Original Article

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Pathology Section

Expression of IDH1, ATRX, p53 in Diagnosis of Gliomas as Per 2016 WHO Classification

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ABSTRACT

Introduction: The 2016 World Health Organisation (WHO) classification of tumours of the Central Nervous System (WHO CNS 2016 classification) is used to classify diffuse gliomas as astrocytoma, Oligodendroglioma (ODG), glioblastoma which are three prognostically distinct groups based on Isocitrate Dehydrogenase (IDH1), alpha thalassaemia/mental retardation, x-linked (ATRX) mutations, p53 and 1p/19q co-deletion status. Although WHO CNS 2022 classification has been brought in use, it is based on molecular studies. In a resource limited setting like in many Indian diagnostic centres it's difficult to apply the WHO CNS 2022 classification. It is felt that WHO CNS 2016 classification has not lost its utility.

Aim: To investigate the Immunohistochemistry (IHC) status of IDH1, ATRX, p53 in diagnosis of diffuse glial tumours and to classify them according to WHO 2016.

Materials and Methods: The present cross-sectional study was conducted at Department of Pathology, Bharati Vidyapeeth Medical College, Hospital and Research Centre, Pune, Maharashtra, India for two years and six months (July 2020 to December 2022). Thirty-two diffuse glioma cases and IHC markers IDH1, ATRX, p53 were evaluated. Ki-67 index was additionally done.

Results: Total 32 cases were studied, 19 cases were male. Mean age of the patients was 40.13 years. Fourteen patients belonged to WHO Grade-II, six to Grade-III, and 12 to Grade-IV. As per the IHC findings and histopathological features, there were 16 (50%) patients with diffuse astrocytoma, while 12 (37.5%) and 4 (12.5%) patients were diagnosed as glioblastoma and ODG, respectively. Reclassification of these cases was done depending on IHC results where IDH1 was positive in 71.9% cases, ATRX was positive in 40.6% cases and p53 was positive in 15.6% cases. This result includes all the cases where these IHC markers showed reactivity. The diagnosis of four patients was modified based on findings of IHC markers.

Conclusion: The study demonstrates subgrouping of gliomas based on IDH1, ATRX, p53. There was no significant association between grade of tumour and Ki-67 expression.

Keywords: Astrocytoma, Isocitrate dehydrogenase, Oligodendroglioma, World health organisation

INTRODUCTION

The annual incidence of primary intracranial tumours is 5 to 10 cases per 100,000 people in India, where it represents 2% of all malignancies [1,2]. Gliomas are the most common and are responsible for nearly 30% deaths due to primary brain tumours [3]. The 2007 WHO classification system puts both diffuse and well-circumscribed gliomas in the same category [4]. However, the 2016 update to the 2007 classification categorises them into well-circumscribed tumours as pilocytic astrocytoma, Pleomorphic Xanthocytoma (PXA), Subependymal Glial Astrocytoma (SEGA) and diffuse tumours as astrocytoma, ODG, and glioblastoma [5]. This updated classification system allows more accurate diagnosis, predicts patient prognosis and better management [6].

The IHC staining of tissue sections can be used to evaluate the expression of mutant proteins such as IDH1, ATRX and p53, which can subtype gliomas. IDH1 mutation, ATRX mutation (loss) and p53 mutation is common in mutant type astrocytoma. IDH1 wild type, ATRX mutation (loss) and p53 mutation is common in wild type glioblastoma. IDH1 mutation, ATRX retained and p53 variable expression is common ODG [7,8]. A 1p19q co-deletion status using Fluorescence In-situ Hybridisation (FISH) is additionally done to confirm ODG. Therefore, the present study was planned to investigate the IHC status of diffuse glial tumours and to utilise the 2016 WHO classification of tumours of the CNS to classify them.

