Gliomas - An experience based on molecular markers

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Abstract

Background: Gliomas account for 45% of all intracranial tumors. Newer technologies have allowed deeper genetic and epigenetic analysis leading to the discovery of IDH (Isocitrate dehydrogenase) mutations and their association with ATRX (alpha-thalassemia/mental retardation syndrome X-linked) and p53, for better diagnosis and prognosis. In this study, we analysed their expression and correlated with various clinicopathological parameters. A follow up to prognosticate gliomas based on the molecular findings is also attempted. **Materials and Method:** During last 5 years both retrospective and prospective cases were included in the study. Immunohistochemistry for IDH1, ATRX, and p53 was done and reported based on intensity and percentage of tumor cells expressing the markers. **Results**: A total of 53 cases of gliomas were included, excluding primary glioblastomas and ependymomas. The patient's age ranged from 10 to 53 years. The male to female ratio was 1.3:1. IDH1 positivity was seen in 88% of diffuse astrocytoma, 80% of anaplastic astrocytoma, 90% of oligodendroglioma, 60% of anaplastic oligodendroglioma, and 54% of glioblastoma. A significant association was seen between positive IDH1 expression and low-grade gliomas (p = 0.028). A combined analysis of expression of IDH1 and ATRX versus IDH1, ATRX, and p53 with WHO grade showed a statistically significant association. A follow-up of 32 patients was available. Out of 24 IDH1+ (positive) cases, 22 patients had a median survival of 21.5 months (92%). Out of 8 IDH1- (negative) cases, 5 had a median survival of 15.8 months (62%). **Conclusion**: Gliomas expressing IDH1 mutation show improved survival of patients. Combined analysis of IDH1, ATRX, and p53 has diagnostic and prognostic significance. For routine cases of gliomas, a combination of IDH1 and ATRX are sufficient; however, the use of p53 is recommended for further prognostication and for possible targeted therapy in the future.

Keywords: ATRX, gliomas, IDH1, p53

Introduction

Glioma is a broad category of tumors originating from the glial cells of the brain. They are found in adults as well as in children, have a slightly higher male preponderance and the most frequent location is the frontal lobe.[1,2]

Gliomas account for 45% of all intracranial tumors with an age-standardized incidence rate of 4.67/100,000 persons per year.[3] The most frequent subtypes include glioblastomas (GB), followed by grade II and III astrocytoma and oligodendroglioma (OG).[2,4] The symptoms, treatment, and prognosis of gliomas depend on the patient's age, location, and type of the tumor and also on some mutations that have been recently introduced.[1]

IDH1 mutations are seen in 70-80% of grades II and III astrocytomas and around 80% of OG. They are also common in secondary GBs (~82%) but are rare in primary GBs and almost always absent in ependymomas.[5-7] Gliomas expressing these mutations are said to be more chemo and radiosensitive.[5,6]

ATRX gene mutation leads to loss of expression of ATRX in tumor cells. ATRX mutation is a specific marker for astrocytomas but it is not mutated in OGs.[7]

The p53 mutation is seen in the range of 58–83% in astrocytomas. Secondary GBs also show p53 mutations but these mutations are rare in primary GBs and OGs.[7]

The new 2016 WHO classification incorporated molecular parameters in addition to histology to define the tumor entities and it is hoped that this will aid both in the diagnosis and prognosis of glioma patients.[8] Patients put a lot of trust in their primary care physicians and look up to them to understand the intricacies of their disease. It is therefore desirable that physicians have a fair understanding of the role of molecular markers in diagnosis and prognosis of the disease.

This study is done to correlate the molecular markers IDH1, ATRX, and p53 with various clinicopathological parameters in gliomas and to follow up these patients to attempt prognostication based on molecular findings.

Material and Methods

The present study includes both retrospective and prospective cases from October 2014 to July 2019. This study has been approved by the Institute's Scientific Advisory Committee and the Institute's Ethics Committee (NEIGR/IEC/M3/T8/17). The relevant clinical and radiological data were obtained for all the cases. The biopsy received in neutral buffered formalin was processed and hematoxylin and eosin (H&E) sections were stained. Light microscopy findings were recorded and WHO grading of tumors was done. Cases of ependymoma and primary GB (denovo GB cases) were excluded. The present study was approved by the Research and Ethics Committee.

