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Investigation of IDH1 in Astrocytoma and Glioblastoma for Reclassification According to 2021 WHO Classification of CNS Tumors

Abstract

The most prevalent neoplasms of the central nervous system are diffuse gliomas. According to the updated 2021 WHO classification of central nervous system (CNS) tumors, Isocitrate dehydrogenase 1 (IDH1) and IDH2 are now included in the classification of gliomas. IDH1 mutation is responsible for early events in the tumoral transformation by producing the oncometabolite 2-hydroxyglutarate (2-HG). This mutation plays a more significant influence on the outcome of patients. This study is the first one that surveys the relationship between IDH1 and demographic characteristics. Also, we intended to reclassify astrocytoma and glioblastoma multiforme(GBM) for the first time in our center based on the 2021 WHO CNS tumors classification.

During two years of cross-sectional research at Al-Zahra hospital in Isfahan, Iran, IDH1 mutation with immunohistochemistry (IHC) staining was identified in all diagnosed patients with gliomas.

Forty-nine samples were collected whereby, 20 (40.8%) were glioblastoma, and 29 (59.2%) were astrocytomas. Out of the 49 samples, 32 (65.3%) were WHO CNS grade 4 (12 astrocytomas and 20 glioblastomas). The p-value of 0.019 (less than 0.05) suggests a statistically significant difference between our variables, age, and grade.

We revealed that the most prevalent brain tumor samples were glioblastoma, WHO CNS grade 4, and IDH-wildtype. The majority of the tumors were frontal lobe-based and IHD-negative. The most of the tumors in patients under 40 were astrocytomas (WHO CNS grade 2,3,4) while glioblastoma (WHO CNS grade 4) was more widespread in those above 55. According to the 2021 WHO CNS tumors classification, glioblastoma IDH-mutant reclassified as astrocytoma, WHO CNS grade 4.

Keywords: *Glioma, Isocitrate Dehydrogenase (IDH), mutation, wildtype*

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Introduction

Astrocytomas, oligodendrogliomas, ependymomas, and all gliomas, comprise the vast bulk of brain tumours. Glioma is divided into four classes (1, 2). In most instances, grade I gliomas could be resected to avoid their progression into high grade conditions. On the other hand, gliomas of WHO grades II and III tend to progress into high-grade lesions and have a poor prognosis. The prognosis for the most aggressive form of the tumor, WHO grade IV (glioblastomas), is dismal (3).

Histological features, growth rate, and genetic markers are used in the new WHO classification for CNS tumors in 2021 to establish a diagnosis. In 2021, the WHO proposed using more objective criteria, such as phenotypic and genotypic characterizations (4). IDH mutation is one of the most critical factors that have been advocated. In addition, it is advantageous to employ the IDH mutation to distinguish between primary and secondary GBMs (5, 6).

Isocitrate dehydrogenase 1 (IDH1) or IDH2 is one of the tumor markers, and it's been linked to diffuse glioma specifically (7-9). Mutated enzymes with neomorphic activity, such as oncometabolite 2-hydroxyglutarate (2-HG), are metabolites of these markers, which result in cellular epigenetic programs and subsequently produce metabolic profiles (10-12).

IDH mutation was reported in most WHO grade II and III gliomas. IDH-wildtype glioma had a poor prognosis compared to mutant types and had less survival rate (13). In addition, several studies hypothesized that these two types are different diseases (14). Although these two patient populations have different biology, genomic changes, and clinical characteristics, they tend to be treated similarly within the medical community. Both types of gliomas interfere with normal brain function in similar ways (15).

Furthermore, according to the same categorization, Glioblastoma, IDH-mutant, occurs in around 10% of cases owing to low-grade gliomas in individuals less than 45 years old. Glioblastoma, IDH-wildtype, appears as the Primary form in roughly 90% of cases and is more prevalent in patients over 55 (7,16,17).

