Acta Scientific CANCER BIOLOGY (ISSN: 2582-4473)

Volume 9 Issue 2 February 2025

Research Article

Correlation Between Ki-67 Proliferation Index and Pathological Grades of Gliomas

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Received: May 23, 2025

Published: June 03, 2025

Abstract

Background: Gliomas comprise approximately 81% of all malignant brain neoplasms diagnosed in adults. The correlation between Ki-67 labelling index in tumour tissues and patient survival has been observed across various cancer types. Research on the prognostic significance of Ki-67 in gliomas has yielded mixed results.

Aim: The present study was conducted to investigate Ki-67 proliferation index in gliomas, aligning with the latest World Health Organization (WHO) CNS classification system.

Materials and Methods: The present study was conducted in the Department of Pathology, Armed Forces Medical College, Pune, India from Oct 2019 to Oct 2024. A comprehensive analysis of 60 gliomas (according to WHO CNS classification system) was conducted. Histomorphological and immunohistochemical techniques were employed to evaluate Ki-67 expression. Pearson's chi-square test, Kruskal-Wallis test, Mann-Whitney U test and Spearman's rank-order correlation were applied for data analysis. P value < 0.05 was considered statistically significant.

Results: Glioblastoma emerged as the most prevalent tumour type (40.0%), with the highest incidence observed in the 51-60-year age group (25.0%). Notably, a male predominance was evident. The mean Ki-67 proliferation index demonstrated a statistically significant positive correlation with WHO grade.

Conclusion: The findings of present research underscore a strong association between Ki-67 expression and tumour aggressive-

Keywords: Brain Tumours; Gliomas; Immunohistochemistry; KI-67 Proliferation Index

Introduction

Gliomas are the most prevalent form of primary brain tumours, starting within the central nervous system (CNS) [1]. These tumours originate from glial cells, which are non-neuronal cells essential for providing support and structure to the nervous system's functioning [2]. Gliomas account for 81% of malignant brain tumours [3]. Several risk factors for gliomas have been identified, including age, exposure to radiation and certain chemicals, and family history. It's important to note that the majority of gliomas occur in individuals without any known risk factors [4,5].

The capacity of gliomas to induce both local and systemic immune suppression hinders the body's natural defences against tumour growth and the effectiveness of immunotherapy. This presents a significant obstacle in developing new treatment strategies [6].

Gliomas can lead to a range of neurological symptoms, primarily depending on the location of the tumour in the brain. This can result in motor dysfunction, cognitive symptoms, and changes in personality. Beyond the impact on the patient, gliomas often significantly affect the patient's family as well [7].

Gliomas are categorized based on their malignancy grade and morphological characteristics. The prognosis for glioma patients is largely determined by the specific type and grade of the tumour. The severity of gliomas increases with grade. This grading system directly influences the patient's survival chances [8,9].

Since 2016, molecular markers have also been considered in the World Health Organization (WHO) classification, aiding in the differentiation of tumours based on their biological characteristics [10].

Ki-67, a cell proliferation marker, has been put forward as a potential supplement to existing methods for grading gliomas. The Ki-67 labelling index (LI) is defined as the percentage of Ki-67-positive tumour nuclei among all tumour nuclei [11]. Ki-67 is a nuclear protein that directly reflects a specific cellular proliferative state. The correlation between Ki-67 LI in tumour tissues and patient survival has been observed across various cancer types. Research on the prognostic significance of Ki-67 in gliomas has yielded mixed results. Some studies have indicated that a high Ki-67 LI is associated with improved overall survival, while others have found a link between high Ki-67 LI and poorer overall survival. However, some studies have reported no association between Ki-67 LI and overall survival [12].

Due to inconclusive prior findings, this research aimed to investigate Ki-67 proliferation index in gliomas, aligning with the latest World Health Organization (WHO) CNS classification system.

Materials and Methods

The present study was conducted in the Department of Pathology, Armed Forces Medical College, Pune, India from Oct 2019 to Oct 2024. All the cases of glioma (according to WHO CNS classification system) reported during the period were included in the study. The non- neoplastic and benign lesions of CNS, non-glial tumours, ependymal tumours and spinal tumours were excluded from the study. The demographic and clinical data pertaining to the cases was collected from the histopathology database and requisition forms sent with the specimen. A written consent was obtained from all the patients for the use of their tissue samples for research purpose. The ethical clearance was obtained from the ethical committee of the institution.