The cases were sub-typed based on the histological features as pilocytic astrocytoma (PA), diffuse astrocytoma (DA) [Figure 1], anaplastic astrocytoma (AA), OG [Figure 2], anaplastic oligodendroglioma (AO), and GB [Figure 3].

Assessment of IDH1, ATRX, and p53 was done on immunohistochemistry (IHC) by the horse radish peroxidase method. The paraffin-embedded sections were stained by mouse monoclonal antibody raised against IDH1 (IDH1 R132H, clone: H09, Dianova), ATRX (clone: BSB-108), and p53 (DO7, Cell Marque). Each IHC slide was examined under a light microscope by 3 independent observers without the knowledge of the patient's other data. All the neoplastic cells were examined.

IDH1-R132H staining was considered positive when it demonstrated moderate to strong cytoplasmic positivity, perinuclear positivity, and weaker nuclear positivity.[9]

ATRX immunostain was considered positive (retained) when cases showed strong nuclear positivity. Endothelial cells and lymphocytes acted as an internal control in case of ATRX staining. A complete absence of nuclear stain was interpreted as a loss of ATRX expression (mutated).
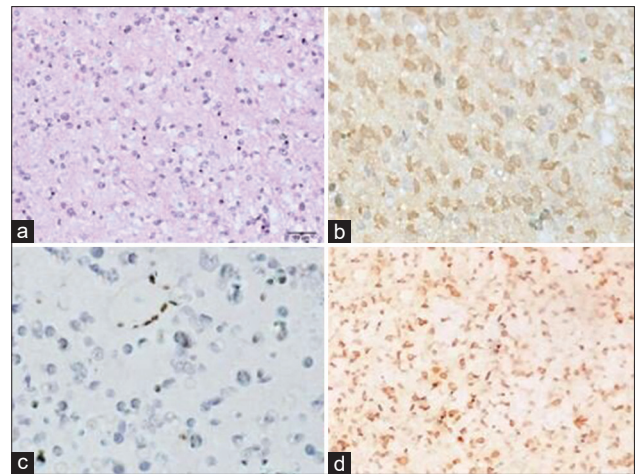

Figure 1: Diffuse Astrocytoma, WHO Grade II, IDH Mutant, ATRX loss, p53 positive (a) Diffuse Astrocytoma (20X, H&E) (b) IDH1 showing cytoplasmic, perinuclear and weak nuclear positivity (40x) (c) ATRX loss with endothelial cells as internal control (40X) (d) p53 showing 3+ nuclear positivity (20x)

Figure 2: Oligodendroglioma, WHO Grade II, IDH mutant, ATRX retained, p53 negative (a) Oligodendroglioma (4X, H&E) (b) IDH1 showing cytoplasmic and perinuclear positivity (40X) (c) ATRX retained (40X) (d) p53 negative (40x)

Figure 3: Glioblastoma, WHO Grade IV, IDH mutant, ATRX loss, p53 positive (a) Glioblastoma (20x, H&E) (b) IDH1 showing cytoplasmic and peinuclear positivity (40X) (c) ATRX loss with endothelial cells as internal control (40X) (d) p53 showing 3+ nuclear positive (40x)
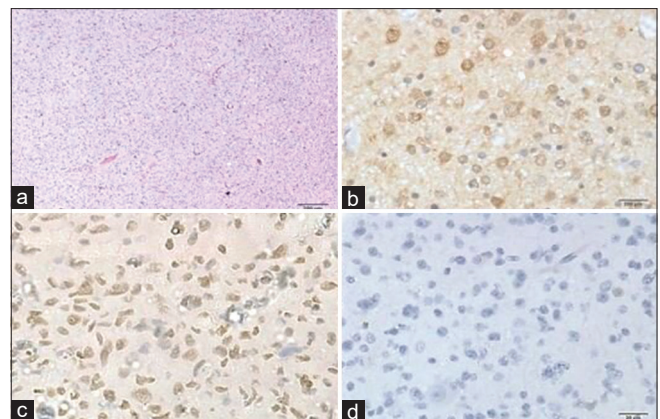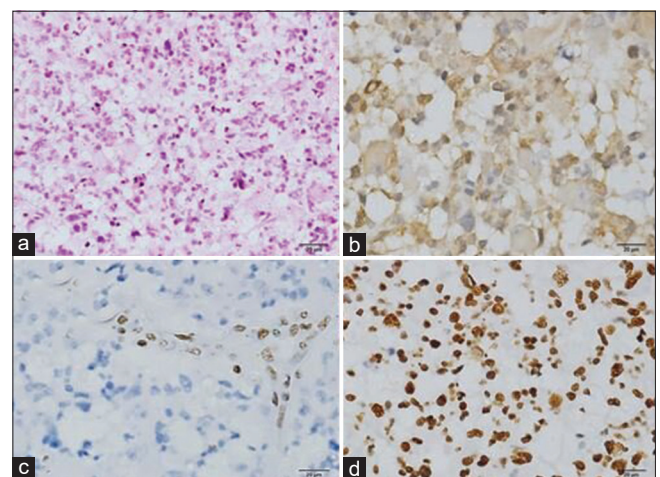
The p53 stain was graded as 0, 1+, 2+, and 3+.[10]

