Section: Pathology

Original Research Article

ANALYSIS OF EXPRESSION OF IDH1 AND EGFR IN WHO GRADE IV GLIOMAS

Niveditha Jayakumar[1], Meenakumari G[2], Shifa S Ibrahim[3]

[1]Assistant Professor, Department of Pathology, Government Sivagangai Medical College, Tamil Nadu, India
[2]Professor, Department of Pathology, Government Theni Medical College, Tamil Nadu, India
3Associate Professor, Government Medical College, Dindigul, Tamil Nadu, India

Received : *XX/XX/2023*
Received in revised form : *XX/XX/2023*
Accepted : *XX/XX/2023*

Keywords:
gliomas – glioblastoma-IDH1 mutant-IDH wild type.

Corresponding Author:
Dr. Niveditha Jayakumar,
Email: neevee89@gmail.com

DOI: 10.47009/jamp.2023.5.3.235

Source of Support: Nil,
Conflict of Interest:None declared

Int J Acad Med Pharm
2023; 5(3); 1145-1149

Abstract

Background: The introduction of molecular markers has restructured the classification of gliomas. IDH1 mutation and EGFR expression have been identified as diagnostic and prognostic markers of significance. This study has analyzed the expression of the same in WHO grade IV gliomas. **Materials and Methods:** Biopsy specimens of 50 glioma cases were processed and histologically assessed. After interpretation of data, appropriate blocks were chosen and sections of 4 micron thickness were cut. Immunohistochemical staining of IDH1 and EGFR were done. **Result:** We found that gliomas occurred commonly in men. They were predominantly located in frontal lobe. IDH wild type glioblastomas were more common than IDH mutant ones. EGFR positivity occurred in 40% IDH wild type glioblastomas. **Conclusion:** IDH1 analysis is mandatory in the diagnosis and prognostication of gliomas. Molecular classification of WHO grade IV gliomas requires IDH1 mutation status.

INTRODUCTION

Gliomas are the most common primary brain tumors among adult population. Their complex pathogenesis and location make gliomas less amenable to surgery and chemotherapy. The relentless search for a new targeted therapy, led researchers in classifying these gliomas based on molecular similarities beyond histomorphological boundaries. The WHO 2016 revised fourth edition has rightly incorporated molecular signature in its classification of CNS tumors. Recent WHO fifth edition has built up on this, by inclusion of key genes and protein to classify the CNS tumors.

Isocitrate dehydrogenase 1(IDH1) is one such key gene which has been the focus of attention since its discovery as an independent diagnostic and theranostic marker in gliomas. The gliomas are restructured into IDH mutant and wild type depending on the presence of this mutation. IDH is a key enzyme in the Krebs cycle. It exists in two forms -NADP dependent IDH1, IDH2 and NAD dependent IDH 3. Mutations in IDH increase the level of 2-hydroxyglutaric acid (2-HG), thereby inhibiting glioma stem cell differentiation. It up regulates vascular endothelial growth factor (VEGF) and increases Hypoxia-Inducible Factor (HIF) levels. This leads to angiogenesis, invasiveness and promotion of tumor micro environment.

Four subtypes are indentified in case of glioblastoma, viz proneural, neural, mesenchymal and classical. Proneural type is associated with PDGFR, IDH and p53 mutations, classical and neural types are associated with EGFR and mesenchymal type isassocaited with NF1.[1]

Studies aimed to assess the role of IDH1 mutation in gliomas have been limited in resource limited settings like ours. Hence this comprehensive study has been undertaken to study the incidence of IDH mutation and EGFR mutation in WHO grade IV gliomas cases.

MATERIALS AND METHODS

Study Design and Study Period

This was a prospective study conducted at the Department of Pathology,
Madurai Medical College from July 2017 to July 2019.

Inclusion Criteria

Operated resection/stereotactic biopsy specimens diagnosed as gliomas.

Exclusion Criteria

Non neoplastic and neoplastic tumors other than gliomas

Ethical Approval

Ethical clearance for the study was obtained from ethical committee of Madurai Medical College, Madurai, Tamil nadu.

