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Research Article

EXPRESSION OF ISOCITRATE DEHYDROGENASE 1 MUTATION BY R 132 H ANTIBODY CLONE H09 IN GRADE II-IV ASTROCYTOMA AND SECONDARY GLIOBLASTOMA

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ABSTRACT

Objective: Based on their development patterns, behaviors, and shared genetic driver mutations in the isocitrate dehydrogenase (*IDH1*) and *IDH2* genes, diffusely infiltrating gliomas are categorized. There are three types of astrocyte tumors: Not otherwise specified (NOS), IDH-mutant, and IDH-wildtype. About 10% of cases of low-grade glioma that arise in young people and have an IDH1 mutation progress to secondary glioblastoma (sGBM). Hence, IDH sequencing is highly recommended. The aim of the study is to show the presence of IDH1 mutation in all grades of astrocytomas and sGBM and classify them using IDH1 mutation as per the new World Health Organization central nervous system classification.

Methods: The study was conducted in the Department of Pathology, Hitech Medical College and Hospital, Rourkela and was a single-center prospective cross-sectional study that started in January 2023 and ended in December 2024. Paraffin-embedded tissue sections were subjected to immunohistochemistry (IHC) using IDH1 R 132 H CLONE H09 as per standard protocol, and slides were studied. All patients are followed through telephonic conversation.

Results: A total of 47 cases were received over the study period. Out of the total cases, 60% of grade 2 astrocytoma and 50% of cases of grade 3 astrocytoma are IDH1 positive. Of 24 cases of grade 4 astrocytoma, one case is IDH1 positive, whereas all 03 cases of sGBM are positive. Statistically significant results were obtained in the distribution of tumors and IDH1 mutation positivity in grade 4 astrocytoma.

Conclusions: Classified astrocytomas into IDH mutant and wild-type categories, thereby reducing NOS categories. IDH1 IHC is considered a surrogate marker in assessing the IDH1 mutational status.

Keywords: Astrocytoma, Glioblastoma, Isocitrate dehydrogenase 1 immunohistochemistry, Prognosis.

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INTRODUCTION

Gliomas, a diverse group of primary brain tumors arising from glial cells, continue to pose formidable challenges in the field of oncology [1]. The global brain cancer incidence is higher in men than in women and in developed than in underdeveloped countries, with incidence and mortality rates of 3.4 and 2.5/100,000 [2]. Grade I gliomas, considered to be benign, were cured with complete surgical resection [3]. By contrast, Grade II or III are invasive, progress to higher-grade lesions, and have a poor outcome. Grade IV tumors are the most invasive form and have a dismal prognosis [4,5]. Secondary glioblastoma (sGBM) is defined as a tumor that was previously diagnosed as a lower-grade glioma and now has all the features of grade 4 astrocytoma. This 2016 update of the World Health Organization (WHO) classification incorporates well-established molecular parameters into the classification of diffuse gliomas, and 2021 introduced epigenetics in the form of methylation signatures to brain tumors. Formerly, all astrocytic tumors were classed together, but currently, regardless of whether they are astrocytic or not, all diffuse gliomas are categorized together based on their development patterns, behaviors, and—most importantly—genetic status isocitrate dehydrogenase (IDH1 and IDH2). Therefore, this offers a dynamic classification based on phenotype, genotype, and epigenetics from a pathogenetic perspective; it groups tumors with similar prognostic markers from a prognostic perspective; and from a therapeutic perspective, it is likely to direct the treatment of biologically similar entities. Mutations of the *IDH1* gene occur in a high percentage of diffuse gliomas and oligodendrogliomas of all grades, and IDH2 in 2–5% of tumors. A surrogate marker for determining the presence of *IDH1* gene mutations is anti-IDH1-R132H immunostaining. The advantages of this method are the easy implementation in laboratories and time and cost efficiency.

METHODS

This prospective cross-sectional study was carried out in the Department of Pathology at Hitech Medical College and Hospital, Rourkela, Odisha, between January 2023 and December 2024. A sample size of 47 instances was chosen at a 95% confidence level, with an observed proportion of 31.91% and a 13.3% margin of error. The study comprises 47 cases of astrocytoma occurring in the frontal, parietal, temporal, and occipital regions of the brain and includes all grades of astrocytoma and sGBM. The study was undertaken after obtaining institutional ethical clearance (HMCHR/Ethics/2023/15).

