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Ki-67 Expression as a Complementary Marker for Histopathological Grading of Astrocytic Tumors

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Abstract

The Ki-67 labelling index (LI) is an important marker used to assess cell proliferation in glioma diagnosis, providing valuable insights into tumor growth and potentially contributing to prognosis. This study investigates Ki-67 expression in astrocytic tumors and its association with clinicopathological features. We documented the clinical and radiological details of 43 glioma patients. Histopathological grade and morphology were assessed by H&E slide examination, while the Ki-67 labelling index was measured using immunohistochemistry in 'hot-spot' regions. Tumors were categorized as high or low grade using a threshold of 4% according to WHO guidelines. Statistical analysis was performed using standard software, with a significance threshold of $P \leq 0.05$. The cohort consisted of 31 males and 12 females, with a mean age of 46.14 years. A significant correlation was found between Ki-67 expression and factors such as older age ($P = 0.01$), higher histological grade ($P < 0.00001$), cellular atypia ($P = 0.0002$), necrosis ($P < 0.00001$), and microvascular proliferation ($P < 0.00001$). The findings suggest that the Ki-67 labelling index is a valuable adjunct to traditional histopathological grading and can be relied upon as a prognostic marker for glioma patients.

Keywords: Ki-67, Astrocytic tumors, Tumor grading, Atypia, Glioma

Introduction

Gliomas are prevalent cancers affecting the central nervous system (CNS) [1, 2]. The Ki-67 antigen, first identified by Scholzer and Gerdes in the early 1980s, exists in two protein forms with molecular weights of 345 and 395 kDa [3]. This protein is expressed during all active phases of the cell cycle (G1, S, G2, and M) but is absent in resting cells (G0) [4, 5]. Ki-67 levels significantly decrease during the later stages of mitosis, particularly in anaphase and telophase [6]. Ki-67 (pKi-67) expression correlates with the proliferation of malignant tumor cells, making it a valuable marker for assessing tumor aggressiveness [7, 8].

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Ki-67 regulates the progression of the cell cycle, the distribution of cells during interphase, and the process of mitosis [9]. In diffuse astrocytic tumors, Ki-67 serves as a marker for cell proliferation, which is important for predicting both the biological characteristics of the tumor and the patient's prognosis [10, 11]. The Ki-67 labeling index (LI) is notably higher in high-grade tumors, such as glioblastomas, compared to low-grade astrocytomas [12].

Gliomagenesis, or the development of gliomas, is largely driven by glial cell proliferation [13, 14]. While histological grading is commonly used to evaluate prognosis in CNS tumors, glioma differentiation can be challenging, especially with small tissue samples obtained through stereotactic biopsies [15, 16]. Proliferation indices, such as Ki-67, have become key tools in predicting disease progression and patient survival [17, 18]. The MIB-1 antibody, which identifies Ki-67 in formalin-fixed paraffin-embedded tissue, has been widely used for classifying and determining the prognosis of astrocytomas [3, 19]. The use of the MIB-1

antibody is vital for developing better prognostic markers to predict tumor behavior and guide treatment decisions [20, 21].

Evaluating Ki-67 as a proliferative index is a supplementary approach to histological grading [22]. Among various biological markers, Ki-67 provides a quantitative measure of tumor proliferation [23]. Several methods have been developed to calculate proliferative indices in CNS malignancies, with Ki-67 standing out as one of the most effective in brain tumor evaluation. Many studies support the utility of the Ki-67 labeling index in assessing cell proliferation in gliomas and other malignancies [24].

This study investigates the expression of Ki-67 in astrocytic tumors and its relationship with clinicopathological features.

Materials and Methods

This study was conducted in the Department of Pathology over two years, from September 2020 to August 2022, following approval by the Institutional Review Board and the Ethics Committee in line with the Declaration of Helsinki. Informed consent was obtained from all participants whose biopsies were included in the study. Formalin-fixed paraffin-embedded (FFPE) tissue blocks from histopathologically confirmed glioma cases were selected, including both prospective and retrospective samples from archival records. Tissues that were inadequately fixed or poorly preserved were excluded from the analysis.

