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ORIGINAL Article

Clinicopathological Features and Outcomes of H3K27-Altered Diffuse Midline Gliomas: An Immunohistochemistry-Based Study from a Tertiary Cancer Centre in Western India

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ABSTRACT

Diffuse midline gliomas (DMGs) arise in central brain structures. The 2021 World Health Organization (WHO) classification redefined "H3K27-mutant DMG" (DMG-H3K27M-mutant) as "H3K27-altered DMG" (DMG-H3K27Alt), encompassing four epigenetically distinct subtypes. This study aimed to identify DMG-H3K27Alt cases in midline locations using H3K27M and H3K27me3 immunohistochemistry (IHC), evaluate the clinicopathological profile, compare them with their H3K27 wild counterparts, and assess their prognostic outcomes.

The study included all midline-located diffuse gliomas (WHO grades 2–4) diagnosed between January 2020 and June 2023, in our institution. Using IHC for H3K27M and H3K27me3, cases were classified as H3K27-altered or wild-type (DMG-H3K27Wt). Statistical analysis was performed using SPSS version 29.0.

Of the 72 cases, 37 (51.4%) were DMG-H3K27Alt, age range 4 to 60 years (mean:28.8years) with a male-to-female ratio of 2.08:1. The thalamus was the most frequent site overall. High-grade morphology was significantly more common in DMG-H3K27Alt ($p = 0.008$), whereas low-grade gliomas were more frequent in DMG-H3K27Wt ($p < 0.001$). Brisk mitosis, necrosis, and microvascular proliferation were significantly associated with H3K27-altered tumours ($p = 0.014$, $p < 0.001$, and $p = 0.017$, respectively). H3K27M positivity was observed in 89.2% of DMG-H3K27Alt cases, while 10.8% were H3K27M-negative and showed isolated H3K27me3 loss. Infratentorial DMG-H3K27Alt were high-grade, occurred in younger patients, and retained ATRX expression. Mean survival was shorter in DMG-H3K27Alt group ($p = 0.009$), although not statistically significant by log-rank test ($p = 0.15$).

Compared with previous reports, our paediatric DMG-H3K27Alt cohort was slightly older than the corresponding wild-type cohort, and low-grade morphology predominantly involved the thalamus rather than the brainstem, highlighting the clinicopathological heterogeneity of DMG-H3K27Alt. This study highlights the utility of combined H3K27M and H3K27me3 immunohistochemistry for identifying DMG-H3K27Alt in routine practice, particularly in resource-limited settings where comprehensive molecular testing is unavailable.

Key words

Brain neoplasms, Diffuse midline glioma, Immunohistochemistry, H3K27 Mutation, Central Nervous System Neoplasms.

Abbreviations

Diffuse midline gliomas (DMG), Diffuse midline glioma, H3K27M-mutant/ H3K27-mutant DMG (DMG-H3K27M-mutant), Diffuse midline glioma, H3K27-altered/ H3K27-altered DMG (DMG-H3K27Alt), Immunohistochemistry (IHC), H3K27-wildtype diffuse midline gliomas (DMG-H3K27Wt), Diffuse intrinsic pontine glioma (DIPG), World Health Organization (WHO), Formalin-fixed paraffin-embedded (FFPE), Haematoxylin and eosin (H&E), Space-occupying lesion (SOL).

INTRODUCTION

Diffuse midline gliomas (DMG) arise in the central locations within the central nervous system, comprising the hypothalamus, thalamus, pineal region, third ventricle, cerebellum, brainstem (midbrain, pons, and medulla), and spinal cord[1]. This entity was previously referred to in the paediatric population as diffuse intrinsic pontine glioma (DIPG)[2]. Genetic analysis has revealed that approximately 80% of DIPG cases carry a mutation in the H3K27 gene, which is linked to a less favourable prognosis. This breakthrough finding led to the incorporation of a new entity in the 2016 World Health Organization (WHO) classification of CNS tumours, namely, diffuse midline glioma, H3K27M-mutant (DMG-H3K27M-mutant)[1,3]. These tumours were considered WHO grade 4 regardless of histologic features, and the term "DIPG " was replaced by the broader term, “DMG” to reflect their occurrence across other midline locations beyond the pons[4,5].

The 2021 WHO classification of CNS tumours, further redefined this entity as diffuse midline glioma, H3K27-altered (DMG-H3K27Alt) to acknowledge the broader range of epigenetic alterations affecting histone modification pathways. Based on integrated molecular and clinical data, four epigenetically distinct subtypes have been identified that includes: (a) H3.3 p. K28M (K27M)-mutant, (b) H3.1 or H3.2 p. K28M (K27M)-mutant, (c) H3-wildtype with EZHIP overexpression, and (d) EGFR-mutant gliomas. Loss of H3K27me3 expression is a unifying feature across these subtypes[6].

