IDH1 Mutations in Diffusely Infiltrating Astrocytomas

Grade Specificity, Association With Protein Expression, and Clinical Relevance

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Abstract

IDH1 mutations are frequent genetic alterations in low-grade diffuse gliomas and secondary glioblastoma (GBM). To validate mutation frequency, IDH1 *gene at codon 132 was sequenced in 74 diffusely infiltrating astrocytomas: diffuse astrocytoma (DA; World Health Organization [WHO] grade II), anaplastic astrocytoma (AA; WHO grade III), and GBM (WHO grade IV). All cases were immunostained with* IDH1-*R132H monoclonal antibody. Mutational status was correlated with mutant protein expression, patient age, duration of symptoms, and prognosis of patients with GBM . We detected 31 (41.9%) heterozygous* IDH1 *mutations resulting in arginine-to-histidine substitution (R132H;CGT-CAT). All 12 DAs (100%), 13 of 14 AAs (92.9%), and 6 of 48 GBMs (12.5%) (5/6 [83.3%] secondary, and 1/42 [2.4%] primary) harbored* IDH1 *mutations. The correlation between mutational status and protein expression was significant (*P < . 001). IDH1 *mutation status, though not associated with prognosis of patients with GBM, showed significant association with younger age and longer duration of symptoms in the whole cohort (*P < .001). *Our study validates* IDH1 *mutant protein expression across various grades of astrocytoma, and demonstrates a high incidence of* IDH1 *mutations in DA, AA, and secondary GBM.*

Somatic mutations of the cytosolic isocitrate dehydrogenase (*IDH1)* gene were initially detected in a fraction of glioblastomas (GBMs).[1] *IDH1* mutations were predominantly found in a group of secondary glioblastomas that harbored *TP53* mutations and were mostly seen in younger patients. These initial findings led to further studies which demonstrated that *IDH1* mutations are present in most cases of diffuse astrocytoma (DA), oligodendroglioma, and mixed oligoastrocytoma of World Health Organization (WHO) grades II and III.[2-6] Although approximately 70% of DAs and secondary GBMs harbor *IDH1* mutations, this alteration is observed in fewer than 10% of primary GBM cases.[1,2,5-7] *IDH1* mutations have been identified as a very early and frequent genetic alteration in the pathway to secondary GBMs because their frequency in low-grade DAs is similar to that in secondary GBMs.[4,5] In contrast, *IDH1* mutations are rare in primary glioblastoma.[1,2,4-6,8,9]

IDH1, a member of the *IDH* gene family, is localized on 2q33.3, and encodes a cytosolic nicotinamide adenine dinucleotide(NADP+)–dependent isocitrate dehydrogenase enzyme. The protein encoded by this gene catalyzes the cytosolic oxidative decarboxylation of isocitrate to α-ketoglutarate, resulting in the production of a reduced form of NADP (NADPH) in the tricarboxylic acid cycle, which is thought to play an important role in the cellular control of oxidative damage and lipid synthesis.[10-15]

Approximately 70% of glioma tumors harbor heterozygous point mutations in *IDH1* codon 132.[16] Among these, more than 90% predominantly affect the amino acid

arginine (arg) at position 132, converting it to histidine (his) (p.R132H) in the amino acid sequence, which belongs to an evolutionarily highly conserved region located at the binding site for isocitrate.[1-6,17] The mutations in *IDH1* have been shown to abrogate enzymatic activity with respect to NADPH generation.[4,6] Recently published data demonstrate *IDH1* codon 132 mutations to be associated with a reduced NADP+-dependent IDH activity in GBM, as a consequence of which, α-ketoglutarate levels are reduced. This in turn increases HIF1-α (hypoxia-inducible factor) levels, thus triggering the transcription of genes involved in angiogenesis, cell motility and invasion, and energy metabolism. Alternatively, *IDH1* codon 132 mutations may be associated with a gain of function, enabling *IDH1* to convert α-ketoglutarate and NADPH into 2-hydroxyglutarate and NADP+.[18-20]

Recently, a mouse monoclonal antibody targeting the *IDH1*-R132H mutation developed by Capper et al[16] was shown to have high specificity and sensitivity in Western blot experiments and immunohistochemistry (IHC) on formalin-fixed paraffin-embedded (FFPE) tumor tissue sections. Previous studies have demonstrated the usefulness of IHC staining for *IDH1*-R132H mutations.[16,21] The antibody m*IDH1*-R132H has been identified as a useful tool for tumor classification, detection of single infiltrating tumor cells, and characterization of the cellular role of mutant *IDH1* protein.

