DOI: 10.7860/NJLM/2021/46719:2548

Original Article

Pathology Section

Determination and Comparison of Ki-67 Index in Astrocytic Tumours: A Tertiary Care Centre Experience

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ABSTRACT

Introduction: The proper management of the astrocytic tumours largely depends on its correct diagnosis. But, smaller size and complicated histomorphology makes it difficult for the histopathologist to conclude the diagnosis. Monoclonal antibody Ki-67 {MIB-1/MIB-1 Labelling Index (LI)} plays a role of diagnostic as well as prognostic marker. It is an important marker, since it helps in deciding malignant potential of the astrocytic tumours where histology alone does not suffice. This study promotes the idea of including Ki-67 in routine practice for astrocytic tumours as it helps in quantifying the growth of the tumour which is of utmost importance in predicting the outcome accurately.

Aim: The aim of this study was to determine the mean and ranges of Ki-67/MIB-1 LI in astrocytic tumours and comparing between different grades.

Materials and Methods: This was a hospital based cross-sectional study with 50 cases of astrocytoma of varying grades over period of six years, retrospective study period started from 1st April 2009 till 30th November 2012 and prospective study period started from 1st December 2012 till 31st March 2014. Immunolabeling was done using Ki-67/MIB-1 antibody. The mean and ranges of Ki-67 was calculated in astrocytic tumours and its correlation with each World Health Organization (WHO) grade of histological diagnosis and clinical presentations were studied. One-way Analysis of Variance (ANOVA) and unpaired t-test were used for statistical analysis.

Results: There were 50 astrocytic tumours, Pilocytic Astrocytoma (PA), grade I-5 (10%) cases; Diffuse Astrocytoma (DA), grade II-8 (16%) cases; Anaplastic Astrocytoma (AA), grade III-10 (20%) cases; and Glioblastoma Multiforme (GBM), grade IV-27 (54%) cases, with mean Ki-67 LI as 2.63%, 2.65%, 18.85% and 30.2%, respectively. The difference in mean Ki-67 LI for low grade astrocytoma and high grade astrocytoma was highly significant statistically ($p<0.0001$). The most common presentation was seizure, which was seen in 26 (52%) cases.

Conclusion: Histological grading with Ki-67 LI can work synergistically to reach the diagnosis, as both are subjected to heterogeneity induced diagnostic accuracy. Ki-67 LI increases with increase in the grade of the astrocytic tumours and hence, its quantification can help histopathologists as well as clinicians to agree on a point, especially in those cases where two differ diagnostically and it effects patients' prognosis.

Keywords: Brain tumours, Grading, Immuohistochemistry, Proliferative index

INTRODUCTION

The most common brain tumours are astrocytomas. For managing the patients properly, it is pertinent to accurately diagnose and grade the tumour [1,2]. There are two main reasons for grading the astrocytic tumours; one that grade helps in predicting the clinical behaviour of the tumour and second, grading method must minimise the inter-observer variation so as to have the maximum reproducibility [3]. It was in the year 1979 that famous neuropathologist; Zulch KJ presented the most widely acceptable classification, World Health Organisation classification [4]. It was later updated in 2007 and lately in 2016 [1,2]. These updates were made mainly because of better understanding of the histomorphology of the different grades of the astrocytoma and enormous development in technical field which provided us with new techniques like Immunohistochemistry (IHC), molecular genetic methods etc., [1,2].

Although Hematoxylin and Eosin (H&E) stained histomorphology is the gold standard method for diagnosing and grading the astrocytomas, but some inevitable reporting errors which might be due to missing tumour behaviour defining microscopic features, regional heterogeneity and inter-observer variation leads to incorrect classification [5]. Also, identifying the mitotic figures on H&E is neither reliable nor reproducible method and is sometimes really difficult to differentiate mitosis from karyorrhectic debris, largely depending on the diligence of the histopathologist [6]. All these lacunae can be filled with upcoming new technologies like IHC which can solve this problem and thus evaluate these tumours. One of these IHC markers is proliferative index marker i.e., Ki-67/MIB-1LI, which can be used as a standard supplement in laboratories [2,5,7,8]. The main objective of this study was to standardise Ki-67/MIB-1 LI to be used in conjunction with histological grading of the tumour, so as to reach the correct diagnosis.

MATERIALS AND METHODS

The present study was retrospective cross-sectional hospital based study, conducted in Department of Pathology and included 50 cases, diagnosed as astrocytic tumours, over a period of six years. This time span included three years eight months of retrospective study and two years four months of prospective study. The retrospective study period started from 1st April 2009 till 30th November 2012 and prospective study period started from 1st December 2012 till 31st March 2014.

Inclusion criteria: Cases which were diagnosed as astrocytic tumour in the Department of Pathology, whose slides and paraffin blocks were available had adequate and satisfactory material for immunostaining were included in the study.

Exclusion criteria: Non astrocytic tumours, inadequate tumour material in paraffin blocks were excluded from the study.