Molecular testing is considered the gold standard for the diagnosis of DMG-H3K27Alt. However, in resource-limited settings, IHC serves as a pragmatic and economical surrogate. A combination of H3K27M immunopositivity and/or loss of H3K27me3 expression helps to identify these tumours[7]. The present study aims to identify DMG-H3K27Alt cases using IHC in radiologically confirmed midline gliomas, evaluate their clinicopathological features, compare them with H3K27-wildtype diffuse midline gliomas (DMG-H3K27Wt), and assess their prognostic outcomes.

MATERIALS AND METHODS

The present study was a prospective study conducted in the Department of Oncopathology of our institution over a period of three and a half years, from January 2020 to June 2023. All midline-located diffuse gliomas (WHO grades 2 to 4), in both paediatric and adult patients, were included. All cases diagnosed as grade 1 astrocytic tumours, ependymal tumours, or gliomas located outside the midline were excluded. Clinical history and radiological findings were retrieved from electronic medical records and case files. IHC was used as a surrogate method for evaluating molecular alterations.

Histopathological examination was performed on formalin-fixed, paraffin-embedded (FFPE) tissue sections stained with haematoxylin and eosin (H&E). Slides were examined under a light microscope.

Immunohistochemistry

IHC was performed on 3 μm FFPE sections using the autostainer. The monoclonal antibodies used included IDH1 R132H (Dilution 1:50, Clone H09), ATRX (Dilution 1:100, Clone CL0537), P53 (Dilution 1:50, Clone D07), Ki67 (Dilution 1:50, Clone MIB1), H3K27M (Dilution 1:50, Clone RM192), and H3K27me3 (Dilution 1:50, Clone MD48R)

Criteria for immunopositivity of IHC markers were as follows:

- IDH1 R132H: Positive for mutation when tumour cells showed robust cytoplasmic and weak nuclear staining.
- ATRX: Loss of nuclear expression in >90% of tumour cells, with retained staining in endothelial and inflammatory cells, indicated ATRX mutation.
- p53: Positive if ≥10% of tumour nuclei showed intense staining.
- H3K27M: Positive if >80% of tumour nuclei stained, with vascular endothelial cells serving as an internal negative control[8] .
- H3K27me3: Loss was defined as absence of nuclear staining in >90% of tumour cells, with

intact internal controls (endothelial, stromal, or inflammatory cells).

Cases showing H3K27M positivity and/or loss of H3K27me3 were classified as DMG-H3K27-altered, while the remaining were designated as DMG-H3K27 wild-type. Analyses were conducted for the entire cohort and separately for paediatric and adult subsets.

Statistical analyses were done using SPSS Version 29.0 software (IBM Company, Armonk, NY) to analyze the data. The statistical analyses included an unpaired Student t-test for continuous variables and a chi-square or Fisher's exact test for categorical variables. Survival was calculated from the patient's first hospital visit to the last follow-up or death. Survival rates and curves were estimated using Kaplan-Meier method, and group comparisons were evaluated using the log-rank test.

RESULTS

Clinicodemographic Findings

A total of 72 patients were analysed, of whom 37 (51.4%) patients were diagnosed as DMGs H3K27Alt (**Table 1**). The age range was 4-60 years, with a mean age of 28.8 years and a median age of 26.0 years. The male-to-female ratio was 2.08:1. Twelve (32.4%) patients were children and 25 (67.6 %) were adults. In the paediatric population, the DMG-H3K27Alt cohort had a slightly higher mean age than the corresponding DMG-H3K27Wt cohort ($p = 0.024$). However, the adult DMG-H3K27Alt cohort was younger than their corresponding DMG-H3K27wt cohort. ($p=0.024$). The thalamus was the most common location (23 cases, 31.9%), particularly in adults, whereas the brainstem was the most common in children. Unusual locations were most frequently observed in adult cohorts, including the third ventricle (two cases), midline cerebrum (one case), corpus callosum (one case) and spinal location (one case) (**Table 2**). Most cases were unifocal (35 cases, 94.6 %) and only two cases were multifocal, and both were adults.

