

# Role of Ki-67 as an Adjunct to Histopathological Diagnosis in the Grading of Astrocytic Tumors

## Abstract

The Ki-67 Labelling Index (LI) is used as an ancillary tool to assess cell proliferation activity in glioma diagnosis. It is a potent biologic marker that estimates the growth of neoplasm and thus will aid in identifying the prognosis in patients with glioma. To study the expression of Ki-67 in astrocytic tumors and its correlation with clinicopathological parameters. Clinico-radiological details of forty-three cases of glioma were documented. H&E slides were reviewed for histopathological grade and morphology. Ki-67 Labelling Index was studied by immunohistochemical analysis at 'hot-spot' areas. Cases were segregated into high and low grades taking 4% as a cut-off as per WHO criteria. Statistical analysis was carried out using standard statistical software with a p-value of  $\leq 0.05$  taken as statistically significant. A total of 43 patients were included comprising 31 males and 12 females with a mean age of 46.14 years. Ki-67 labeling index showed a statistically significant relationship with the advanced age ( $p=0.01$ ), higher histological grade ( $p<0.00001$ ), presence of cellular atypia ( $p=0.0002$ ), necrosis ( $p<0.00001$ ), and microvascular proliferation ( $p<0.00001$ ). Ki-67 assessment supplements standard histopathological grading. It is a simple and reliable method. This study demonstrates that it can serve as an important prognostic marker. It can be used as an adjunct to histopathological diagnosis.

**Keywords:** Astrocytic tumors, Ki-67, Grade, Atypia

## Introduction

Gliomas are common malignancies of the central nervous system (CNS).<sup>[1, 2]</sup> Scholzer and Gerdes discovered the Ki-67 antigen in the early 1980s, which expresses two protein variants with molecular weights of 345 and 395 kDa.<sup>[3]</sup> It is present at all active cell cycle phases (G1, S, G2, and M) but absent in resting cells (G0).<sup>[4, 5]</sup> In the later stages of mitosis significant decrease in Ki-67 (during anaphase and telophase) levels takes place.<sup>[6]</sup> The Ki-67 protein (pKi-67) expression has been linked to the proliferation of intrinsic cell components in malignant tumors enabling its use as a measure of tumor aggressiveness.<sup>[7, 8]</sup>

The cell cycle's advancement, changes in cellular distribution, interphase, and process of cell mitosis are all regulated by the expression of Ki-67.<sup>[9]</sup> Ki-67 is utilized in diffuse astrocytic tumors as a biomarker of tumor cell proliferation that forecasts the biological characteristics of the tumor and the patient's prognosis.<sup>[10, 11]</sup> Low-grade astrocytomas have a considerably different mean Ki-67 LI compared to anaplastic astrocytomas and glioblastoma.<sup>[12]</sup>

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Glial cell proliferation is the foundation of gliomagenesis.<sup>[13, 14]</sup> It is well established that histological grading can be used to assess a patient prognosis in CNS neoplasm but histological differentiation in glioma may not always be evident, particularly when tiny tissue pieces from stereotactic guided needle biopsies are available.<sup>[15, 16]</sup> Proliferation indices have thus been utilized for prognostic purposes in both disease progression and patient survival.<sup>[17, 18]</sup> The MIB-1 antibody recognizes the Ki-67 antigen in formalin-treated paraffin-embedded tissue.<sup>[3, 19]</sup> Classification and prognosis of astrocytomas have been determined using the MIB-1 antibody because in order to predict tumor behavior, it is crucial to develop better prognostic markers.<sup>[20, 21]</sup>

Evaluation of the tumor's proliferative index (Ki-67) is one such complementary method.<sup>[22]</sup> Among the many useful biological markers available, the proliferative index provides a quantitative measure of tumor proliferation.<sup>[23]</sup> Several techniques have been used to calculate the

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proliferative index of malignancies in the CNS. In this group, the Ki-67 labeling index stands out as one of the most effective techniques for many human malignancies, particularly brain neoplasm. Numerous studies have demonstrated the value of Ki-67 LI in assessing cell proliferation.<sup>[24]</sup>

## Materials and Methods

The study was conducted in the Department of Pathology, over two years between September 2020 to August 2022 after approval from the Institute Review Board as well as Institute Ethical Committee, as per the Declaration of Helsinki. Before, the initiation of the study, informed consent was taken from all the participants whose biopsies were considered for the study. Formalin-fixed paraffin-embedded (FFPE) blocks of histopathologically proven cases of glial tumors were included both prospectively and retrospectively from the archives. Inadequate and poorly-fixed tissues were excluded from the study. After taking informed consent, detailed clinical and radiological information was documented.

