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Mutations in IDH1/2 Genes Predict Better Disease Outcome of Glioma Patients-A Study from Western India

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ABSTRACT

Introduction: Isocitrate Dehydrogenase (IDH) plays an important role in cellular metabolism. In gliomas, the mutational status of IDH1/2 genes have paramount significance, however, study from Western India is limited. Therefore, the current study we sought to explore the clinical impact of IDH1/2 mutations for glioma patients from Western India.

Materials and Method: A total of 50 pre-therapeutic, histopathologically confirmed patients with astrocytoma tumors were included and IDH1/2 mutations were detected using real-time PCR. IDH1/2 mutations were correlated with clinicopathological parameters and disease outcome. Data was evaluated by SPSS software.

Results: The overall incidence of IDH1/2 mutations was noted in 24% (12/50) of glioma patients. Out of 12 patients whose tumors showed IDH mutations, 83% patients have IDH1 mutations, whereas 17% showed IDH2 mutation. Further, in IDH1 mutations, IDH1 R132H & IDH1 R132C mutations were noted in, 80% and 20% of patients, respectively. When correlated with clinicopathological parameters, significant inverse correlation was found with patients age ($\chi 2$= 9.75, r = -0.476, p=0.001) and grade of tumors ($\chi 2$=17.51, r =-0.636, p=0.0001). In Kaplan-Meier survival analysis, a part from age (Log rank=5.443, p=0.020), IDH mutation status (Log rank=3.855, p=0.050), and both, IDH mutation and low grade glioma tumors (Log rank=6.492, p=0.039) remained significant parameters for predicting better 24 months PFS and OS of glioma patients. However, in multivariate survival analysis using Cox Proportional Hazard Forward Stepwise Model, only combination of low grade glioma with presence of IDH mutation emerged at step one as positive significant independent prognostic factor that predict better PFS (HR=2.92, 95% CI=1.12-7.61, p=0.028) and OS (HR=3.0, 95% CI=1.45-6.19, p=0.003).

Conclusion: Based on this data, we concluded that for glioma patients, apart from patients age, low grade tumors with presence of IDH mutations remained significant independent positive prognosticators and would be helpful to clinicians for better management of glioma patients.

Keywords: Glioma, IDH, Univariate survival analysis, Multivariate analysis, PFS, OS

INTRODUCTION:

Glioma tumors are the most prevalent primary malignant brain tumors, arise from the glia, the supporting cells of central nervous system (CNS). These tumors denotes a major threat to the public health system not only worldwide, but including India due to its high level of morbidity and mortality. Glial malignancies develop as a result of molecular alterations that continuously accumulate with tumor progression. These tumors are characterized by complex biological behavior with a heterogeneous molecular background. Fortunately, for this devastating neoplasm, more and more research on the intra-tumoral heterogeneity has been specified over the years (1). However, From India very few research centres are working on brain tumors with the myth that brain tumor is a Western malignancy therefore, data regarding this malignancy is very rare from India. However, according to currently published data [1], India is the 3rd most common country in the world, where the cases of CNS tumors are escalating, and it is really worrisome and challenging for our clinicians to manage patients with these complex tumors. Even at Gujarat Cancer & Research Institute (GCRI), based on population based cancer registry, the cases of brain tumors registered were 1.81% of all malignancies. Therefore, to understand the molecular mechanism of this devastating malignancy which could be useful in future for targeted therapy.

