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UNRAVELLING GLIOMA COMPLEXITY: THE UTILITY OF IMMUNOHISTOCHEMICAL MARKERS

Histopathology

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ABSTRACT

Introduction: The refinement of molecular criteria in the 2021 5th edition of the WHO classification of central nervous system tumors has improved diagnosis, prognostication, and eventually treatment of Diffuse Gliomas. This study assesses IDH mutation, ATRX loss, p53 overexpression, and Ki67 expression in adult diffuse gliomas, correlating them with clinicopathological features. **Methods:** All histopathologically diagnosed cases of diffuse gliomas, received over a duration of 18 months from February 2023 to July 2024, were assessed for immunohistochemical expression of IDH1, ATRX, p53 and Ki67. 6 monthly follow-ups were done about prognosis for a duration of 18 months. **Results:** In the study of 35 diffuse glioma cases, mean age was 53.4 years with a male-to-female ratio of 1.69:1. Glioblastoma was the most common histological subtype in the study, comprising 40% of the cases. IDH1 positivity in a total of 22 cases, mostly Oligodendroglioma and Astrocytoma. A significant correlation was seen between the diffuse glioma cases with older age, Male gender, positive IDH1 expression, Ki67 LI>5%, and ATRX loss. A total of 11 cases were lost to death during follow-up. Significant association was found between WHO grade, p53 expression, and Ki67 LI with mortality. Survival differed significantly based on WHO grade and IDH status. **Conclusion:** The findings of our study suggest that IHC may serve as a potential tool to assess the genetic profile of diffuse glioma cases in a resource-constrained tertiary healthcare center. IHC may serve as a reliable and cost-effective method to evaluate these mutations.

KEYWORDS

INTRODUCTION

CNS tumours make up about 1-2% of all cancerous growths[1]. While not very common, the morbidity and mortality caused by this condition greatly impact a significant number of young to middle-aged individuals, resulting in a high number of years of life lost compared to other types of cancer. In adults, diffuse gliomas are the most prevalent tumours that originate in the central nervous system (CNS)[2]. Before the World Health Organization (WHO) revised the 4th edition of the classification of CNS tumours in 2016, the main method for diagnosing diffuse gliomas relied on examining the histopathological characteristics. The World Health Organization (WHO) has included mutations in isocitrate dehydrogenase (IDH) and the co-deletion of 1p19q, along with certain histopathological characteristics, in their updated classification of central nervous system tumours[3]. This classification includes Grade 2 and 3 astrocytomas, oligodendroglioma, and Grade 4 glioblastoma (GBMs) in the category of diffuse glioma. Among various molecular factors, the main markers used to categorize diffuse gliomas are IDH mutation, 1p19q co-deletion, and mutation in the alpha thalassemia mental retardation syndrome X-linked (ATRX) gene, along with other molecular markers such as p53 and Ki-67[4,5].

MATERIAL AND METHODS

This 18-month prospective study (February 2023–July 2024) conducted at a tertiary pathology department in India included all histopathologically diagnosed diffuse glioma cases. Detailed patient demographics, clinical presentations, radiological findings, pathological parameters, treatment protocols, and survival follow-up records were meticulously compiled. Biopsy specimens were formalin-fixed, fully embedded, routinely processed, and sectioned at 4–6 µm for standard Hematoxylin and Eosin staining. For molecular profiling, 4 µm serial sections were evaluated via immunohistochemistry for IDH1 mutation, ATRX expression, p53 mutation, and the Ki67 labeling index, which quantified the percentage of positive tumor nuclei. Statistical analysis was performed using IBM SPSS Version 20, utilizing Chi-square tests to determine significance and Kaplan-Meier curves to map patient survival trends. Table 1 summarizes various antibodies used for IHC and their interpretation. Appropriate internal and external controls were used for validation.

Table 1: Various Antibodies used for IHC and their Interpretation

Marker	Source	Interpretation	Internal/Batch controls
IDH-1	Anti-IDH1 R132H (Rabbit monoclonal, BioGenex)	Mutant: >10% tumor cells with strong cytoplasmic positivity	Normal glial and vascular endothelial cells (Negative control)
ATRX	Anti-ATRX (Mouse monoclonal, PathnSitu)	Mutant (Loss): <10% nuclear staining Wildtype (Retained): >10% nuclear staining	Endothelia, microglia, lymphocytes, and astrocytes (Positive control)
P53	p53 protein (Mouse monoclonal, PathnSitu)	Mutant: Intense nuclear staining in >10% of tumor cells	Parallel positive and negative controls
Ki67	MIB1 (Mouse monoclonal, PathnSitu)	Positive: ≥5% tumor cells with nuclear staining Negative: <5% tumor cells	Endothelial cells, Lymphocytes, and reactive astrocytes (Negative control)

RESULTS

The study evaluated 35 cases of diffuse glioma (February 2023–July 2024) with a mean age of 53.4 years (range: 8–78 years), predominantly affecting individuals in the 46–75 age group (68.57%). A male predominance observed(62.86%; M:F ratio 1.69:1). Tumors mostly occurred in the frontal (28.57%) and temporal (20%) lobes, with no significant differences in laterality.