All the information regarding the demographics, age, gender and clinical features were noted. Findings of Magnetic Resonance Imaging (MRI), surgical procedure and operative findings were recorded.

Processing of the Brain Biopsies

One paraffin block containing tumour with characteristic histological features for each type of astrocytoma was selected, sectioned at

5µ thickness, stained with H&E stain and 5 µ unstained sections were cut for use in the immunostaining [9]. The PA and DA were grouped together as low grade astrocytoma. The AA and GBM were grouped together as high grade astrocytoma [10].

Immunohistochemical staining was done by "Novocastra Novolink Polymer Detection System" supplied by Leica Biosystems Newcastle Ltd., UK. Paraffin blocks for all cases were retrieved and IHC for Ki-67/MIB-1 LI formed by the standard procedure [11]. Tonsils were taken as positive control and sections without primary antibody was used as negative control. Nuclei showing obvious brownish staining were considered positive.

Study Procedure

All the immunostained sections of Ki-67 in each grade were scanned randomly at 100X magnification by routine microscope for areas of tissue where most dense positively stained nuclei were evenly distributed. Where an uneven distribution of Immunolabelling was seen, fields from areas of maximal labelling were chosen for counting, as choosing areas at random underestimates the growth fraction by combining active and quiescent areas [12]. The total number of nuclei in each field was determined using the method of Going JJ [13]. Positively stained brown granular Ki-67 tumour cell nuclei were counted in a high power field (400X) in a systematic manner using an ocular grid. Care was taken not to repeat tumour cell nuclei while counting. 1000 tumour cells were counted in each case as was done in various studies [7,8]. Percentage of reactive nuclei for Ki-67 LI was calculated as, number of positive cells divided by the estimated number of total cells (1000 in present study) multiplied by 100. Cells' nuclei which were positive for Ki-67 were expressed as a percentage of total number of cells. Range and mean±SD were noted and calculated respectively for each grade. Vascular endothelial cells, lymphocytic cells, and necrotic areas were excluded from the counts. Therefore, areas which were free from necrosis and endothelial proliferation were chosen for LI of GBM.The infiltrative edge of the tumour where tumour cells surround normal neurons and glia were also avoided.

STATISTICAL ANALYSIS

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software version 16 and Microsoft Excel 2010 programs. For all categorical variables like sex, morphology of tumour, frequencies and percentages were calculated. Mean, median and standard deviation were estimated for quantitative variables like age and Ki-67 LI. For comparison of the mean Ki-67 LI values in low grade astrocytoma, AA and GBM, unpaired t-test was applied and p-value was calculated. For the overall correlation between histological grade and Ki-67 LI value, One-way ANOVA was applied and p-value was calculated. Values were considered statistically significant when p-value was less than 0.05.

RESULTS

Fifty astrocytic tumours included in the study were classified histologically according to the WHO classification, 2007 [2] tumours of the central nervous system into PA, grade I-5 cases; DA, grade II-8 cases; AA, grade III-10 cases; and GBM, grade IV-27 cases. The youngest patient was two-year-old boy while the oldest was 75-year-old man. The mean age was 43.4±17.7 years. Of these, majority, 39 (78%) cases were between fourth to eight decades of life. The demographic data related to all four grades is shown in [Table/Fig-1]. The most common presentation was seizure, seen in 26 (52%) cases, alongwith following as other common clinical manifestations [Table/Fig-2].

MRI Findings

In all the cases of PA, a well-defined lesion ranging from 4.5 to 7 cm in largest dimension was seen. All of these showed a cystic component, of which 2 (40%) in addition showed solid component and 3 (60%) showed a well enhanced mural nodule in the cystic component. In all grades of astrocytoma ranging from grade II to grade IV, an ill-defined lesion was noted in MRI. The lesion showed hyperintense signals in T2 weighted sequence, hypointense on T1 weighted sequence. On postcontrast study, enhancement was observed in a single case of DA, all cases of AA and GBM. Ring enhancement was seen in 17 (63%) cases of GBM. In all 50 cases of astrocytoma included in the present study, craniotomy followed by near total excision of the lesion was done.

Histopathology

Gross findings: In 46 (92%) cases, the specimen was received as multiple greyish white to greyish brown to dark brown tissue pieces which were soft in consistency. In all the 5 (10%) cases of PA, occasional cystic areas were evident among the tissue pieces. In 24 (48%) cases of suspected GBM, friable and necrotic soft tissue