The objective of the current study was to validate the frequency of *IDH1* mutations across different grades of diffusely infiltrating astrocytomas, correlate the mutational status with the expression of mutant *IDH1* protein, and evaluate its possible association with clinical variables in diffusely infiltrating astrocytomas and prognosis in patients with GBM. To accomplish this, we determined *IDH1* mutational status and evaluated *IDH1* mutant protein expression in tissue specimens of diffusely infiltrating astrocytoma, including diffuse astrocytoma (DA; WHO grade II), anaplastic astrocytoma (AA; WHO grade III), and GBM (WHO grade IV). Subsequently, we determined the correlations of *IDH1* mutational status with its mutant protein expression in the tumor tissues across the different grades of astrocytoma and clinical variables (age of the patient and duration of symptoms). Further, in GBM cases, the possible association of *IDH1* mutational status with patient prognosis was assessed.

Materials and Methods

Patient Population and Tissue Samples

Tumor samples (n = 74) of diffusely infiltrating astrocytoma of different grades: DA (n = 12), AA (n = 14), GBM (n = 48) were obtained from patients who underwent surgery at the National Institute of Mental Health and Neurosciences (NIMHANS) and Sri Satya Sai Institute of Higher Medical Sciences (SSIHMS), Bangalore, India. Control samples (n = 2) comprised a portion of anterior temporal cortex resected from patients who underwent surgery for intractable epilepsy. The 48 GBM tissue specimens were collected from a prospectively studied cohort of adult patients with newly diagnosed GBM. These patients underwent maximal safe resection of a supratentorial lobar tumor and had a postoperative Karnofsky performance score of 70 or higher. This cohort was considered for survival analysis. Uniform adjuvant therapy was administered to all the patients in this cohort, which included radiotherapy (total dose of 59.4 Gy, given in 33 fractions) with concomitant temozolomide (100 mg/day for 45 days). Subsequently, 5 cycles of temozolomide were administered at a dose of 150 mg/m^2 of body surface area for 5 days every 28 days. The patients were followed up prospectively and their clinical status was documented regularly. The patient cohort had a mean follow-up of 16.8 months (range, 2-46 months), with a maximum follow-up of 46 months. Overall survival was defined as the duration between surgery and death of the patient. GBM samples were classified as primary (de novo) (n = 42) and secondary (n = 6) based on their clinical profile only.[22,23] Patients with primary GBM had a short duration of symptoms with no clinical or histologic evidence of a preexisting, less malignant precursor lesion, whereas those with secondary GBM had a longer duration of symptoms and/or histologic/clinical evidence of a preceding lower-grade astrocytoma.

All the tissue samples were fixed in 10% buffered neutral formalin and processed routinely. FFPE sections were used for IHC and genomic DNA extraction. The *IDH1* mutational status was determined with direct DNA sequencing and its mutant protein expression was evaluated by IHC in 74 samples.

DNA Extraction, PCR Amplification, Purification, and Direct DNA Sequencing

Genomic DNA was extracted from FFPE sections using the QIAamp DNA FFPE Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The DNA concentration was estimated with spectrophotometry (NanoDrop 1000 Spectrophotometer, Thermo Scientific, Wilmington, DE). The extracted DNA sample was used for determining *IDH1* mutational status with direct DNA sequencing.

Exon 4 of the *IDH1* gene was amplified with a polymerase chain reaction (PCR) assay, as described previously.[8] Primer design was based on the accession number, NM_005896.2 exon 4 (http://www.ncbi.nlm.nih.gov). A fragment of 129–base pair (bp) length spanning the sequence encoding the catalytic domain of *IDH1*, including codon 132, was amplified using 10 μmol/L each of the sense primer *IDH1*f: CGGTCTTCAGAGAAGCCATT and the antisense primer *IDH1*r: GCAAAATCACATTATTGCCAAC. PCR using standard buffer conditions, 100 ng of DNA and *Taq* DNA polymerase with ThermoPol Buffer (No. M0267X, New England Biolabs Inc, Ipswich,

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MA), involved 35 cycles with denaturation at 95°C for 45 seconds, annealing at 56°C for 45 seconds, and extension at 72°C for 45 seconds in a total volume of 50 μL.

The products were visualized on 2% agarose gels to confirm the presence of *IDH1* PCR amplicons. PCR products were purified using QIAquick PCR Purification Kit (No. 28104, Qiagen). All purified PCR amplicons of *IDH1* along with its PCR sense primers were screened for specific mutations.