MATERIALS AND METHODS

The present cross-sectional study was conducted in the Department of Pathology at Bharati Vidyapeeth, Deemed to be University, Medical College, Pune, Maharashtra, India after approval by the Institutional Ethics Committee (IEC) (BVDUMC/IEC/141). The period of the study was two years and six months (July 2020 to December 2022).

Inclusion criteria: All cases of diffuse glioma were included in the study.

Exclusion criteria: Inadequate and non representative samples were excluded from the study.

Study Procedure

Data for age, sex and location of tumour was collected from histopathology registers and test requisition forms. All retrospective cases were reviewed to confirm diagnosis of diffuse glioma. For the prospective study, specimens were collected in 10% buffered formalin and processed using routine histotechnique and stained with Haematoxylin and Eosin (H&E) for histopathological diagnosis. IHC was done using IDH1 (IDH1 R132H point mutated form, H09, RTU master diagnostic), ATRX (Flex mono, MAD-0007826D-R-3, polyclonal master diagnostica), p53 (MAD-000479QD, polyclonal master diagnostica), Ki-67 (clone SP6, MAD-000310QD, master diagnostica). All four IHC markers were studied in all cases. Preparation of the tissue, antigen retrieval, inhibition of non-specific binding sites, incubation with primary and secondary antibodies, and detection of the labelled antibodies are the fundamental steps in IHC staining.

Using antibodies that recognise these proteins, IHC markers were evaluated by examining the presence and distribution of particular proteins in tissue samples. These antibodies are frequently marked with a chromogen or a fluorescent chemical, enabling microscopic observation of the target protein. Grading of CNS tumours was done according to WHO 2016 classification [4].

STATISTICAL ANALYSIS

The data was analysed using Statistical Package for Social Sciences (SPSS) version 26.0 software. For qualitative data various rates, ratios, and percentages (%) were calculated. Chi-square test/ Fisher's-exact test was used to find the association between two or more attributes for qualitative data variables. The p-value <0.05 was considered as significant.

RESULTS

A total of 32 cases with diffuse gliomas were studied. The mean age of the patients was 40.13 years with age range from 7-70 years and male to female ratio of 1.46:1. Maximum tumours were located in the frontal lobe (37.5%) followed by temporal lobe (12.5%), parietal lobe (9.4%), Insular region (9.4 %), parasagittal region (9.4%), thalamus (9.2%), Cerebro-pontine (CP) angle angle (6.3%), brain stem (6.3%), cases were diagnosed as diffuse astrocytoma (50%), 12 as glioblastoma (37.5%) and 4 as ODG (12.5%). Fourteen patients belonged to WHO Grade-II (43.8%) and 6 (18.7%) patients belonged to WHO Grade-III while there were 12 patients who belonged to WHO Grade-IV (37.5%). The IHC expression of IDH1, ATRX and p53 was positive in 23 (71.9%), 13 (40.6%) and 5 (15.6%) of patients, respectively. The distribution of patients according to IHC findings is shown in [Table/Fig-1].

Antibody	Results	N (%)
IDH1	Positive (mutant)	23 (71.9)
	Negative	9 (28.1)
ATRX	Positive	13 (40.6)
	Negative (loss/mutant)	19 (59.4)
p53	Positive	5 (15.6)
	Negative	27 (84.4)
Ki-67	<5	11 (34.4)
	6-10	15 (46.8)
	11-20	3 (9.4)
	>21	3 (9.4)

[Table/Fig-1]: Distribution of cases according to IHC findings.

IHC findings related to IDH1 mutation status of diffuse glioma cases: The IHC findings revealed that IDH1 was mutant in 23 (71.9%) patients, while in 9 (28.1%) was wild type. Ideally, genetic testing should be done before labelling cases as IDH wild type. But when patient age is more than 54 years and there is no previous history of glioma, absence of IDH1 mutation can be treated as IDH1 wild. Hence, three cases could be labelled as glioblastoma wild type. Rest 6 (18.8%) patients, the IDH1 was labelled as Not Otherwise Specified (NOS).

