RESEARCH ARTICLE

Editorial Process: Submission:10/02/2023 Acceptance:01/25/2024

Evaluation of Immunohistochemical Expression of *ALK-1* in Gliomas, WHO Grade 4 and Its Correlation with IDH1-R132H Mutation Status

Rasha Ahmed Khairy*, Eman Mohamed Momtaz, Ahmed Mahmoud Abd El Aziz, Passant Essam ELdin Shibel

Abstract

Background: Glioblastoma (GB), a grade 4 glioma is the most common primary malignant brain tumor in adults. Recently, the mutation status of isocitrate dehydrogenase (IDH) has been crucial in the treatment of GB. IDH mutant cases display a more favorable prognosis than IDH-wild type ones. The anaplastic lymphoma kinase (ALK) is expressed as a receptor tyrosine kinase in both the developing central and peripheral nervous systems. Increasing lines of evidence suggest that ALK is over-expressed in GB and represents a potential therapeutic target. **Objectives:** The goal of the current study was to investigate *ALK-1* immunohistochemical expression in gliomas, grade 4, besides its correlation with *IDH1*-R132H mutation status and the clinicopathological parameters of the tumors. Material and methods: Seventy cases of gliomas, grade 4 were tested for immunohistochemical expression of *ALK-1* & *IDH1*-R132H in the tumor cells. **Results:** *ALK-1* immunoexpression was detected in 22.9% of our cases and *IDH1*-R132H mutation was detected in 12.9% of them. *ALK-1* expression (100%) was only detected in the more aggressive IDH R132H-negative GBs. *ALK-1* expression was also noted in the larger-sized tumors, more in males and patients older than the mean age. **Conclusion:** Our results suggest that mutations in *ALK-1* may predict a more dismal prognosis since ALK expression was only noted in IDH-R132H negative GBs known to have a considerably poorer outcome compared to IDH-R132H mutant cases. GBs with detectable ALK-protein expression could potentially experience substantial clinical advantages through the utilization of newly introduced ALK inhibitors allowing personalized treatment to a subset of patients. Hence, future studies targeting ALK in IDH wildtype Glioblastomas including clinical trials on larger scales are recommended.

Keywords: Glioma grade 4- Glioblastoma- Immunohistochemistry- *ALK-1- IDH-1*

Asian Pac J Cancer Prev, **25 (1)**, 317-323

Introduction

Glioblastoma (GB), classified by the World Health Organization (WHO) as a grade 4 glioma, is the most common and deadly primary malignant tumor affecting the central nervous system (CNS) in adults accounting for approximately 60% of all CNS tumors [1]. It has a grim prognosis, with an average survival time of 15 months [2].

The development of glioblastoma involves a complex array of genetic and epigenetic alterations, which result in various subsets of mutations. Some of these mutations have been shown to impact survival rates and response to therapy [3]. IDH mutations have been identified in more than 80% of WHO grade II/III gliomas. In grade IV gliomas, IDH mutations are also prevalent in secondary GBs (73%), while being less common in primary GBs (3.7%) [4]. Furthermore, the presence of IDH mutations has been associated with a more favorable disease outcome, leading to extended median survival in glioblastoma patients [5]. The most prevalent IDH mutation is *IDH1*-R132H, present in approximately 90% of IDH-mutant cases [6].

Targeted therapy approaches for IDH-mutant gliomas involve direct targeting of mutant IDH and epigenetic modulators. Additionally, targeting essential metabolic enzymes, DNA repair enzymes, redox homeostasis, and immunotherapies in order to improve treatment outcomes [7]. The anaplastic lymphoma kinase (ALK) gene, located on chromosome 2p23 belongs to the insulin receptor superfamily of receptor tyrosine kinases [8]. Full-length ALK is specifically expressed during embryogenesis in the developing central and peripheral nervous systems and seems to play a role in regulating cell proliferation and differentiation [9].

Studies have indicated that the presence of ALK expression alone can activate tumorigenic functions in GB. In pre-clinical models, depleting ALK through knockdown or inhibitory antibodies has resulted in reduced tumor

*Department of Pathology, Faculty of Medicine, Cairo University, Egypt. *For Correspondence: Rasha.jawad@kasralainy.edu.eg*

growth, inhibited invasion, and induced apoptosis in GB cells [10].

Developing effective ALK inhibitors that can penetrate the blood-brain barrier poses a significant challenge. If researchers can identify ALK overexpression as a predictive marker for sensitivity to new ALK inhibitors, it would have important implications for the treatment of GB. This additional information could help guide targeted therapies and improve patient outcomes in the future [11].