Immunohistochemical Analysis

FFPE sections (4 μm) from the tumor tissues and control samples were collected on silane-coated slides and IHC was performed to evaluate the expression of *IDH1*-R132H mutant protein. Tissue sections were stained using primary monoclonal antibody (mouse antihuman *IDH1* R132H, DIA clone H09, Dianova, Hamburg, Germany) at a 1:30 dilution. The antigen retrieval was performed with heat treatment of the deparaffinized sections in a microwave oven for 30 to 35 minutes at 850 W in citrate buffer (10 mmol/L, pH 6.0). After the initial processing steps, sections were incubated overnight with primary antibody at room temperature. This was followed by incubation with supersensitive nonbiotin HRP detection system (QD440-XAK, Biogenex, Fremont, CA). 3,3′-Diaminobenzidine (Sigma-Aldrich, St Louis, MO) was used as the chromogenic substrate. A negative control slide in which the primary antibody was excluded was incorporated with each batch of staining. The staining pattern of *IDH1* mutant protein was assessed by 2 neuropathologists (V.S., Y.T.C.). A visual semiquantitative grading scale was applied to assess the immunoreactivity. A strong cytoplasmic staining of tumor cells for *mIDH1R132H* was scored positive. A weak diffuse staining and staining of macrophages were not scored positive.[24]

Statistical Analyses

Data were analyzed using the statistical package SPSS (version 15.0, SPSS, Chicago, IL). Variables were tested for normal distribution and nonparametric tests were used wherever required. Fisher exact test was used to correlate *IDH1* mutational status with its mutant protein expression and determine the mutation frequencies across different grades of diffusely infiltrating astrocytoma. Mann-Whitney *U* test was used to determine the possible association of *IDH1* mutant protein expression with patient age and duration of symptoms in the entire cohort. $P < .05$ was considered significant and all exact 2-sided *P* values were reported.

Survival Analysis in GBM Cases

The extent of surgical resection was uniform for all patients in the prospective cohort and postoperative Karnofsky performance score was uniformly ≥70, therefore the only clinical variable included for analyses was patient age. Univariate Cox regression analysis was performed to test for associations of continuous variables (patient age) with survival. Because the results of *IDH1* mutation and IHC assays were categorical variables, Kaplan-Meier survival analysis followed by the log-rank test for pair-wise comparisons was used to analyze the influence of *IDH1* mutational status/protein expression on overall survival.

Results

Mutational Analysis

We examined 74 diffusely infiltrating astrocytomas and detected mutations at codon 132 of the *IDH1* gene in 31 samples (41.9%). All identified mutations affecting codon 132 were heterozygous and resulted in amino acid sequence alteration, with an amino acid substitution of arginine to histidine (CGT-CAT) **Figure 1**. Other amino acid substitutions were not detected in the current study. All DA samples (12/12; 100%), 13 of 14 AA samples (92.9%) and 6 of 48 GBM samples (12.5%) harbored *IDH1* mutations. Among the GBM samples, mutations were detected in 5 of 6 secondary GBM samples (83.3%) and 1 of 42 primary GBM samples (2.4%).

Immunohistochemical Analysis

All samples (n = 74) examined for mutations at codon 132 of the *IDH1* gene were analyzed for *IDH1*-R132H mutant protein expression. Representative micrographs of IHC in different grades of astrocytoma (DA, AA, and GBM) are shown in **Image 1**. Positive cases showed a strong cytoplasmic and

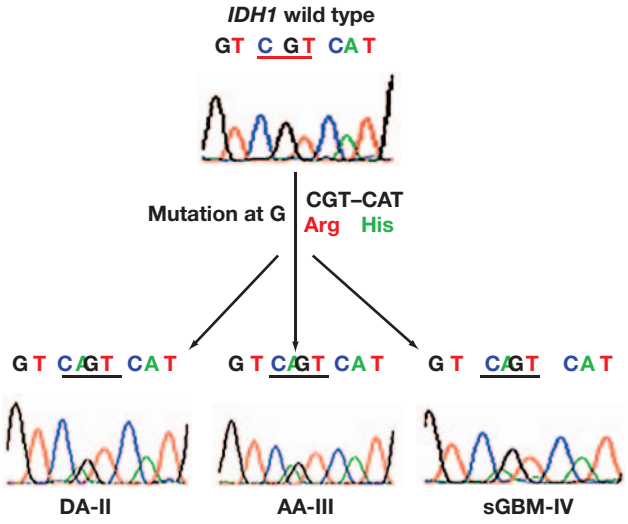

Figure 1 A resultant chromatogram profile after direct DNA sequencing showing *IDH1* mutations in diffuse astrocytoma (DA), anaplastic astrocytoma (AA) and secondary glioblastoma (sGBM) tumors. The mutation affects codon 132, which results in amino acid sequence alteration with an amino acid substitution of arginine to histidine (CGT-CAT).