The association between diagnosis and expression of IDH1, ATRX, p53 and Ki-67: [Table/Fig-2] shows the IHC diagnosis results and p-values for diffuse gliomas. The results are based on the analysis of three antibodies-IDH1, ATRX, p53. The p-values indicate the statistical significance of the differences between the groups. For IDH1, the positive result was seen in a higher percentage of diffuse astrocytoma cases (87.5%) compared to glioblastoma (41.7%). The difference in IDH1 positivity between diffuse astrocytoma and glioblastoma was statistically significant (p-value=0.012). ATRX loss was seen in a higher percentage of diffuse astrocytoma cases (87.5%) compared to glioblastoma (33.3%) and ODG (25%) cases. The difference in ATRX negativity between diffuse astrocytoma and glioblastoma was statistically significant (p-value=0.005). p53 was negative in all cases of diffuse astrocytoma (100%), in a higher percentage of glioblastoma cases (75%) compared to ODG cases (50%). The difference in p53 negativity between diffuse astrocytoma and glioblastoma was statistically significant (p-value=0.025). Ki-67 was between 5-10% in most gliomas irrespective of their grades.

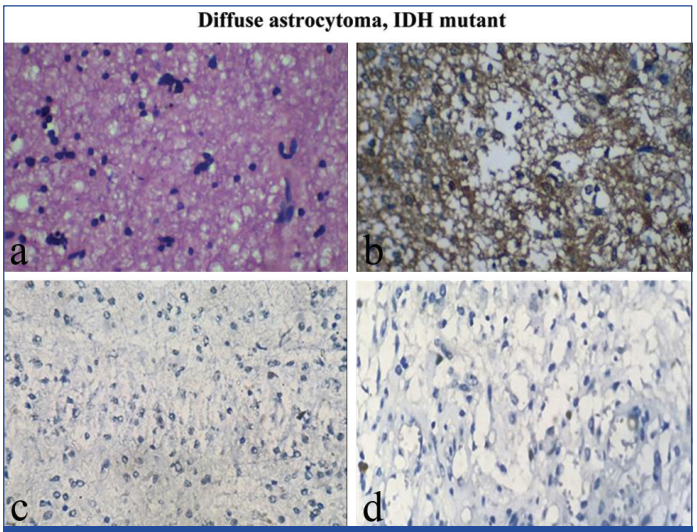
Antibody	Result	IHC diagnosis						p-values
		Diffuse astrocytoma, n %		Glioblastoma, n %		Oligodendroglioma (ODG), n %		
IDH1	Negative	2	12.5	7	58.3	0	0	0.012*
	Positive	14	87.5	5	41.7	4	100.0	
ATRX	Negative	14	87.5	4	33.3	1	25.0	0.005*
	Positive	2	12.5	8	66.7	3	75.0	
TP53	Negative	16	100.0	9	75.0	2	50.0	0.025*
	Positive	0	0	3	25.0	2	50.0	
Ki-67	<5	8	50.0	3	25.0	0	0	0.084
	6-10	6	37.5	6	50.0	3	75.0	
	11-20	2	12.5	0	0	1	25.0	
	>21	0	0	3	25.0	0	0	

[Table/Fig-2]: The association between diagnosis and expression of IDH1, ATRX, p53 and Ki-67.

Among 16 cases of astrocytoma, diagnosis of two cases was modified to ODG because IDH1 and ATRX expression were positive in both cases. Among four cases of ODG, diagnosis of two cases was modified to astrocytoma because IDH1 mutation and ATRX mutation was present in both cases [Table/Fig-3-5]. Histopathological and IHC diagnosis was similar in all 12 cases of glioblastoma [Table/Fig-3,6].

Total cases (32)	Histopathology diagnosis	IHC diagnosis
14	Astrocytoma	Astrocytoma
12	Glioblastoma	Glioblastoma
2	Astrocytoma	Oligodendroglioma
2	Oligodendroglioma	Oligodendroglioma
2	Oligodendroglioma	Astrocytoma

[Table/Fig-3]: Concordance/discordance after comparing H&E and IHC diagnosis.