0: when no cell stained nuclear positive.

1+ : <10% cells showing nuclear positivity

2+: clusters of cells showing nuclear positivity

3+: all tumor cells showing nuclear positivity.

0, 1+ score was considered negative and 2+, 3+ score was considered positive

Results

A total of 53 histologically diagnosed cases of PA, DA, AA, OG, AO, and secondary GB were obtained fulfilling the inclusion and exclusion criteria. The patient's age ranged from 10 to 53 years with a mean of 32 years. Out of the 53 cases, 30 (56.6%) were male while 23 (43.39%) were female, with a male-female (M: F) ratio of 1.3:1. Of the 53 cases studied, 34% presented with frontal lobe lesion followed by parietal, fronto-parietal, and temporal lobes. Patients presented with multiple symptoms with headache being the most common followed by vomiting and altered sensorium. Out of 53 cases, 2 patients were diagnosed as PA, 9 as DA, 10 as OG, 5 each as AA and AO, and 22 patients as secondary GB. WHO grade I tumors were seen in 2 cases (3.7%), grade II in 19 cases (35.8%), grade III in 10 cases (18.8%), and grade IV in 22 cases (41.5%), respectively.

IDH1 mutation

IDH1mutation (IDH1 positivity) was observed in 8/9 DA (88.8%), 4/5 AA (80%), 9/10 OG (90%), 3/5 AO (60%) and 12/22 GB (55%). Overall, positivity was seen in 20/30 males and 16/23 females.

ATRX mutation

ATRX mutation (loss of staining) was observed in 9/9 DA (100%), 4/5 AA (80%), and 12/22 GB (55%), whereas non-mutated ATRX status (retained staining) was seen in all the cases of PA, OGs, and AOs.

p53 mutation

p53 mutation was seen in 8/9 DA (88.8%), 4/5 AA (80%), 14/22 GB (63.6%) and 1/5 AO (20%), whereas none of the OG and PA showed p53 mutated.

Statistical analysis

Statistical analysis was calculated by IBM SPSS statistics software and *P* value of <0.05 considered as statistically significant. The association of IDH1 expression with age, sex, location, symptoms, WHO grade, and histological lineage are enumerated in Table 1. The association of ATRX expression with age, sex, WHO grade, and histological lineage are enumerated in Table 2. The association of p53 mutation with age, sex, WHO grade, and histological lineage are enumerated in Table 3. The association of IDH and ATRX with WHO grading and the association of IDH, ATRX and, p53 with WHO grading are illustrated in Tables 4 and 5.

Follow-up

A follow-up of 32 patients was available out of 53, which included 11-GB, 6-DA, 8-OG, 3-AA, 3- AO, and 1- PA. Of the 11 GB cases, 7 were IDH1+ (positive) and 6 were alive till the date of the last follow-up. One IDH1 + GB patient succumbed within 4 months, giving an average survival of 14.8 months. Among the 4 IDH1- (negative) GB cases, only 1 survived for 24 months and the other 3 patients succumbed within 6 months of diagnosis.