This study aimed to investigate *ALK-1* immunohistochemical expression in gliomas, grade 4, besides its correlation with *IDH1*-R132H mutation status and the clinicopathological parameters of the tumors.

Materials and Methods

Retrieval of Cases and Material Collection

This cross-sectional analytical study included 70 formalin-fixed, paraffin-embedded full-face tumor tissue sections of retrospective cases that were diagnosed as glioma grade 4 (GB). The cases were collected from the Pathology Department at EL Kasr El Aini Hospital over a 19-month period between July 2021 and January 2023.

The clinical data including patient age, gender, tumor site and size, and history of recurrence were recorded from the Pathology requests and reports of the cases. For the sake of confidentiality, the names of the patients were replaced by an ID number and this number was used afterward on the glass slides and in the datasheet. The study was approved by the Kasr Alainy Research Ethics Committee (REC) with an approval number code: MD-218-2021.

Exclusion criteria were cases with missing data and biopsies with extensive crushing, cautery artifacts, or insufficient material for immunohistochemistry.

Histopathological Examination

Each paraffin block was cut by rotatory microtome at 4 μm thickness then mounted on glass slides, stained with hematoxylin and eosin (H&E) for routine histopathological examination, and on charged slides for immunostaining. Confirmation of the histologic diagnosis and grading and recording of the presence of any histologic variant were done according to the WHO classification of central nervous system tumors [12].

ALK-1 & IDH-1 Immunohistochemical Staining & Evaluation

Immunostaining was done using *IDH-1* R132H ready-to-use monoclonal mouse antibody (#400100295, clone QM002, isotype IgG1, Dako, Denmark) and *ALK-1* ready-to-use monoclonal mouse antibody (#05278783001, clone ALK01, Roche diagnostics, USA). Staining was carried out using an automated immunostainer from Dako. Negative and positive control sections were included for each assay. Sections obtained from oligodendroglioma were used as the positive control for *IDH-1* and sections obtained from a known ALK protein-positive anaplastic lymphoma were used as the positive control for *ALK-1* according to the manufacturer's recommendations.

For *IDH-1* evaluation, the immuno-expression was determined by assessing the proportion of positive cytoplasmic staining in tumor cells; cases in which ≥10 % of cells were stained were defined as positive [13]. Cases showing *IDH-1* immunoexpression were termed astrocytoma, IDH-mutant, WHO grade 4, instead of the formerly used term; IDH-mutant glioblastoma. However, the term glioblastoma IDH wild type can't be used in our study as it refers to a histologically WHO grade 4 astrocytic tumor which lacks mutations in *IDH1*/IDH2 and Histone 3 genes, while only *IDH1*-R132H mutation was examined *IDH1* in our study.

As for *ALK-1* evaluation, the granular cytoplasmic staining of any number of neoplastic cells was considered positive. Weak or equivocal staining was considered negative [14].

Statistical Analysis

The histopathological and immunohistochemical data were imported into the statistical analysis software SPSS, version 26 (Armonk, New York, United States), for further statistical analysis. Simple descriptive statistics such as the arithmetic mean and standard deviation were used to summarize the quantitative data, while frequencies were employed for qualitative data. For comparison, the Chi-square ($\chi 2$) test was performed. A significance level of p-value less than 0.05 was considered statistically significant.

Results

This study included 70 cases of gliomas, WHO grade 4. Their age ranged from 5 to 76 years with a mean of 48.04 years. Regarding the gender of the studied cases, females were 48% and males were 52 %. The clinicopathological characteristics of the studied cases are summarized in Table 1, Figure 1 illustrates the basic histological features and variants of Gliomas, WHO grade 4.

In our study, we observed cytoplasmic positivity of *IDH-1* in 9 out of 70 cases. Hence 12.9% of our cases were classified as Astrocytoma, IDH mutant, WHO grade 4. We found a significant association between *IDH-1* expression and age of patients (P value= 0.007) as well as with the mean larger size of the tumor (P value= 0.011). However, we did not report any significant correlation between *IDH-1* expression and gender (0.815), tumor site (0.520), histological variants (0.879), or history of recurrence (0.167).

Regarding *ALK-1* expression, we detected positive cytoplasmic expression in 16 out of 70 cases (22.9%). We did not report any significant correlation between *ALK-1* expression and any of the studied parameters: age (0.533), gender (0.082), tumor site (0.096), tumor size (0.064), histological variants (0.354), or history of recurrence (0.188). However, it is worth noting that a majority of the *ALK-1* positive cases were older than the mean age, and *ALK-1* expression was more frequently observed in larger tumors.