Histopathological and Immunohistochemical Findings

Histologically, grade 4 tumours (27 cases, 73%) predominated, followed by grade 3 (8 cases, 21.6%) and grade 2 tumours (5.4%). Brisk mitotic activity was more common in DMG-H3K27Alt tumours (40.3%, $p=0.014$) and these tumours more frequently showed necrosis and microvascular proliferation ($p <0.001$ and $p = 0.017$, respectively). Grade 4 morphology was significantly more common in DMG-H3K27Alt tumours (**Figure 1**; p-value = 0.008), whereas grade 2 gliomas were more frequently observed in the DMG-H3K27Wt cohort ($p < 0.001$).

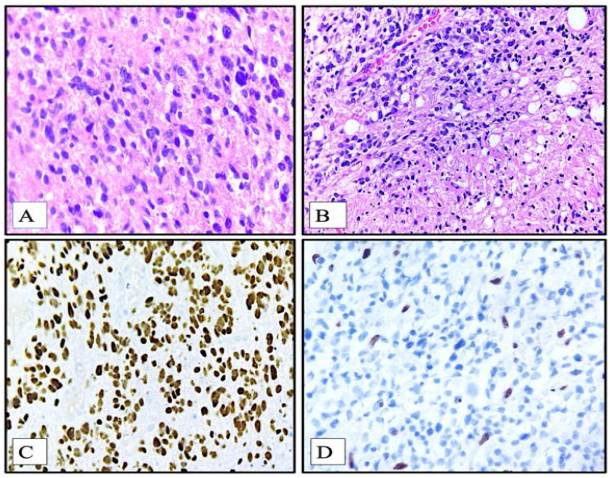

Figure 1. DMG-H3K27Alt with high grade morphology (A) displaying necrosis (B) with H3K27M mutant (C) & H3K27 Me3 loss (D) on IHC.

On immunohistochemistry, ATRX loss was identified in 08 cases (25.8 %). p53 mutation was observed in 21 cases (60 %). H3K27M positivity was seen in 33 cases (89.2 %). Only four cases (10.8%) were H3K27M wild-type but showed isolated H3K27me3 loss (**Figure 2**), three of which were paediatric and one was adult.

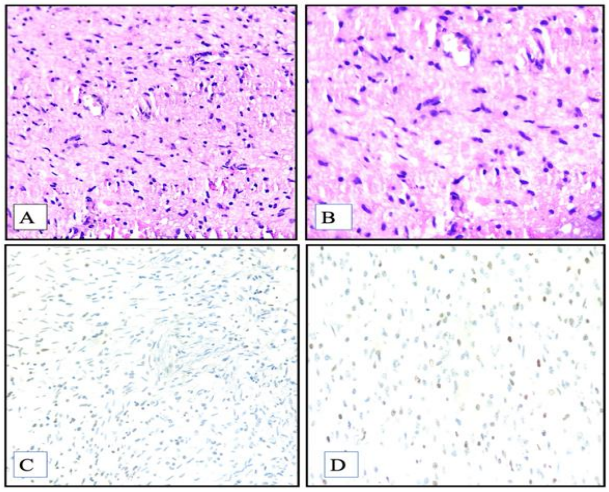

Figure 2. DMG-H3K27Alt, WHO grade 4, displaying low grade morphology (A and B), H3K27M wild type (C) & H3K27 Me3 loss (D) on IHC.

