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Prognostic significance of HIF1-α immunohistochemical expression in gliomas and it's relation to IDH1 mutation status

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Abstract

Background: Gliomas are the commonest primary adults' brain tumors. Hypoxia performs an essential role in gliomas' initiation as well as progression through hypoxia inducible factor (HIF-1α) activation, which could serve as a promising target in treatment of gliomas. Our study aimed to evaluate types and grades of glioma cases and detect isocitrate dehydrogenase 1 (IDH1) mutation status and expression of HIF-1α in all included cases and its correlation with clinical data and pathological parameters.

Results: Samples from 71 patients who were diagnosed with glioma were studied immunohistochemically for IDH1-R132H (if indicated) and HIF-1α expression. Expression of HIF-1α was detected in 73.2% of the included 71 gliomas. HIF-1α expression significantly increased in older patients, in high-grade gliomas and in tumors positive for necrosis. We studied IDH1 mutation in the histologically diagnosed grade 2, 3and 4 astrocytic and oligodendroglial tumors (51 cases out of the included 71 gliomas). IDH1-R132H immunohistochemical expression was positive in 62.7% of cases. IDH1 mutation was significantly higher with younger age. IDH1 mutation was noted also with lower tumor grade. A statistically significant relation was detected between negative IDH1-R132H expression and high level of HIF-1α immunohistochemical expression.

Conclusion: Absence of IDH1 mutation with increased HIF-1α expression among high-grade gliomas suggesting both as predicting indicators for poor prognosis.

Keywords: Gliomas, IDH1-R132H mutation, HIF-1α expression

1 Background

Gliomas are tumors derived from supporting glial tissue. They are the commonest intra-axial 1ry CNS tumors and are classified according to cell type, WHO grade and also location [1].

Hypoxia is one of the most important features of tumor's micro-environment driving aggressiveness of tumors [2]. Hypoxia inducible factor (HIF) is activated in response to hypoxic status. In the presence of oxygen, HIF levels are down regulated. Normally, HIF-1α subunits are unstable. HIF-1α activation in hypoxia causes increased nutrient and oxygen supply to tumor cells via formation of new blood vessels which is associated with activation of glucose transporters and glycolytic enzymes [3].

Hypoxia is an important factor in aggressiveness and treatment resistance in gliomas. HIF-1α down regulation could increase the effect of gliomas therapy by cell cycle and apoptosis mechanisms modulation [4, 5].

This study aimed to analyze the immunohistochemical expression of HIF-1α in included glioma cases and study the relation between IDH1 mutation and immunohistochemical expression of HIF-1α.

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2 Methods

A total of 71 cases (formalin fixed and paraffin embedded tumor tissue of space occupying lesion (SOL) specimens diagnosed as gliomas) included in the present study. Cases were collected retrospectively from pathology lab at specialized medical centre (SMC), faculty of medicine Beni-Suef University, Egypt during period from Sep. 2017 to April 2021.

- *Study design* Cross sectional analytical study.
- *Sample size* It was calculated by G*Power (3.1.9.4) software using a priori analysis with a size = 0.6 for contingency tables using χ^2 tests. A total sample size of *minimally 50* cases estimated for 96% power, α- error probability 0.05.
- *Inclusion criteria* All glioma cases with full available clinical data and adequate viable tumor tissue.
- *Exclusion criteria* Cases with insufficient clinical data, cases with extensive necrosis or hemorrhage with scanty viable tumor tissue and blocks inadequate for multiple sectioning.

The data including age, gender, tumour size, location and recurrence were recorded from patients' files.

2.1 Histopathological evaluation

Blocks of the tumors were sectioned (4 μm); then, they were stained by H and E stain for histopathological evaluation, morphologic classification and grading according to 2021 WHO classification and grading system of CNS tumors [6].

2.2 Immunohistochemical examination

2.2.1 IDH1-R132H

- IDH1-R132H immunohistochemistry was performed for astrocytic tumors (51 cases).

2.2.1.1 Tissue microarray (TMA) construction A qualified area from each tumor tissue was marked on H&E sections and blocks. 5 mm diameter area was extracted out of the marked zone in blocks using an arraying device (Tissue-Tek Quick-Ray™, Sakura Finetek USA). Each core was implanted in a recipient block. 4-μm-thick serials were cut from new blocks. A representative serial was stained by H&E to assess suitability of each core (Fig. 1). Section from each TMA block (4 μm in thickness) was mounted on positively charged slide and stained by anti-IDH1-R132H antibody *(Mouse, monoclonal, 7 ml of ready to use antibody from Medaysis, Livermore, CA 94,551, USA).* Slides were stained using auto-stainer Link48 (Agilent Dako, Santa Clara, CA 95,051. USA). Since automated technique gave no staining of positive control cases, manual technique was used.