Out of 6 DA (5 IDH1 + case and 1 IDH1 – case), all were alive and had a median survival of 37.8 months. Out of 8 OG

Table 1: Association of IDH1 with various clinico-pathological parameters in CNS Gliomas

Characteristics	Status of IDH1		*P*
	Mutated (*n*=36)	Non-mutated (*n*=17)	
Age			
<40 years	29	14	0.8
>40 years	7	3	
Gender			
Male	20	10	0.8
Female	16	7	
Location			
Frontal lobe	14	4	0.5
Other sites	22	13	
Symptoms			
Headache +	21	12	0.39
Headache-	15	5	
Vomiting+	9	4	0.9
Vomiting-	27	13	
Altered sensorium+	6	6	0.13
Altered sensorium-	30	11	
WHO Grade			
WHO grade II	17	2	0.028
WHO grade III + IV	19	13	
Histological Lineage			
Astrocytic tumors	24	14	0.23
Oligodendroglial tumors	12	3	

Table 2: Association of ATRX with various clinico-pathological parameters in CNS Gliomas

Characteristics	ATRX status		*P*
	Mutated (*n*=25)	Non-mutated (*n*=28)	
Age			
<40 years	19	24	0.2
>40 years	06	04	
Sex			
Male	16	14	0.3
Female	9	14	
WHO Grade			
II	9	10	0.85
III + IV	16	16	
Histological Lineage			
Astrocytic tumors	25	13	<0.0001
Oligodendroglial tumors	0	15	

(7 IDH1+ and 1 IDH1-), all were alive and had a median survival of 13.57 months. Out of 3 AA (all IDH1+), 2 were alive and median survival was 21 months. 1 patient of AA survived for only 10 months from the time of diagnosis. All 3 AO cases were alive (2 IDH1+ and 1 IDH1-) and the median survival was 17 months. One PA case survived for 23 months.

Therefore, of the 24 IDH1+ cases which were followed-up, 22 (91.6%) were alive allowing a median survival of 21.5 months and of the 8 IDH1- cases, 5 (62.5%) were alive and had a median survival of 15.8 months.

Discussion

In the present study, the patient's ages ranged from 10 to 53 years with a mean age of 32 years. The maximum number of patients were in the age group of 31-40 accounting for 37.7%. Thambi *et al.*, Krishnatreya *et al.*, Jaiswal J *et al.*, found similar findings.[11-13]

The M: F ratio of 1.3:1 was similar to other studies,[12,14,15] whereas in the study by Thambi *et al.*, a slightly higher female preponderance was noted with a M: F ratio of 0.9:1.[11]

Table 3: Association of p53 with various clinico-pathological parameters in CNS Gliomas

Characteristics	p53 Status		P
	Positive (*n*=27)	Negative (*n*=26)	
Age			
<40 years	22	21	0.6
>40 years	05	05	
Sex			
Male	16	14	0.6
Female	11	12	
WHO Grade			
II	8	11	0.2
III + IV	19	13	
Histological Lineage			
Astrocytic tumors	26	12	0.0000135
Oligodendroglial tumors	1	14	

Table 4: Association of IDH1, ATRX with WHO Low Grade vs WHO High Grade Gliomas

WHO GRADE	IDH1 + ATRX-L	IDH1 + ATRX-R	IDH1- ATRX-L	IDH1- ATRX-R	P (Fisher)
I+II	8	11	1	3	0.01
III+IV	14	3	2	11	

ATRX-L=Loss, ATRX-R=Retained

Frontal lobe lesions were most commonly seen (34%) similar to other studies.[2,11,16] The present study had one patient each, presenting with occipital and temporo-occipital glioma whereas another study in the northeast done by Krishnatreya *et al.* found no cases of occipital glioma.[12] In our study, patients presented with multiple symptoms, the headache was the most common followed by vomiting, altered sensorium, seizures, and fever.

Mondal *et al.* also found the most common presentation of brain tumors is a headache (63 cases, 48.46%).[16] In a study done by Grier *et al.* the seizure was the presenting symptom in 72%–89% of patients while in the study by Rasmussen *et al.* the focal deficit was the most common symptom seen in 64% of the patients.[17,18]

The WHO grading of gliomas in our study had similar findings to the study conducted by Mondal S *et al.*[16]

Comparison of WHO grading with IDH1 showed that IDH1mutation is more commonly seen in WHO grade II low-grade gliomas, 17/19 cases (89%) although WHO Grade I tumors like PA lacked this mutation.

In the study done by Agarwal S *et al.* immunoreactivity for IDH1 was noted in 30/50 cases accounting for 60%.[19] Capper *et al.* and Cai *et al.* also found findings similar to the present study.[9,20] Both these studies had cases of oligoastrocytoma (OA) and anaplastic oligoastrocytoma (AOA) which showed IDH1 mutation but as the present study did not have any OA and AOA cases, IDH1 mutation in these categories could not be assessed.