A summary of the correlation between *ALK-1* and *IDH-1* expression and various pathological characteristics is presented in Table 2, Figure 2 displays

Table 1. The Clinicopathological Characteristics of the Studied Cases

Parameter	Number (%)
Age (years)	
≥48	44 (62.9)
<48	26 (37.1)
Gender	
Male	49 (70)
Female	21 (30)
Site	
Frontal lobe	28 (40)
Parietal lobe	18 (25.7)
Temporal lobe	12 (17.1)
Occipital lobe	6 (8.6)
Corpus callosum	4 (4.7)
Third ventricle	1 (1.4)
Thalamus	1 (1.4)
Size (cm)	
≥5	43 (61.4)
<5	27 (38.6)
Histological variant	
Classic	55 (78.6)
Oligodendroglial features	10 (14.3)
Gliosarcoma	3 (4.3)
Giant cell	1 (1.4)
Epithelioid	1 (1.4)
Recurrence	
Recurrent	12 (17.1)
Non recurrent	58 (82.9)

the immunostaining of Gliomas, WHO grade 4 with *IDH-1* and *ALK-1*. Among the cases studied, 45 showed negative staining for both *ALK-1* and *IDH-1*. Interestingly, *ALK-1* expression (100%) was exclusively observed in the more aggressive IDH-R132H negative GB cases, as shown in Table 3.

Discussion

In some studies, overexpression of ALK in GB has been associated with increased tumor cell proliferation, poorer overall survival, and progression-free survival, suggesting a potential prognostic role. However, the available data remains limited and conflicting [8].

Despite the positive outcomes observed in pre-clinical and in vivo studies conducted on GB when ALK inhibitors were applied, there have been no clinical trials demonstrating a significant impact on the survival of GB patients with the use of ALK inhibitors, likely due to their limited ability to penetrate the blood-brain barrier and the challenges in achieving therapeutic concentrations in the brain [8, 11]. However, the second and third-generation ALK inhibitors have exhibited notable effectiveness in treating CNS metastases in ALK-rearranged non-small cell lung cancers (NSCLC), indicating their ability to penetrate the brain effectively [10, 15].

In our study, we examined 70 cases of gliomas, WHO grade 4 for ALk-1 by immunohistochemistry and correlated it with *IDH1*-R132H immunohistochemical expression and other clinicopathologic variables. As for *ALK-1* immunoexpression in our study, granular cytoplasmic positivity was detected in 16 cases (16/70; 22.9%). This finding was in harmony with Kulig et al.