Methodology and Techniques

The study material included 50 gliomas. Clinical and morphological details were recorded. Operated

ISSN(O): 2687-5365; ISSN(P): 2753-6556

resection/stereotactic biopsy specimens were collected and fixed in 10 % neutral buffered formalin. After fixation, the specimens were fully embedded and processed routinely. Multiple 4 to 6 micron thin paraffin sections were obtained. Staining was done by Hematoxylin and Eosin staining technique.

Histomorphological Evaluvation

Stained slides were evaluated under light microscopic examination by our expert panel of pathologists. Tumors were classified and categorized according to their pattern of differentiation. Tumors were graded based on cellularity, nuclearatypia, mitotic activity and necrosis.

Immunohistochemical Evalulation

Paraffin blocks with 4 micron thick serial sections from the biopsy specimens were used for IDH1 mutation and epidermal growth factor receptor (EGFR) protein expression. All the cases of WHO grade IV gliomas diagnosed were subjected to immune histochemical evaluvation with monoclonal antibody IDH1 R132H. The presence of strong cytoplasmic staining and weaker nuclear staining in the tumor cells with R132H mutated peptide was taken as positive.

For analysis of EGFR, all IDH1 negative WHO grade IV gliomas were selected. In case of EGFR, the tumor cell membrane staining is considered to be specific for interpretation of the result.

Score 0 was given for tumors that had no staining of the tumor cell membrane.

Score 1 was given for tumors that had weak membrane staining of more than 10% of the tumor cells

Score 2 was given for tumors that had moderate membrane staining of more than 10 % of the tumor cells.

Score 3 was given for tumors that had intense membrane staining of more than10% of the tumor cells.

For the gliosarcoma cases, immunohistochemical evalulation with markers glial fibrillary acidic protein (GFAP), vimentin and special stain study with reticulin were done. In case of GFAP and vimentin, intense cytoplasmic staining of the respective tumor cells was taken as positive. The presence of black reticular fibers with red nuclei was taken as reticulin positivity.

Statistical Analysis

Data obtained was entered into the Microsoft excel spread sheet. The data was analysed using ratios and percentage. Spearman's Rho and Pearman's Coefficient correlation studies were done and p value was derived to determine the statistical significance of the study. Observations and results were compared with other studies and inferences drawn.

RESULTS

We studied and reported 50 gliomas. Assignment of CNS grade was in accordance with the WHO CNS tumors 2021 classification.CNS grade 4(42%) tumors predominated, followed by grade 2(32%), grade 3 (18%) and grade I (8%)[Table 1].The age of the patients in our study ranged from 1-79 years. The highest incidence of gliomas occurred in the age group 50-59 years (26%) [Table 2]. Gliomas were commoner in men (62 %) compared to women. In our study, out of the 50 gliomas, 44 occurred in the cerebrum, accounting for 88%. The frontal lobe was the commonest site of occurrence (30%) and laterality was confined more to right (46%) [Table 2]. Age, sex and location predilection in glioblastomas was similar to gliomas[Table 3]. In the analysis of expression of IDH1 (R132H) antibody in all grade 4 gliomas, we found 29% were IDH mutant (IDH1 positive) and 71% were IDH wildtype (IDH1 negative) [Table 4&Figure 1]. The results were statistically significant with a p value of 0.0327.We further analyzed the IDH wild type grade 4 gliomas for expression of EGFR for categorization into primary and secondary cases. Six cases (40%) of IDH wild type grade 4 gliomas were EGFR positive amongst which three cases had intense(3+) staining[Table 4&Figure 1]. The location predilection of both IDH mutant and wildtype grade 4 gliomas was frontal lobe. Significantly, 26.7% of elderly population above 60 years was exclusively seen in IDH wild type. The cases with unique histomorphological features –gliosarcoma and glioblastoma with giant cells were both IDH wild type. We confirmed gliosarcoma with positivity of vimentin, GFAP and reticulin stain.