Glioblastoma was the most common subtype (40%), followed by oligodendroglioma (34.29%), astrocytoma (20%), and pilocytic astrocytoma (5.71%). Grade 4 lesions predominated (42.86%; 14 glioblastomas, 1 astrocytoma), followed by Grade 2 (31.43%), Grade 3 (20%; 4 astrocytomas, 3 oligodendrogliomas), and Grade 1 (5.71% pilocytic astrocytomas). Oligodendrogliomas, astrocytomas, and glioblastomas predominantly presented as Grade 2, 3, and 4, respectively.

IDH1 positivity was seen in a total of 22 cases, the majority of which were Oligodendroglioma (11 cases) and Astrocytoma (9 cases). Of 7 cases of Astrocytoma and 2 cases of pilocytic astrocytoma, loss of ATRX expression was seen in 6 and 2 cases respectively. Majority of glioblastoma cases were IDH1 negative with Ki 67 labelling Index (LI) of >5%. IDH1 positive cases of Glioblastoma were categorized as Grade 3 Astrocytoma. Table no.2 summarizes distribution of

histological types of Diffuse glioma based on IDH1, ATRX, p53, and Ki67 expression.

Table 2: Distribution of Histological Types of Diffuse Glioma Based on IDH1, ATRX, p53, and Ki67 Expression.

Histologic type	No. of cases	IDH1		ATRX		P53		Ki6 7 LI> 5%
		Positive	Negative	Retained	Loss	Positive	Negative	
ODG	12	11	1	12	0	3	9	6
PA	2	1	1	0	2	1	1	0
A	7	7	0	1	6	6	1	5
GBM	14	3	11	4	10	7	7	13
Total	35	22	13	17	18	17	18	26

A significant correlation was seen between the diffuse glioma cases with older age, Male gender, positive IDH1 expression, KI67 LI>5%, and ATRX loss. A significant correlation was seen between WHO grade, IDH expression, and Ki67 expression in the present study. Table 3 and 4 summarizes them respectively.

Table 3: Distribution of Study Subjects Based on WHO Grade and IDH1 Expression.

IDH1	WHO Grade >2	WHO Grade< 2	P value
Positive	11	11	0.040572
Negative	11	2	

Table 4: Distribution of Study Subjects Based on WHO Grade and Ki67 LI

Ki67%	WHO Grade 1	WHO Grade 2	WHO Grade 3	WHO Grade 4	P value
Ki67 LI<5%	2	7	1	1	0.001921
Ki67 LI>5%	0	4	6	14	

The study subjects were followed-up at every 6 months, for a period of 18 months. Total 11 study subjects were lost to death during follow up.

Significant association was found between WHO grade, p53 expression, and Ki67 LI with mortality, as shown in table no.5.

When Kaplan Meier Survival curves were plotted and the Log-rank test performed, a significant difference in survival rates was seen between: Low-grade and high-grade tumour with a p-value of 0.027072 (Chart no.1), and in cases with Positive IDH1 mutation and Negative IDH1 mutation with a p-value of 0.022692 (Chart no.2). No significant difference in survival rates of the cases based on Ki67 expression as p value was 0.661594.

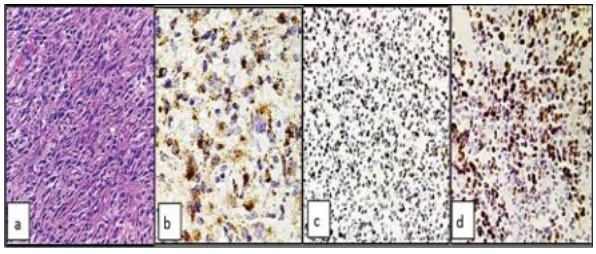

Figure1: (a) Astrocytoma; WHO Grade 3 (H&E; 400x), (b) Positive IDH1 Expression in Astrocytoma; WHO Grade 3 (IHC; 400x), (c) Positive p53 Expression in Astrocytoma; WHO Grade 3 (IHC; 400x), (d) High Ki67 Expression in Astrocytoma; WHO Grade 3(IHC; 400x)