Positive control Oligodendroglioma (Fig. 1).

IDH1-R132H mutant protein expression mainly detected in cytoplasm with weak nuclear staining and evaluated only as positive or negative. IDH1-R132H Positive cases were considered (IDH1-mutant) while IDH1-R132H negative cases were labeled as (NOS) as IDH1 DNA sequencing was unavailable.

2.2.2 HIF-1α

Section from each case paraffin block (4 μm in thickness) was mounted on positively charged slide and stained by anti-HIF1α antibody (Rabbit monoclonal (RM0374), 1 ml of concentrated antibody in PBS pH 7.4, with BSA and ≤ 0.09% sodium azide (NaN3), Medaysis, Livermore, CA 94551, USA). Slides were stained manually.

Positive control Human breast carcinoma tissue (Fig. 2).

HIF-1α staining detected in tumor cells nuclei and extent of positivity was scored according to positive cells percentage as follows:

− Negative: no positive cells, +1 score: positive cells were more than 1% and ≤ 10%, +2 score: were more than 10 and ≤ 50% and +3 score: > 50% [7].

2.3 *The procedures of manual immunohistochemical staining

1. Sections were de-paraffinized in xylene and hydrated through graded alcohols (100%–95%–70%) 5 min each, distilled water for 5 min and phosphate buffered saline (PBS) for 5 min.
2. 3% hydrogen peroxide (H_2O_2) was used for blocking endogenous peroxidase activity.
3. For antigen retrieval, the slides were treated by microwave heating in citrate buffer (pH 6.0) for 10 min.
4. The sections were then incubated with primary antibody and incubated in humid chamber at room temperature overnight.
5. After washing in phosphate buffered saline, the samples were incubated with a biotin conjugated secondary antibody and then incubated for 30 min at room temperature.
6. The reactions became visible after immersion of the slides in 3,3- diaminobenzidine tetra hydrochloride (DAB).
7. Counter staining was done by hematoxylin and washed in tap water.