Isocitrate Dehydrogenases (IDHs) play significant roles in cellular metabolism. Three enzymes are included in the IDH family, and all catalyze the oxidative decarboxylation of isocitrate to 2-oxoglutarate [2]. However, in human malignancies, two mutations, IDH1 and IDH2 have been reported [3-4]. In normal cells, both IDH1 and IDH2 play a major role in cellular respirations and protect against oxidative stress [4-5]. Currently, many studies have been reported the important of these two genes mutations. Even, in recurrent samples of somatic mutations have been noted. As a consequences, failure in cell maintenance mechanism, proliferation, and eventually develop malignant tumors [6-8]. Thus, due to IDH genes mutations are associated for an abnormal enzymatic gain of function that catalyzes αKG to 2HG [9-12]. IDH mutation was first detected in 2008 in cancer, then after the somatic mutations were identified in even recurrent tissue samples as well as in other malignancies, including glioma tumors [2]. With this background, in the current study, our aim was to determine the frequency of IDH1/2 mutations in different grades of glioma tumors by evaluating IDH1/2 mutations using real time PCR technique and to identify the clinical significance of IDH ½ mutations by correlating them with clinico-pathological parameters and disease outcome of patients registered at the Gujarat Cancer & Research Institute.

Materials and Methods:

Patients

A total 50 untreated histologically confirmed glioma patients with astrocytoma tumors registered at The Gujarat Cancer & Research Institute from January 2018 to January 2021 were enrolled in the current study. The study was approved by the Institute's Ethics Committee Board and written consent forms were obtained from all the patients prior to treatment administration. Detailed clinical and pathological history of the patients was obtained from the case files maintained at the Medical Record Department of our institute. The clinico-pathological characteristics of the enrolled patients are enlisted in Table 1. In the present study, 7 patients were in the age paediatric age group (age range 6 years-16 years) and 43 patients were adults (Age range between 17 years-67 years) with median age 47 years. Majority of patients were male in gender (60%) and 46% of patients have location of glioma tumors were in frontal lobe of brain. According to histological grade of tumors, 28% patients had grade II tumors and 12% patients had grade III tumors and 60% of patients had grade IV astrocytoma tumors. All patients underwent for surgery as primary treatment. Fifty-two percent of patients underwent for only surgery, whereas, 40% patients were underwent for surgery followed by radiotherapy and chemotherapy as adjuvant therapy (Table 1).

As paediatric patients were very less in number, data was analysed for adult group of patients only (n=43).

Table 1. Patient and Tumors Characteristics

Characteristic	N	%
Total Patients	50	
Age groups		
Paediatric (Range:6-16 years)	07	14
Adult (Range: 17-67 years; Median 47 years)	43	86
≤ 47 years	23	54
>47 years	20	46
Gender		
Female	20	40
Male	30	60
Location of tumors		
Frontal	23	46
Parietal	18	36
Temporal	09	18
Grades of tumors		
II	14	28
III	06	12
IV	30	60
Treatment		
Surgery (S)	21	42
Followed by		
S + Radiotherapy (RT)	05	10
S + Chemotherapy (CT)	04	08
S + RT + CT	20	40
Progression free survival (n=40)		
GC Well	27	67
Recurrence	13	33
Overall survival (n=40)		
Alive	21	52
Died	19	48
IDH1/2 Mutation Status		
Absent	38	76
Present	12	24

Out of a total of 43 patients, 40 patients could be followed for a minimum period of 24 months or until their death within that period. Progression-free survival (PFS) and overall survival (OS) was evaluated. Within 24 months, 33% (13/40) patients had developed recurrent disease and 48% (19/40) of patients died within 24 months (Table 1).

DNA Extraction:

Genomic DNA was extracted from histopathology confirmed astrocytoma FFPE blocks retrieved from histopathology department of our institute. DNA isolation was done using the Qiagen DNA extraction Kit, according to the manufacturer's instructions. The concentration, purity and quality of the extracted DNA were determined

by Qubit 3.0 Fluorometer (Invitrogen, USA) and 0.8% gel agarose electrophoresis, respectively.

Real-time PCR for IDH1/2 mutation detection:

IDH1/2 mutations was detected using ARMS PCR using therascreen IDH1/2 RGQ PCR kit following manufacturer's instructions (Qiagen). Qualitative detection of 6 mutations within IDH1 codon 132 (c.395G>A for R132H, c.394C>T for R132C, c.394C>A for R132S, c.394C>G for R132G, c.394G>T for R132L and c.394_395CG>GT for R132V) one within IDH1 codon 100 (R100Q) and 5 within IDH2 codon 172 (c.515G>A for R172K, c.515G>T for R172M, c.514A>T for R172W, c.516G>T for R172S and c.514A>G for R172G) were noted.