Formaldehyde-fixed biopsy specimens were received and were subjected to routine histopathological examination (Leica tissue processor and Leica embedding station). Paraffin-embedded tissues were sectioned, and slides were stained with hematoxylin and eosin stain. All the cases were classified and graded morphologically according to the WHO Diagnostics [6]. Immunohistochemistry (IHC) was done using the standard protocol for the anti-IDH1 R132H by manual method. The control was the color card supplied with the kit. Since it's a cytoplasmic stain, a single positivity is also considered positive. Further, the slides were subjected to glial fibrillary acidic protein staining and Ki67 staining. A 3-tiered semiquantitative scoring system [7] was used for scoring of anti-IDH stain, where staining in ≥50% of tumor cells is considered diffusely positive, staining in <50% of tumor cells is patchy or focal positive, and negative staining is in 0% of tumor cells. Out of

47 astrocytic tumor cases, 12 cases showed a strong cytoplasmic and often a weak nuclear staining of tumor cells with diffuse staining of the fibrillary tumor matrix. In positive cases, all tumor cells are marked positive by the R132H antibody, whereas endothelial cells, perivascular lymphocytes, and residual brain glial cells are negative. All 03 negative controls (reactive gliosis, meningioma, ependymoma) do not show binding to the R132H antibody with a weak diffuse background staining.

Limitation of the study: Grade 2 and 3 astrocytic tumors take a period of 8–10 years to develop into grade glioblastoma (GBM) [8]. Long follow-up results are not available.

RESULTS

A total of 47 cases were studied, which were 15 cases of grade 2 astrocytoma, 5 cases of grade 3 astrocytoma, 24 cases of grade 4 astrocytoma, and 3 cases of sGBM. Regarding the distribution of these tumors, it was found that 37 out of 47 cases were supratentorial, which accounts for 79% of total cases. The remaining 10 cases (21%) were infratentorial. This shows a statistically significant difference in IDH1 mutation frequency among the different tumor types ($p<0.0001$). Out of 47 cases, 18 cases (38%) belonged to the age group of 50–60 years. The youngest was a 2-year-old male child, and the oldest was 65 years old. Out of 47 cases, 24 (59%) cases were male and 23 (41%) were female. The male-to-female ratio was 1.42:1, depicting a male predominance. This suggests no significant association in age distribution between male and female patients.

IDH1-R132H immunostaining of tumor cells was found in 15 cases (31.9%) out of the 47 cases. Diffuse staining of all tumor cells was seen in 12 cases, and 3 cases showed a patchy and single-cell pattern of immune positivity. In this study of 47 cases, grade 2 astrocytomas were 15 cases, which is 32% of the study population, and the male-to-female ratio is 2:1. Out of 10 cases of grade 2 astrocytic tumors in male patients, 7 cases were IDH1-positive, which is 60% of grade 2 astrocytomas. 05 cases were female, two showing IDH1 positivity. This suggests no significant association between gender and IDH1 mutation positivity in grade 2 astrocytoma ($p=0.26$).

Grade 3 astrocytomas were 05 cases, which is 11% of the study population. Grade 3 astrocytoma was 5 cases, of which the male-to-female ratio is 4:1. In 4 cases of grade 3 astrocytic patients, two patients are IDH1 positive, which is 40% of total grade 3 astrocytic patients and 13% of the total study population. One case of grade 3 astrocytoma is in a female, which is not showing IDH1 positivity. This suggests no significant association between gender and IDH1 mutation positivity in grade 3 astrocytoma ($p=0.36$).

A total of 27 (57%) cases of grade 4 astrocytomas were in the study, out of which, three cases are sGBM. The male-to-female ratio is 8:1. In 18 cases of male patients, one case is IDH1 positive, and out of 09 cases of female patients, 03 cases are IDH1 positive. Hence, out of 27 cases of grade 4 astrocytoma, 4 cases are IDH1 positive, which is 15% of grade 4 astrocytoma patients and 12% of the total study population. Of 17 cases of grade 4 astrocytoma, one case comes to be IDH1 positive, whereas all 3 cases of sGBM are IDH1 positive. This suggests a marginal association between gender and IDH1 mutation positivity in grade 4 astrocytoma ($p=0.055$). This warrants further study with a greater number of cases.