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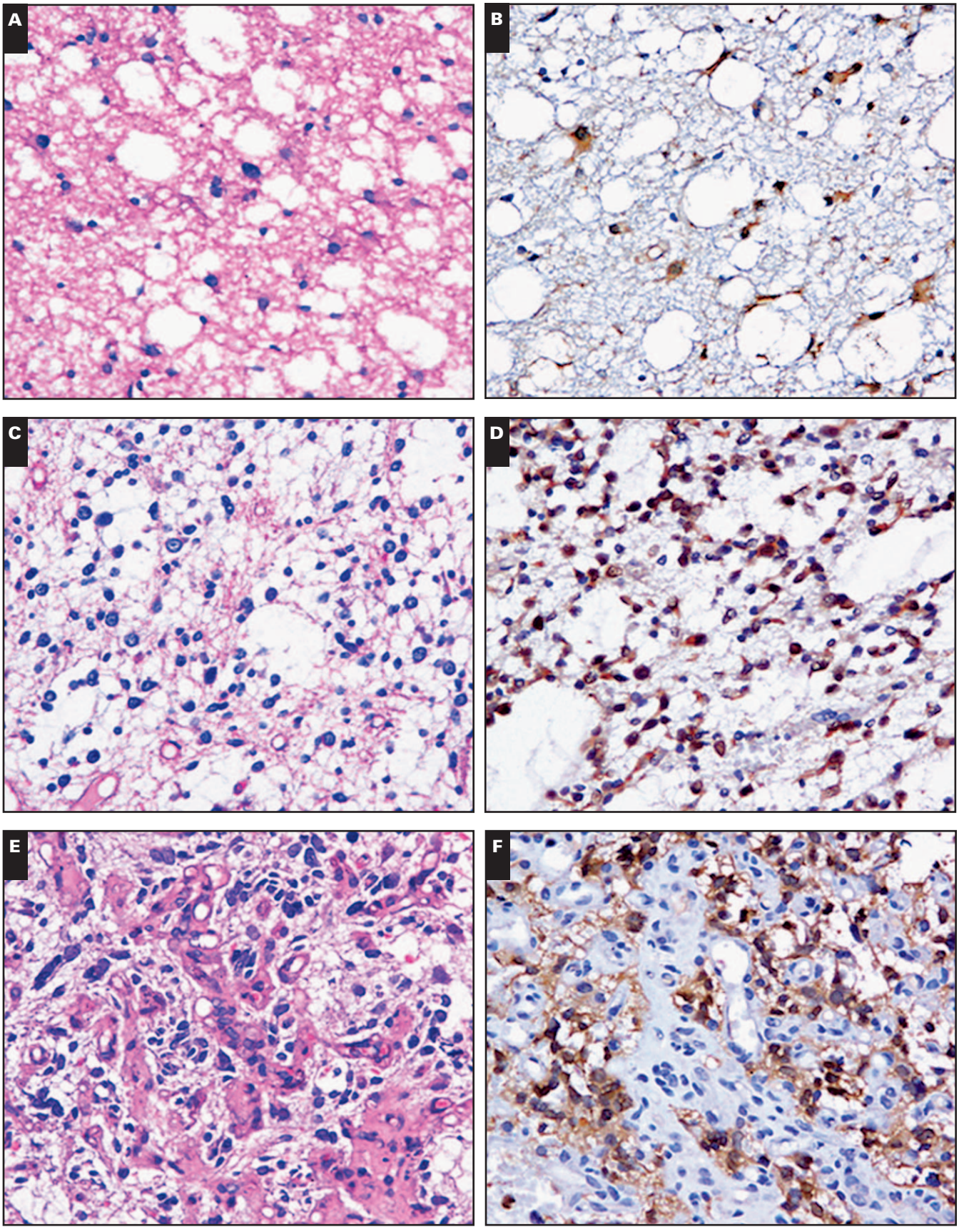

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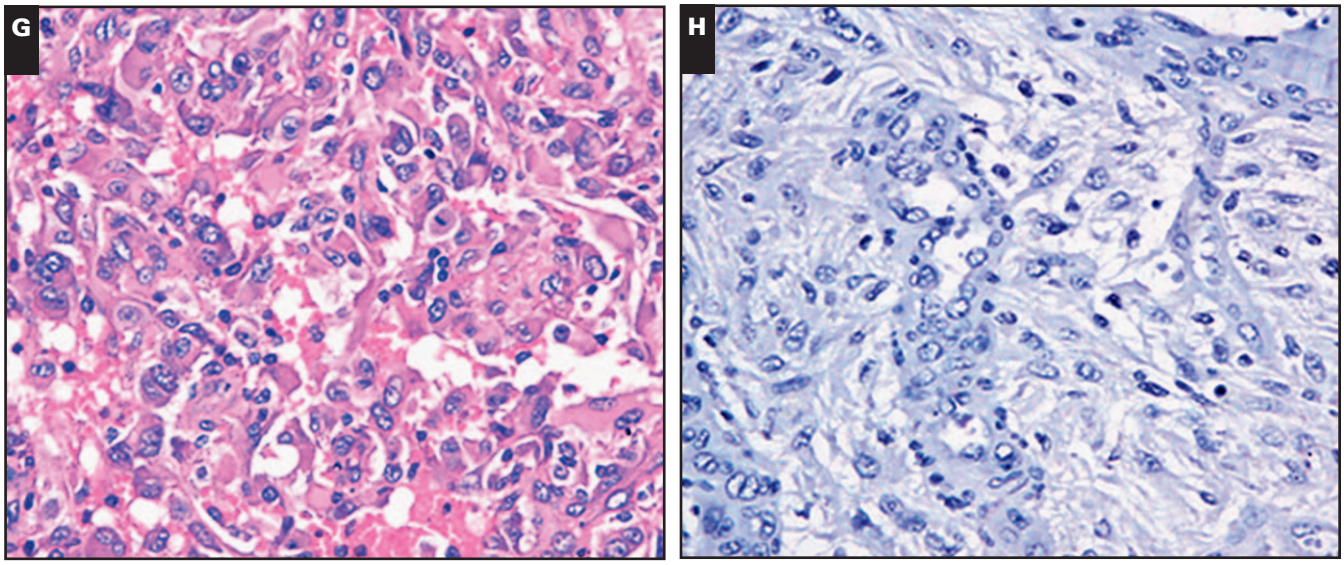