Procedure

From all the paraffin-embedded tissue blocks, sections of 3-4 micron thickness were made on separate glass slides. The slides to be used for Hematoxylin and eosin (H and E) stain were coated with egg albumin and the section from tumour was taken on the glass slide. All the sections stained with H and E were reviewed. The Blocks and stained sections with tumour were selected for immunohistochemistry (IHC) and assessment of Proliferation index done by using a Ki67 marker. The IHC was performed using Pathn-situ IHC kit for representative tumour section.

Statistical analysis

Data was entered in Microsoft Excel 2021 for Windows. Pearson's chi- square test, Kruskal-Wallis test, Mann-Whitney U test and Spearman's rank-order correlation were applied for data analysis. P value < 0.05 was considered statistically significant. Data analyses were performed using version 21.0 of the Statistical Package for Social Sciences (IBM Corporation, Armonk, New York, USA).

Results

Among the total 60 cases, maximum cases (n = 24, 40.00%) were observed in glioblastoma (Table 1). Maximum number of cases (n = 15, 25.00%) were observed in the age group 51-60 years (Figure 1). Among total 60 cases, 43 (71.67%) were males and 17 (28.33%) were females. Male to female ratio of glioma cases was approximately 2.5:1. Site-wise, maximum number of cases (n = 22, 36.67%) were observed for temporal region (table 2). Among a total of 60 cases, microcystic changes were observed in 04 (6.67%) cases, vascular spaces containing glomeruloid structures formed by capillaries were observed in 25 (41.67%) cases, vascular proliferations were observed in 47 (78.33%) cases, cytological atypia was observed in 55 (91.67%) cases, increased mitotic activity was observed in 47 (78.33%) cases and necrosis was observed in 25 (41.67%) cases.

In the present study grade 4 gliomas accounts for maximum number of cases (n = 24, 40%), followed by grade 3 (n = 14, 23.33%) and 2 (n = 13, 21.67%) gliomas which accounts for 45% of cases combinedly. In grade 1 gilioma, there were 9 (15%) of cases (Figure 2).

Diagnosis	Frequency	Percentage
Anaplastic astrocytoma	06	10.00
Anaplastic oligoastrocytoma	01	1.67
Anaplastic oligodendroglioma	04	6.67
Astrocytoma	01	1.67
Central neurocytoma	01	1.67
Diffuse astrocytoma	06	10.00
Diffuse glioma (NOS)	02	3.33
Dysembryoplastic neuroepithelial tumor	02	3.33
Ganglioglioma	01	1.67
Glioblastoma	24	40.00
Oligoastrocytoma NOS	01	1.67
Oligodendroglioma	05	8.33
Pilocytic astrocytoma	06	10.00
Total	60	100.00

Table 1: Distribution of glioma cases according to diagnosis.

Site	Frequency	Percentage
Basifrontal region	01	1.67
Cerebellum	04	6.67
Corpus callosum	03	5.00
Frontal region	12	20.00
Fronto-parietal region	02	3.33
Fronto-temporal region	05	8.33
Insula	03	5.00
Intraventricular	01	1.67
Parieto-occipital region	04	6.67
Suboccipital region	01	1.67
Supra seller mass	01	1.67
Temporal region	22	36.67
Third ventricle	01	1.67
Total	60	100.00

Table 2: Distribution of glioma cases according to site.