According to the latest WHO 2021 classification, Diffuse astrocytoma IDH-mutant tumors are considered as s single type called Astrocytoma, IDH-mutant, and include all three WHO CNS grades 2, 3, 4. Accordingly, Diffuse glioma tumors are classified as Adult-type, including IDH-mutant Astrocytoma, IDH-mutant Oligodendroglioma, and IDH-wildtype Glioblastoma (18).

IDH1 mutations may lead to metabolic alterations critical in therapeutic approaches (19). Several clinical trials have recently surveyed IDH1 mutation in diffuse glioma as a therapeutic target (20-22).

Method:

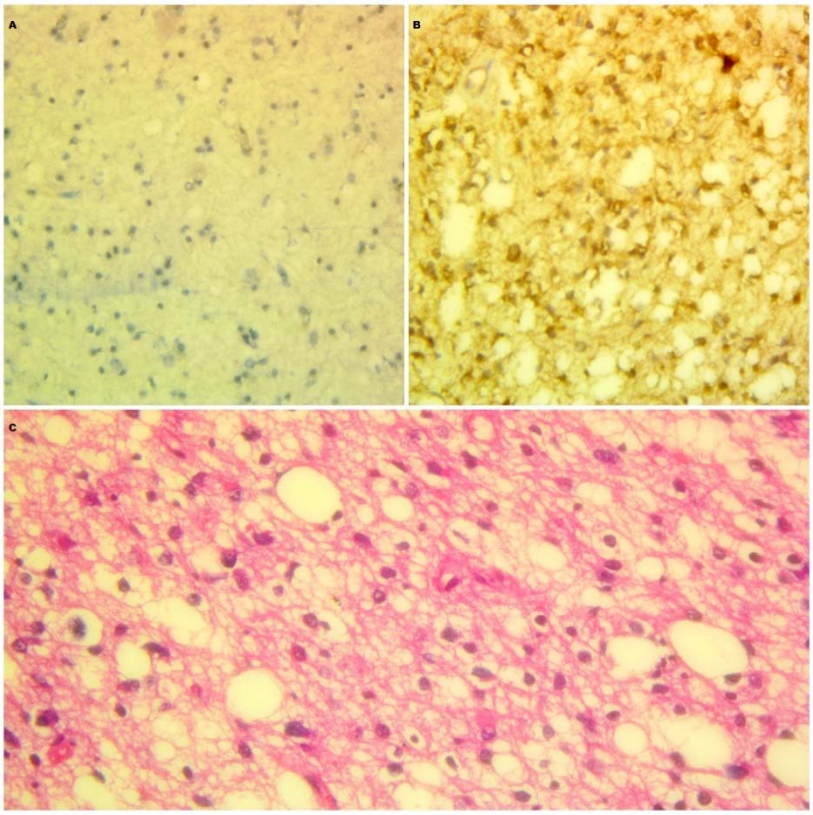
We conducted a cross-sectional study from April 2020 to April 2022 in Al-Zahra hospital, Isfahan, Iran. All patient samples

previously diagnosed with diffuse glioma, based on the WHO 2016, were included in the study. Samples of patients with a history of chemotherapy and radiotherapy before sampling, and those improperly fixed in the paraffin block were excluded from the study.

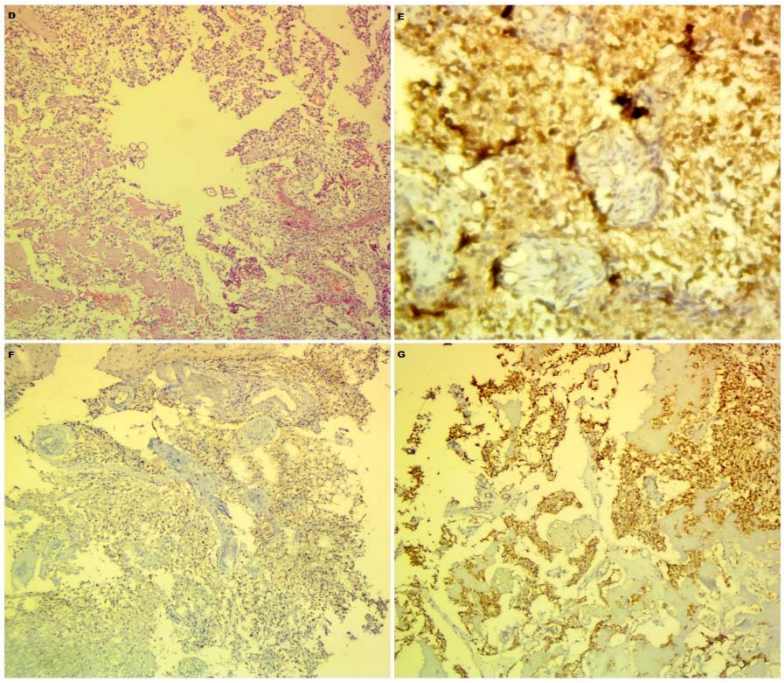
Immunostaining:

