J Radiat Oncol Biol Phys. 2006;65(3):788-99. https://doi.org/10.1016/j.ijrobp.2006.01.026.
24. Edwards LA, Woo J, Huxham LA, Verreault M, Dragowska WH, Chiu G, et al. Suppression of vegf secretion and

changes in glioblastoma multiforme microenvironment by inhibition of integrin-linked kinase (ilk). Mol Cancer Ther. 2008;7(1):59-70. https://doi.org/10.1158/1535-7163.Mct-07-0329.
25. Reifenberger g, blümcke i, pietsch t, paulus w. Pathology and classification of tumors of the nervous system. In: Tonn jc, westphal m, rutka jt, editors. Oncology of cns tumors. Springer, berlin, heidelberg, 2010; 3-75. Https://doi.Org/10.1007/978-3-642-02874-8_1.
26. Xie Q, Wu TP, Gimple RC, Li Z, Prager BC, Wu Q, et al. N(6)-methyladenine DNA modification in glioblastoma. Cell. 2018;175(5):1228-43.e20. https://doi.org/10.1016/j.cell.2018.10.006.
27. Petterson SA, Dahlrot RH, Hermansen SK, S KAM, Gundesen MT, Wohlleben H, et al. High levels of c-met is associated with poor prognosis in glioblastoma. J Neurooncol. 2015;122(3):517-27. https://doi.org/10.1007/s11060-015-1723-3.
28. Miller JJ, Gonzalez Castro LN, McBrayer S, Weller M, Cloughesy T, Portnow J, et al. Isocitrate dehydrogenase (idh) mutant gliomas: A society for neuro-oncology (sno) consensus review on diagnosis, management, and future directions. Neuro Oncol. 2023;25(1):4-25. https://doi.org/10.1093/neuonc/noac207.
29. Cambruzzi E. The role of *IDH1/2* mutations in the pathogenesis of secondary glioblastomas. J Bras Patol Med Lab. 2017;53(5):338-44. https://doi.org/10.5935/1676-2444.20170055.
30. Zeng A, Hu Q, Liu Y, Wang Z, Cui X, Li R, et al. Idh1/2 mutation status combined with ki-67 labeling index defines distinct prognostic groups in glioma. Oncotarget. 2015;6(30):30232-8. https://doi.org/10.18632/oncotarget.4920.
31. Xia L, Wu B, Fu Z, Feng F, Qiao E, Li Q, et al. Prognostic role of idh mutations in gliomas: A meta-analysis of 55 observational studies. Oncotarget. 2015;6(19):17354-65. https://doi.org/10.18632/oncotarget.4008.
32. de Quintana-Schmidt C, Alvarez-Holzapfel MJ, Nomdedeu-Guinot J, Bague-Rosell S, Gallego-Rubio O, Leidinger A, et al. Isocitrate dehydrogenase type i mutation as a prognostic factor in glioblastoma and a literature review. Neurocirugia (Astur). 2015;26(6):276-83. https://doi.org/10.1016/j.neucir.2015.04.001.
33. Juratli TA, Lautenschläger T, Geiger KD, Pinzer T, Krause M, Schackert G, et al. Radio-chemotherapy improves survival in idh-mutant, 1p/19q non-codeleted secondary high-grade astrocytoma patients. J Neurooncol. 2015;124(2):197-205. https://doi.org/10.1007/s11060-015-1822-1.
34. Kurdi M, Alkhotani A, Alsinani T, Alkhayyat S, Katib Y, Jastaniah Z, et al. Effects of radiotherapy alone versus concomitant radiotherapy with temozolomide chemotherapy on the outcome of idh-wildtype glioblastoma patients. Clin Oncol (R Coll Radiol). 2025;38:103741. https://doi.org/10.1016/j.clon.2024.103741.
35. Higuchi F, Fink AL, Kiyokawa J, Miller JJ, Koerner MVA, Cahill DP, et al. Plk1 inhibition targets myc-activated malignant glioma cells irrespective of mismatch repair deficiency-mediated acquired resistance to temozolomide. Mol Cancer Ther. 2018;17(12):2551-63. https://doi.org/10.1158/1535-7163.Mct-18-0177.
36. Caccese M, Ius T, Simonelli M, Fassan M, Cesselli D, Dipasquale A, et al. Mismatch-repair protein expression in high-grade gliomas: A large retrospective multicenter study. Int J Mol Sci. 2020;21(18). https://doi.org/10.3390/ijms21186716.
37. Kawaguchi K, Otani R, Kikuchi M, Kushihara Y, Funata N, Yamada R, et al. Genetic characteristics of mismatch repair-deficient glioblastoma. NMC Case Rep J. 2021;8(1):565-71. https://doi.org/10.2176/nmccrj.cr.2020-0366.
38. McCord M, Steffens A, Javier R, Kam KL, McCortney K, Horbinski C. The efficacy of DNA mismatch repair enzyme immunohistochemistry as a screening test for hypermutated gliomas. Acta Neuropathol Commun. 2020;8(1):15. https://doi.org/10.1186/s40478-020-0892-2.
39. Briggs M, Bruty J, O'Donovan D, Liu H, Tischkowitz M, Tarpey P, et al. Lynch/turcot syndrome and sporadic mismatch repair deficient high-grade gliomas – a case series. Neuro-Oncology. 2022;24:iv16-iv. https://doi.org/10.1093/neuonc/noac200.071.