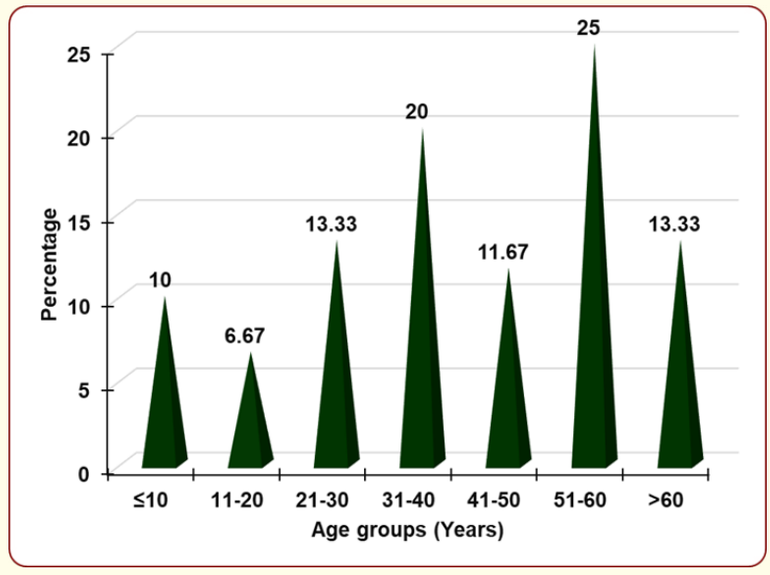

Figure 1: Age-wise distribution of glioma.

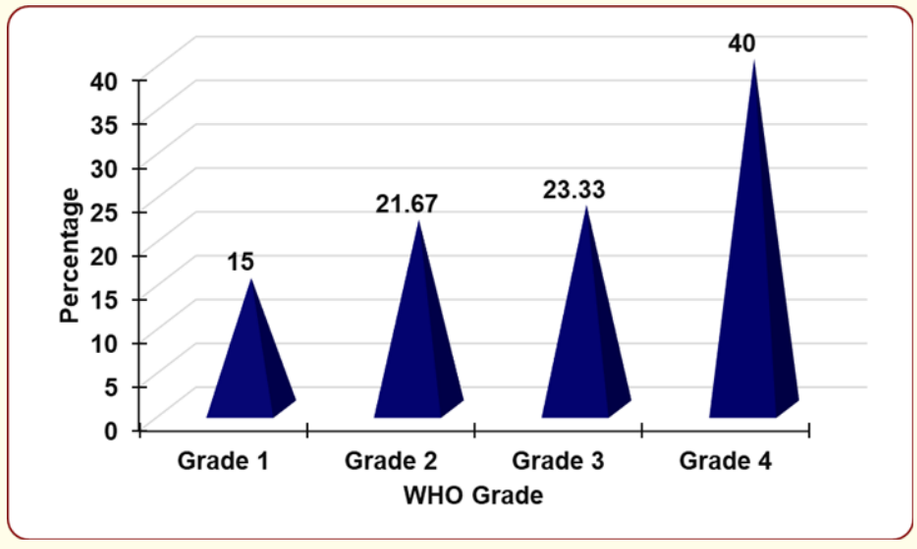

Figure 2: Distribution of who grade of glioma.

Table 3 show the comparison of Ki67 proliferation index in different WHO grades. Mean ± SD of Ki67 proliferation index values in grade 1, grade 2, grade 3 and grade 4 were 1.22 ± 0.44, 2.69 ± 1.93, 13.57 ± 11.02 and 28.00 ± 18.91, respectively. Kruskal-Wallis test showed significant difference between WHO grades for Ki67 proliferation index values ($X^2 = 43.656$, df = 3, $P < 0.001$). After that Mann-Whitney U test was applied for pairwise comparison, which showed following observations

- Ki67 proliferation index values in grade 4 were significantly higher than grade 1 MW = 0.000, $P < 0.001$), grade 2 (MW = 0.000, $P < 0.001$) and grade 3 (MW = 64.500, $P < 0.01$).
- Ki67 proliferation index values in grade 3 were significantly higher than grade 1 (MW = 5.500, $P < 0.001$) and grade 2 (MW = 13.500, $P < 0.001$).
- Ki67 proliferation index values in grade 2 were significantly higher than grade 1 (MW = 27.000, $P < 0.05$).

WHO Grade	Ki-67 Proliferation Index (%)	
	Mean ± SD	Min-Max
Grade 1	1.22 ± 0.44	1-2
Grade 2	2.69 ± 1.93	1-7
Grade 3	13.57 ± 11.02	1-40
Grade 4	28.00 ± 18.91	10-85
Kruskal-Wallis test	$X^2 = 43.656$, df = 3, P < 0.001, Very high sig.	
Mann-Whitney U test	Grade 1 and Grade 2: MW = 27.000, P < 0.05, Sig. Grade 1 and Grade 3: MW = 5.500, P < 0.001, Very high sig. Grade 1 and Grade 4: MW = 0.000, P < 0.001, Very high sig. Grade 2 and Grade 3: MW = 13.500, P < 0.001, Very high sig. Grade 2 and Grade 4: MW = 0.000, P < 0.001, Very high sig. Grade 3 and Grade 4: MW = 64.500, P < 0.01, Highly sig.	