Table 1. Clinicopathological profile of DMG-H3K27Alt and DMG-H3K27Wt

Variable		Total	DMG-H3K27Alt(n=37)	DMG-H3K27Wt (n=35)	P value
Age (years)	Mean ± SD	34.9 ± 20.3	28.8 ± 16.9	41.6 ± 21.5	0.067
	Median	34.5	26.0	45.0	
	Range	2–69	4–60	2–69	
Age group based on median age	<34.5 years	36/72 (50%)	23/37 (62.2%)	13/35 (37.1%)	0.034
	>34.5 years	36/72 (50%)	14/37 (37.8%)	22/35 (62.9%)	
Age group (Paediatric vs Adult)	<18 years	19/72 (26.4%)	12/37 (32.4%)	7/35 (20%)	0.232
	>18 years	53/72(73.6%)	25/37 (67.6%)	28/35 (80%)	
Gender	Male:female ratio	49:23	25:12	24:11	0.930
Size (cm)	Mean ± SD	4.3 ± 1.4	4.6 ± 1.4	3.9 ± 1.5	0.495
	Median	4.45	4.7	3.7	
	Range	1.5–7.3	2.1–7.3	1.5–7.3	
Focality	Unifocal	67 (93.1%)	35/37 (94.6%)	32/35 (91.4%)	0.670
	Multifocal	5 (6.9%)	2/37 (5.4%)	3/35 (8.6%)	
Location					
Supratentorial	Pineal	2 (2.8%)	0/37 (0.0%)	2/35 (5.7%)	0.233
	Thalamus	39 (54.2%)	23/37 (62.2%)	16/35 (45.7%)	0.237
	Third ventricle	3 (4.2%)	2/37 (5.4%)	1/35 (2.9%)	1.000
	Mid cerebrum	2 (2.8%)	1/37 (2.7%)	1/35 (2.9%)	1.000
	Corpus callosum	5 (6.9%)	1/37 (2.7%)	4/35 (11.4%)	0.193
	Basal ganglia	3 (4.2%)	0/37 (0.0%)	3/35 (4.2%)	0.110
Infratentorial	Brainstem	12 (16.7%)	7/37 (18.9%)	5/35 (14.3%)	0.754
	Cerebellum	3 (4.2%)	2/37 (5.4%)	1/35 (2.9%)	1.000
	Spinal	3 (4.2%)	1/37 (2.7%)	2/35 (5.7%)	0.609
Morphological features					
Nuclear pleomorphism	Mild to moderate	63 (87.5%)	31/37 (83.8%)	32/35 (91.4%)	0.483
	Marked	9 (12.5%)	6/37 (16.2%)	3/35 (8.6%)	
Mitosis	Brisk	46 (63.9%)	29/37 (78.4%)	17/35 (48.6%)	0.014
	Occasional	10 (13.9%)	6/37 (16.2%)	4/35 (11.4%)	0.736
	Absent	16 (22.2%)	2/37 (5.4%)	14/35 (4.0%)	<0.001
Necrosis	Present	34 (47.2%)	25/37 (67.6%)	9/35 (25.7%)	<0.001
	Absent	38 (52.8%)	12/37 (32.4%)	26/35 (74.3%)	
Microvascular proliferation	Present	40 (45.6%)	26/37 (70.3%)	14/35 (40%)	0.017
	Absent	32 (44.4%)	11/37 (29.7%)	21/35 (60%)	
Histological grade	Grade 2	15/72 (20.8%)	2/37 (5.4%)	13/35 (37.1%)	<0.001
	Grade 3	16/72 (22.2%)	8/37 (21.6%)	8/35 (22.9%)	0.900
	Grade 4	41/72 (56.9%)	27/37 (73%)	14/35 (40%)	0.008
Grade group	Low grade	15/72 (20.8%)	2/37 (5.4%)	13/35 (37.1%)	<0.001
	High grade	57/72 (79.2%)	35/37 (94.6%)	22/35 (62.9%)	
Immunohistochemistry					
IDH	Mutant	5/72 (6.9%)	0/37 (0.0%)	5/35 (14.3%)	0.023
	Wild-type	67/72 (93.1%)	37/37 (100%)	30/35 (85.7%)	
ATRX	Retained	47/62 (75.8%)	23/31 (74.2%)	24/31 (77.4%)	0.767
	Loss	15/62 (24.2%)	8/31 (25.8%)	7/31 (22.6%)	
p53	Mutant	41/69 (59.4%)	21/35 (60.0%)	20/34 (58.8%)	0.921
	Wild-type	28/69 (40.6%)	14/35 (40.0%)	14/34 (41.2%)	
H3K27M	Mutant	33/72 (45.8%)	33/37 (89.2%)	0/35 (0.0%)	<0.001
	Wild-type	39/72 (54.2%)	4/37 (10.8%)	35/35 (100%)	
H3K27me3	Loss	37/72 (33.3%)	37/37 (100%)	0/35 (0.0%)	<0.001
	Retained	35/72 (66.7%)	0/37 (0.0%)	35/35 (100%)	
Overall survival (months)	Mean survival	3.97 ± 0.842	2.86 ± 4.38	5.17 ± 9.12	0.010

P value <0.05 were considered significant. Diffuse midline glioma H3K27 altered (DMG-H3K27Alt); Diffuse midline glioma H3K27 wild type (DMG-H3K27Wt); Immunohistochemistry (IHC)

The infratentorial DMG-H3K27Alt cohort predominantly showed high-grade morphology, occurred in younger patients (mean age:14.8 years), and displayed ATRX retention in all cases. Low-grade morphology was observed only in the supratentorial location. Except for the predilection of tumours for the brainstem in the paediatric age group (p = 0.002), adult and paediatric DMG-H3K27Alt cohorts had similar clinicopathological profiles.