Table no. 5: Distribution of Study Subjects Who Survived During Follow up, Based on Grade, IDH1, ATRX, p53 and Ki67 Expression

Parameters	No. of cases died	No. of cases survived	P value
Grade >2	10	12	0.0200576
Grade <2	1	12	
IDH1+	6	16	0.4908434
IDH1-	5	8	
ATRX +	5	11	0.9833388
ATRX -	6	13	
p53 +	8	9	0.0528955
p53 -	3	15	
Ki 67>30%	9	0	0.0000002
Ki 67<30%	2	24	

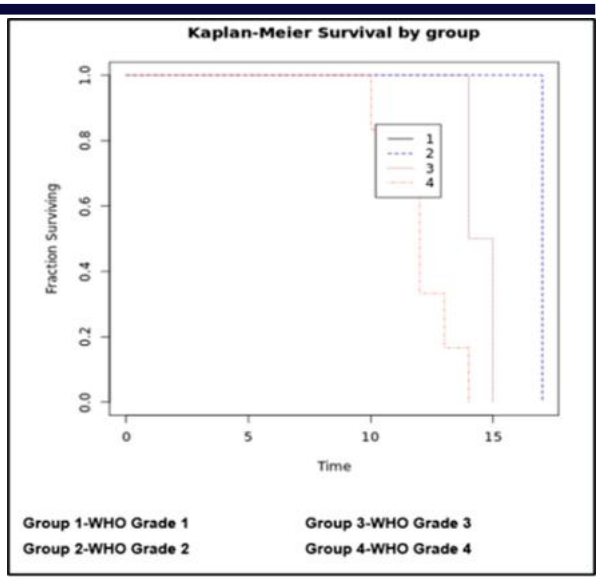

Chart no 1: Kaplan-Meier Survival Analysis Curve by WHO Grade

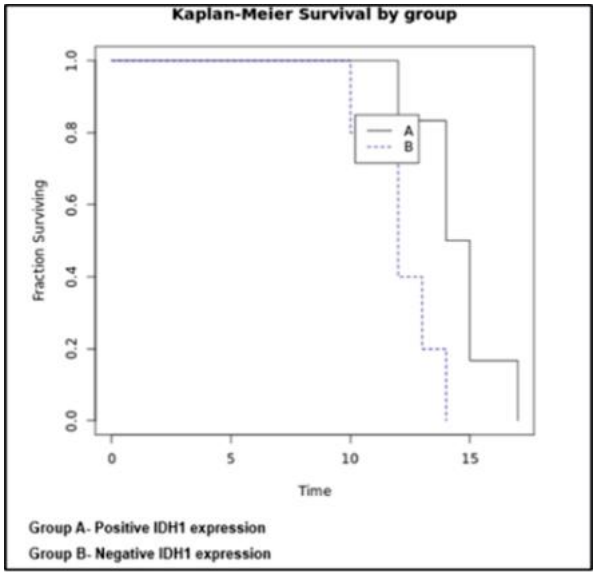

Chart no.2: Kaplan-Meier Survival Analysis Curve by IDH1 Expression

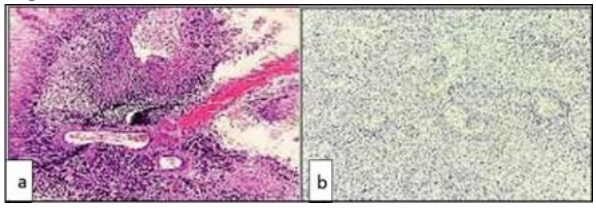

Figure 2: (a) Glioblastoma; WHO Grade 4 (H&E; 100x), (b) Negative IDH1 Expression in Glioblastoma; WHO Grade 4 (IHC; 100x)

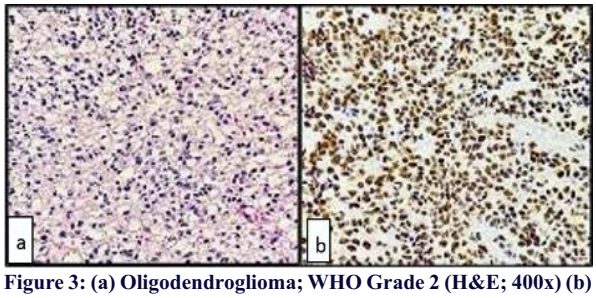

Figure 3: (a) Oligodendroglioma; WHO Grade 2 (H&E; 400x) (b) Retained Expression of ATRX in Oligodendroglioma; WHO Grade 2 (IHC; 400x)