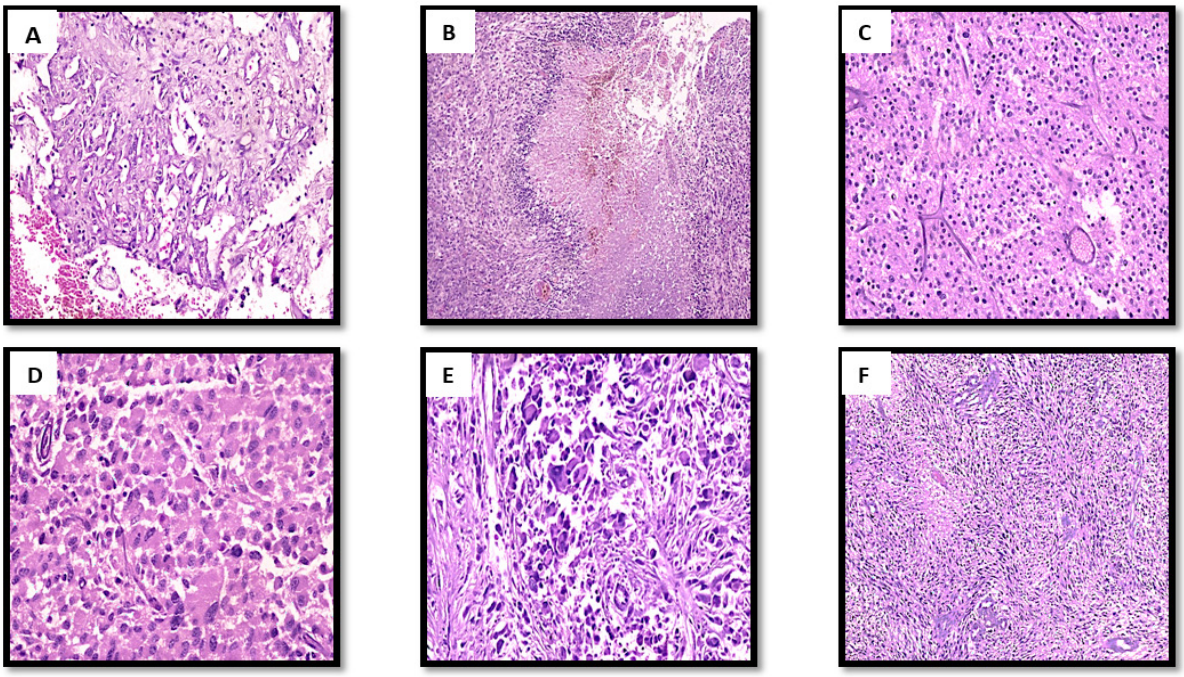

Figure 1.Basic Histological Features and Variants of Gliomas, WHO Grade 4; (A) Microvascular proliferation, (B) Palisading necrosis, (C) oligodendroglial features, (D) epithelioid cells, (E) Giant cell (F) sarcomatous component formed of densely packed spindled cells. (H&E; (A)X400, (B)X200 (C)X200, (D)X400, (E)X 400 & (F)X200 original magnification).

Table 2. Correlation of *IDH-1* and *ALK-1* Expression with Various Clinicopathological Characteristics among the Studied Gliomas, WHO Grade 4 Cases

Parameter	*IDH-1*		P value	*ALK-1*		P value
	Positive (% within parameter)	Negative (% within parameter)		Positive (% within parameter)	Negative (% within parameter)	
Age (years)			0.007*			0.533
≥48	2 (4.5)	42 (95.5)		9 (20.5)	35 (79.5)	
<48	7 (26.9)	19 (73.1)		7 (26.9)	19 (73.1)	
Gender			0.815			0.082
Male	6 (12.2)	43 (87.8)		14 (28.6)	35 (71.4)	
Female	3 (14.3)	18 (85.7)		2 (9.5)	19 (90.5)	
Site			0.52			0.096
Frontal lobe	3 (10.7)	25 (89.3)		7 (25)	21 (75)	
Parietal lobe	3 (16.7)	15 (83.3)		2 (11.1)	16 (88.9)	
Temporal lobe	0 (0)	12 (100)		4 (33.3)	8 (66.7)	
Occipital lobe	2 (33.3)	4 (66.7)		0 (0)	6 (100)	
Corpus callosum	1 (25)	3 (75)		1 (25)	3 (75)	
Third ventricle	0 (0)	1 (100)		1 (100)	0 (0)	
Thalamus	0 (0)	1 (100)		1 (100)	0 (0)	
Size (cm)			0.011*			0.064
≥5	9 (20.9)	34(79.1)		13 (30.2)	30 (69.8)	
<5	0 (0)	27 (100)		3 (11.1)	24 (88.9)	
Histological variant			0.879			0.354
Classic	7 (12.7)	48 (87.3)		11 (20)	44 (80)	
Oligodendroglial features	2 (20)	8 (80)		3 (30)	7 (70)	
Gliosarcoma	0 (0)	3 (100)		1 (33.3)	2 (66.7)	
Giant cell	0 (0)	1 (100)		1 (100)	0 (0)	
Epithelioid	0 (0)	1 (100)		0 (0)	1 (100)	
Recurrence			0.167			0.188
Recurrent	3 (25)	9 (75)		1 (8.3)	11 (91.7)	
Non-recurrent	6 (10.3)	52 (89.7)		15 (25.9)	43 (74.1)	

*Statistically Significant

2012 [16] where *ALK-1* was expressed in 17.9% of their GB cases. However, Chiba et al. 2017 [14], Karagkounis et al. 2017 [17], Elsers et al. 2021 [18] showed much higher positivity rates; 84.9%, 49.2% & 70.6% respectively. Conversely, Hakeem et al. 2021 [19] showed *ALK-1* positivity in only 3 of their 60 studied astrocytoma cases, 2 of which were grade 4. These different findings can be attributed to tissue fixation, immunohistochemistry methodology, and cut-offs used for positivity.

In the present study, *ALK-1* expression was higher in cases older than the mean age compared to those younger than the mean age, disagreeing with Ferguson et al. 2016 [20], yet, consistent with Karagkounis et al. 2017 [17], Elsers et al. 2021 [18], Hakeem et al. 2021 [19] as they all noted more frequent ALK overexpression in older cases. This difference might be ascribed to variations in environmental factors and sample size. As for the correlation of *ALK-1* immunoexpression with the gender of the studied cases, we reported higher expression in males (14/16); a finding consistent with Karagkounis et al. 2017 [17], Elsers et al. (021 [18], and Hakeem et al. 2021 [19]. The established male predominance in GBs might clarify those concordant findings.