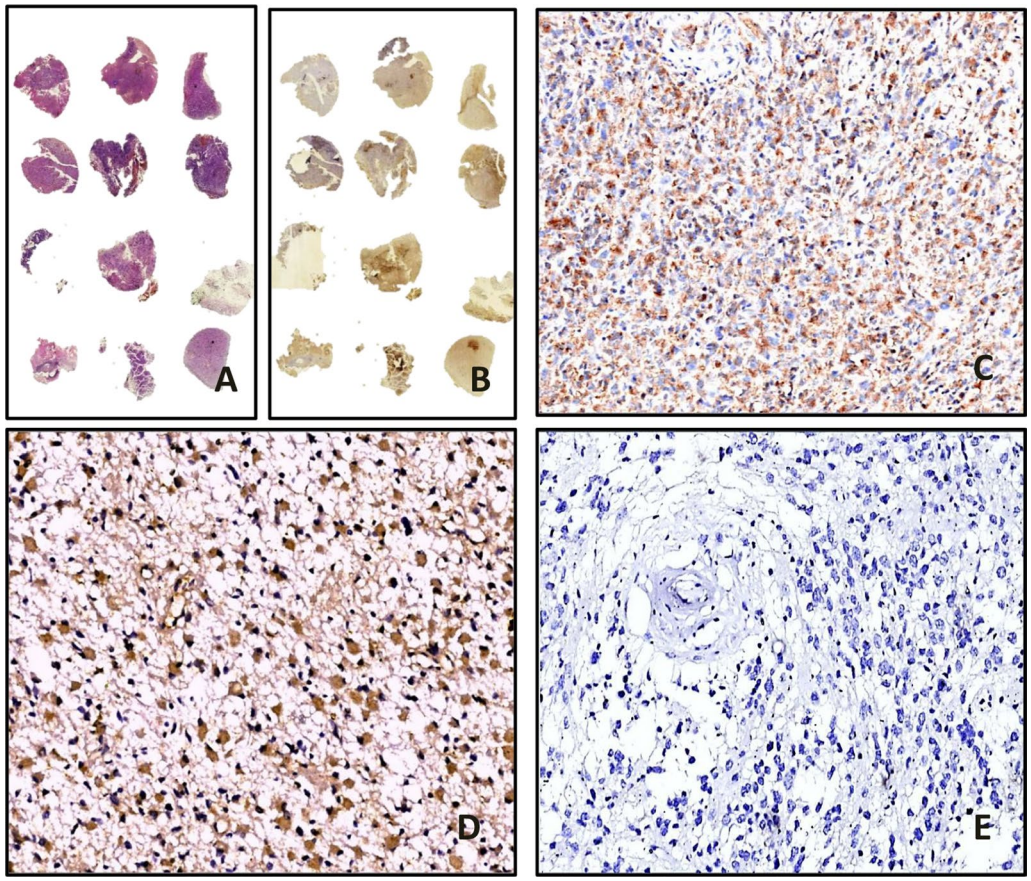

Fig. 1 Overall image of sections from a TMA block. H&E stained section (**A**) and IDH1-R132H immunohistochemically stained section (**B**). Oligodendroglioma grade 2, IDH1-mutant showing positive cytoplasmic staining by IDH1-R132H (positive control), original magnification ×200 (**C**). Astrocytoma WHO grade 2, IDH1-mutant with gemistocytic differentiation, showing positive cytoplasmic staining by IDH1-R132H, original magnification ×200 (**D**). Glioblastoma NOS, WHO grade 4, showing negative staining by IDH1-R132H, with endothelial proliferation, original magnification ×200 (**E**)

8. The slides were placed in two changes of 95% ethyl alcohol then two changes of absolute alcohol, each for 2 min.
9. The slides were dried and cover slips were fixed by DPX.

2.3.1 Slides examination and imaging

Slides were examined by (Olympus model BX53) light microscopy and photos were captured by Leica digital pathology slide scanner (APERIO LV1) at Pathology lab, Beni-Suef University.

2.4 Data analysis

The data were coded then analyzed by the SPSS version 22 (Statistical package for social science).

Descriptive statistics for characteristics of the cases were analyzed.

Description of qualitative measurements by frequency and percentage.

Description of quantitative measurements in the form of mean and standard deviation (mean ± SD).

Graphs were used in illustration of information. Suitable tests were performed (Chi-square (χ^2) and non-parametric Spearman correlation). *P*-values ≤ 0.05 were considered significant.

3 Results

The clinico-pathological variables of the studied glioma cases are represented in Table 1. Median age of current study patients was **42 years**. 87.3% of the studied cases were adults (more than 18 year old). As regarding gender of our study cases, females represented 52.1%. Out