PCR was performed using the Rotor-Gene Q 5-plex HRM instrument (Qiagen). Quality control was seen using CT values of controls. With each assay, we run positive, negative and no template control to ensure that acceptable Ct values were met and that the reactions were performed correctly. The PCR condition used was: 95°C Time: 10 min Cycling 40 times 95°C for 15 sec 60°C for 60 sec with an acquisition of FAM™ fluorescence in channel Green: Single. Sample ΔCt values were calculated as the difference between the mutation assay Ct and respective total assay Ct from the same sample. Samples were classified as mutation positive if the ΔCt value was less than or equal to the ΔCt cut-off value of the respective mutation assay. The cut-off values used were as follows: ΔCT IDH1 R132 Mut-≤4.25, ΔCT IDH1 Mut R132H-≤4.40, ΔCT IDH1 Mut R132C-≤5.80, ΔCT IDH1 R100 Mut-≤4.22, ΔCT IDH2 R172 Mut-≤4.00, ΔCT IDH2 Mut R172K-≤5.70 (1).

Statistical Analysis:

The data was analyzed statistically using SPSS Inc. version 25 software. The correlation between IDH mutational status with clinicopathological parameters of glioma patients was determined by two-tailed chi square test ($\chi 2$) and spearman's correlation. Survival analysis was performed using Kaplan-Meier survival function and the differences in survival were tested for statistical significance using log-rank statistic. Multivariate survival analysis was performed using Cox forward stepwise proportional hazard regression model. $p \leq 0.05$ was considered to be statistically significant.

Results:

Incidence of IDH ½ mutations in glioma patients:

MThe overall frequency of IDH1/2 gene mutations were noted in 24% (12/50) of glioma patients and 76% (38/50) patients showed absence of IDH1/2 mutations. The frequency of IDH1 mutations was noted in 83%