Concerning the relationship between the site of the tumor and *ALK-1* immunoexpression, the frontal lobe showed the most frequent expression (7/16), disagreeing with Elsers et al. 2021 [18] where the parieto-occipital region showed the highest *ALK-1* expression.

In our study, higher *ALK-1* immunoexpression was noted in the tumors larger than the mean size (13/16);

Table 3. The Relationship between *IDH-1* and *ALK-1* Expression.

ALK-1	Positive	Negative	Total	P
IDH-1	Expression	expression	(n:70)	value
Positive expression	0 (0%)	9 (12.90%)	9 (12.90%)	
Negative expression	16 (22.90%)	45 (64.30%)	61 (87.10%)	0.08
Total	16 (22.90%)	54 (77.10%)	70 (100%)	

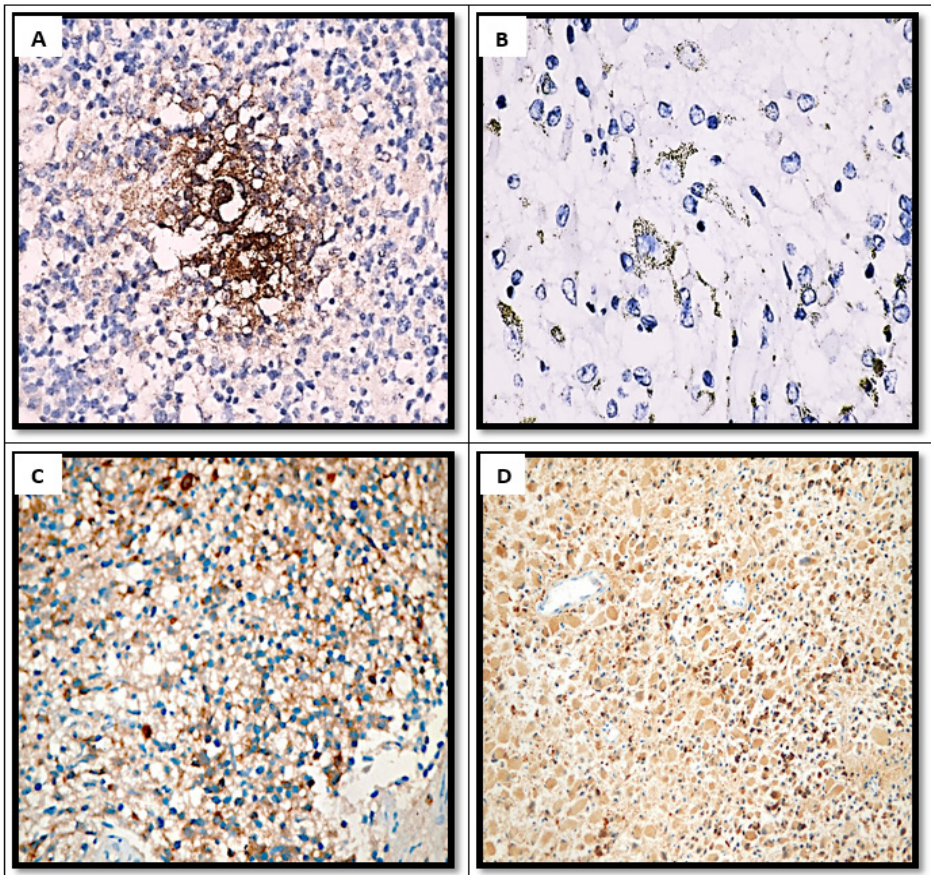

Figure 2. (A&B). GB, NOS Showing Positive Granular Cytoplasmic staining for *ALK-1*. (C). Astrocytoma grade 4, *IDH* mutant with oligodendroglial features showing positive *IDH-1* cytoplasmic staining & (D) Astrocytoma grade 4, *IDH* mutant with focal gemistiocytic changes showing positive *IDH-1* cytoplasmic staining. (DAB (A) X200, (B) X400 (C) X200, (D) X200 original magnification).