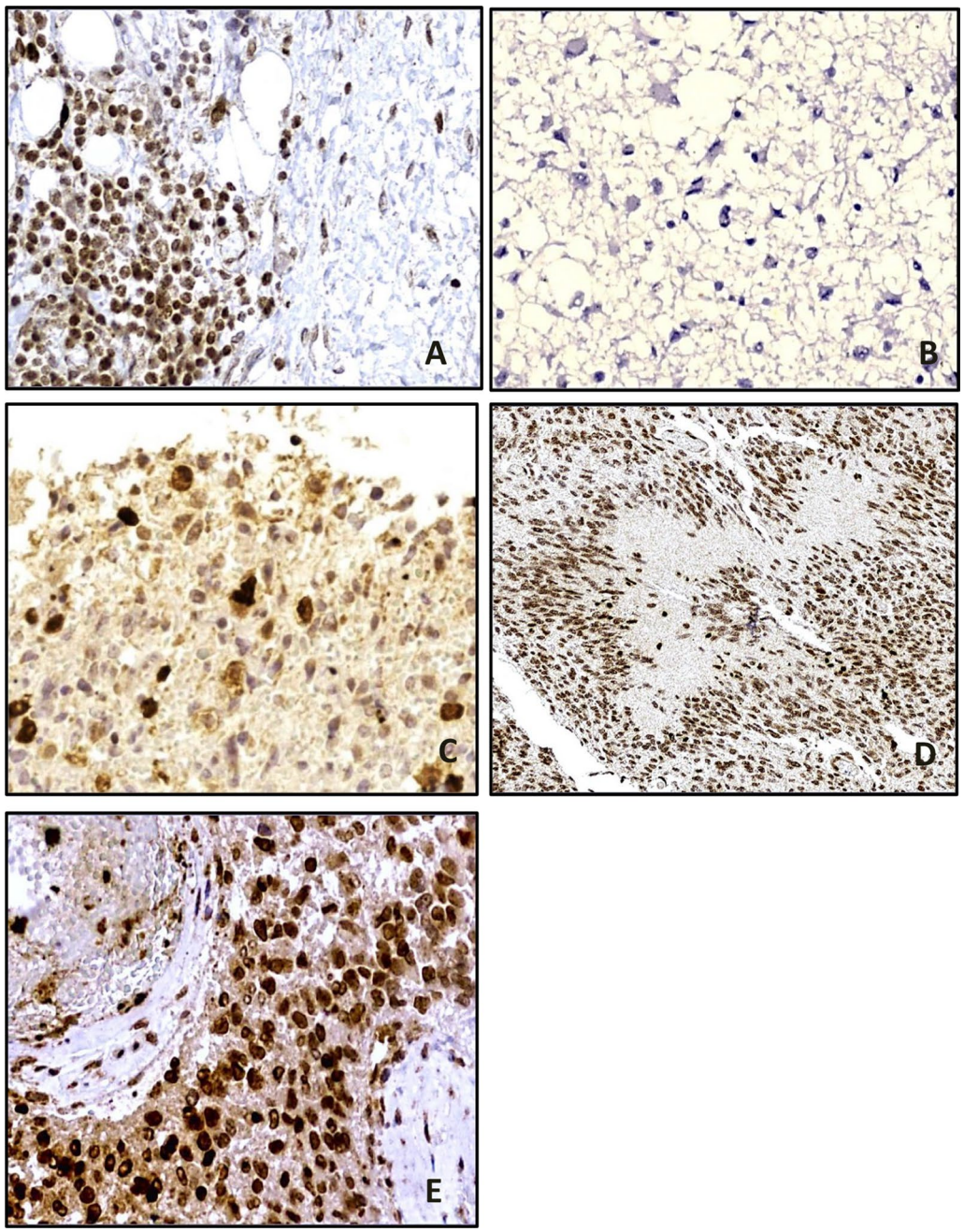

Fig. 2 HIF-1 α immunohistochemistry positive control (breast invasive duct carcinoma), ×400 (**A**). Astrocytoma, IDH1 mutant, WHO grade 2, negative HIF1 α immunohistochemistry, ×400 (**B**). Astrocytoma, IDH1-mutant, WHO grade 3 with abnormal mitosis, HIF1-α positive nuclear staining (score 2), ×400 (**C**). Astrocytoma, IDH1-mutant, WHO grade 4, HIF1 α positive, palisading necrosis (score-3) ×200 (**D**). Glioblastoma NOS, WHO grade 4. HIF1α positive nuclear staining. Cells around blood vessels ×400 (**E**)

of the studied 71 gliomas in current study, 22.5% were extended to more than one brain lobe, while single lobe tumors represented 55% (of them; 21% were limited to frontal lobe followed by 15.5% limited to temporal lobe). Our study showed that the mean of largest diameter of studied tumors was **4.22 cm**. As regard to diagnosis of gliomas included in our work, glioblastoma NOS cases were the most frequent (26.8%), followed by grade 2 IDH-mutant astrocytoma and grade 4 IDH-mutant astrocytoma (15.5% each). Four cases were astrocytoma IDH-mutant grade 3, 6 cases were oligodendroglioma IDH-mutant, 7 cases were Pilocytic

Table 1 Clinico-pathological data of the studied cases; (*N* = 71)

Variables		Frequency	Percent (%)
Age in years (*N* = 71)	**≤ 18**	**9**	12.7
	> 18	**62**	87.3
	≤ 60	50	
	> 60	12	
Age: Median (IQR*): 42.00 (30.00) Range: 1.5–75 years			
Gender (*N* = 71)	Male	34	47.9
	Female	37	52.1
Side (*N* = 71)	Right	28	39.4
	Left	32	45.1
	Midline	11	15.5
Relation to tentorium cerebelli (*N* = 69)**	Supra-tentorial	59	85.5
	Infra-tentorial	10	14.5
Site (*N* = 71)	Frontal	15	21.1
	Temporal	11	15.5
	Parietal	7	9.9
	Occipital	6	8.5
	Corpus Callosum	3	4.2
	More than one lobe***	16	22.5
	Lumbar	2	2.8
	Posterior fossa	11	15.5
Grade (*N* = 71)	1	8	11.3
	2	27	38.0
	3	6	8.5
	4	30	42.3
Recurrence (*N* = 71)	First time	59	83.1
	Recurrent	12	16.9
Necrosis (*N* = 71)	Negative for necrosis	30	42.3
	Positive for necrosis	41	57.7