Before initiating the study, clinical and radiological details of the patients were documented. Consecutive H&E-stained slides were examined for histomorphological features, including tumor type, grade, cellularity, atypia, mitotic activity, presence of necrosis, and microvascular proliferation. Grade I and II tumors were categorized as low-grade, while Grade III and IV tumors were classified as high-grade. Tumor sections from the paraffin blocks with viable and highly cellular tumor areas, including necrotic regions when present, were selected for immunohistochemical (IHC) analysis.

Immunohistochemical analysis

The immunohistochemical staining for Ki-67 was performed using deparaffinized tissue sections mounted on albumin-coated slides. A two-step indirect method was employed with a pre-diluted Ki-67 monoclonal antibody (CAT-P-K001-30, Clone QR015, Berlin, Germany). Placenta tissue served as a positive control, while the negative control was prepared by excluding the primary antibody step. The stained slides were independently evaluated by two pathologists, who were blinded to the clinical details. Any discrepancies between the evaluations were resolved by reviewing the slides together with an expert pathologist using a multi-head microscope.

Evaluation of Ki-67

The “hotspot” area, which showed the highest density of immunostained tumor nuclei, was selected for analysis. Adjacent fields were then examined until 1,000 tumor cells were counted. Positive Ki-67 staining was determined by the presence of distinct nuclear labeling in tumor cells. The Ki-67 index was calculated as the percentage of positively stained tumor cells among the 1,000 examined. According to WHO criteria, low-grade gliomas are classified as having a Ki-67 index of less than 4%, while high-grade gliomas have a Ki-67 index of 5% or more. Based on this threshold, the cases were categorized into low or high Ki-67 expression.

Statistical analysis

The data were entered into Microsoft Excel 2010 for analysis. Continuous data were presented as mean ± standard deviation (SD), median, or range, while categorical data were analyzed using Pearson’s chi-square test or Fisher’s exact test. The statistical software used for analysis included Microsoft Excel 2010, GraphPad QuickCalcs ©2021 GraphPad Software, and Social Science Statistics©2021 Jeremy Stangroom. A p-value of ≤ 0.05 was considered statistically significant.

Results and Discussion

The study included 43 patients, with a mean age of 46.14 years and a male-to-female ratio of 2.5:1. The frontal lobe was the most common site for tumor development (12 out of 43 cases). Of the total number of tumors, 15 were classified as low-grade gliomas, including pilocytic and diffuse astrocytomas, accounting for 25% of the cases **(Figure 1)**.