a finding in harmony with Elsers et al. 2021 [18], and Hakeem et al. 2021 [19]. No statistically significant correlation was be detected between *ALK-1* expression and the histologic variants of the tumors in our study. Regarding the association between tumor recurrence and *ALK-1* immunoexpression, we observed positivity in only one recurrent case (8.3%), and the association was not statistically significant, which aligns with the findings of Hakeem et al. 2021 [19]. However, Azab 2023 [21] demonstrated a significant correlation between tumor recurrence and the ALK staining score of tumors. This difference may be attributed to the smaller number of recurrent cases in our study compared to non-recurrent cases. In view of *IDH-1* immunoexpression in the present study, 12.9% of our cases were revealed to be Astrocytoma, IDH mutant, WHO grade 4, as cytoplasmic positivity of *IDH-1* was detected in 9 cases (9/70; 12.9%), in consistency with Liu et al. 2016 [22], and Houssaini et al. 2022 [23] where *IDH-1* was expressed in 12.8% & 11.8% of their GB cases respectively.

Regarding the correlation of *IDH-1* expression with the mean age, we found a statistically significant association, and most *IDH-1* negative cases were relatively older age, consistent with Liu et al. 2016 [22], WHO (2021) [12], Houssaini et al. 2022 [23] and Kiraz et al. 2022 [24]. However, the latter's results didn't reach statistical significance. Regarding the relationship between the gender of the studied cases and *IDH-1* immunoexpression, we found a statistically insignificant association, being compatible with Liu et al. 2016 [22], and Houssaini et al. 2022 [23].

As for the correlation between the site of the tumor and *IDH-1* immunoexpression, the frontal lobe showed the most frequent expression (3/9), unlike Liu et al. 2016 [22] who noted the most frequent expression in the temporal lobe. Even though, these relations were statistically insignificant in both studies. Concerning the relationship between the mean size of the tumor (5 cm) and *IDH-1* immunoexpression, we found a statistically significant association and positivity was noted only in tumors larger than the mean size and none of the smaller ones, being inconsistent with WHO CNS5 by WHO (2021) [12], and Kiraz et al. 2022 [24] who observed that most larger tumors are *IDH-1* negative. This difference might be attributed to the difference in the sample size.

With respect to the relationship between the histological variants of the tumor and *IDH-1* immunoexpression, *IDH-1* expression was expressed in 20% of GB with oligodendroglial features (GB-Os) and in 12.7% of GB, classic cases. This finding was consistent with Hinrichs et al. 2016 [25], and Appin et al. 2013 [26] who noted that GB-Os show consistently increased frequencies of *IDH-1* mutations compared to classic GBs. This has received particular attention given the

association of *IDH-1* mutations with secondary GBs & oligodendrogliomas with improved clinical outcomes according to Riemenschneider et al. 2010 [27], and Appin et al. 2013 [26] studies. With respect to the relationship between tumor recurrence and *IDH-1* immunoexpression, the frequency of *IDH-1* expression was higher in the recurrent cases (25% of recurrent cases while 10.3% of non-recurrent cases), this finding was compatible with Choi et al. 2018 [5], Han et al. 2020 [4] and, WHO (2021) [12] who stated that lower-grade glioma with IDH mutation often recur with having undergone malignant transformation to a higher grade.

In our study, we detected ALK expression (16/16; 100%) in IDH-R132H negative GB cases, which is known to have a significantly worse prognosis compared to IDH-mutant grade 4 astrocytomas, as reported by Gritsch et al. 2022 [28]. Our findings align with the studies conducted by Ferguson et al. 2016 [20], Karagkounis et al. 2017 [17], and Bu et al. 2021 [29], all of which observed higher *ALK-1* expression in *IDH1*-R132H negative or wild-type GBs. The survival analysis performed by Bu et al. 2021 [29] confirmed that the presence of ALK mutation was associated with shorter overall survival in glioma patients, particularly in IDH-WT-GB patients. Therefore, it is crucial to identify ALK mutations in the care and management of glioma patients due to the potential for a worse prognosis and the possible eligibility for ALK-targeting therapies. However, it is worth noting that another study reported that despite observing high ALK expression in their GB cases, ALK overexpression did not correlate with prognosis in their study [17].

In conclusion, current evidence reveals that ALK mutations are absent in IDH mutant, astrocytoma grade 4 cases, exclusively positive in the more aggressive IDH-negative-GBs and likely play a role in glial tumor cell proliferation, as their expression was significantly higher in larger tumors, indicating a worse prognosis. Furthermore, new targeted ALK inhibitors could offer significant clinical benefits for GB cases with detectable ALK-protein expression, allowing personalized treatment for a subset of patients. Further studies, including clinical trials, are needed to elucidate the modality of selecting the candidates among GB patients that are most likely to benefit from *ALK-1* targeted immunotherapy. This includes which antibody clone to be used and the threshold for positivity. As for the prognostic impact of *ALK-1* expression, more studies addressing the effect of *ALK-1* immunoexpression on GBs outcome are still required, to resolve this controversial issue in the literature.