Table 3: Comparison of Ki-67 proliferation index in different WHO grades of gliomas.

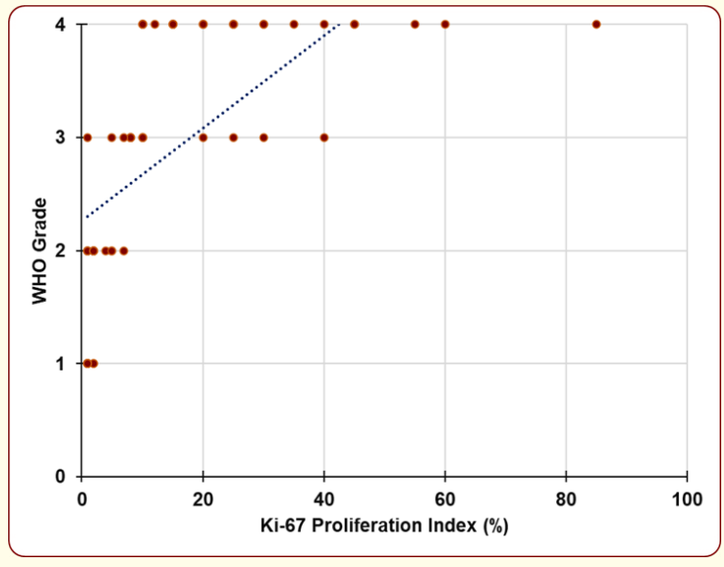

Figure 3: Correlation between Ki-67 proliferation index (%) and WHO grades of gliomas.

Highest Ki67 proliferation index value was observed in grade 4 and lowest in grade 1.

There was a statistically significant strong positive relationship (Spearman's rho = 0.852, P < 0.001) between Ki-67 proliferation index (%) and who grades (Figure 3).

Discussion

Gliomas constitute a heterogeneous group of primary brain tumours characterised by distinct genetic and epigenetic profiles. The aggressive clinical course of gliomas, characterised by high recurrence rates and significant mortality, presents a substantial challenge for neuro- oncologists [5]. Gliomas are classified by the WHO into grades I-IV utilizing standardized histopathological criteria. Given the potential for intertumoral heterogeneity, particularly in smaller biopsy specimens, the incorporation of ancillary diagnostic tools is essential for ensuring accurate tumour grading [11].

The nuclear protein Ki-67 serves as a well-established marker of cellular proliferation and a valuable tool in immunohistochemistry applications. In the context of gliomas, Ki-67 expression has emerged as a promising ancillary marker for tumour grading [11,13]. The Ki- 67 exhibits nuclear localization [11]. It is a non-histone protein, serves as a DNA-binding nuclear protein expressed throughout the cell cycle in actively proliferating cells, excluding quiescent (G0) cells [14].

The Ki-67 labeling index (LI), defined as the percentage of Ki-67 positive tumor nuclei among all tumour nuclei [11]. Its utility lies in distinguishing growing cells from non- growing ones [14]. Ki-67 proliferation index stands out as one of the most valuable markers for assessing cellular proliferation in a variety of human neoplasms, including lung, bladder, breast, cervical, urothelial carcinomas, upper urinary tract, lymphoma, cervical, renal and intracranial tumours [13,15]. Despite numerous studies conducted on glioma patients, the prognostic significance of Ki-67 for survival remains a topic of controversy [14]. Hence the present study aimed to investigate Ki-67 proliferation index in gliomas, aligning with the latest World Health Organization (WHO) CNS classification system.

Comparison between age incidence of gliomas

In our study, the maximum number of cases were observed in the age group 51-60 years (n = 15, 25.00%) with a median age of 50.5 years. It was similar to earlier conducted studies [13,16-19].