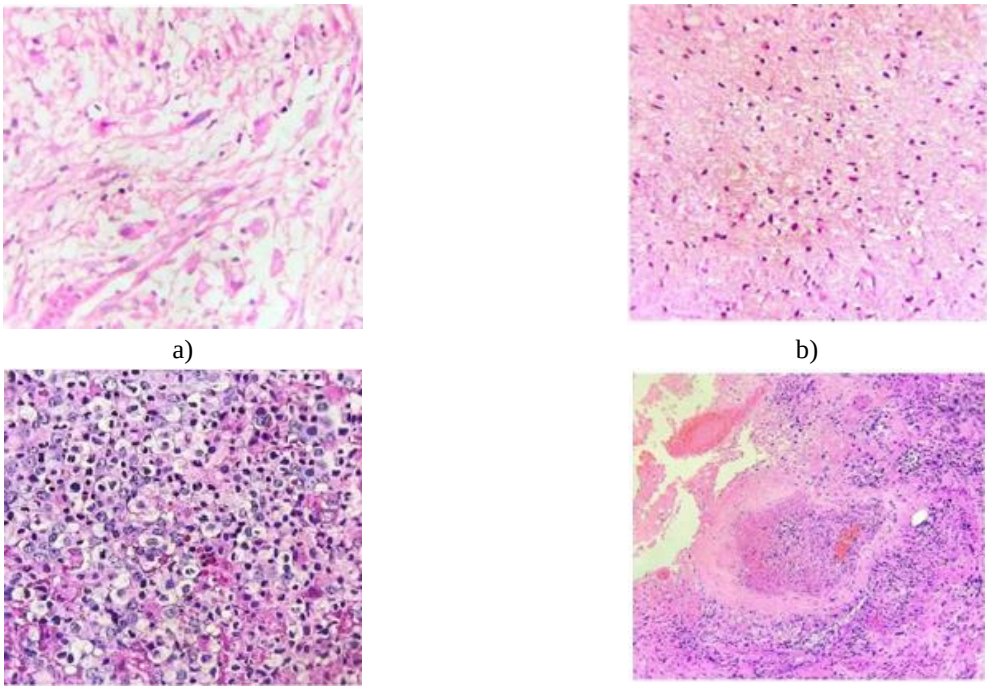


Figure 1. Different grades of glial tumors were encountered in the study; a) grade I-pilocytic astrocytoma (H&E,400X); b) grade II-diffuse astrocytoma (H&E, 100X); c) grade III- anaplastic astrocytoma (H&E, 400X), and d) grade IV-glioblastoma (H&E, 100X)

Ki-67 expression in glioma

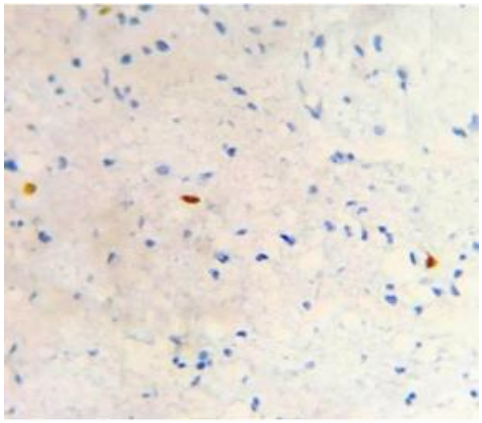
The Ki-67 labeling index (LI) was observed in 95.3% of the cases, with values ranging from 0 to 80% (Mean: 22.65 ± 24.28%). Strong nuclear positivity was observed in the majority of samples. Low Ki-67 expression (< 4%) was detected in 14 out of 43 cases, accounting for 32.6%. The association between Ki-67 and clinicopathological characteristics is detailed in **Table 1**.

A significant relationship was found between Ki-67 LI and various clinicopathological factors, including patient age, histological grade, cellular atypia, necrosis, and microvascular proliferation **(Table 1)**. Higher Ki-67 LI was correlated with advanced age, higher histological grade, and increased cellular atypia **(Figure 2)**.

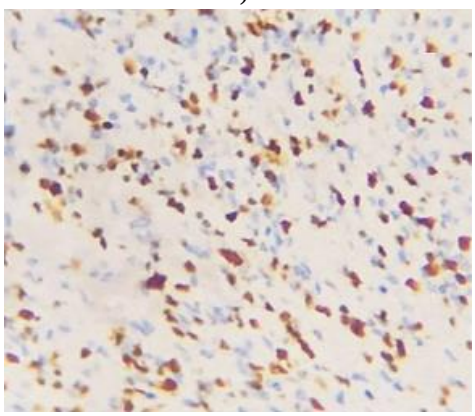
Table 1. Correlation of PD-L1 and Ki-67 with clinicopathological parameters

Sl. No.	Parameters	Category (n)	Ki-67 expression		P-value
			Low	High	
1	Age (years)	< 46 (19)	10	09	0.01
		> 46 (24)	04	20	
2	Cellularity	Reduced (27)	13	17	0.22
		Increased (16)	04	12	
3	Atypia	Minimal (12)	09	03	0.0002
		Marked (31)	05	26	
4	MVP**	Absence (19)	13	06	<0.00001
		Noted (24)	01	23	
5	Necrosis	Absence (19)	13	06	<0.00001
		Presence (24)	01	23	
6	Histologic grade (WHO)	Grade I/Grade II (15)	12	03	<0.00001
		Grade III/Grade IV (28)	02	26	