Consecutive H&E sections slides were reviewed for histomorphological features including-histological type and grade of tumors, degree of cellularity, atypia, mitosis, presence of necrosis, and microvascular proliferation. Grade I and Grade II tumors were referred to as low-grade tumors, while Grade III and Grade IV were tumors referred to as high-grade tumors. The corresponding sections from paraffin block with a viable tumor in highly cellular areas (including necrosis if present) were chosen for IHC.

## Immunohistochemical analysis

IHC for Ki-67 was performed on deparaffinized sections taken on albumin-coated slides using two-step indirect methods. Prediluted ready-to-use Ki-67 monoclonal antibody CAT-P-K001-30 (Clone QR015, Berlin, Germany) was used. Placenta tissue was taken as a positive control. For negative control, the same sections were used by omitting the primary antibody step. The observations were taken independently by two pathologists blinded to the clinical details. The discrepant cases were reviewed and evaluated over a multiheaded microscope with an expert for consensus observations.

## Evaluation of Ki-67

A hot spot (area with the highest density of immunostained nuclei) was selected and adjacent fields were counted to include 1000 nuclei. Distinct nuclear staining of tumor cells was recorded as positive. Ki-67 labelling index was recorded as a 'percentage' of positively stained nuclei in 1000 tumor cells. The Ki-67 cut-off value given by WHO for glioma is less than 4% for low-grade gliomas and more than or equal to 5% for high-grade gliomas. We categorically divided Ki-67 expression into low and high expression taking 4% as cut off.

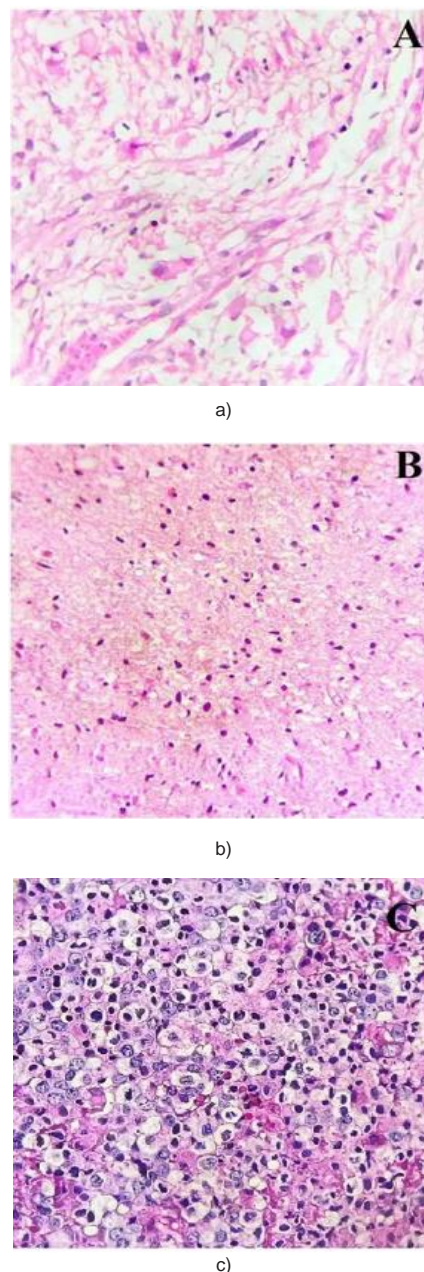
## Statistical analysis

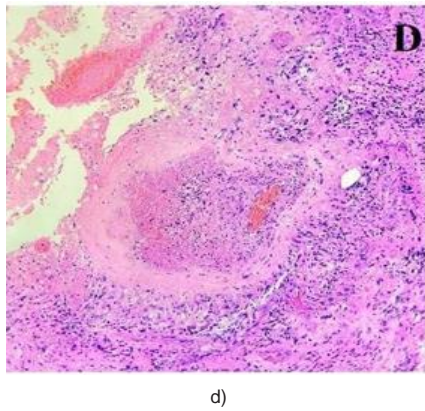
For statistical analysis, data were entered into a Microsoft Excel spreadsheet in 2010. Quantitative data were expressed as range, mean±SD, or median. The categorical variables were analyzed using Pearson's chi-square test or Fisher's exact test.