Image 1 Immunohistochemical expression of *IDH1*-R132H mutated protein in different grades of astrocytoma. **A**, Diffuse astrocytoma, World Health Organization (WHO) grade II (H&E) stained section. **B**, Positively stained diffuse astrocytoma cell, WHO grade II (immunohistochemical staining with m*IDH1*R132H). **C**, Anaplastic astrocytoma, WHO grade III (H&E). **D**, Positively stained anaplastic astrocytoma, WHO grade III (immunohistochemical staining with m*IDH1*R132H). **E**, **G**, Glioblastoma, WHO grade IV (H&E). **F**, Positively stained, and **H**, negatively stained glioblastoma, WHO grade IV (immunohistochemical staining with m*IDH1*R132H) (**A**- **H**, ×400).

often a weak nuclear staining of tumor cells. A diffuse background fibrillary staining was noted in a few cases. The diffuse staining was, however, restricted to tumor-containing areas, and was thus considered a specific antibody staining reaction as in previous studies.[16] Cases with isolated nuclear staining were not observed. In positive cases, most of the histologically identifiable tumor cells showed staining, whereas endothelial cells, perivascular lymphocytes, and reactive astrocytes were not stained. Heterogeneity of staining was noted in individual tumor samples which showed variations in intensity. This antibody seems to possess a strong "on-off" quality, in which the intensity of antibody binding in cases with R132H mutation is very strong and all negative cases were easily distinguishable from positive cases as observed in a previous study.[24]

Correlation of *IDH1* Mutational Status With Its Mutant Protein Expression: Grade-Specific Expression Pattern

Irrespective of the grade of the tumor, all tumors that harbored the *IDH1* mutations were also stained positive by IHC, thus demonstrating a statistically significant correlation between the mutational status and protein expression across different grades of diffusely infiltrating astrocytomas ($P < .001$, Fisher exact test) **Table 1**. *IDH1* mutant protein expression was observed in all DA samples (100%) (Image 1A and Image 1B), 13 of 14 AA samples (92.9%) (Image 1C and Image 1D), and 6 of 48 GBM samples (12.5%) (Image 1E-Image 1H). *IDH1* mutations and mutant protein expression were predominantly seen in DA and AA compared with GBM ($P < .001$). However, there was no significant difference between DA and AA ($P = 1.000$) **Table 2**.

Correlation of *IDH1* Mutational Status With Clinical Variables

A statistically significant association of *IDH1* mutations with younger age and longer duration of symptoms was noted in diffusely infiltrating astrocytomas. The median (± standard deviation [SD]) ages of patients with and without *IDH1* mutations were 33.00 ± 8.7 and 46.63 ± 14.5 years, respectively ($P < .001$; Mann-Whitney test). The median ± SD duration of symptoms of patients with and without *IDH1* mutations was 18.6 ± 24.4 months and 2.2 ± 2.8 months, respectively ($P < .001$; Mann-Whitney test).

Table 1
Frequency of *IDH1* Mutations and Mutant Protein Expression in Diffusely Infiltrating Astrocytomas

IDH1 Mutant Protein Expression	*IDH1* Mutation Status		
	Mutated	Nonmutated	Total
Positive	31	0	31
Negative	0	43	43
Total	31	43	74

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Table 2
Individual Group Differences in *IDH1* Mutations/Mutant Protein Expression

Variables	DA-II (n = 12)	AA-III (n = 14)	GBM-IV (n = 48)	*P*†
IDH1 mutation/protein expression				
Positive	12 (100)	13 (92.9)	6 (12.5)	1.000‡
Negative	0 (0)	1 (7.1)	42 (87.5)	<.001§ <.001‖

AA, anaplastic astrocytoma; DA, diffuse astrocytoma; and GA, glioblastoma.
* Data are given as number (percentage) unless otherwise indicated.
† Fisher exact test.
‡ DA vs AA.
§ DA vs GBM.
‖ AA vs GBM.