Table 1: Distribution of Gliomas according to the WHO CNS tumors (2021) grade

Gliomas (WHO grade)	Frequency	Percentage
Grade 1	04	08
Grade 2	16	32
Grade 3	09	18
Grade 4	21	42

Table 2: Background characteristics of 50 Gliomas

S no	Parameter		Frequency	Percentage
1.	Gender	Male	31	62
		Female	19	38
2.	Age	1-9	03	06
		10-19	06	12
		20-29	05	10

ISSN(O): 2687-5365; ISSN(P): 2753-6556

		30-39	08	16
		40-49	10	20
		50-59	13	26
		60-69	04	08
		70-79	01	02
3.	Location	Frontal &frontopareital	23	46
		Parietal &pareito occipital	10	20
		Temporal &tempopareital	05	10
		Occipital	04	08
		Cerebellum	03	06
		Corpus callosum	02	04
		Ventricle	01	02
		Filum terminale	02	04
4.	Laterality	Right	23	46
		Left	20	40
		Others	07	14

Table 3: Background characteristics of WHO grade IV gliomas

S no	Parameter		Frequency	Percentage
1.	Gender	Male	15	71.4
		Female	06	28.6
2.	Age	10-19	01	4.8
		30-39	03	14.3
		40-49	05	23.8
		50-59	08	38.1
		60-69	03	14.3
		70-79	01	4.8
3.	Location	Frontal &frontopareital	12	57.1
		Parietal &pareito occipital	04	19.1
		Temporal &tempopareital	02	9.6
		Cerebellum	02	9.5
		Corpus callosum	01	4.8
4.	Laterality	Right	11	52.4
		Left	07	33.3
		Others	03	14.3

Table 4: Frequency of expression of IDH1 and EGFR in WHO grade IV gliomas

S .no	Parameter		Frequency	Percentage
1.	WHO grade IV gliomas (n=21)	IDH mutant	06	28.6
		IDH wild type	15	71.4
2.	IDH wild type (n=15)	EGFR Positive	06	40
		EGFR Negative	09	60

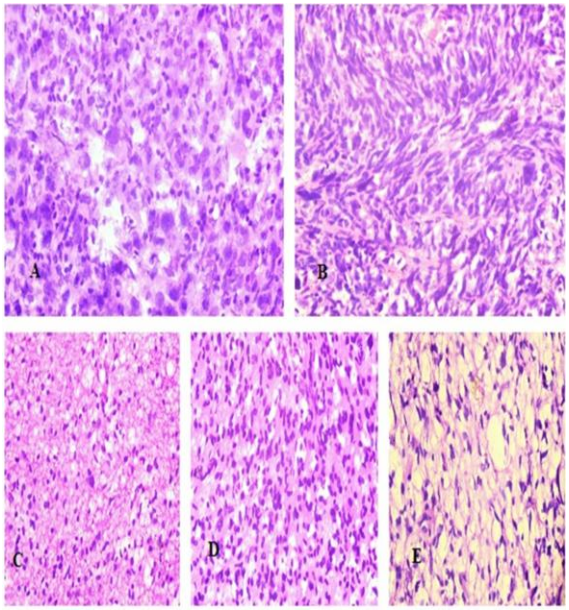

Figure 1: [Gliomas] A-CNS Grade 4, B- Gliosarcoma, C- CNS grade 3, D- CNS grade 2, E- CNS Grade 1