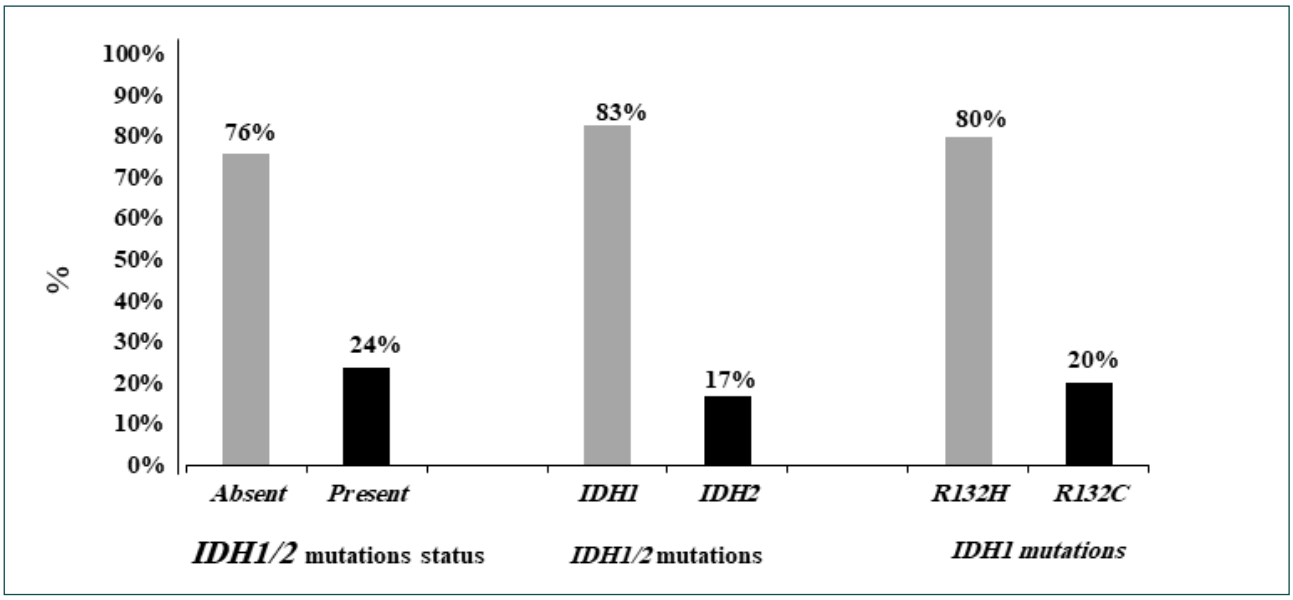

Figure 1. Overall frequency of IDH1/2 mutations in glioma tumors

patients and 17% patients showed IDH2 mutations. In IDH1 gene, the frequency of IDH1 R132H was noted in 80%, (8/10) & IDH1 R132C mutation was present in 20% (2/10) of patients (Figure 1).

Correlations between IDH1/2 mutations with clinico-pathological parameters:

A significantly high incidence of IDH mutation was observed in patients having ≤47 years, compared to 5% (1/20) patients with age group >47 years (r =-0.476, $\chi2$=9.752, p =0.001). Thus, we found inverse correlation between age of patients and IDH mutations status. Also, this finding indicating that age is significant factor for glioma patients. Similar significant difference we noted between grade of tumors and IDH mutation (r=-0.636, $\chi2$=17.51, p =0.0001). In patients with grade II glioma tumors showed high incidence of IDH mutation (67%) followed by in grade III (50%) and grade IV tumors (4%) (Table 2). Thus, as grade of tumors increased, the incidence of IDH mutations were decreased.

Survival Analysis:

Univariate and multivariate survival analysis was carried out for PFS and OS of glioma patients. The median months used was 24 months.

Univariate Survival Analysis

Univariate survival analysis for 24 months PFS and OS was performed using Kaplan-Meier survival analysis for all clinicopathological parameters and IDH mutational status and it is depicted in Table 3.

Progression free survival

Univariate Kaplan-Meier survival analysis for PFS demonstrated that apart from patients who were in the age group ≤47years (Log rank=5.443, df=1, p=0.020), presence of IDH1/2 mutations (Log rank=3.855,df=1, p=0.050) and combination of grade II tumors with presence of IDH ½ mutation (Log rank=6.492, df=2, p=0.039) showed significantly longer PFS in comparison to patients with their respective counterparts (Table 3). The Kaplan-Meier Survival curve for PFS indicated that the incidence of recurrence was 39% within 24 in patients whose tumors showed absent of IDH1/2 mutations as compared to 17% recurrence was observed in patients with IDH1/2 mutations in their tumors (Figure 2A). Similar significant difference was noted in Kaplan-Meier survival curve for combination of grade II glioma tumors and presence of IDH1/2 mutations. Patients with grade II glioma tumors and presence of IDH1/2 mutation status, showed only 17% recurrence incidence than patients with either only grade II tumors or only IDH mutations presence (22%) followed by neither grade II tumors nor IDH mutations were present (43%) (Figure 2B).

Overall survival

Univariate Kaplan-Meier survival analysis for OS indicated that patients with ≤47years age, (Log rank=4.959, df=1, p=0.026), low grade of tumors (Log rank=11.716, df=2, p=0.003), presence of IDH1/2 mutations (Log rank=9.793,df=1, p=0.002) and combination of grade II tumors with presence of IDH ½ mutation (Log

Table 2. Correlation between IDH1/2 Gene Mutations with Age and Grade of Tumor

Parameters	N	IDH1/2 Mutations		r	$\chi2$	p value
Age	43	Absent N (%)	Present N (%)			
≤47	23	12 (52)	11 (48)	-0.476	9.752	0.001
>47	20	19 (95)	01 (05)			
Grade of Tumors						
II	12	04 (33)	08 (67)	-0.636	17.51	0.0001
III	06	03 (50)	03 (50)			
IV	25	24 (96)	01 (04)			