Bold indicates differentiate between pediatric and adult age

*IQR: interquartile range

**Two cases were excluded from total as they were spinal tumors (extracranial)

***11 tempro-parietal, 2 fronto-tempro-parietal, 1 fronto-temporal, 2 fronto-parietal

astrocytomas, 7 cases were Pleomorphic xanthoastrocytomas (PXAs), 5 cases were ependymomas and one case was ganglioglioma. The diagnosis that showed the strongest predilection for pediatric patients in our study was pilocytic astrocytoma which was mainly found in the posterior fossa. Concerning grades, less than half of current study cases were grade 4 gliomas (42.3%) followed by grades 2, 1 then grade 3. About 17% of our study glioma cases were recurrent after previous surgical excision. 57.7% of our study cases were positive for microscopic necrosis.

In current study we performed IDH1-R132H immunohistochemistry for 51 cases (grade 2, 3 and 4 astrocytic and oligodendroglial tumors) for adequate diagnosis according to the most recent WHO classification (fifth edition/2021) (Fig. 1). 62.7% of cases were IDH-mutant while 37.3% of cases were IDH 1- R132H negative Table 2, Fig. 1. Since molecular testing was unavailable we labeled IDH 1- R132H negative cases with histological features of glioblastoma as "Glioblastoma NOS". All the examined histologically diagnosed grade 2 and 3 astrocytoma cases were IDH1-R132H positive so there was no need for further analysis. Regarding HIF-1α expression and scores of our studied cases (Fig. 2), 73% were positive and the most frequent score was score + 2 (32% of the total number). Tables 2 and 3 demonstrate association between HIF1α expression scores and clinico-pathological data of the studied cases. Table 2 demonstrates a significant association between IDH1-R132H expression and HIF-1α expression; IDH1 mutation was noted with lower HIF-1α staining scores.

4 Discussion

Gliomas are the most common intra-axial primary CNS tumors and are classified by cell type, WHO grade and by location. Our study found a statistically significant relation between necrosis in gliomas and relation to tentorium cerebelli; 64% of supra-tentorial tumors were positive for necrosis (***p*-value = 0.031**). We also found a statically significant association between relation to tentorium cerebelli and tumor grade (***p*-value = 0.001**). This result was matching with the data from Dallabona et al. [8] and Roux et al. [9] reporting that the majority of high-grade gliomas were supra-tentorial.

In our study necrosis significantly increased with higher grades (***p*-value = 0.001**). Similarly, Rodríguez-Florido et al. [10] also reported a significant relation between necrosis and high-grade gliomas (**p-value < 0.0001**).

IDH1 mutations contribute in development of gliomas making them valuable targets for glioma prevention and also for treatment [11].

62.7% of cases were IDH-mutant while 37.3% of cases were IDH1-R132H negative. In parallel to our results Fukuya et al. [12] observed IDH1-R132H mutation in 79% of cases, while Rathore et al. [13] reported IDH1-R132H mutation in only about half of 735 glioma cases. In a cohort of 544 diffuse glioma cases (WHO grade 2, 3 and 4), the positive percent of IDH1-R132H mutation was 43.3% [14]. These variations could be due to difference in samples size.

Our results demonstrated that IDH1 mutation was significantly higher with younger age, with a statistically significant *p*-value **(0.015)**. Similarly, Robinson et al. [15] reported that the vast majority of IDH1-mutated glioma occurs in patients ≤ 55 years old. Barresi et al. [16] conducted a study on 273 diffuse glioma cases in which they also found that IDH1 mutation in diffuse gliomas was

Table 2 Association between HIF1α expression scores and Clinico-pathological data of the studied cases; (*N* = 71)