Comparison between gender wise incidence of gliomas

In our study, there were 43 (71.67%) males and 17 (28.33%) females in the study population. Male to female ratio of glioma cases was approximately 2.5:1. The findings were consistent with previous research [13,16,19-22].

Comparison between the site of occurrence of gliomas

In our study, maximum number of cases were observed for temporal region (n = 22, 36.67%), followed by frontal region (n = 12, 20.00%) and fronto-temporal region (n = 05, 8.33%). Zeng., *et al.* categorized gliomas into supratentorial and infratentorial regions, with 199 cases in the supratentorial area and 30 cases in the infratentorial area [16]. Meanwhile, Hwang., *et al.* reported 49 cases of temporal lobe, 31 cases of frontal lobe, and 16 cases of parietal lobe pediatric gliomas [23]. Shivaprasad., *et al.* within a cohort of 30 patients of astrocytomas found that the frontal lobes harbored the highest tumour burden, with a right frontal predilection (11 cases, 36.7%) [19].

Comparison between WHO grading of glioma

In our study among 60 cases, grade 4 gliomas accounts for maximum number of cases (n = 24, 40%), followed by grade 3 (n = 14, 23.33%), grade 2 (n = 13, 21.67%) and grade 1 gilioma (n = 9, 15%). A study by Zeng., *et al*. revealed 10 cases of grade 1, 83 of grade 2, 74 of grade 3, and 62 of grade 4 gliomas [16]. Studies by Han., *et al.* (54 cases) [17], Miyazaki., *et al.* (16 cases) [24], Pratt., *et al*. (183 cases) [20], and Nduom., *et al* (92 cases) [25] all found glioblastoma to be Grade 4. Shivaprasad *et al.* reported that the most common histological grade of astrocytic tumours in their study was grade IV (n=16, 53.3%) followed by grade II (n = 7, 23.3%), grade III (n = 6, 20.0%) and grade I (n = 1, 3.3%) [19].

Ki-67 proliferation index and different WHO grades of gliomas

In our study, mean ± SD of Ki67 proliferation index values in grade 1, grade 2, grade 3 and grade 4 were 1.22 ± 0.44, 2.69 ± 1.93, 13.57 ± 11.02 and 28.00 ± 18.91, respectively. There was a statistically significant strong positive relationship between Ki-67 proliferation index (%) and WHO grades. With the increase in Ki-67 proliferation index (%) there will be an increase in WHO grades and vice versa.

In a study, Shivaprasad., *et al.* observed a gradually increasing trend in the mean Ki-67 LI across grades I to IV [19]. In a study Skjulsvik., *et al.* found that Ki-67/MIB-1 PIs correlated well with histological malignancy grade in all glioma subtypes. Still, a notable degree of overlap persisted between the malignancy groups [26].

Similarly, Xu., *et al.*, observed among 45 glioma patients that postoperative Ki-67 expression are associated with pathological glioma grade [27].

Das., *et al.* reported that the mean Ki-67 labelling index values in Astrocytoma grade I, II, III, IV were 4.66, 8.07, 13.5, 22.93 respectively. They found that the Ki-67 labeling index (LI) increases with histological grade, and there is a significant difference between low-grade (I and II astrocytomas) and high-grade (grade III and IV) tumors. In their study, Ki-67 LI is not influenced by factors such as age and sex; it is solely dependent on histological grade [21]. This result agrees with the studies done by Wakimoto., *et al.* (28) and Ambroise., *et al.* [29].

Limitations of the Study

Though the present research includes contemporary data based on a meticulous clinicopathological analysis, there are certain limitations. This was a retrospective study from a single centre which limit the generalizability of the findings to the broader population. The sample size of 60 patients may not be sufficient to detect subtle associations between subgroups within the study population. A larger, prospectively designed study would be required to enhance the generalizability and robustness of the findings. The study design does not incorporate survival analysis, hindering a comprehensive assessment of patient prognosis and the clinical course of the disease.

Conclusion

The present study's findings, demonstrates a statistically significant positive correlation between the Ki-67 proliferation index and WHO grades, lend strong support to the potential utility of Ki-67 as a prognostic indicator in gliomas. This finding aligns with the growing emphasis on incorporating molecular markers alongside traditional histological grading for a more comprehensive assessment of glioma aggressiveness and patient outcomes.

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