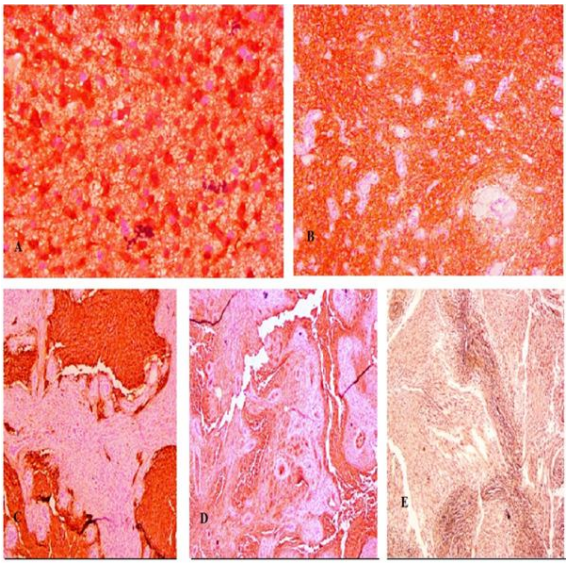

Figure 2: a- glioma grade IV IDH1 positive, B- EGFR positive (3+intensity), C- gliosarcoma glial fibrilliary acidic protein (positive in glial areas) D- vimentin, E- reticulin (both positive in sarcomatous areas)

ISSN(O): 2687-5365; ISSN(P): 2753-6556

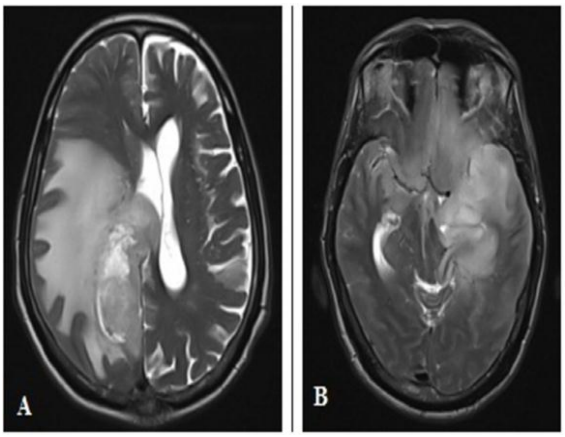

Figure 3: A- pareito occipital glioblastoma (IDH1 negative) – ring enhancement present, B-temporal grade IV astrocytoma (IDH positive) – ring enhancement absent

DISCUSSION

Brain tumors are among the rarest malignancies of the human body accounting for about less than 2% of all the tumors.[2] Their privileged status is due to location, complex functionality and their rarity in causing extra-neural metastasis (<2%).[3]According to the Global Burden of Diseases, Injuries, and Risk Factors Study2016, the incidence rates of CNS cancers that were age standardized increased globally by 17.3%.[4] India was one of the countries that topped the list with increased incidence rates.

The frequency of occurrence was comparable to the study by Jung KW et al (2016), where the commonest glioma was WHO grade 4 gliomas accounting for 40.4%.[5] In our study, lateralisation of gliomas was increased in the right side of the brain, comparable to Coluccia D et al (2018).[6] This study had analysed the neurological outcome, survival and extent of resection in relation to tumor lateralization. The Karnofsky performance score (KPS) was used in all neurology patients to assess their functional impairment and prognosis. The study reported that all patients with left hemisphere tumors had a diminished KPS, six months postoperatively, increase in dysphasia and lack of complete resection.

According to Jalali et al (2008),[7]the most common age group of presentation of all gliomas was 41-60 years (37.3%) and M:F ratio was 2.3:1 which was comparable to our study. IDH mutant astrocytoma patients were a decade lesser than wild type ones. Age was an independent predictor of prognosis, with patients more than 60 years carrying a dismal outcome. Frontal lobe of the cerebrum was the predilected site, similar to Larjavaara S et al (2007).[8] Patients with frontal lobe involvement had better survival rates.[9]The preference of frontal lobe delineates the IDH mutant astrocytoma from the wild type as the former has proneural type signature as against the mesenchymal signature of the wild type glioma. The importance of this molecular signature is that all the intra tumoral regions sampled from the same anatomic location of gliomas were homogenous, regardless of the patient. Therefore, our results can elucidate and provide newer insights into tumorigenesis, presurgical clinical decision and personalized treatment.

Our results of IDH1 expression in WHO grade 4 gliomas, were near comparable to UNO M et al (2011).[10] This study stated that out of 161 glioblastoma cases, only 19 (11.8%) were IDH1 positive. This was also in correlation with the WHO 2016 classification of CNS tumors which states that IDH wild type gliomas were the commonest malignant gliomas accounting for 90% while the rest 10 % are IDH mutant tumors.[11]IDH mutation in glioma is always associated with secondary type and it is proneural.