Variables		Negative (19)	Score +1 (18)	Score +2 (23)	Score +3 (11)	Total 71	*P*-value
Gender (*N* = 71)	Male	9 (26.5%)	8 (23.5%)	10 (29.4%)	7 (20.6%)	34	0.689
	Female	10 (27.0%)	10 (27.0%)	13 (35.1%)	4 (10.8%)	37	
Side (*N* = 71)	Right	8 (28.6%)	4 (14.3%)	13 (46.4%)	3 (10.7%)	28	0.041*
	Left	6 (18.8%)	9 (28.1%)	9 (28.1%)	8 (25.0%)	32	
	Midline	5 (45.5%)	5 (45.5%)	1 (9.1%)	0 (0.0%)	11	
Relation to tentorium cerebelli (*N* = 69)**	Supra-tentorial	13 (22.0%)	13 (22.0%)	22 (37.3%)	11 (18.6%)	59	0.037*
	Infra-tentorial	6 (60.0%)	3 (30.0%)	1 (10.0%)	0 (0.0%)	10	
Recurrence (*N* = 71)	First time	18 (30.50%)	15 (25.40%)	18 (30.50%)	8 (13.60%)	59	0.383
	Recurrent	1 (8.30%)	3 (25.00%)	5 (41.70%)	3 (25.00%)	12	
Necrosis (*N* = 71)	Negative for necrosis	13 (43.3%)	11 (36.7%)	6 (20.0%)	0 (0.0%)	30	< 0.001*
	Positive for necrosis	6 (14.6%)	7 (17.1%)	17 (41.5%)	11 (26.8%)	41	
IDH1-R132H expression (*N* = 51)	Negative	0 (0.0%)	3 (15.8%)	10 (52.6%)	6 (31.6%)	19	0.012*
	Positive	11 (34.4%)	8 (25.0%)	8 (25.0%)	5 (15.6%)	32	

P*-value ≤ **0.05 is considered significant

**Two cases were excluded from total as they were spinal tumors (extracranial)

Table 3 Correlation between HIF1α expression scores with age, tumor size and grade

clinicopathological parameters	HIF1α scores
Age (years)	
r	0.400*
p-value	0.001**
N	71
Tumor's size	
*r**	0.124
p-value**	0.303
N	71
Tumor's grade	
*r**	0.648
p-value**	< 0.001
N	71

**r*: Spearman's rank correlation coefficient

***P*-value ≤ 0.05 is considered significant

related to younger age. Previously mentioned studies explained these results by increasing glioma grade with older patient's age.

We reported a statistically significant association between IDH1-R132H mutation and low tumor grade **(*p*-value < 0.001)**. All the included grade 2 and grade 3 and 36.7% of grade 4 glioma cases were positive for IDH1 mutation. We also reported a statistically significant association between IDH1-R132 mutation and necrosis **(*p*-value = 0.005)**. Parallel to our results, in Hu et al. [14] study there was also a statistically significant relation between IDH1-R132H mutation and tumor grade **(*p*-value < 0.001)**, they also reported that IDH1-R132H + ve gliomas had a longer survival than IDH1-R132H wild-type cases. Di Carlo et al. [17] perform a study on 2592 grade 2 gliomas in which more than 80% of cases were IDH1-R132H mutant, and reported in their meta-analysis that IDH1 mutation has been identified in a range from 50 to 90% of low-grade glioma patients. Deng et al. [18] recognized IDH1 mutation as a positive prognostic biomarker.

Suh et al. [19] included only patients with grade 4 gliomas, with the percentage of cases with IDH1 mutation was 20.9%. While Potharaju et al. [20] demonstrated that in grade 4 gliomas, 91% cases were wild type IDH1. Hao et al. [21] have reported that IDH1 mutated grade 4 gliomas have a favorable prognosis when compared to IDH1 non-mutant glioblastomas. On same side, the analysis of 585 grade 4 glioma patients by Gately et al. [22] revealed that IDH1mutation was a good prognostic biomarker, with IDH-wildtype tumors having a much worse outcome than IDH1-mutant tumors.

The expression of HIF-1α indicates tumor hypoxia, which plays an essential role in initiation, progression and also therapy resistance. High HIF-1α is associated with bad prognosis in many tumors [23].

Regarding HIF-1α expression and scores of our studied cases, 73% were positive. Other studies showed wide variation in HIF-1α positivity (32%, 42% and 50% of glioma cases [20, 25] and [24] respectively).

There was a statistically significant positive correlation between HIF-1α score and patients' age ($r = 0.400$, $p = 0.001$). Same results were found by Ibrahim et al. [26]. On the other hand, [4] and Potharaju et al. [20] results showed no association between HIF-1α score and patient's age.