Table 3. Univariate survival analysis for PFS and OS using Kaplan-Meier Analysis

		PFS 24 Months		OS 24 Months	
Parameters		**Patients relapsed**	**P value Log rank**	**Patients died**	**P value Log rank**
	N	**N (%)**		**N (%)**	
Age					
≤47	24	05 (21)	0.020 5.443	10 (42)	0.026 4.959
>47	16	08 (50)		09 (56)	
Gender					
Female	16	04 (25)	NS	09 (56)	NS
Male	24	09 (37)		10 (42)	
Location of tumors					
Frontal	22	06 (27)	NS	09 (41)	NS
Parietal	11	05 (45)		07 (64)	
Temporal	07	02 (28)		03 (43)	
Grade of tumors					
Grade II	13	02 (15)	0.063 5.534	04 (31)	0.003 11.716
Grade III	05	02 (40)		02 (40)	
Grade IV	22	09 (41)		13 (59)	
IDH1/2 mutation status					
Absent	28	11 (39)	0.050 3.855	16 (57)	0.002 9.793
Present	12	02 (17)		03 (25)	
Grade of tumors and IDH1/2 mutation status					
Grade II & IDH Mt*	08	01 (12)	0.039 6.492	02 (25)	0.001 13.164
Grade II & IDH Mt	09	02 (22)		03 (33)	
Grade III/IV & IDH Wt**	23	10 (43)		14 (61)	

*IDH mutant, **IDH wild type tumors

rank=13.164, df=2, p=0.001) showed significantly better OS in comparison to patients with their respective counterparts (Table 3).

The Kaplan-Meier Survival curves for OS demonstrated that the death incidence was 31% which was significantly low in patients with grade II glioma tumors compared to 40% in grade III tumors followed by 59% in grade IV glioma tumors (Figure 2C). Similar results we noted with IDH1/2 mutations and OS (Figure 2D) and combination of both, grade II tumors and presence of IDH1/2 mutations (Figure 2E). Thus, with IDH mutation status and grade of tumors we observed inversed correlation with disease outcome of glioma patients.