IDH1 expression can be combined with certain radiological parameters – tumor contrast enhancement, multifocality, location, edema and cystic degeneration for prognostication. Wang K et al (2016) observed 73.3% of IDH1 mutant astrocytomas had contrast enhancement as against 94.9% of IDH wild type tumors.[12] Among the IDH mutant astrocytomas, the ones with non-enhancement had a longer duration of median progression free survival and overall survival.

Avenues of usage of IDH1 mutation study includes:

-Differentiation of infiltrating gliomas from localized ones.[13]

-Improvement of diagnostic accuracy in suboptimal biopsies or resections less than 20 ml.[14,15]

-Amenability to resection.[16]

-Better responsiveness and sensitivity to chemotherapy.[17]

-Intraoperative enhancement of tumor demarcation and visualization of 5-amino levulinic acid (5-ALA) in fluorescence guided neurosurgery.[18]

-As non-invasive method of monitoring IDH mutant tumor cells, by detecting the oncometabolite 2 – hydroxyl glutarate (2 HG) that accumulates in IDH mutant gliomas.[19]

-Potential target for immunotherapy due to uniform penetrance in all tumor cells.[20]

According to Ohgaki H et al (2013),[21] 30-45% of IDH wild type gliomas were EGFR positive like our study. EGFR analysis has paved the way for many novel treatment modalities like EGFR-targeted monoclonal antibodies, tyrosine kinase inhibitors (TKIs). Correlation studies of EGFR over expression, EGFR vIII mutation and gene amplification, the sensitivity of EGFR IHC, in predicting EGFR gene amplification was 100%.[22] The tumors with EGFR amplification showed intense EFGR IHC staining (3+). Therefore in our study, we were able to identify glioblastomas with EGFR amplification. Tripathy K et al (2017),[23]has stated, responders to treatment were 86.4% among EGFR negative cases. Hence the analyses of EGFR positive cases help identify non responders to treatment.

ISSN(O): 2687-5365; ISSN(P): 2753-6556

Furthermore, mutational studies like – TERT promoter mutation, chromosome +7 /-10 copy number variations for IDH wild type glioma and CDKN2A/B homozygous deletion for IDH mutant astrocytoma can be done.

CONCLUSION

The incorporation of molecular signature has broadened the horizons of the knowledge of gliomas. The analysis of IDH1 and EGFR expression in our study population has helped us establish awareness of differences in approach to diagnosis of the disease and provide ground for novel techniques in treatment. This progress will hopefully take us one step closer in providing full fledged benefits to patients treated for CNS tumors.