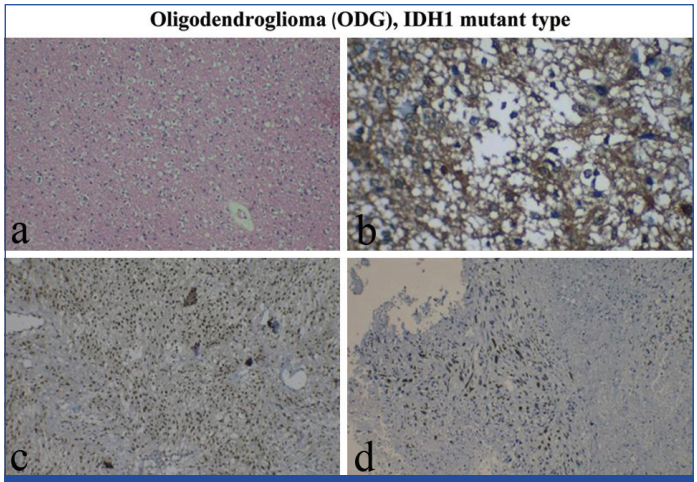


[Table/Fig-4]: a) Diffuse astrocytoma (H&E, 400x); b) IDH1 staining positive, (IHC 400x); c) ATRX staining loss (IHC 400x); d) p53 staining complete loss (IHC 400x).

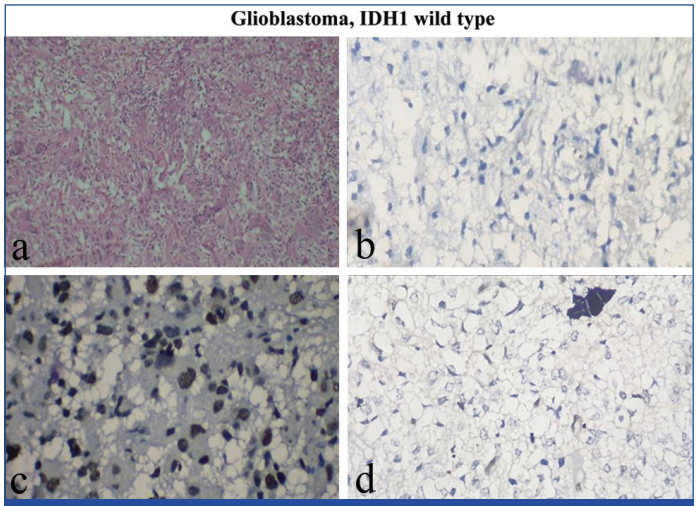
DISCUSSION

The understanding of molecular changes in CNS tumours has significantly grown over the past 10 years. Due to these efforts, the classification of CNS tumours has undergone a fundamental change. Now, the definition of several diagnostic categories may be partially based on genotype, and in some cases, genotype may even take precedence over a histological diagnosis.

A total of 32 patients with diffuse gliomas were included in the present study. The mean age of the patients was 40.13 years. There were total 19 (59.4%) male patients and 13 (40.6%) females patients, with male to female ration of 1.46:1. Sarma S et al., included



[Table/Fig-5]: a) Oligodendroglioma (ODG) (H&E, 400x); b) IDH1 staining positive, (IHC 400x); c) ATRX staining positive, (IHC 400x); d) p53 variable expression (IHC 400x).



[Table/Fig-6]: a) Glioblastoma, (H&E, 400x); b) IDH1 staining negative (IHC 400x); c) ATRX staining negative, (IHC 400x); d) p53 negative (IHC 400x).

53 glioma patients, 30 (56.60%) of whom were male and 23 (43.39%) were female with a mean age of 32 years [9]. According to Arshad H et al., of the 50 glial cases evaluated, 35 (70%) cases were male, while 15 (30%) were female with mean age of 35 years [10].