For immunohistochemistry (IHC) examination, first, a paraffin block selected from each patient's sample was cut to a thickness of 3 µm and placed on a glass slide, then a slide was set in an oven at 60°C for 45 minutes, placed in 100% xylene and then in graded alcohol(100%, 95%, 85%, 75% ethanol) to deparaffinize and rehydrate the tissue, rinsed with distilled water, applied TE (Tris-EDTA) buffer at PH 9 as antigen retrieval solution and put in microwave for 20 min, cooled down at room temperature, covered tissue with 3% H2O2 for 10 min to inactivate endogenous hydrogen peroxidase, washed with PBS(phosphate-buffered saline) and distilled water, applied dilute primary antibody(1:100) anti-IDH1 RH132/clone H09 and incubated 60 min at room temperature, washed with PBS, applied secondary antibody anti-mouse/rabbit polymer HRP-label and incubated for 20 min at room temperature, washed with PBS, covered tissue with diaminobenzidine-chromogen(DAB) solution and incubated for 5 min to allow for brown color development, rinsed with distilled water, counterstained with Hematoxillyne for 30 seconds, rinsed with running water, dehydrated tissue with graded alcohol(75%, 85%, 95%, 100% ethanol) and xylene, mounted coverslip on slide, allowed slide to dry at room temperature and analyzed the results with microscope. IDH1 marker color scoring is as follows:

Score 0: No staining, score +1: Nuclear staining (weak or ambiguous), Score +2: Cytoplasmic staining (strong) scattered and non-uniform, score +3: Cytoplasmic staining (strong) diffuse and Uniform. In case of cytoplasmic staining with a score of +2 and +3 in more than 10% of tumor cells, IDH1 marker staining is considered positive. Also, in each shift, prostate cancer or colon cancer tissue is considered positive external control, and reactive gliosis tissue is considered negative external control (23).



A: astrocytoma (IDH-wildtype), x10 – B And C: astrocytoma (WHO CNS grade 2, IDH-mutant), IHC, H&E, x10



D: glioblastoma multiforme (GBM), H&E, x10- E: astrocytoma (WHO CNS grade 4, IDH-mutant), x40- F: glioblastoma (WHO CNS grade 4, IDH-wildtype), x10- G: astrocytoma (WHO CNS grade 4, IDH-mutant), x10

Data analysis:

The results were described using mean±SD. Frequencies and percentages were used to describe categorical data. Quantitative data was analyzed using an independent t-test, and qualitative data were evaluated using the chi-square test. All the information was analyzed with the SPSS version 25 software, and a p-value of less than 0.05 was used to establish the statistical significance threshold.

Results:

During two years, 49 samples were collected. 20 (40.8%) samples were glioblastoma, and 29 (59.2%) were astrocytoma. Of the 49 samples, 32 (65.3%) were WHO CNS grade 4 (12 astrocytomas and 20 glioblastomas).

The p-value of 0.019 (less than 0.05) suggests a statistically significant relationship between our variables, age, and grade. More details are shown in table 1.

Table1. Demographic and histopathological characteristics of samples.