IDH1 mutations were more prevalent in younger patients as seen in the present study but no statistically significant relationship between age and IDH1 mutation ($p = 0.864$) was seen. The study done by Yusoff *et al.* also found no significant relation with age and IDH1 mutation while the significant association with younger age was found in the study done by Das *et al.*[21,22]

Sex distribution in IDH1 mutation was not found to be statistically significant. There were 66.6% males and 69.5% females with IDH1 mutation indicating a slightly higher value in females which correlates with the findings of Jaiswal *et al.* and Das *et al.*[7,22] Yosoff *et al.* found IDH1 mutations to be more common in males.[21]

The most common symptom in our study was headache (63.6% in IDH1+ cases), followed by vomiting (69% in IDH1+ cases) and altered sensorium (50% in IDH1+ cases) but none were

Table 5: Association of IDH1, ATRX and p53 with WHO Low Grade vs WHO High Grade Gliomas

WHO GRADE	IDH1 + ATRX-L P53+ (*n*=21)	IDH1 + ATRX-R P53+ (*n*=2)	IDH1 + ATRX-L P53- (*n*=1)	IDH1 + ATRX-R P53- (*n*=12)	IDH1- ATRX-L P53+ (*n*=1)	IDH1- ATRX-R P53+ (*n*=3)	IDH1- ATRX-L P53- (*n*=2)	IDH1- ATRX-R P53- (*n*=11)	P (Fisher)
I + II	7	2	1	9	1	0	0	3	0.02
III + IV	14	0	0	3	0	3	2	8	

ATRX-L=Loss, ATRX-R=Retained

statistically significant with an IDH1 mutation. Jaiswal *et al.* in their study found seizure as the most common symptom and a significant association was found between seizures and IDH1 mutation in their study.[7]

The association of IDH1 mutation was also not statistically significant with the site of the tumor though 77.7% of IDH1 positive cases were found in the frontal lobe. In the study done by Jaiswal *et al.*, 71% of all frontal gliomas had IDH1 expression and were statistically significant.[7]

In the present study, even though not statistically significant, IDH1 mutations were seen more often in younger patients, in females, with vomiting being the most common presenting symptom and frontal lobe the most common location. Statistically significant association of IDH1 mutation with low-grade glioma (WHO grade II) versus high-grade gliomas (WHO grade III and IV) was obtained with a *P* value of 0.028, showing that IDH1 mutations are more common in low-grade gliomas and therefore low-grade gliomas have a better prognosis. Jaiswal *et al.* in their study found a higher association of WHO grade II and III tumors (p-value of 0.0008) with IDH1 mutations.[7] Similar results were also found in studies done by Yan *et al.* Hartmann *et al.*[23,24]

ATRX mutation was not seen in any case of PA, OG, AO while mutation was seen in all the cases of DA (100%). ATRX mutation was also seen in 4/5 (80%) cases of AA and 12/22 (54.54%) cases of secondary GB. Tumors of the oligodendroglial lineage did not show ATRX mutation in the present study, which infers that ATRX retained status could be used for confirming an oligodendroglial lineage in centers where advanced techniques like FISH (fluorescent *in situ* hybridization) for detecting 1p19q mutation are not available. ATRX mutation compared with age, sex, and WHO grading did not show any statistical significance.

The histological lineage showed a sole statistically significant relation with ATRX status in the present study (p-value=<0.0001), reiterating their importance in detecting oligodendroglial tumors. Ebrahimi *et al.*, and Liu *et al.*, also found similar findings.[25,26] Ebrahimi *et al.* found that ATRX loss was significantly associated with astrocytic lineage tumors (p < 0.001) which was similar to the present study.[25]

Liu *et al.*, also assessed the effects of age, sex, and WHO classification with ATRX status.[26] The results indicated that sex and WHO classification did not significantly affect ATRX status, which was similar to the present study; however, age was significantly associated which was not seen in the present study.

The histological lineage had sole statistical significance in relation to p53 mutation (p-value = 0.0000135) signifying that they are more prevalent in astrocytic tumors. No statistical significance of the association of p53 was seen between age, sex, and WHO grade in the present study. The p53 mutations were more prevalent in tumors of astrocytic lineage and are rare in grade I tumors like PA and OG tumors.

Kyritsis *et al.*, and Sipayya *et al.*, found similar findings.[27,28] Sipayya *et al.* also found p53 positivity in 41% of OG.[28] In the present study, however, p53 mutation was absent in grade II OG but was seen in 20% of AO.