Multivariate Survival Analysis for Progression-free and Overall Survival

To assess the dependence of the predictive value of

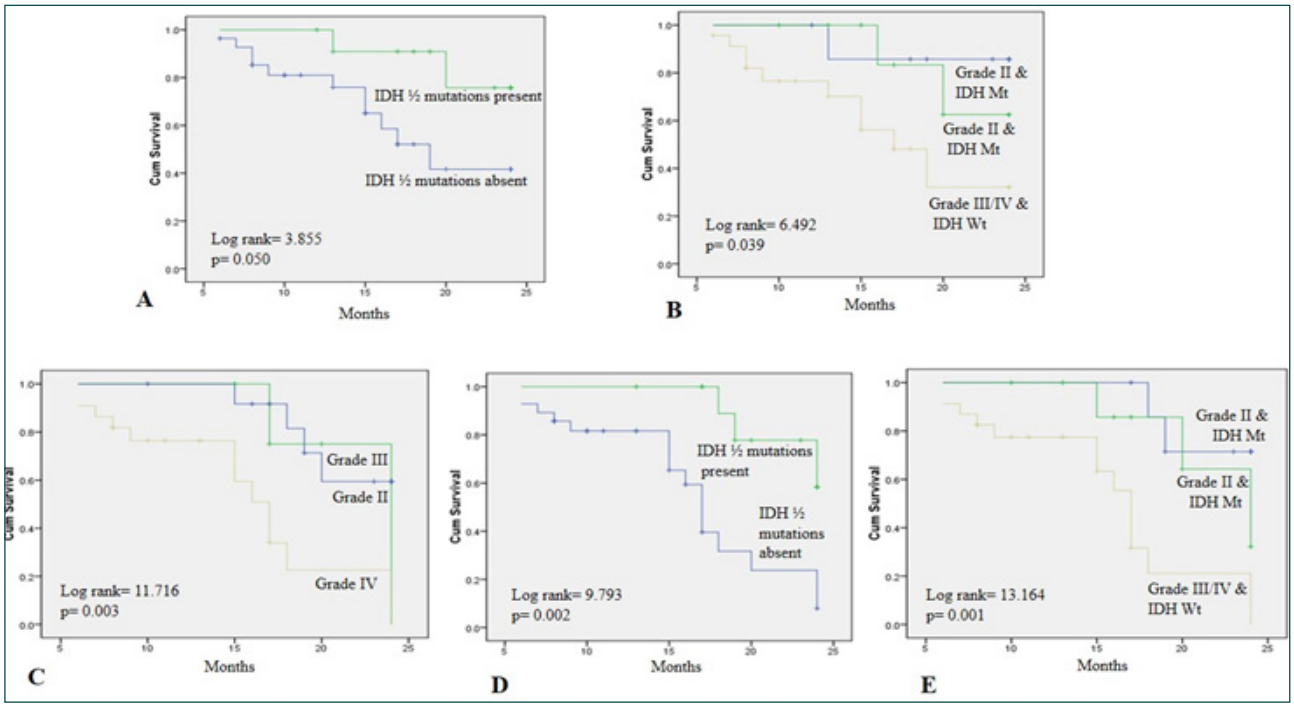

Figure 2. Progression-free survival: A IDH1/2 mutations for PFS, B-Combination of grade II tumors with IDH1/2 mutations for PFS. Overall survival: C-Grade of tumors for OS, D- IDH1/2 mutations for OS, E- Combination of grade II tumors with IDH1/2 mutations for OS.

IDH1/2 mutations with other known prognostic factors (age, gender, location of tumors, histologic grade), a multivariate Cox Forward Stepwise Proportional Hazard Regression analysis for PFS and OS was performed. We observed that for PFS and OS both, grade II glioma tumors with presence of IDH1/2 mutations together entered the equation at step 1. Thus, glioma patients with grade II tumors and presence of IDH1/2 mutations remained a significant positive prognosticator risk factor for recurrence of disease (HR=2.92, 95% CI=1.12-7.61, p=0.028; Table 4) and better OS (HR=3.00, 95% CI=1.45-6.19, p=0.003) (Table 4). This indicated that grade II tumors and present of IDH mutations could serve as an independent prognostic factor for predicting longer 24 months PFS and better OS.

Discussion:

Currently, 5S 2021 WHO classification for CNS tumors has incorporated molecular aberrations that might help to resolve the discrepancy between classification and clinical outcome of astrocytic glioma tumors. For glioma patients, IDH mutation is emerged as prognostic and predictive parameter independent of the WHO grade of tumors. However, based on IDH status, till date, no novel therapeutic targeted therapy is translated at clinic. On the contrary, in many cases, WHO grade II or III IDH-wild-type infiltrating astrocytoma patients have worse outcomes than IDH-mutant glioblastomas (grade IV), reflecting that their tumors are likely to behave in a manner similar to IDH-wild-type glioblastoma. This is creating a significant problem in the current grading criteria. Keeping this in mind, in the current study, we evaluated the clinical significance of IDH1/2 mutations using real time PCR for glioma patients with astrocytic tumors.

Currently, IHC covers the most prevalent IDH1 R132H mutation, and various molecular techniques are used to assess the IHC-negative cases, leading to a two-step diagnostic algorithm with possibly long timelines to complete a full IDH profiling. Using one step real time PCR kit (Qiagen) detection of IDH1/2 mutations (12 mutations) is a sensitive method for detection of IDH1/2 mutations for glioma patients [13]. Using this kit, we identified IDH1/2 mutations in total 24% of glioma patients.