Type	glioblastoma	20(40.8%)
	astrocytomas	29(59.2%)
Grade	2	12(24.5%)
	3	5(10.2%)
	4	32(65.3%)
Age-group	<40y	19(38.8%)
	40-55y	12(24.5%)
	>55y	18(36.7%)
Gender	male	27(55.1%)
	female	22(44.9%)
IDH	positive	24(49%)
	negative	25(51%)
Location	frontal	21(42.9%)
	temporal	8(16.3%)
	parietal	5(10.2%)
	occipital	1(2%)
	Frontotemporal	9(18.4%)
	Temporoparietal	3(6.1%)
	Frontotemporoparietal	2(4.1%)

Table 2. Relation between age, grade, and other variables

The findings of the chi-square test demonstrate a significant difference between the levels of the two variables, age and tumor type. This discrepancy indicated that glioblastoma was the most prevalent tumor type in individuals aged 55 and older. In comparison, in the age group less than 40 years, they were more in the astrocytoma group. In addition, the p-value was found to be 0.019 (less than 0.05), indicating a significant difference between the age and grade. Compared to other age groups, a higher proportion of patients over 55 were in Grade 4. IDH positivity was more prevalent in age groups under 40, whereas IDH negativity was more frequent in older age groups. Consequently, the age group impacts whether IDH is positive or negative. Details are shown in table 2.

		<40y	40-55y	>55y	p-value	2	3	4	p-value
Tumor type	glioblastoma	3	5	12	0.007				
	astrocytoma	16	7	6					
Grade	2	7	5	0	0.019				
	3	3	1	1					
	4	9	6	17					
Gender	male	11	7	9	0.861	7	3	17	0.928
	female	8	5	9		5	2	15	
IDH	positive	14	4	6	0.023	8	4	12	0.077
	negative	5	8	12		4	1	20	
Location	frontal	7	5	9	0.824	5	2	14	0.538
	temporal	3	2	3		3	1	4	
	parietal	2	2	1		0	0	5	
	occipital	0	0	1		0	0	1	
	Frontotemporal	6	1	2		4	2	3	
	Temporoparietal	1	1	1		0	0	3	
	Frontotemporoparietal	0	1	1		0	0	2	

According to the results of the chi-square test, there is a statistically significant correlation between IDH levels and tumor type (p-value = 0.00). Atrocytoma showed a higher percentage of IDH-positive cases. Also, the p-value for the chi-square test is 0.01 however, a statistical difference distribution between the two categories of location and IDH is seen. The majority of the tumors were frontal lobe-based and IHD-negative (Table 3).

Table 3. Relation between gender, IDH, and other variables.

		female	male	p-value	positive	negative	p-value
Tumor type	Glioblastoma	9	11	0.99	0	20	0.00*
	Astrocytoma	16	13		24	5	
IDH	Positive	14	10	0.656			
	Negative	13	12				
Location	Frontal	12	9	0.731	8	13	0.01**
	Temporal	4	4		7	1	
	Parietal	3	2		0	5	
	Occipital	1	0		0	1	
	Frontotemporal	5	4		7	2	
	Temporoparietal	2	1		2	1	
	Frontotemporoparietal	0	2		0	2	

*Fisher's Exact Test: .000 (0 cells (.0%) have an expected count of less than 5)

**12 cells (85.7%) have an expected count of less than 5

The p-value for the chi-square test is 0.005 however, a statistical difference distribution between the two categories of location and tumor type is seen. The astrocytomas and glioblastoma are mostly located in the frontal lobe. (Table 4).

Table 4. Relation between tumor type and location

		glioblastoma	astrocytoma	p-value
Location	Frontal	9	12	0.005*
	Temporal	0	8	
	Parietal	5	0	
	Occipital	1	0	
	Frontotemporal	2	7	
	Temporoparietal	1	2	
	Frontotemporoparietal	2	0	

* 11 cells (78.6%) have an expected count of less than 5

Discussion:

According to the new WHO classification for 2021, significant changes have been made to the classification of tumors of the

central nervous system. A new section entitled "integrated diagnosis" has been added, integrating morphological and genetic findings to provide a proper diagnosis from a biological standpoint. Various genetic and epigenetic alterations contribute to the development of gliomas, with spontaneous mutations in the IDH gene complex representing the earliest and most prevalent genetic alterations (24).