Survival Analysis

The mean survival duration of DMG-H3K27Alt was significantly shorter than DMG-H3K27Wt (p = 0.009). The mean overall survival duration for DMG-H3K27Alt cohort was 2.86 ±4.38 months and for DMG-H3K27Wt, it was 5.17±9.12 months. A total eight in-hospital deaths were reported, six in DMG-H3K27Alt and two in the DMG-H3K27Wt cohort. Within the first month of admission, 18 patients in the DMG-H3K27Alt cohort were lost to follow-up. Follow-up duration corresponded to the survival period, as patients were followed from the time of first hospital visit until death or last contact. Univariate survival analysis by using the log-rank test showed poor survival in the DMG-H3K27Alt cohort, but it was not statistically significant (p=0.15) (**Figure 3A**), both in paediatric (p=0.168) and adult (p=0.332) age groups.

Among the high-grade tumours, DMG-H3K27Alt and DMG-H3K27Wt cohorts showed similar survival rates (p = 0.623). Within the DMG-H3K27Alt cohort, H3K27M-negative cases exhibited significantly poorer survival than H3K27M-positive cases on univariate analysis (p = 0.040; **Table 3** and **Figure 3B**). However, this difference lost statistical significance after adjustment for age, gender, and tumour size in the multivariate analysis (**Table 4**).

Table 2. Distribution DMG-H3K27Alt in different midline location

Characteristic		Total (n = 37)	Supratentorial (n = 27)	Infratentorial (n = 9)	Spinal (n = 1)
Age (years)					
	Mean ± SD	28.7 ± 16.9	32.9 ± 15.7	14.8 ± 13.9	41.0
	Median	26.0	34.0	10.0	41.0
Gender, n (%)					
	Male	25/37 (67.6%)	18/37 (48.6%)	6/37 (16.2%)	1/37 (2.7%)
	Female	12/37 (32.4%)	9/37 (24.3%)	3/37 (8.1%)	0
Size (cm)					
	Mean ± SD	4.6 ± 1.3	4.67 ± 1.5	4.5 ± 0.9	NA
	Median	**4.7**	4.7	4.8	NA
Location, n (%)					
Supratentorial	Thalamus	—	23 (62.2%)	—	—
	Third ventricle	—	2 (5.4%)	—	—
	Corpus callosum	—	1 (2.7%)	—	—
	Mid cerebrum	—	1 (2.7%)	—	—
Infratentorial	Brainstem	—	—	7 (18.9%)	—
	Cerebellum	—	—	2 (5.4%)	—
Spinal	D6–D12	—	—	—	1 (2.7%)
Histological grade, n (%)	Grade 2	2/37 (5.6%)	2 (5.6%)	0 (0%)	0 (0%)
	Grade 3	7/37 (19.4%)	6 (16.7%)	1 (2.8%)	0 (0%)
	Grade 4	27/37 (75.0%)	18 (50.0%)	8 (22.2%)	1 (2.8%)
IHC profile, n (%)					
p53	Mutant	21/35 (60.0%)	13/35 (37.1%)	7/35 (20.0%)	1/35 (2.9%)
ATRX	Loss	8/31 (25.8%)	7/31 (22.6%)	—	1/31 (3.2%)
H3K27M	Mutant	33/37 (89.2%)	24/37 (64.9%)	8/37 (21.6%)	1/37 (2.7%)
	Wild-type	4/37 (10.8%)	3/37 (8.1%)*	1/37 (2.7%)	0/37 (0%)
Overall survival(months)	Mean ± SD	—	1.56 ± 2.07	3.15 ± 4.94	—

Diffuse midline glioma H3K27 altered (DMG-H3K27Alt); Immunohistochemistry (IHC)

Table 3. Univariate analysis by Log-Rank Test for Categorical Variables and Cox Proportional Hazards Model for Continuous Variables

Variables	P value	
DMG-H3K27Alt vs DMG-H3K27 Wt	0.126	81.1 % VS 94.3%
Paediatric (DMG-H3K27Alt vs DMG-H3K27 Wt)	0.111	66.7% vs 100%
Adult (DMG-H3K27Alt vs DMG-H3K27 Wt)	0.705	88.0 % vs 92.9%
DMG-H3K27Alt	P value	
M:F	0.178	84.0% vs 75.0%
Paediatric vs Adult	0.200	69.4% vs 84.2 %
H3K27M IHC (mutant vs wild)	**0.040**	**85.6%vs 33.3%**
ATRX (loss vs retained)	0.569	75.0% vs 82.6%
P53 (Mutant vs Wild)	0.279	85.7% vs 71.4%
Histological grade (HG vs LG)	0.759	80.0% vs 100.0%
Thalmus location vs others	0.066	86.9% vs 67.3%
Brainstem location vs other	0.200	44.7 % vs 67.2%
Supratentorial vs infratentorial	0.189	85.2% vs 66.7%
Biopsy vs Resection	0.393	76.9% vs 90.9%
RT vs non RT	0.077	100.0% vs 68.8%
Chemo vs non Chemo (TMZ)	0.054	100.00% vs 66.7%
≤4.45 cm vs > 4.45 cm	0.150	68.5% vs 48.6%
Ki67 ≤15% VS >15%	0.838	80.0% vs 81.8%
Unifocal vs multifocal	0.691	80.0% vs 100%