IDH1 Mutational Status and Survival Analysis in Newly Diagnosed Glioblastoma

The median survival of patients with newly diagnosed GBM in the current study was 14 months (Kaplan-Meier analysis). Univariate Cox regression analysis revealed patient age (hazard ratio, 1.028; $P = .001$; confidence interval, 1.009-1.048) to be associated with shorter survival. However, *IDH1* mutational status did not correlate with overall survival. In the present cohort, we could not demonstrate any predictive value of *IDH1* mutation in patients who received uniform adjuvant radiotherapy along with both concurrent and cyclical chemotherapy with temozolomide. The median survival of patients with GBM with and without mutations was 14 months for both groups ($P = .39$).

Discussion

Cytosolic *IDH1* mutation has emerged as a major diagnostic and prognostic biomarker for gliomas. Important earlier findings in a fraction of GBM tumors[15,17,20] led to the identification of *IDH1* mutations in a vast majority of diffuse astrocytomas, oligodendrogliomas, and mixed oligoastrocytomas of WHO grades II and III.[2-6,9]

The current study is the first of its kind from India to validate the IHC expression of *IDH1* mutant protein across various grades of glioma. We detected *IDH1* mutations and its mutant protein expression in diffusely infiltrating astrocytoma samples of different grades. Notably, 31 of 74 samples (41.9%) analyzed harbored heterozygous mutations at codon 132 of the *IDH1* gene. In line with several previous studies that reported a higher frequency of *IDH1* mutations in grade II gliomas compared with that of grade III, the results of our study show that *IDH1* mutations are common in DA (100%), AA (92.8%), and secondary GBM (83.3%) but infrequent in primary GBM tumors (2.4%).[3-5,25,26] However, in contrast to other studies that reported frequencies ranging from 59% to 83.5% in DA tumors[3-6,17,27-29] and 52% to 87.5% in AA tumors,[3-6,24,27-29] our results show a higher frequency of *IDH1* mutations in individual grades. This could be because of the presence of a smaller number of tumor samples in individual grades. Our results thus indicate that *IDH1* mutations are frequent in the progressive pathway to secondary glioblastomas and further aids in distinguishing primary GBM from others. It has been well established that *IDH1* mutations occur more frequently in secondary GBM than in primary GBM tumors, and therefore have been considered the best available molecular markers for secondary GBM tumors.[4-6,8,30,31]

In line with many studies, including that of Parsons et al,[1] the current study showed that all the samples identified with *IDH1* mutations occurred at position 395 (codon 132) of the IDH transcript which resulted in G-A transition, and subsequently led to an amino acid sequence alteration, with a substitution of arginine to histidine (p.R132H).[1,16,17,20] Nonetheless, the results of a few other studies also showed cases with amino substitutions: p.R132S, p.R132C, p.R132G, and p.R132L.[3-5,25] Interestingly, the current study demonstrated that all the samples of diffusely infiltrating astrocytomas that harbored an *IDH1* mutation, irrespective of grade, were positive for the mutant protein by IHC making it evident that *IDH1* mutation is associated with its mutant protein expression. Several reports showed a similar correlation of *IDH1* mutational status by direct sequencing with its mutant protein expression using an *IDH1* mutation-specific monoclonal antibody (*mIDH1R132H*).[3,20,24] The *IDH1* mutation-specific monoclonal antibody specifically recognizes the substitution of amino acid arginine by histidine (R132H) at the catalytically active domain, which is mediated by a base-pair exchange of guanine to adenine (G395A). The findings of our study validate and further strengthen the value of IHC detection as an important diagnostic tool. This is further corroborated by the fact that previous studies that used IHC also demonstrated the usefulness of *IDH1* mutation-specific monoclonal antibody in distinguishing tumor cells from reactive astrocytes and diffuse astrocytomas from pilocytic astrocytomas, ependymomas, and glioneuronal tumors,[16,21,32] thus aiding in making a diagnosis across all grades of diffuse glioma.[33]

The current study clearly established a statistically significant grade-specific expression of *IDH1* mutant protein together with the occurrence of *IDH1* mutations in

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diffusely infiltrating astrocytomas. Our study showed that *IDH1* mutations commonly occur in DA tumors suggesting the plausible role of *IDH1* in the pathogenicity and malignant progression of astrocytomas. *IDH1* mutations have been identified as early molecular events in the evolution of gliomas and are known to occur before the acquisition of a p53 mutation or 1p/19q codeletion.[5]

The results of our study also showed a significantly longer median duration of symptoms in patients with *IDH1* mutations compared with those without these mutations ($P < .001$), indicating that these mutations are frequently seen in low-grade diffuse astrocytomas and secondary GBMs. Furthermore, our study demonstrated that *IDH1* mutations/mutant protein is commonly expressed in younger patients ($P < .001$). Accordingly, the association of *IDH1* mutations with younger age of patients was reported in several previous studies.[1,4,6,7,17,26,28,34] In a study by Parsons et al,[1] the median age of patients with and without *IDH1* mutations was 33 and 53 years, respectively. Similarly, a study by Nobusawa and colleagues[31] showed that patients with GBM tumors harboring *IDH1* mutations were significantly younger (mean, 47.9 years) than those without the mutation (mean, 60.6 years).