The IDH mutation due to the synthesis of 2-hydroxyglutarate instead of alpha-ketoglutarate and the reduction of NADPH, as a possible oncometabolite, may be associated with the transformation of CNS neoplasia. This hypothesis suggests that mutated IDH is an oncogene that causes cell proliferation and the inability to differentiate cells. Hence, IDH mutation plays a crucial and primary role in glioma conversion (25, 26). The IDH mutation is present in approximately 80% of WHO grade II and III gliomas and secondary GBM. Mutations in IDH2 have been observed in less than 3% of all glial neoplasms (27, 28).

In a study by Khan et al., an examination of the evidence reveals the prognostic significance of the IDH1 mutation in GBM. Numerous studies have demonstrated that the IDH1 mutation is a significant and independent predictor of extended overall survival and progression-free survival in patients with GBM (29).

In a study by Eduardo Cambruzzi, gliomas with mutated IDH with better prognosis were often secondary GBM and WHO grade II and III gliomas and more common in the frontal lobe (30).

In a study by Hao-YuanWang et al., among 811 gliomas, 55.2% had IDH1 mutation, 2.2% had IDH2 mutation, and 42.6 % had wild-type IDH1 / 2 (31). These were similar to our study.

Several studies have investigated the survival of IDH mutant and wild type, and fewer studies have targeted the relationship between mutation and demographics as we have done. Parsons et al. reported IHD1 mutation in secondary GBM and demonstrated better overall survival in this group (14). Yan et al. surveyed 445 CNS and 494 non-CNS tumors, showing that 161 samples had IDH mutation. This study also showed that patients with anaplastic astrocytomas or glioblastomas with IDH1 or IDH2 mutations were significantly younger than patients with tumors carrying wild-type IDH1 and IDH2 genes (median age, 34 years vs. 56 years for patients with anaplastic astrocytomas and 32 years vs. 59 years for those with glioblastomas; $P<0.01$). Even though patients with IDH1 or IDH2 mutations tend to have a younger median age, no mutations were found in the glioblastomas of the 15 patients under-age 21. In addition, patients with IDH mutant compared to wild type had more median overall survival rate (13). In patients with WHO grade II / III glioma and GBM, IDH mutations have been associated with a younger age at diagnosis and a limited number of genetic changes, suggesting that they may impact the course of the disease (32-34). Similar to the aforementioned research, ours showed that an IDH mutation was more strongly associated with younger ages. Still, we did not include a survey of survival outcomes. For the first time, we also demonstrated the tumors tend to be located in the frontal lobe and astrocytomas tend to be IDH-positive.

As per our knowledge, this is the first study to investigate the demographic and histopathologic associations between IDH and diffuse glioma**.** We revealed that the most prevalent brain tumor samples were glioblastoma, WHO CNS grade 4, and IDH-wildtype. Out of 49 cases, 55.1% were men (n=27) and 44.9% were women (n=22), Most of them were younger than 40. A significant proportion of the malignancies was frontal lobe-based and IDH-negative. In addition, we showed that the majority of tumors in patients under 40 were astrocytomas, WHO CNS grade 2,3,4, and IDH-mutant (Figure B, E, G), whereas glioblastoma, WHO CNS grade 4, and IDH-wildtype (Figure F). was more prevalent in those over 55.

Previous studies have shown that both low-grade and high-grade glioma tumors with IDH gene mutation are associated with favorable prognosis in case of early and extensive surgical resection.The study by Pierre A. Robe showed that early aggressive surgical treatment of IDH mutant low-grade glioma results in longer life expectancy and faster return to work (35). Patel SH. stated that the extent of surgical resection differentially affects the overall survival of patients with IDH-mutated LGGs. Also, surgical resection of IDH-wildtype LGGs has no survival advantage (36).