Maximum tumours (12 cases) were located in the frontal lobe (37.5%). Sarma S et al., Wang Z et al., also found frontal lobe to be the commonest site [9,11]. In the present study, most common histological type was diffuse astrocytoma 16 (50%) followed by glioblastoma 12 (37.5%) and the least common type was ODG in 4 (12.5%) of patients. In the study by Wang Z et al., and Popova SN et al., showed 58% and 51% astrocytoma cases [11,12]. In the present study, 14 (43.8%), 12 (37.5%) and 6 (18.7%) patients belonged to WHO Grade-II, III and IV, respectively. In the study by Popova SN et al., 76 (42.22%), 32 (17.77%), 72 (40%) patients belonged to WHO Grade-II, III and IV, respectively [12].

The IHC expression of IDH1, ATRX, and p53 was positive in 23 (71.9%), 13 (40.6%), and 5 (15.6%) of patients, respectively. As per these IHC findings, there were 16 (50%) patients with diffuse astrocytoma, while 12 (37.5%) and 4 (12.5%) patients were diagnosed as glioblastoma and ODG, respectively. Present study results showed 14 cases (87.5%) with diffuse astrocytoma had IDH1 mutation and ATRX mutation (loss). Remaining two cases of astrocytoma IDH wild type were put in NOS category because IDH status could not be genetically assessed. In the studies by Sarma S et al., Wang Z et al., and Larjavaara S et al., IDH1 mutation was seen in 69.69%, 32.56% and 87% of cases, respectively [9,11,13]. Present study results showed IDH1 mutation, IDH wild and ATRX loss in 41.7%, 58.3% and 33.3% of glioblastoma. The percentages of IDH mutation and ATRX mutation in other studies were different, possibly due to the small number of glioblastoma cases in the present study. The study suggests that genetic testing should ideally be performed before labelling cases as IDH wild type.

The present study found that 100% of the ODG cases had IDH1 mutation (positive) and 75% showed ATRX retention. In the study by Sarma S et al., Broggi G et al., and Dahuja G et al., [9,14,15]. IDH1 mutation and ATRX retention was seen in 83.33% and 100%, 100% and 100%, 80% and 100% of ODG cases. Ideally cases with ATRX loss in ODG morphology should undergo 1p19q co-deletion by FISH, which was not done in the present study.

All 16 cases of diffuse astrocytoma showed p53 mutation. In glioblastoma, out of total 12 patients, 75% cases showed p53 mutation. Among four cases of ODG, 50% cases show p53 mutation. No significant association was found between tumour grade and Ki-67 expression. IDH1 mutation, ATRX mutation (loss) and p53 mutation is common in mutant type astrocytoma. IDH1 wild type, ATRX mutation (loss) and p53 mutation is common in wild type glioblastoma. IDH1 mutation, ATRX retained and p53 variable expression is common ODG.

Modification of diagnosis after IHC study: As per the findings of IHC in present study, the diagnosis of four patients was modified. All four cases showed IDH1 mutation. The diagnosis of diffuse astrocytoma was modified to ODG, IDH mutant, Grade-II, NOS in two patients. This was done because IHC expression of ATRX was positive (retained). As genetic study (1p,19q co-deletion) was not done in ODG cases they were classified as ODG, NOS type. Diagnosis of ODG Grade-II was modified to diffuse astrocytoma, IDH mutant, Grade-II because IHC expression of ATRX was negative (loss).

Limitation(s)

Genetic testing was not performed before labelling cases as IDH wild type. FISH for 1p19q co-deletion was not done for cases with ATRX loss in ODG morphology.

CONCLUSION(S)

The study demonstrated subgrouping in diffuse gliomas based on IDH1, ATRX, p53. However, 1p19q co-deletion study using FISH is necessary to confirm diagnosis of ODG. Glioblastoma generally have high Ki-67 index as compared to astrocytoma and ODG. Ki-67 do not have a role in reclassification of CNS tumour.

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