Radiotherapy (RT); Temozolomide (TMZ); Immunohistochemistry (IHC); Confidence Interval (CI), Diffuse midline glioma H3K27 altered (DMG-H3K27Alt); Diffuse midline glioma H3K27 wild type (DMG-H3K27Wt).

Table 4. Multivariate Cox Regression Analysis of Overall Survival in H3K27-Altered DMG Cohort

Characteristics	Hazard ratio (95% CI)	*p*-Value
Age (Adult vs paediatric)	0.62 (0.09-4.08)	0.620
Gender (Male vs female)	0.98 (0.09-10.20)	0.987
Tumour size	1.26 (0.63-2.54)	0.516
H3K27M IHC (Wild vs mutant)	5.01 (0.61-41.27)	0.134

Immunohistochemistry (IHC); Confidence Interval (CI), Diffuse midline glioma H3K27 altered (DMG-H3K27Alt).

Given the small number of events included in the multivariate Cox regression analysis, the findings should be interpreted with caution. The treatment details within the DMG-H3K27Alt cohort, including the number of patients receiving surgery, radiotherapy, chemotherapy, and combined treatment modalities, along with survival outcomes, are illustrated in **Figure 4**.

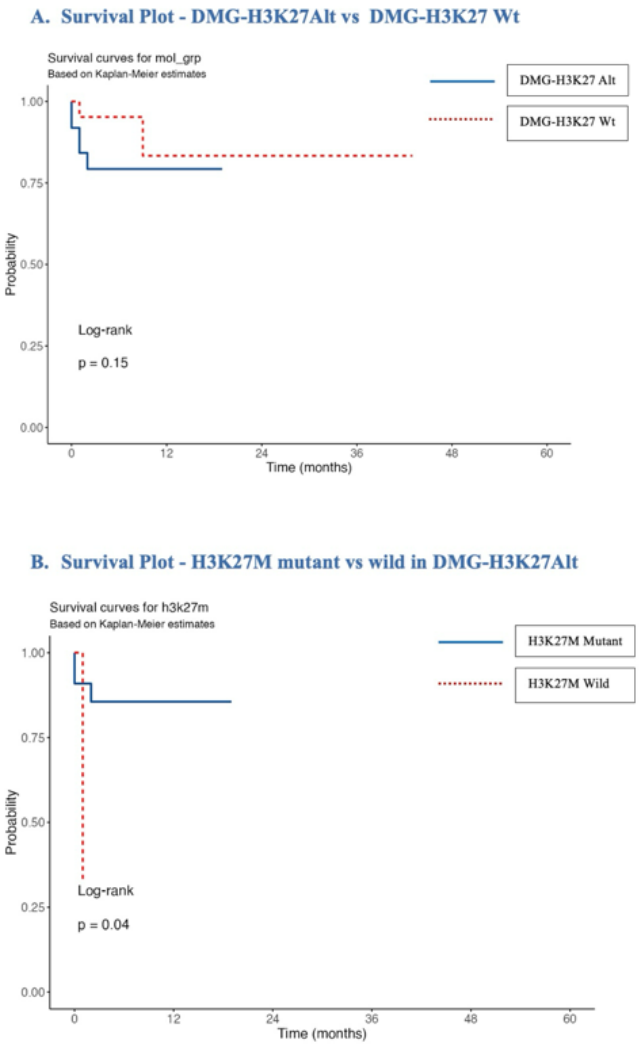

Figure 3. Kaplan-Meier survival analyses
(A) Kaplan meier plot showing poor overall survival in DMG-H3K27Alt compared to DMG-H3K27Wt. though not statistically significant (B) Kaplan meier plot showing poor survival in H3K27M negative DMG-H3K27Alt cohort compared to H3K27M positive.