Author Contribution Statement

All authors contributed efficiently to the study: Rasha Ahmed Khairy shared in the study design, interpretation of results and, writing the manuscript. Eman Mohamed Momtaz shared in data collection, data analysis, interpretation of results and, writing the manuscript. Ahmed Mahmoud Abd El Aziz, shared in the research idea, and revising the manuscript. Passant Essam ELdin Shibel shared in the interpretation of results and revising the manuscript

Acknowledgements

Scientific approval

This study was approved by the scientific committee of the pathology department, faculty of medicine, Cairo University.

Conflict of interest

The authors declare there is no conflict of interest

Approval of ethical committee

This study was approved by the Kasr Alainy Research Ethics Committee (REC) with an approval number code: MD-218-2021.

Availability of data

The data is available upon request according to the institution's guidelines and approval.

References

1. Hanif F, Muzaffar K, Perveen K, Malhi SM, Simjee Sh U. Glioblastoma multiforme: A review of its epidemiology and pathogenesis through clinical presentation and treatment. Asian Pac J Cancer Prev. 2017;18(1):3-9. https://doi.org/10.22034/apjcp.2017.18.1.3.
2. Grochans S, Cybulska AM, Simińska D, Korbecki J, Kojder K, Chlubek D, et al. Epidemiology of glioblastoma multiforme-literature review. Cancers (Basel). 2022;14(10). https://doi.org/10.3390/cancers14102412.
3. Stoyanov GS, Dzhenkov D, Ghenev P, Iliev B, Enchev Y, Tonchev AB. Cell biology of glioblastoma multiforme: From basic science to diagnosis and treatment. Med Oncol. 2018;35(3):27. https://doi.org/10.1007/s12032-018-1083-x.
4. Han S, Liu Y, Cai SJ, Qian M, Ding J, Larion M, et al. Idh mutation in glioma: Molecular mechanisms and potential therapeutic targets. Br J Cancer. 2020;122(11):1580-9. https://doi.org/10.1038/s41416-020-0814-x.
5. Choi S, Yu Y, Grimmer MR, Wahl M, Chang SM, Costello JF. Temozolomide-associated hypermutation in gliomas. Neuro Oncol. 2018;20(10):1300-9. https://doi.org/10.1093/neuonc/noy016.
6. Gondim DD, Gener MA, Curless KL, Cohen-Gadol AA, Hattab EM, Cheng L. Determining idh-mutational status in gliomas using idh1-r132h antibody and polymerase chain reaction. Appl Immunohistochem Mol Morphol. 2019;27(10):722-5. https://doi.org/10.1097/pai.0000000000000702.
7. 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.
8. Kim JH. Prognostic and predictive markers in glioblastoma and alk overexpression. J Pathol Transl Med. 2021;55(3):236-7. https://doi.org/10.4132/jptm.2021.04.29.
9. Hallberg B, Palmer RH. Mechanistic insight into alk receptor tyrosine kinase in human cancer biology. Nat Rev Cancer. 2013;13(10):685-700. https://doi.org/10.1038/nrc3580.
10. Berberich A, Schmitt LM, Pusch S, Hielscher T, Rübmann P, Hucke N, et al. Cmyc and erk activity are associated with resistance to alk inhibitory treatment in glioblastoma. J Neurooncol. 2020;146(1):9-23. https://doi.org/10.1007/