DISCUSSION

IDH mutation has emerged as a major diagnostic and prognostic biomarker for gliomas. The goal of this study is to confirm that the IHC expression of the mutant IDH1 protein is the same in different types of glioma. We detected IDH1 expression in grade 2 astrocytoma samples of different grades. In our study, grade 2 astrocytomas were 15 cases, of which the male-to-female ratio was 2:1. Out of 10 cases of grade 2 astrocytoma tumors in male patients, 7 cases were IDH1 positive, which is 60% of grade 2 astrocytoma. 05 cases were female; two showed IDH1 positivity. As per the recent classification of central nervous system (CNS) tumors, these 09 cases are IDH mutants, as they show immunochemistry for R132H IDH1 protein expression. The rest of the 06 cases are IDH1 wild type, as they do not show IDH1 immunopositivity. These cases should be subjected to IDH 2 codon 172 immunochemistry followed by DNA sequencing before stamping it as a wild type. This study does not corroborate Capper *et al*.'s [9] study, where the positivity of grade 2 astrocytoma tumors was 83% and 73% in the Pressure *et al*. study. This may be due to a small sample size and a short duration of the study. Furthermore, in the above two studies, DNA sequencing along with IHC (for both IDH 1 and 2) was done to assess IDH status; Whereas in our study we did only IDH1 IHC by pure manual method, which may be another cause for the variation of results between my study and the other two studies.

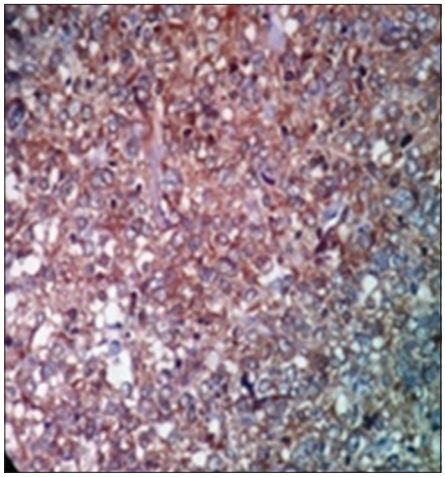

Fig. 1: Isocitrate dehydrogenase 1 positivity in grade 3 astrocytoma

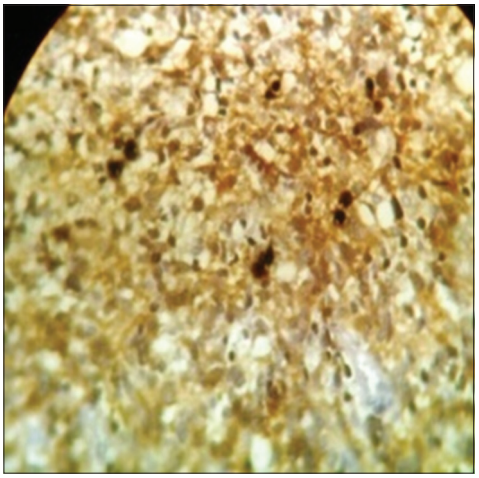

Fig. 2: Isocitrate dehydrogenase 1 positivity in grade 4 astrocytoma

In our study, grade 3 astrocytoma had 5 cases, of which the male-to-female ratio was 4:1. In 4 cases of grade 3 astrocytoma patients, two patients were IDH1 positive, which is 40% of total grade 3 astrocytoma patients and 13% of the total study population. One case of grade 3 astrocytoma is in a female who does not show IDH1 positivity. As per the recent classification of CNS tumors, these two cases are IDH mutants, as they show immunochemistry for R132H IDH1 protein expression. The remaining 03 cases are IDH1 wild type, as they do not show IDH1 immunopositivity. These cases should be subjected to IDH2 codon 172 immunochemistries followed by deoxyribonucleic acid (DNA) sequencing before stamping it as wild type. This study does not corroborate Capper *et al*.'s study, where the positivity of

grade 3 astrocytoma was 81%. This study nearly matches with Hartmann *et al*. [10], where patients with grade 3 astrocytoma carried IDH1 mutations in 60%. In the pre-IDH era, the median survival of grade 3 astrocytoma patients was reported to be in the range of 3–5 years with individual variation. But once the tumor becomes IDH positive, the median survival time becomes 9.3 years [11]. Even if the prognosis for IDH-mutant cases seems better in both classes, it is advised that WHO grading be kept for both IDH-mutant and IDH-wild-type astrocytomas.

Out of 27 cases of grade 4 astrocytoma, 4 cases are IDH1 positive, which is 15% of grade 4 astrocytoma patients and 12% of the total study population. Of the 17 cases of grade 4 astrocytoma, one case appears IDH1 positive, whereas all 3 cases of sGBM are IDH1 positive. According to a recent categorization, instances that test positive for IDH1 are classified as grade 4 astrocytomas, while those that test negative for IDH1 are classified as GBMs. This study supports the findings of Capper *et al*.