DISCUSSION

The present study is among the few studies from Western India evaluating the clinicopathological characteristics of H3K27-altered diffuse midline gliomas using immunohistochemistry as a surrogate diagnostic method. DMG-H3K27Alt tumours more frequently showed high-grade morphology, brisk

mitotic activity, necrosis, and microvascular proliferation and had shorter overall survival than DMG-H3K27Wt tumours, although the survival difference was not statistically significant on log-rank analysis. Isolated H3K27me3 loss in H3K27M-negative tumours was uncommon and was observed predominantly in paediatric patients[4].

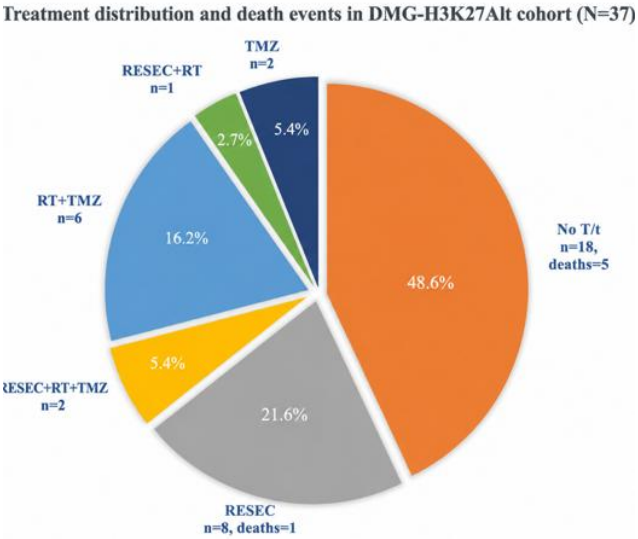

Figure 4. Pie chart showing treatment distribution and death events in the DMG-H3K27Alt cohort (N=37)

Due to the recent recognition of DMG-H3K27Alt, epidemiological data remain limited. Consistent with previous studies, our DMG-H3K27Alt cohort tended to be younger than the DMG-H3K27Wt cohort. Interestingly, unlike prior reports, the paediatric H3K27-altered cohort in our study was slightly older than the wild-type group[1,9-16].

Most tumours presented as solitary space-occupying lesions (SOLs), while multifocality was uncommon and restricted to adults, similar to the findings of Gong et al. 2023[17].

In the paediatric patients, the brainstem was the predominant location in the DMG-H3K27Alt group, whereas the thalamus predominated in adults irrespective of H3K27 status. Unusual tumour locations were almost exclusively observed in adults, consistent with previous reports[11,18-22].

DMGs with H3K27Alt tumours demonstrated a broad histological spectrum, although high-grade morphology predominated in our cohort. Low-grade morphology was uncommon and was predominantly observed in thalamic tumours. Similar variability in grade distribution has been reported in previous studies[11,12,14, 23, 24]. However, unlike Wang et al, who reported a predominance of low-grade tumours in the brainstem[12].

The mutations found in midline gliomas involve variants of the H3 gene, specifically H3.3 and H3.1. The H3F3A gene, which encodes the H3.3 protein, has 80%–90% of these mutations. The remaining 10%–20% of mutations occur in the HIST1H3B gene, which encodes the H3.1 protein. The coding gene HIST1H3C is also present, albeit in rare occurrences[25,26].

H3K27M immunohistochemistry is highly sensitive and specific for detecting K27M mutations in both the H3.1 and H3.3 genes but does not identify rare H3K27 mutations (HK27I), EZHIP overexpression, and EGFR mutations. Loss of H3K27me3 expression helps identify these additional subgroups and has been associated with poor prognosis in a few studies[6]. However, in the absence of EZHIP immunohistochemistry and molecular testing, the four H3K27M-negative cases showing H3K27me3 loss could not be further classified according to their underlying molecular alteration, which may have affected the distribution of the molecular subtypes within the DMG-H3K27Alt group.

Recently, Aboubakr et al. 2024 found OLIG2 and SOX10 positivity in H3K27 mut DMG and EZHIP-overexpressed DMG, but SOX10 was always negative in EGFR-mutant DMG. In the absence of EZHIP IHC and molecular studies to classify DMG-H3K27Alt, Olig 2 and SOX 10, in addition to H3K27M and H3K27me3 are useful determining the aforementioned three broad categories[27]. In most of our cases, immunohistochemical expression was widespread and uniform. However, the H3 histone mutation may show mosaic expression, suggesting it may be the initial factor in tumour development or a subclone mutation[28]. The current study found 89.2% of H3K27M mutant cases and these findings align with certain prior studies[19,22].