In contrast to other studies that showed *IDH1* mutations to be associated with an increase in the overall survival of patients with GBM, the current study could not elicit the predictive value of these mutations on overall survival.[35] The lack of statistical effect on the overall survival might be attributed to the smaller number of patients in our prospective cohort and a few cases of secondary GBM among patients with the mutations. Several studies demonstrated the prognostic significance of *IDH1* mutations in grade II and III astrocytic and oligodendroglial neoplasms.[6,7,36,37] It has been identified that *IDH1* mutations independently predicted longer survival for patients with both low- and high-grade gliomas.[16,37]

In summary, the current study validates the frequency of *IDH1* mutations across different grades of diffusely infiltrating astrocytomas, establishes grade-specific expression of these mutations, and further demonstrates a significant correlation between the *IDH1* mutational status (p.R132H) by direct DNA sequencing and its mutant protein expression by IHC. This study further demonstrates that *IDH1* mutations are frequent in the progressive pathway to secondary GBM, thus differentiating primary GBM tumors from the others. Our study highlights the usefulness of a simple laboratory technique, IHC, as a valuable diagnostic tool in assessing the *IDH1* mutational status and suggests the need for future studies on larger prospective cohorts to elicit the prognostic significance of this molecular marker in patients with newly diagnosed GBM.

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References

1. Parsons DW, Jones S, Zhang X, et al. An integrated genomic analysis of human glioblastoma multiforme. *Science*. 2008;321:1807-1812.
2. Balss J, Meyer J, Mueller W, et al. Analysis of the IDH1 codon 132 mutation in brain tumors. *Acta Neuropathol*. 2008;116:597-602.
3. Hartmann C, Meyer J, Balss J, 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;118:469-474.
4. Ichimura K, Pearson DM, Kocialkowski S, et al. IDH1 mutations are present in the majority of common adult gliomas but rare in primary glioblastomas. *Neurol Oncol*. 2009;11:341-347.
5. Watanabe T, Nobusawa S, Kleihues P, et al. IDH1 mutations are early events in the development of astrocytomas and oligodendrogliomas. *Am J Pathol*. 2009;174:1149-1153.
6. Yan H, Parsons DW, Jin G, et al. IDH1 and IDH2 mutations in gliomas. *N Engl J Med*. 2009;360:765-773.
7. Van den Bent MJ, Dubbink HJ, Marie Y, et al. IDH1 and IDH2 mutations are prognostic but not predictive for outcome in anaplastic oligodendroglial tumors: a report of the European Organization for Research and Treatment of Cancer Brain Tumor Group. *Clin Cancer Res*. 2010;16:1597-1604.
8. Bleeker FE, Lamba S, Leenstra S, et al. IDH1 mutations at residue p.R132 (IDH1 R132) occur frequently in high-grade gliomas but not in other solid tumors. *Hum Mutat*. 2009;30:7-11.
9. Kang MR, Kim MS, Oh JE, et al. Mutational analysis of IDH1 codon 132 in glioblastomas and other common cancers. *Int J Cancer*. 2009;125:353-355.
10. Geisbrecht BV, Gould SJ. The human PICD gene encodes a cytoplasmic and peroxisomal NADP (+)-dependent isocitrate dehydrogenase. *J Biol Chem*. 1999;274:30527-30533.
11. Margittai E, Bánhegyi G. Isocitrate dehydrogenase: a NADPH-generating enzyme in the lumen of the endoplasmic reticulum. *Arch Biochem Biophys*. 2008;471:184-190.
12. Narahara K, Kimura S, Kikkawa K, et al. Probable assignment of soluble isocitrate dehydrogenase (IDH1) to 2q33.3. *Hum Genet*. 1985;71:37-40.