We found no statistically significant relation between HIF-1α expression and gender among current study (***p*-value > 0.05**); expression was slightly higher in females than that males (75% vs. 71.4%). In a study by [4] including 223 male patients and 210 female patients showed the frequency of HIF-1α score was higher in males than females (68.2% vs. 63.8%). We simply can explain that by the variation in numbers of the included both genders.

We also reported a moderate positive correlation between HIF-1α staining scores and tumor's grade (***r* = 0.648, *p* = 0.001**). In a meta-analysis including 24 studies on 1422 gliomas [4] reported that HIF-1α was more expressed in high-grade glioma cases than in low-grade glioma cases (86.0% vs. 44.0%), indicating HIF expression was related to high WHO glioma grades (***P*-value < 0.00001**).

In our study, 93% of grade 4 gliomas (astrocytomas grade 4 and glioblastomas) and all glioblastoma NOS cases were positive for HIF-1α expression (54.5% were score +3). Agreeing with our findings, Chen et al. [27] noted high score of HIF-1α expression in IDH-wildtype glioblastomas. Potharaju et al. [20] also found that all glioblastomas had a degree of HIF-1α expression, so, hypoxia may play a role in glioblastomas progression. Liu et al. [28] compared the levels of HIF-1α expression between glioblastomas and low-grade gliomas, HIF-1α scores were higher in glioblastomas (***p*-value = 0.008**).

We reported a significant association between HIF-1α and presence of necrosis (***p*-value < 0.001**). In parallel, Ding et al. [29] performed a multivariate analysis and indicated that HIF-1α expression were independent poor prognostic factor and associated with presence of necrosis.

Previously mentioned results could be explained by data from Domènech et al. [30]; they reported that hypoxia has a central role in gliomas pathogenesis. HIF-1α is related to high-grade gliomas pathophysiology and affects angiogenesis, immunosuppression, cell invasion and cell survival under hypoxic conditions.

We reported a non-significant association between HIF-1α and tumor recurrence, (***p*-value > 0.05**). On the other hand, Liu et al. [28] reported that after tumor recurrence, levels of HIF-1α expression started to increase. That can be explained by the relative small number of the included recurrent cases in our study.

In our study we noted that IDH1 mutation was detected with lower HIF-1α expression score, 34.4% of negative HIF-1α expression showed positive IDH1-R132H mutation, while 15.6% of score +3 showed positive IDH1-R132H mutation (***p*-value = 0.012**). In accordance, Li et al. [31] results also showed reduced HIF-1α in cases with IDH1 mutation compared to IDH1-wildtype gliomas (***p*-value < 0.001**). Liu et al. [28] study also found a significant difference of the HIF-1α expression and IDH1 mutation (p-value = 0.012). In grade 4 gliomas studied by Sfifou et al. [23] most cases expressed HIF-1α (70%) were IDH1 R132H -ve.

Disagreeing with our results, in Zhao et al. [32] study HIF-1α expression was increased in IDH1-mutant gliomas. However, Meng et al. [33] performed on 8 cases and indicated that HIF-1α score was not affected by IDH1 mutation status and HIF-1α was increased in glioma cells compared to other brain cells. These different results could be explained by the relative small sample size of the two mentioned studies.

5 Conclusion

- IDH1-R132H mutation among gliomas is associated with younger age of patients, lower WHO grade of the tumor and absence of necrosis, indicating that IDH 1 mutation is a marker of good prognosis in glioma.
- HIF-1α expression is associated with older patients' age, supratentorial location of the tumor, high WHO grade of glioma and presence of necrosis.
- HIF-1α might be an important biomarker for glioma prognosis and a therapeutic target for manipulation of tumor growth.
- HIF-1α expression is associated with negative IDH1-R132H mutation.

Abbreviations

SOL: Space occupying lesion; TMA: Tissue microarray; WHO: World Health Organization; HIF-1α: Hypoxia-inducible factor 1α; IDH 1: Isocitrate dehydrogenase 1.

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Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by EA, SD, ZM and EM. The first draft of the manuscript was written by EA and all authors commented on previous versions of the manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
This study was performed in line with the principles of the Declaration of Helsinki.
Approval was granted by the Ethics Committee of Beni-Suef University, Jan 2020, Approval number: FMBSUREC/05012020/Mahmoud.
We enclosed a copy of the ethical approval.

Consent for publication
Not applicable.

Competing interests
We have no relevant financial or non-financial interests to disclose.

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