Charlotte Dufour et al. 2020 found H3K27me3 loss in five (10.2%) H3-wild group patients. Three individuals had ACVR1 mutations, while two had TP53 mutations. The median overall survival for this group of five patients was 13.2 months[14]. In our cohort, 10.8% of DMG-H3K27Alt cases demonstrated isolated H3K27me3 loss despite being H3K27M wild-type, and most occurred in paediatric patients. This subgroup may represent biologically

distinct tumours requiring further molecular characterisation.

Unlike Gong et al, we did not observe significant differences in p53 overexpression or ATRX loss between paediatric and adult DMG-H3K27Alt cohorts or between H3K27-altered and wild-type tumours. However, IDH mutation was identified exclusively in the H3K27 wild-type group[17].

The recent CNS WHO 2021 also recognises associated collaborating mutations like PDGFRA, FGFR1, and ACVR1 in DMG-HK27-mutant cases further representing sub-clonal populations and their recognition allowed for more precise classification and potential targeted therapeutic approaches[29, 30]. In the present study, further molecular studies were not performed because of resource and technical limitations. Consequently, alterations such as H3.3/H3.1 variants, EZHIP overexpression, EGFR mutation, and rare H3 alterations could not be evaluated. Nevertheless, immunohistochemistry served as a practical and reliable surrogate for identifying H3K27 alterations in our setting.

Although the DMG-H3K27Alt cohort showed poorer overall survival than the DMG-H3K27Wt cohort, statistical significance was not reached on univariate analysis. This may be attributed to the limited sample size, shorter follow-up duration, and loss to follow-up in a substantial proportion of patients. Notably, 18 of 37 DMG-H3K27Alt patients (48.6%) were lost to follow-up within the first month, which may have biased the Kaplan–Meier estimates either toward or away from statistical significance Furthermore, as 95% confidence intervals were not calculated for the Kaplan–Meier estimates, the results should be interpreted with caution. Previous studies have demonstrated poorer outcomes in H3K27-altered gliomas, particularly in paediatric and brainstem tumours[11, 21, 18,32]. Adult gliomas are less aggressive and have a longer median overall survival[18,33]. Multiple studies have shown that adult H3K27M-mutant gliomas have the same prognosis as their wild-type counterparts in similar locations[33- 35]. However, H3-mutant thalamic gliomas have a better prognosis than H3-mutant brainstem gliomas in terms of location[33, 35, 36]. ATRX loss was more common in thalamic and spinal tumours and thalamic gliomas had the most p53 overexpression. The aforementioned observations indicate that there is molecular heterogeneity in tumours, which is influenced by the anatomical location and is present within each age group[33].

In our study, patients with H3K27M-wild DMG-H3K27Alt showed poorer survival compared to those with H3K27M mutations, which contrasts with Ajuyah et al., who found similar outcomes between the two[37]. Since this entity is newly defined, data are still limited, and more studies are needed to understand the true prognostic differences.

At the moment, effective targeted therapies for DMG-H3K27Alt remain limited. In our study, patients receiving surgery, radiotherapy, and/or chemotherapy demonstrated relatively better survival, although the difference was not statistically significant. Owing to the critical location of these tumours, surgical intervention is often limited to biopsy, while radiotherapy remains the primary treatment modality[38].

CONCLUSION

A significant proportion of both paediatric and adult DMGs are H3K27 altered and usually display high-grade morphology. Low-grade morphology is less frequent and has a predilection for the thalamus. Unusual location and multifocality are more common in adult cohorts. Standalone loss of H3K27me3 expression is less frequent and was majorly found in the paediatric population and was associated with poorer survival on univariate analysis. This study highlights the utility of combined H3K27M and H3K27me3 immunohistochemistry for identifying DMG-H3K27Alt in routine practice, particularly in resource-limited settings where comprehensive molecular testing is unavailable.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Author Contributions

Concept: VR PT, Design: VR, PT, Supervision: PT, Data collection and/ or processing: VR, PM, AS and PM Analysis and/or interpretation: VR, PM Literature search: VR Writing: VR, Approval: PT, PM, AS, PM. The manuscript has been read and approved by all the authors, the requirements for authorship as stated earlier in this document have been met, and that each author believes that the manuscript represents honest work, if that information is not provided in another form.

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Declaration of Generative AI and AI-assisted technologies

The authors utilised the AI tool QuillBot solely for language enhancement and not for generating any of the scientific ideas, interpretations, analyses or conclusions presented in this paper. The authors reviewed and edited the manuscript and take full responsibility for the final content.

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