Jason Beiko, concluded that the therapeutic benefit of maximal surgical resection in IDH mutant malignant astrocytomas may be associated with a better prognosis (37).

Furthermore, in some studies, the importance of IDH immunohistochemical staining compared to the genetic method in cases of tumor recurrence, especially in the low cellular area, has been mentioned.

In a study by David capper et al., IDH1 mutation was considered a useful molecular marker for differentiating glioma edge from gliosis in post-therapy specimens with extensive reactive changes (38).

Juliet Rashidian et al. demonstrated that identifying IDH1 mutation status by IHC is useful when genetic testing is inaccurate due to the low tumor cell content. IHC can detect single infiltrating tumors cells in otherwise normal-appearing brain tissue (39).

Sandra Camelo-Piragua showed IDH1 was a valuable immunohistochemical marker for distinguishing less cellular areas of glioma from non-neoplastic conditions in stereotactic biopsy and is also essential in sequencing to detect mutant IDH1 that may not produce sufficient tumor DNA after extraction (PCR-based assay) (40).

Diagnostic findings in imaging studies of gliomas have been associated with IDH gene mutation.

In a study by Guiquan Shen et al., MRI scanning in gliomas with IDH1 mutation was more likely to exhibit homogeneous signal intensity, less contrast enhancement, and more inclined to cross the midline (41).

Luca Pasquini's MR images showed a correlation between IDH mutation and neoangiogenesis in gliomas through the hypoxia-inducible factor (42).

Due to the important role of the IDH gene in the diagnosis, prognosis, and treatment of glioma, the studied samples have been reclassified according to the 2021 WHO classification of central nervous system tumors. 12 out of 32 cases of glioblastoma were IDH-mutant (secondary GBM) and reclassified as astrocytoma WHO CNS grade 4, which has a better prognosis than primary GBM (IDH-wildtype). In addition, 5 out of 17 astrocytoma patients were IDH-wildtype. Some papers have reported that in the presence of 1 or more of 3 genetic parameters (TERT promoter mutation, EGFR gene amplification, combined gain of the entire chromosome 7 and loss of the entire chromosome 10 ([+7/-10]), astrocytoma IDH-wildtype in adults should be considered the highest WHO grade. Therefore, glioblastoma IDH-wildtype can be diagnosed in the setting of astrocytoma IDH-wildtype by considering the aforementioned genetic parameters or microvascular proliferation or necrosis(18). The diagnosis of astrocytoma instead of glioblastoma can be a result of sampling in the early stage of the tumor or insufficient sampling. However, in younger age groups, rare pediatric molecular subtypes of diffuse glioma may also be relevant(24).

One of the most significant drawbacks of this study was the lack of a comparable report with which to compare the findings, particularly in terms of demographic data. Therefore, more investigation is recommended.

Conclusion:

This study explored IDH mutation in astrocytoma and glioblastoma, two frequent forms of glioma. We demonstrated the association between IDH mutation, tumor location, tumor type and patient age. We found no statistically significant correlation between IDH and gender. Due to a lack of proof, we encourage more investigation into this topic.

According to the 2021 WHO CNS tumors classification, we reclassified glioblastoma IDH-mutant (secondary GBM) as astrocytoma, WHO CNS grade 4 which has a better prognosis than primary GBM (IDH-wildtype).

To add additional information, grade 2 and 3 astrocytomas IDH-wildtype in adults, in the presence of known genetic parameters or diagnostic histological features of glioblastoma, were considered as the highest WHO grade. Of course, in younger age groups, rare pediatric molecular subtypes of diffuse glioma are also discussed.

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Conflict of interest:

None.

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Ethical consideration:

The Ethics Committee of Isfahan University of Medical Sciences (https://ethics.research.ac.ir/PortalCommittee.php) approved this study. The institutional ethical committee at Isfahan University of Medical Sciences approved all study protocols (IR.MUI.MED.REC.1400.628). Accordingly, written informed consent taken from all participants before any intervention. This study was extracted from M.D/MSc thesis of Fereshteh Ayati at this university (Thesis#3400635).

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