s11060-019-03348-z.
11. Goodwin CR, Rath P, Oyinlade O, Lopez H, Mughal S, Xia S, et al. Crizotinib and erlotinib inhibits growth of c-met(+)/egfrviii(+) primary human glioblastoma xenografts. Clin Neurol Neurosurg. 2018;171:26-33. https://doi.org/10.1016/j.clineuro.2018.02.041.
12. Who classification of tumors editorial board. World health organization classification of tumors of the central nervous system. 5th ed. International agency for research on cancer. lyon; 2021.
13. Takano S, Ishikawa E, Sakamoto N, Matsuda M, Akutsu H, Noguchi M, et al. Immunohistochemistry on idh 1/2, atrx, p53 and ki-67 substitute molecular genetic testing and predict patient prognosis in grade iii adult diffuse gliomas. Brain Tumor Pathol. 2016;33(2):107-16. https://doi.org/10.1007/s10014-016-0260-x.
14. Chiba R, Akiya M, Hashimura M, Oguri Y, Inukai M, Hara A, et al. Alk signaling cascade confers multiple advantages to glioblastoma cells through neovascularization and cell proliferation. PLoS One. 2017;12(8):e0183516. https://doi.org/10.1371/journal.pone.0183516.
15. Gil M, Knetki-Wróblewska M, Niziński P, Strzemski M, Krawczyk P. Effectiveness of alk inhibitors in treatment of cns metastases in nsclc patients. Ann Med. 2023;55(1):1018-28. https://doi.org/10.1080/07853890.2023.2187077.
16. Kulig K, McLendon RE, Locke SC. Met and alk in glioblastoma multiforme (gbm): Comparison of ihc and fish. J Clinic Oncol. 2012;30:2021.
17. Karagkounis G, Stranjalis G, Argyrakos T, Pantelaion V, Mastoris K, Rontogianni D, et al. Anaplastic lymphoma kinase expression and gene alterations in glioblastoma: Correlations with clinical outcome. J Clin Pathol. 2017;70(7):593-9. https://doi.org/10.1136/jclinpath-2016-204102.
18. Elsers D, Temerik DF, Attia AM, Hadia A, Hussien MT. Prognostic role of alk-1 and h-tert expression in glioblastoma multiforme: Correlation with alk gene alterations. J Pathol Transl Med. 2021;55(3):212-24. https://doi.org/10.4132/jptm.2021.03.15.
19. Hakeem AHIA, Khaled RST, Ismail MS. Expression of anaplastic lymphoma kinase in astrocytic tumors (histopathological and immunohistochemical study). Open Access Maced J Med Sci. 2021;9:911-23.
20. Ferguson SD, Xiu J, Weathers SP, Zhou S, Kesari S, Weiss SE, et al. Gbm-associated mutations and altered protein expression are more common in young patients. Oncotarget. 2016;7(43):69466-78. https://doi.org/10.18632/oncotarget.11617.
21. Azab MA. Expression of anaplastic lymphoma kinase (alk) in glioma and possible clinical correlations. A retrospective institutional study. Cancer Treat Res Commun. 2023;36:100703. https://doi.org/10.1016/j.ctarc.2023.100703.
22. Liu N, Wang P, Song H, Kong L, Yao K, Qi X, et al. Immunostaining of idh-1 r132h and atrx proteins in the classification of adult glioblastomas. Int J Clin Exp Pathol. 2016;9:12849-54.
23. Houssaini AS, Lamrabet S, Senhaji N, Seka M, EL Hejjioui B, Karkour M, et al. Immunohistochemical and molecular evaluation of idh in glioblastomas as prognostic marker in moroccan population. Teikyo Medical J. 2022;45:7490-99.
24. Kiraz U, Vural C, Turan G, Alparslan B, Dervisoglu E. The value of immunohistochemical methods and preoperative magnetic resonance imaging findings in diagnosis of idh1 mutant glioblastomas. Turk Neurosurg. 2022;32(2):228-36. https://doi.org/10.5137/1019-5149.Jtn.33708-21.3.
25. Hinrichs BH, Newman S, Appin CL, Dunn W, Cooper L, Pauly R, et al. Farewell to gbm-o: Genomic and transcriptomic profiling of glioblastoma with oligodendroglioma component reveals distinct molecular subgroups. Acta Neuropathol Commun. 2016;4:4. https://doi.org/10.1186/s40478-015-0270-7.
26. Appin CL, Gao J, Chisolm C, Torian M, Alexis D, Vincentelli C, et al. Glioblastoma with oligodendroglioma component (gbm-o): Molecular genetic and clinical characteristics. Brain Pathol. 2013;23(4):454-61. https://doi.org/10.1111/bpa.12018.
27. Riemenschneider MJ, Jeuken JW, Wesseling P, Reifenberger G. Molecular diagnostics of gliomas: State of the art. Acta Neuropathol. 2010;120(5):567-84. https://doi.org/10.1007/s00401-010-0736-4.
28. Gritsch S, Batchelor TT, Gonzalez Castro LN. Diagnostic, therapeutic, and prognostic implications of the 2021 world health organization classification of tumors of the central nervous system. Cancer. 2022;128(1):47-58. https://doi.org/10.1002/cncr.33918.
29. Bu L, Hameed NUF, Luo C, Hong P, Zhou X, Wang S, et al. Germline alk variations are associated with a poor prognosis in glioma and idh-wildtype glioblastoma. J Neurooncol. 2021;152(1):27-36. https://doi.org/10.1007/s11060-020-03676-5.