Statistical analysis was carried out using Microsoft Excel spreadsheet 2010, GraphPad QuickCalcs ©2021 GraphPad Software, and Social Science Statistics©2021 Jeremy Stangroom. A p-value of  $\leq 0.05$  was taken as statistically significant.

## Results and Discussion

A total of 43 patients were included with a Mean age of 46.14y with M:F=2.5:1. Tumours most frequently involved the frontal lobe (12/43) with 15low-grade gliomas comprising pilocytic and diffuse astrocytoma (11/43) 25% (**Figure 1**).





d)

Figure 1. Showing different grades of glial tumors 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), d) Grade IV-Glioblastoma (H&E, 100X)

**Ki-67 expression in glioma**

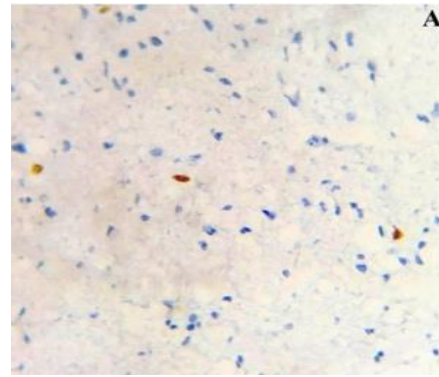
The Ki-67 LI was found to be in 95.3% of cases ranging from 0-80% (Mean: 22.65±24.28%) with strong nuclear positivity. Low Ki-67(<4%) was seen in 14 out of 43 cases (32.6%). The correlation of Ki-67 with the clinicopathological parameters is summarized in **Table 1**.

Ki-67 LI showed a statistically significant relationship with age, histomorphological grade, presence of cellular atypia, necrosis, and microvascular proliferation (**Table 1**). Higher Ki-67 LI was associated with higher age of the patient, higher histologic grade, and a greater degree of cellular atypia (**Figure 2**).

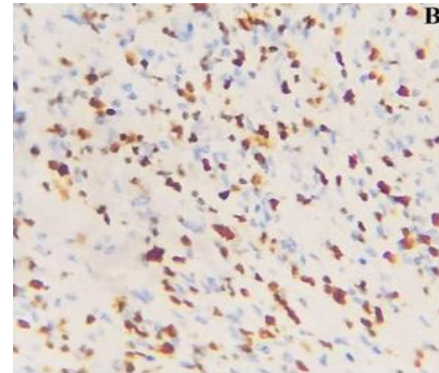
**Table 1. Correlation of PD-L1 and Ki-67 with clinicopathological parameters**

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

\*MVP=Microvascular proliferation.



a)



b)

Figure 2. Ki-67LI showing low (<4%) nuclear expression, a) in a case of low-grade glial tumor (Pilocytic Astrocytoma, WHO Grade I) while a case of glioblastoma (WHO grade IV)shows higher (>4%) nuclear staining in b) (IHC for Ki-67, A,B:100X)

Ki-67 is a biological marker that is used for grading astrocytoma. It estimates the growth of neoplasm quantitatively as well as predicts the progress accurately. Through qualitative and quantitative estimation of mitosis is important to know the proliferative rate of all tumors but in CNS tumors the mitotic rate is very less and difficult to quantify accurately also. A variety of methods have been used to know the proliferative rate in CNS tumors. Out of these, the most widely used is based on immunohistochemistry with Ki-67. Ki-67 has been established to be a standalone indicator of tumor development and prognosis in glioma patients.<sup>[25, 26]</sup> HGG (WHO grade III/IV) has a much higher Ki-67 labeling index than Low-grade glioma (LGG) (WHO grade I/II).<sup>[27]</sup> Therefore, although histological grade and clinical characteristics are significant prognostic factors in astrocytomas, Ki-67 LI can be utilized in addition to histopathological diagnosis when clinical characteristics and histomorphological grade do not correlate with one another.<sup>[10]</sup>