*MVP = microvascular proliferation.



a)



b)

Figure 2. Ki-67 LI demonstrates low (< 4%) nuclear staining in a case of low-grade glioma (pilocytic astrocytoma, WHO grade I) in panel (a), conversely, a glioblastoma (WHO grade IV) displays higher (> 4%) nuclear staining in panel (b) (IHC for Ki-67, A, B: 100X).

Ki-67 expression in gliomas

Ki-67 serves as an essential biomarker for grading astrocytomas by quantitatively assessing tumor growth and predicting its progression. Although assessing the mitotic rate is crucial for understanding the proliferation of tumors, accurately quantifying mitosis in central nervous system (CNS) tumors can be challenging due to the typically low mitotic rate. A variety of methods are used to assess proliferation in CNS tumors, with Ki-67 immunohistochemistry being the most widely applied. Ki-67 has proven to be a reliable indicator of glioma progression and patient prognosis. High-grade gliomas (WHO grade III/IV) exhibit significantly higher Ki-67 labeling indexes compared to low-grade gliomas (WHO grade I/II). Therefore, while histological grading and clinical characteristics are important prognostic factors, Ki-67 LI can provide valuable supplementary information, particularly in cases where clinical and histological findings do not align [25-29].

Skjulsvik *et al.* [26] conducted a cohort study of 267 glioma cases, observing that Ki-67 expression was significantly higher in high-grade gliomas (grades III/IV) compared to low-grade tumors (grades I/II). Other researchers investigating the role of immunohistochemical markers, such as p53 and Ki-67, in glioma grading found a statistically significant increase in Ki-67 expression with tumor grade. A similar study by Hu *et al.* [29] showed that cell proliferation rates (Ki-67) significantly increased with the grade of gliomas. Thotakura *et al.* [10] also explored the use of Ki-67 as an adjunct to histopathological grading and reported a clear increase in Ki-67 LI with tumor grade. For instance, grade I astrocytomas had a mean Ki-67 LI of 3.36%, grade II tumors had 7.05%, grade III had 28.24%, and grade IV tumors had 38.7%.

Previous studies have shown that Ki-67 expression is highly present in glioma cells, unlike normal brain tissue, and its levels rise as the tumor progresses. Ki-67 also serves as an indicator of cellular replication. Furthermore, stem cells contribute to tumor progression through the epithelial-mesenchymal transition (EMT), and Ki-67 is expressed in these cells, further supporting its role as a proliferation marker. High Ki-67 levels in gliomas are correlated with more aggressive malignancy, making it a useful tool for both grading and prognostication. Studies have also indicated that increased Ki-67 expression is associated with poor prognosis, suggesting that it may help in predicting therapeutic response [30-34].

Despite its potential, morphological grading in gliomas can be challenging due to tumor heterogeneity and sampling bias, especially in small biopsy specimens. Ki-67 LI offers a more consistent grading approach and can complement other markers like p53 expression and IDH mutation studies to provide a more definitive diagnosis. However, several factors, including tissue quality, immunohistochemistry techniques, and interpretation methods, can affect the grading results. Although the manual hotspot technique, as used in this study, is common, some suggest that averaging the entire slide for Ki-67 assessment may offer a more reliable approach, given the uncertainty of hotspot location in biopsy samples [35-39].

Conclusion

Unlike breast cancer, gliomas lack established screening methods for early diagnosis and treatment. This study concludes that the Ki-67 proliferation index correlates significantly with age, histological grade, and the degree of cellular atypia. Although clinical parameters and histopathological grading are important for prognosis, Ki-67 serves as a useful adjunct, particularly when discrepancies exist between clinical findings and histopathological grade, especially in small biopsy samples.

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