Table 4. Multivariate survival analysis using all parameters for PFS and OS

Survival	Step	Parameters	Wald	HR	95% Confidential Interval		p value
					Lower	Upper	
PFS	1	Co-detection IDH Mt & Grade II	4.80	2.92	1.12	7.61	0.028
OS	1	Co-detection IDH Mt & Grade II	8.84	3.00	1.45	6.19	0.003

Similar to our findings, the prevalence of IDH mutations was reported in 18.7% and 17% by Pashmina et al. and BR das et al, respectively from India [14-15]. However, the frequency of IDH mutation in diffuse glioma is variable, ranging from 54% to 90% [16-19]. Further, out of 12 patients whose tumors showed IDH mutations, 83% patients had IDH1 mutation, whereas 17% had IDH2 mutations. Most of the available reports on IDH1 and IDH2 mutations are from Western countries, while there is very few data are available that showed data from India [20]. Also, corroborate to our results, various other studies have been observed IDH1/2 mutations in different population. Data from Netherland (20%) and Germany (19%) were corroborate to our results. However, varied reports were noted from Brazil (11.8%), and USA (45.3%) and Japan (29%) [21-25]. Thus, these findings clearly indicated that the ethnic racial differences and methodology used may impact on prevalence of IDH mutations.

In this study, we correlated IDH mutation with clinicopathological parameters. A significant correlation was observed with age of patients. Patients with <47 years, showed significantly high incidence of IDH mutations than patients with >47 years. The similar findings were reported by BR das et al (2013) and Susmita et al (2020) from Indian population [15, 26]. In these lines, BR das et al., have reported that IDH1 mutations were significantly higher in younger patients when compared with patients who were above 50 years which tallies with several previous studies [15,20, 22]. Thus, younger age glioma patients have better outcome as they have more prevalence of IDH mutation.

Further, we noted inverse correlation between IDH mutation and grade of glioma tumors. Thus, as tumor grades increased, the frequency of IDH mutations were decreased. This means patients with low grade glioma tumors have high incidence of IDH mutations than patients with high grade glioma tumors. The similar results were obtained by Susmita et al. where they found more prevalence of IDH mutations in low grade glioma tumors [26]. Contradictory to our results, BR das et al., have not found any significant association between IDH mutation and grade of glioma tumors [15]. From Susmita et al [26] Further, Jaiswal et al [27] in their study found a higher association of WHO grade II and III tumors with IDH1 mutations. Similar results were also found in studies done by Yan et al. Hartmann et al [28-29].

On survival analysis, our results demonstrated that patients with presence of IDH1/2 mutations showed significantly longer PFS and better OS. Similar findings were reported by Agarwal Shilpra et al (2013), Chatterjee et al (2018) and Susmita et al (2021) [19,26,30]. Also, another study by Xi et al (2015) have in one meta-analysis study shown that glioma patients with presence of IDH mutations had significantly better PFS and OS compared to patients with absence of IDH mutations.

Mohrenz et al (2013) and Li et al (2013) have shown that mutant IDH may increase the survival of glioma patients as mutant IDH enhanced cellular oxidative stress and simultaneously reduced NADPH levels [32-33]. However, as a result of IDH mutations, 2 hydroxyglutarate (2 HG) produced and that might enable in disease progression [9, 34]. In line of this, the negative effect of 2 HG may be abolished by the favorable effect of IDH mutations. This is demonstrated that mutant IDH enzymes may af-

fect multiple pathways in glioma, which coordinate to influence patient prognosis [35]. Overall, for glioma patients, IDH1/2 mutation status could be a useful positive prognosticator and could be useful as targeted molecule [36]. We also noted that glioma patients with not only IDH mutations or only low grade tumors, but the combination of presence of IDH mutation and grade II glioma tumors showed significantly longer PFS and better OS than their respective counterparts.

Conclusion:

In conclusion, the current study demonstrated that R132H is the most frequent IDH1 mutation while the incidence of IDH2 mutation was less frequent. The fact that the value of IDH 1/2 mutations relates to prognosis, as demonstrated in terms of longer survival in patients with IDH mutation. Also, grade II glioma tumors and presence of IDH1/2 mutations could be a positive prognosticators. Thus, though this is known fact that IDH1/2 are positive prognosticator for glioma patients, with this study, we generated data from Western India and we also make a punch line that for routine clinical testing of IDH mutations will allow assignment of patients to better-defined risk categories.

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