Skjulsvik *et al.* in 2014 studied KI-67 immunostaining in a cohort study of glioma in 267 cases. They observed that Ki-67 expression for high-grade gliomas (grade III/IV) was significantly higher than in low-grade (grade I/II) tumors.<sup>[26]</sup> Authors who studied the role of immunohistochemical markers p53 and Ki-67 in the grading of glial tumors in a statistically significant rise in Ki67 with grade.<sup>[28]</sup> Hu *et al.* in 2013 studied the expression of p53, epidermal growth factor receptor, Ki-67, and O6-methylguanine-DNA methyltransferase in human gliomas and also found that the



rate of cell proliferation (Ki-67) increased significantly with the gliomas grade. A statistically significant difference was observed among all WHO grades.<sup>[29]</sup> Thotakura *et al.* in 2014 studied the role of the Ki-67 labeling index as an adjunct to the histopathological diagnosis and grading of astrocytomas in cases of astrocytoma and found that mean Ki-67 LI in Grade I astrocytomas was  $3.36 \pm 4.59$  standard deviation (SD),  $7.05 \pm 4.16$  SD in Grade II astrocytomas,  $28.24 \pm 6.23$  SD in Grade III astrocytomas and  $38.7 \pm 7.19$  SD in Grade IV astrocytomas. P values were significant between all grades of astrocytomas except between Grade I and Grade II tumors.<sup>[10]</sup>

Prior studies have shown that the protein Ki-67 is greatly expressed in glioma cells, however, not in normal brain tissue, therefore, the degree of positive expression increases as the tumor develops.<sup>[30]</sup> In addition to complementing postoperative pathology in the prediction of tumor biological behavior, the identification of the Ki-67 antigen may be utilized to determine the grade of glioma.<sup>[31]</sup> Ki-67 is seen in dividing cells and offers an indicator of cellular replication.<sup>[30, 32]</sup> Tumor progression is facilitated by stem cells which is a part of the epithelial-mesenchymal transition (EMT).<sup>[33]</sup> Ki 67 expression has been documented in stem cells further reinforcing the same.<sup>[34]</sup> Studies have highlighted that higher Ki-67 in gliomas increases the severity of malignancy. It helps in grading as well as prognostication. Also, solid malignancies are accompanied by chronic inflammation and raised CRP which is associated with high lymphocyte levels.<sup>[35]</sup> High Ki-67 levels are associated with poor prognosis and may respond to immunotherapeutic intervention.<sup>[3]</sup> Ki-67 is seen in dividing cells and offers an indicator of proliferation.<sup>[30, 36]</sup> Studies have highlighted higher Ki-67 in the gliomas increases the severity of malignancy. Prognostic markers are of relevance for glioblastoma, particularly for survival. Few authors who have studied Ki-67 LI in gliomas have found Ki-67 to be associated with an increase in grade, but they did not show a significant association with isocitrate dehydrogenase (IDH) or with survival.<sup>[37]</sup> However, few authors have correlated the Ki-67 LI with the volume of the edema zone in radiology.<sup>[38]</sup>

Morphologic grading of gliomas is fraught with limitations especially due to heterogeneity leading to sampling bias in small biopsy specimens. Ki-67 LI offers a more unified grade for the assessment. Though the Ki-67 LI may also be high in cases of reactive gliosis, p53 expression in conjunction with morphological parameters and IDH mutation studies can help in arriving at a definitive diagnosis.<sup>[38]</sup> Several pre- and analytical factors affect the grading of gliomas ranging from technical factors like the tissue and paraffin block quality to the IHC procedure and interpretation. There are no unified guidelines available for the Ki-67 LI assessment and there are various methods for it. Theresia *et al.* who used a method similar to the present study have highlighted the low inter-observer variability while counting 1000 cells.<sup>[13]</sup> The authors also mention that though the common practice is to use the manual hotspot technique, as done in the present study, the average of the entire slide is a better approach. The reason for the same as discussed is the lack of certainty that the actual hotspot exists in the supplied tissue. The present study holds

merit in a reliable and reproducible method of Ki-67 LI and is limited by the lack of survival data and a low number of cases per category for statistical analysis.

## Conclusion

Gliomas, unlike breast cancer, have no screening methods for early diagnosis and treatment.<sup>[39]</sup> It can be concluded from the present study that the Ki-67 Proliferation index correlates significantly with higher age, higher histological grade, and a greater degree of cellular atypia. Clinical parameters and histopathological grade are important prognostic indicators, however, Ki-67 can be used as an adjunct to histopathological diagnosis where there is discordant among clinical parameters and histopathological grade, especially in small biopsies.

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None.

## Conflict of interest

None.

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None.

## Ethics statement

The study was approved by the Institutional Ethics Committee (IEC) and informed consent was taken from all participants (IEC 493 /2020).

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