Komori *et al.* found that the combined IHC for IDH1, p53, and ATRX improves the molecular classification of diffuse gliomas in daily practice.[29] However, in the present study, an association of IDH1, ATRX with WHO low grade versus high grade and IDH1, ATRX, p53 with WHO low grade versus high grade both showed statistically significant *P* value, enhancing the fact that using 2 IHC markers for confirming the subtype of gliomas is equally good for routine practices.

A follow-up of 32 patients out of 53 was obtained in the present study. A total of 24 IDH1 + cases were present in the follow-up, 22 (91.6%) of whom were alive and had a median survival of 21.5 months. IDH1- cases were 8 in number. 5 (62.5%) were alive with a median survival of 15.8 months. This clearly shows that IDH1 mutation is associated with increased survivability in glioma patients. The statistical significance of survival with IDH1 mutation could not be evaluated due to inadequate number of cases that had follow up in each glioma category. Lv *et al.*, and Jones *et al.*, also found that IDH1 mutated cases had improved overall survival and progression-free survival in cases of gliomas as compared to IDH1 wild type cases.[30,31]

This study is limited by its small sample size, unavailability of FISH, and Next Generation Sequencing for confirming cases of OG and IDH1 negative cases, respectively.

Conclusion

Majority of the gliomas expressing IDH1 mutation are associated with improved survival even though this was not statistically proven due to the inadequate number of cases in each category. All OGs had retained ATRX status, and maybe used for confirming an oligodendroglial lineage in centers where advanced techniques like FISH for detecting 1p19q mutation is not available. The IHC status of 3 markers IDH1, ATRX, and p53 versus 2 markers IDH1 and ATRX with WHO grade of gliomas were both found to be statistically significant. Therefore, for routine cases of gliomas, a combination of IDH1 and ATRX is sufficient; however, the use of p53 is recommended for further prognostication and for possible targeted therapy in the future.

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Conflicts of interest
There are no conflicts of interest.

References

1. Schwartzbaum JA, Fisher JL, Aldape KD, Wrensch M. Epidemiology and molecular pathology of glioma. Nat Clin

Pract Neurol 2006;2:494–503.

2. Larjavaara S, Mäntylä R, Salminen T, Haapasalo H, Raitanen J, Jääskeläinen J, *et al.* Incidence of gliomas by anatomic location. Neuro Oncol 2007;9:319–25.

3. Yi FX, Ma J, Ni WM, Chang R, Liu W Da, Han X Bin, *et al.* The top cited articles on glioma stem cells in Web of Science. Neural Regen Res 2013;8:1431–8.

4. Khonglah Y, Shangpliang D, Mishra J, Mustafa A, Kakoti A, Phukan P. Histological spectrum of Central Nervous System lesions at a tertiary care centre in India. Clin Cancer Investig J 2020;9:175-81.

5. Cohen AL, Holmen SL, Colman H. IDH1 and IDH2 mutations in gliomas. Curr Neurol Neurosci Rep 2013;13:345.

6. Cairncross JG, Wang M, Jenkins RB, Shaw EG, Giannini C, Brachman DG, *et al.* Benefit from procarbazine, lomustine, and vincristine in oligodendroglial tumors is associated with mutation of IDH. J Clin Oncol 2014;32:783–90.

7. Jaiswal S. Role of immunohistochemistry in the diagnosis of central nervous system tumors. Neurol India 2016;64:502–12.

8. 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;131:803–20.

9. Cai J, Zhu P, Zhang C, Li Q, Wang Z, Li G, *et al.* Detection of ATRX and IDH1-R132H immunohistochemistry in the progression of 211 paired gliomas. Oncotarget 2016;7:16384–95.

10. Wang YY, Zhang T, Li SW, Qian TY, Fan X, Peng XX, *et al.* Mapping p53 mutations in low-grade glioma: A voxel-based neuroimaging analysis. Am J Neuroradiol 2015;36:70–6.

11. Thambi R, Kandamuthan S, Sainulabdeen S, Vilasiniamma L, Abraham TR, Balakrishnan PK. Histopathological analysis of brain tumours- A seven year study from a tertiary care centre in South India. J Clin Diagnostic Res 2017;11:EC05–8.