DISCUSSION

Among the gliomas, glioblastoma was the commonest histological type accounting for 40% and the least common was pilocytic astrocytoma comprising 5.71% of all gliomas. The frequency of occurrence was comparable to the study by Dahuja et al[6] and Jain, S et al[7] where the commonest glioma was glioblastoma accounting for 53.3% and 39.13% respectively. An analysis was done to assess the distribution of gliomas as per the WHO grade. In our study, Grade 4 cases(42.86%) were in the majority followed by Grade 2 (31.43%). This was compared to the study of Sarma, S et al[8], Jain, S et al[7]and Dahuja, G. et al[6]. In all these studies, the most common grade was Grade 4, followed by Grade 2, Grade 3, and Grade 1 respectively. Study conducted by Rasmussen, B.K et al[9] and Anand, N et al[10] showed more cases of Grade 3. A total of 12 cases belonged to Oligodendroglioma, of which 11 cases (91.6%) showed a positive IDH1 expression on Immunohistochemistry and only a single case was IDH1 negative. None of the cases showed a loss of ATRX expression. These results were comparable to Dahuja, G et al[6], Sarma, S et al[8]. Also, the majority of oligodendroglioma cases showed negative p53 expression. Sonoda et al. developed a practical procedure in which immunohistochemistry was used as the next step after a simple, but not recommended, diagnosis by pathology alone, and ATRX and p53 IHC could be used as a surrogate for 1p/19q. Both cases of Pilocytic astrocytoma showed loss of ATRX expression. This result was not in concordance with studies conducted by Sarma, S. et al[8] and Dahuja, G. et al[6]. All 7 cases of Astrocytoma were IDH1 positive and 6 of 7 Astrocytoma cases (85.71%) showed loss of ATRX expression and positive p53. All the cases of glioblastoma were categorised as Grade 4. Only 3 cases (21.43%) are IDH1 positive on IHC (IDH mutant) while the remaining 11 cases are IDH1 negative (78.57%). This is comparable to a study conducted by Anand, N et al[10] in which 94.9% of grade 4 tumours were IDH1-negative. Similar results were obtained by Jain, S. et al[7] in which 27 (84.37%) cases of GBM were IDH1-negative. ATRX loss was seen in the majority of glioblastoma cases in our study (71.42%). This was not comparable to the results of Jain, S. et al[7]where 31 (96.9%) cases of GBM are ATRX positive; i.e. showed ATRX expression.

Of 15 Glioblastoma patients, 6 expired in 18 months of follow-up. Out of 6 patients, 5 were IDH1-negative and these results were suggestive of better survival among IDH1-positive patients. These results were consistent with other studies conducted by Dahuja, G.et al[6].

Out of 11 cases that died during follow-up, 6 cases were IDH1 positive, and 5 cases were IDH1 negative. Kaplan Meier survival analysis was plotted to compare the survival of cases based on IDH mutation and further log-rank test was performed and the p-value was 0.022692[Median overall survival=14 months for IDH positive mutation vs 12 months for IDH negative mutation respectively]. As the p-value is less than 0.05, it indicates that there is a significant difference in survival rates of the glioma cases based on IDH expression. This was comparable to a study conducted by Takano, S. et al[11].

All Grade 1 patients were alive with no reoccurrence during 18 follow-up months. Among Grade 2 out of 11 patients, 1 expired. Among the living patients, there was no case of reoccurrence. In Grade 3 patients out of 7, 4 patients expired. Of 15 Grade 4 patients, 6 expired during 18 months of follow-up. This was comparable with Dahuja, G et al[6]. Among Grade 2 and Grade 3 tumors, Grade 2 tumors had better prognosis in Dahuja, G et al study, consolidating the importance of grading on the basis of histo-morphological features.

CONCLUSION

The results of the present study indicate that IHC panel of IDH1, ATRX, p53, and Ki67 is useful for the molecular classification of diffuse gliomas, which could be useful for evaluating prognosis.

Although the immunohistochemical approach does not replace genetic testing completely, it is a practical and powerful means of assessing molecular genetic changes.

IDH mutations are the established markers of better prognosis in diffuse gliomas.The findings of our study suggest that IHC may serve as a potential tool to assess the genetic profile of diffuse glioma cases in a resource-constrained tertiary healthcare center.

Limitations

Our study is limited by its small sample size, unavailability of higher molecular methods like FISH to check for 1p/19q co-deletion in Oligodendroglioma cases, and of DNA sequencing to confirm IDH1 mutation in IDH1-negative Oligodendroglioma and Astrocytoma cases.

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