While comparing our study with other studies, the difference in IDH1 mutation percentages across the studies is statistically significant (p=0.0021). This indicates that the observed variances are not likely the result of chance.

The criteria for IDH evaluation may differ for older patients compared to younger ones. For example, in patients 55 years of age and older, sequencing may not be necessary if R132H IDH1 IHC is negative.

This antibody seems to be useful for the identification of infiltrating tumor cells and the assessment of patterns of tumor infiltration in the brain tumor [11]. According to some authors [7], antibody-based detection of the mIDH 132 H grade mutation appears to be superior in sensitivity compared with direct sequencing, especially in low-grade diffuse astrocytoma.

Survival data

All IDH-positive cases are followed for a period of 1 year via telephonic conversation. A few grade 4 astrocytoma patients (04) and one grade 3 astrocytoma patient died during this study period due to some complications.

CONCLUSION

An important biomarker for glioma diagnosis and prognosis is the IDH mutation. IDH1 mutations are expressed in the vast majority of oligodendrogliomas and astrocytomas of all grades. In my study, we used IDH1 R132 H 09 stain IHC by the manual method and classified grade 2, 3, and 4 astrocytomas into IDH mutant and wild-type categories, thereby reducing not otherwise specified categories. The practical aspects of routine IDH1 R 132 H IHC are clear—faster turnaround time, lower costs, and the ability to detect just a few single positive cells. The study indicates that future research on larger prospective cohorts is necessary to determine the prognostic significance of this molecular marker in patients with newly diagnosed astrocytoma. It also shows that IHC, a straightforward laboratory technique, is a useful diagnostic tool in assessing the IDH1 mutational status.

AUTHORS' CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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Nil.

REFERENCES

1. Mohamed AA, Alshaibi R, Faragalla S, Mohamed Y, Lucke-Wold B. Updates on management of gliomas in the molecular age. World J Clin Oncol. 2024 Feb 24;15(2):178-94. doi: 10.5306/wjco.v15.i2.178, PMID 38455131
2. Giunchedi P, Gavini E, Bonferoni MC. Nose-to-brain delivery. Pharmaceutics. 2020;12(2):138. doi: 10.3390/pharmaceutics12020138, PMID 32041344
3. Burger PC, Scheithauer BW, Paulus W, Szymas J, Giannini C, Kleihues P. Pilocytic astrocytoma. In: Kleihues P, Cavenee WK, editors. Pathology and Genetics of Tumours of the Nervous System. Lyon, France: IARC Press; 2000. p. 45-51.
4. Stupp R, Mason WP, Van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, *et al*. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005;352(10):987-96. doi: 10.1056/NEJMoa043330, PMID 15758009
5. Wen PY, Kesari S. Malignant gliomas in adults. N Engl J Med. 2008;359:492-507. [Erratum. N Engl J Med. 2008;359(877)].
6. Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature. 2008;455(7216):1061-8. doi: 10.1038/nature07385, PMID 18772890
7. Preusser M, Capper D, Hartmann C, Euro-CNS Research Committee. IDH testing in diagnostic neuropathology: Review and practical guideline article invited by the Euro-CNS research committee. Clin Neuropathol. 2011 Sep-Oct;30(5):217-30. doi: 10.5414/np300422, PMID 21955925
8. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, *et al*. IDH1 and IDH2 Mutations in gliomas. N Engl J Med. 2009 Feb 19;360(8):765-73.
9. Louis DN, Perry A, Reifenberger G, Von Deimling A, Figarella-Branger D, Cavenee WK, *et al*. The 2016 World health organization classification of tumors of the central nervous system: A summary. Acta Neuropathol. 2016;131(6):803-20. doi: 10.1007/s00401-016-1545-1, PMID 27157931
10. Hartmann C, Meyer J, Balss J, Capper D, Mueller W, Christians A, *et al*. Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: A study of 1,010 diffuse gliomas. Acta Neuropathol. 2009;118(4):469-74. doi: 10.1007/s00401-009-0561-9, PMID 19554337
11. Capper D, Weissert S, Balss J, Habel A, Meyer J, Jäger D, *et al*. Characterization of R132H mutation-specific IDH1 antibody binding in brain tumors. Brain Pathol. 2010;20:245-54.