DOI: 10.1309/AJCPZOIY3WY4KIKE

13. Koshland DE Jr, Walsh K, LaPorte DC. Sensitivity of metabolic fluxes to covalent control. *Curr Top Cell Regul.* 1985;27:13-22.
14. Shechter I, Dai P, Huo L, et al. IDH1 gene transcription is sterol regulated and activated by SREBP-1a and SREBP-2 in human hepatoma HepG2 cells: evidence that IDH1 may regulate lipogenesis in hepatic cells. *J Lipid Res.* 2003;44:2169-2180.
15. Lee SM, Koh HJ, Park DC, et al. Cytosolic NADP(+)-dependent isocitrate dehydrogenase status modulates oxidative damage to cells. *Free Radic Biol Med.* 2002;32:1185-1196.
16. Capper D, Zentgraf H, Balss J, et al. Monoclonal antibody specific for IDH1 R132H mutation. *Acta Neuropathol.* 2009;118:599-601.
17. Sanson M, Marie Y, Paris S, et al. Isocitrate dehydrogenase 1 codon 132 mutation is an important prognostic biomarker in gliomas. *J Clin Oncol.* 2009;27:150-154.
18. Xu X, Zhao J, Xu Z, et al. Structures of human cytosolic NADP-dependent isocitrate dehydrogenase reveal a novel self-regulatory mechanism of activity. *J Biol Chem.* 2004;279:33946-33957.
19. Dang L, White DW, Gross S, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature.* 2009;462:739-744.
20. Bleeker FE, Atai NA, Lamba S, et al. The prognostic IDH1 (R132) mutation is associated with reduced NADP+-dependent IDH activity in glioblastoma. *Acta Neuropathol.* 2010;119:487-494.
21. Takano S, Tian W, Matsuda M, et al. Detection of IDH1 mutation in human gliomas: comparison of immunohistochemistry and sequencing. *Brain Tumor Pathol.* 2011;28:115-123.
22. Ichimura K, Ohgaki H, Kleihues P, et al. Molecular pathogenesis of astrocytic tumours. *J Neurooncol.* 2004;70:137-160.
23. Ohgaki H, Kleihues P. Epidemiology and etiology of gliomas. *Acta Neuropathol.* 2005;109:93-108.
24. Capper D, Weissert S, Balss J, et al. Characterization of R132H mutation-specific IDH1 antibody binding in brain tumors. *Brain Pathol.* 2010;20:245-254.
25. Gravendeel LA, Kloosterhof NK, Bralten LB, et al. Segregation of non-p.R132H mutations in IDH1 in distinct molecular subtypes of glioma. *Hum Mutat.* 2010;31:E1186-E1199.
26. Sonoda Y, Kumabe T, Nakamura T, et al. Analysis of IDH1 and IDH2 mutations in Japanese glioma patients. *Cancer Sci.* 2009;100:1996-1998.
27. Jha P, Suri V, Sharma V, et al. IDH1 mutations in gliomas: first series from a tertiary care centre in India with comprehensive review of literature. *Exp Mol Pathol.* 2011;91:385-393.
28. Felsberg J, Wolter M, Seul H, et al. Rapid and sensitive assessment of the IDH1 and IDH2 mutation status in cerebral gliomas based on DNA pyrosequencing. *Acta Neuropathol.* 2010;119:501-507.
29. Mellai M, Piazzi A, Caldera V, et al. IDH1 and IDH2 mutations, immunohistochemistry and associations in a series of brain tumors. *J Neurooncol.* 2011;2:345-357.
30. Loenarz C, Schofield CJ. Expanding chemical biology of 2-oxoglutarate oxygenases. *Nat Chem Biol.* 2008;3:152-156.
31. Nobusawa S, Watanabe T, Kleihues P, et al. IDH1 mutations as molecular signature and predictive factor of secondary glioblastomas. *Clin Cancer Res.* 2009;19:6002-6007.
32. Hartmann C, Hentschel B, Wick W, et al. Patients with IDH1 wild type anaplastic astrocytomas exhibit worse prognosis than IDH1-mutated glioblastomas, and IDH1 mutation status accounts for the unfavorable prognostic effect of higher age: implications for classification of gliomas. *Acta Neuropathol.* 2010;120:707-718.
33. Weller M, Wick W, von Deimling A. Isocitrate dehydrogenase mutations: a challenge to traditional views on the genesis and malignant progression of gliomas. *Glia.* 2011;59:1200-1204.
34. Weller M, Felsberg J, Hartmann C, et al. Molecular predictors of progression-free and overall survival in patients with newly diagnosed glioblastoma: a prospective translational study of the German Glioma Network. *J Clin Oncol.* 2009;27:5743-5750.
35. Uno M, Oba-Shinjo SM, Silva R, et al. IDH1 mutations in a Brazilian series of glioblastoma. *Clinics (São Paulo).* 2011;66:163-165.
36. Wick W, Hartmann C, Engel C, et al. NOA-04 randomized phase III trial of sequential radio chemotherapy of anaplastic glioma with procarbazine, lomustine, and vincristine or temozolomide. *J Clin Oncol.* 2009;27:5874-5880.
37. Hofer S, Lassman AB. Molecular markers in gliomas: impact for the clinician. *Target Oncol.* 2010;5:201-210.

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