REFERENCES

1. Verhaak RG, Hoadley KA, Purdom E, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell. 2010; 17(1):98–110.
2. Love.S, Louis D.N, Ellison D.W, Greenfield Neuropathology, Hodder Arnold, 8th edition , 2008,1833-1898
3. Beauchesne P., Extra-neural metastases of malignant gliomas: Myth or Reality? Cancers (Basel), 2011, 3(1):461–477.
4. Global, Regional, and National burden of brain and other CNS cancer, 1990-2016: A systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol., 2019, 18(4):376-393
5. Jung KW, Ha J, Lee SH, Won YJ, Yoo H. An updated nationwide epidemiology of primary brain tumors in republic of Korea. Brain Tumor Res. Treat. 2013, 1(1):16–23
6. Coluccia D, Roth T, Marbacher S, Fandino J. Impact of Laterality on Surgical Outcome of Glioblastoma Patients: A Retrospective Single-Center Study. World Neurosurg, 2018, 114:121-128.
7. Jalali, Rakesh, Dutta, Debnarayan., Prospective analysis of incidence of central nervous tumors presenting in a tertiary cancer hospital from India. Journal of Neuro-oncology, 2008, 87:111-114.
8. Larjavaara S, Mäntylä R, Salminen T, et al., Incidence of gliomas by anatomic location. Neuro Oncol. 2007, 9 (3):319–325.
9. Simpson JR, Horton J, Scott C, Curran WJ, Rubin P, Fischbach J, Isaacson S,Rotman M, Asbell SO, Nelson JS., Influence of location and extent of surgical resection on survival of patients with glioblastoma multiforme: results of three consecutive Radiation Therapy Oncology Group (RTOG) clinical trials. Int. J.Radiat. Oncol. Biol. Phys., 1993, 26 (2):239-244.
10. UNO, Miyuki et al., IDH1 mutations in a Brazilian series of Glioblastoma. Clinics, 2011, 66 (1):163-165.
11. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK: WHO classification of tumours of the central nervous system. Revise 4th edition. Lyon: IARC Press, 2016
12. Wang K, Wang Y, Fan X, et al. Radiological features combined with IDH1 status for predicting the survival outcome of glioblastoma patients. Neuro. Oncol., 2016, 18 (4): 589–597
13. Capper, D, Zentgraf, H, Balss, J, Hartmann, C, & Von Deimling, A., Monoclonal antibody specific for IDH1 R132H mutation. Acta Neuropathol., 2009, 118: 599-601
14. Horbinski, C., Kofler, J., Kelly, L. M., Murdoch, G. H., & Nikiforova, M. N.,Diagnostic use of IDH1/2 mutation analysis in routine clinical testing of formalinfixed, paraffin-embedded glioma tissues. Journal of Neuropathology and Experimental Neurology, 2009, 68 (12):1319-1325
15. Kim BY, Jiang W, Beiko J, Prabhu SS, DeMonte F, Gilbert M R, et al., Diagnostic discrepancies in malignant astrocytoma due to limited small pathological tumor sample can be overcome by IDH1 testing. J. Neuro. Oncol., 2014, 118:405 – 412.
16. Beiko J, Suki D, Hess KR, et al., IDH1 mutant malignant astrocytomas are more amenable to surgical resection and have a survival benefit associated with maximalsurgical resection. Neuro Oncol., 2014, 16(1): 81–91.
17. Houillier C, Wang X, Kaloshi G, Mokhtari K, Guillevin R, Laffaire J, Paris S,Boisselier B, Idbaih A, Laigle-Donadey F, Hoang-Xuan K, Sanson M, Delattre JY.IDH1 or IDH2 mutations predict longer survival and response to temozolomide in low-grade gliomas. Neurology, 2010,75 (17):1560-1566.
18. Hadjipanayis CG, Widhalm G, Stummer W., What is the surgical benefit of utilizing 5 aminolevulinic acid for fluorescence-guided surgery of malignant gliomas? Neurosurgery, 2015, 77(5): 663 – 673.
19. Choi C, Ganji SK, De Berardinis RJ, Hatanpaa KJ, Rakheja D, Kovacs Z, et al., 2-hydroxyglutarate detection by magnetic resonance spectroscopy in IDH-mutated patients with gliomas. Nat. Med., 2012, 18:624–629.
20. Kaminska B, Czapski B, Guzik R, Król SK, Gielniewski B., Consequences ofIDH1/2 Mutations in Gliomas and an Assessment of Inhibitors Targeting Mutated IDH Proteins. Molecules, 2019, 24 (5): 968
21. Ohgaki H, KleihuesP.,The Definition of Primary and Secondary Glioblastoma. Clin. Cancer Res., 2013, 19 (4):764 -772
22. Lee M, Kang SY, Suh YL Genetic Alterations of Epidermal Growth Factor Receptor in Glioblastoma: The Usefulness of Immunohistochemistry. Appl. Immunohistochem. Mol. Morphol., 2019, 27(8): 589-598.
23. Tripathy K, Das B, Singh AK, Misra A, Misra S, Misra SS,Prognostic Significance of Epidermal Growth Factor Receptor in Patients of Glioblastoma Multiforme. J Clin. Diagn. Res., 2017, 11(8): 05-08.

ISSN(O): 2687-5365; ISSN(P): 2753-6556