12. Krishnatreya M, Kataki AC, Sharma JD, Bhattacharyya M, Nandy P, Hazarika M. Brief descriptive epidemiology of primary malignant brain tumors from North-East India. Asian Pac J Cancer Prev 2014;15:9871–3.

13. Jaiswal J, Shastry AH, Ramesh A, Chickabasaviah YT, Arimappamagan A, Santosh V. Spectrum of primary intracranial tumors at a tertiary care neurological institute: A hospital-based brain tumor registry. Neurol India 2016;64:494–501.

14. Thakur AS, Gahine R, Kulkarni V. A study on morphologic and histological pattern of the central nervous system tumors. Int J Res Med Sci 2018;6:3879-82.

15. Ostrom QT, Kinnersley B, Wrensch MR, Eckel-Passow JE, Armstrong G, Rice T, *et al.* Sex-specific glioma genome-wide association study identifies new risk locus at 3p21.31 in females, and finds sex-differences in risk at 8q24.21. Sci Rep 2018;8:1–15.

16. Mondal S, Pradhan R, Pal S, Biswas B, Banerjee A, Bhattacharyya D. Clinicopathological pattern of brain tumors: A 3-year study in a tertiary care hospital in India. Clin Cancer Investig J 2016;5:437-40.

17. Grier JT, Batchelor T. Low-Grade Gliomas in adults. Oncologist 2006;11:681–93.

18. Rasmussen BK, Hansen S, Laursen RJ, Kosteljanetz M, Schultz H, Nørgård BM, *et al.* Epidemiology of glioma: Clinical characteristics, symptoms, and predictors of glioma patients grade I-IV in the the Danish Neuro-Oncology Registry. J Neurooncol 2017;135:571–9.

19. Agarwal S, Sharma MC, Jha P, Pathak P, Suri V, Sarkar C, *et al.* Comparative study of IDH1 mutations in gliomas by immunohistochemistry and DNA sequencing. Neuro Oncol 2013;15:718–26.

20. 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.

21. Yusoff AA, Zulfakhar FN, Sul'ain MD, Idris ZI, Abdullah JM. Association of the IDH1 C.395G>A (R132H) mutation with histological type in Malay brain tumors. Asian Pac J Cancer Prev 2016;17:5195–201.

22. Das BR, Tangri R, Ahmad F, Roy A, Patole K. Molecular investigation of isocitrate dehydrogenase gene (IDH) mutations in gliomas: First report of IDH2 mutations in Indian patients. Asian Pac J Cancer Prev 2013;14:7261–4.

23. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, *et al.* Mutations in Gliomas. N Engl J Med 2009;360:765–73.

24. Hartmann C, Hentschel B, Wick W, Capper D, Felsberg J, Simon M, *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;120:707–18.

25. Ebrahimi A, Skardelly M, Bonzheim I, Ott I, Mühleisen H, Eckert F, *et al.* ATRX immunostaining predicts IDH and H3F3A status in gliomas. Acta Neuropathol Commun 2016;4:60.

26. Liu J, Zhang X, Yan X, Sun ME, Fan Y, Huang Y. Significance of TERT and ATRX mutations in glioma. Oncol Lett 2019;17:95–102.

27. Kyritsis AP, Bondy ML, Hess KR, Cunningham JE, Zhu D, Amos CJ, *et al.* Prognostic significance of p53 immunoreactivity in patients with glioma. Clin Cancer Res 1995;1:1617–22.

28. Sipayya V, Sharma I, Sharma KC, Singh A. Immunohistochemical expression of IDH1 in gliomas: A tissue microarray-based approach. J Cancer Res Ther 2012;8:598–601.

29. Komori T, Nitta M, Maruyama T, Muragaki Y, Kawamata T. P03.10 Combined immunohistochemistry for IDH1R132H, p53 and ATRX improves the molecular classification of diffuse gliomas in adults. Neuro Oncol 2017;19:iii35.

30. Lv S, Teugels E, Sadones J, Quartier E, Huylebrouck M, DU Four S, *et al.* Correlation between IDH1 gene mutation status and survival of patients treated for recurrent glioma. Anticancer Res 2011;31:4457–63.

31. Jones PS, Carroll KT, Koch M, DiCesare JA, Reitz K, Frosch M, *et al.* Isocitrate dehydrogenase mutations in low-grade gliomas correlate with prolonged overall survival in older patients. Neurosurgery 2019;84:519–28.