	PA (n=5)				DA (n=8)				AA (n=10)				GBM (n=27)			
			Sex				Sex				Sex				Sex	
Age range (year)	n	%	M	F	n	%	M	F	n	%	M	F	n	%	M	F
1-10	3	60	3	-	-	-	-	-	-	-	-	-	-	-	-	-
11-20	1	20	-	1	1	12.5	-	1	-	-	-	-	2	7.5	1	1
21-30	1	20	-	1	2	25	2	-	1	10	-	1	-	-	-	-
31-40	-	-	-	-	4	50	2	2	-	-	-	-	6	22.2	5	1
41-50	-	-	-	-	-	-	-	-	4	40	2	2	6	22.2	4	2
51-60	-	-	-	-	-	-	-	-	3	30	2	1	6	22.2	4	2
61-70	-	-	-	-	-	-	-	-	2	20	-	2	6	22.2	4	2
71-80	-	-	-	-	1	12.5	1	-	-	-	-	-	1	3.7	1	-
Total	5	100	3	2	8	100	5	3	10	100	4	6	27	100	19	8

Names	Mean age with SD	Ratios
Low grade astrocytoma:	**27.9±19.3 years**	**1.6:1**
Pilocytic astrocytoma:	12±9.9 years	1.5:1
Diffuse Astrocytoma:	37.8±17.1 years	1.7:1
High grade astrocytoma:	**49.4±13.3 years**	**1.6:1**
Anaplastic astrocytoma:	51.1±9.29 years	0.7:1
Glioblastoma multiforme:	51.1±9.29 years	2.4:1

[Table/Fig-1]: Histopathological diagnosis, age and sex distribution in different grades of astrocytic tumours.
AA: Anaplastic astrocytoma; GBM: Glioblastoma multiforme; PA: Pilocytic astrocytoma; DA: Diffuse astrocytoma; M: Male; F: Female; SD: Standard deviation

Symptoms	PA	DA	AA	GBM
Headache	5 (100%) (5d-1yr)	5 (63%) (5d-8)	3 (30%) (7d-1 yr)	16 (59%) (7d-2 yr)
Seizures	2 (40%) (6 m)	7 (88%) (5d-15 yr)	3 (30%) (2d-1 yr)	14 (52%) (1d-2 yr)
Weakness	-	-	1 (10%) (Lt side)	4 (15%) (15d)
Vision abn	Diplopia (20%) 1 (10d)	-	Vision loss: 1 (10%)	Haemianopia: 2 (7%)
Speech abn	-	Aphasia: 1 (13%)	Slurred speech: 2 (20%)	Slurred speech: 2 (7%); Aphasia: 1 (4%)
Others	Vomiting: 1 (20%) (↑ICT)	-	Urinary Incontinence:1 (10%), Vomiting: 2 (20%) (↑ICT)	Vomiting: 6 (22%)

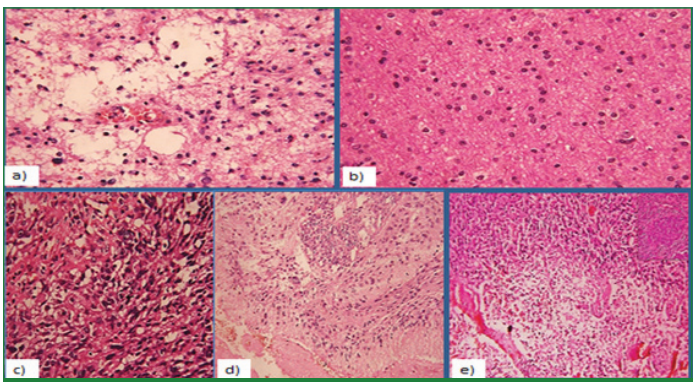
[Table/Fig-2]: Clinical features of different grades of astrocytomas.
*d: Days; yr: Year; m: Months; abn: Abnormality; AA: Anaplastic astrocytoma; GBM: Glioblastoma multiforme; PA: Pilocytic astrocytoma; DA: Diffuse astrocytoma; Lt: Left; ICT: Intra-cranial tension

pieces were also seen. The size of the tissue pieces received ranged from 9.5×7×0.3 cm to 1×0.8×0.4 cm depending on the size of the lesion removed.

There were four main histologic features namely mitosis, nuclear atypia, endothelial proliferation and necrosis along with morphology that formed the basis of WHO system of classification 2007 and 2016 [1,2]. In 2016, WHO defined grade I as those tumours not having any of these, grade II are those which have one criterion i.e., only cytological atypia, grade III are those having two features i.e., anaplasia and mitosis and grade IV as those which have three or four features i.e., anaplasia, mitosis, microvascular proliferation and necrosis [1]. Microscopically, tumours were graded according to this [Table/Fig-3,4].

Microscopy	PA (n=5)	DA (n=8)	AA (n=10)	GBM (n=27)
Cellularity				
Low	5	1	-	-
Moderate	-	7	2	5
Marked	-	-	8	22
Nuclear atypia				
Mild	04	5	-	-
Moderate	01	3	5	20
Marked	-	-	5	7
Mitosis	Absent	Absent	Frequent	Brisk
Necrosis	Not seen	Not seen	Not seen	Seen
Tumour necrosis	-	-	-	12
Palisadingnecrosis	-	-	-	15
Microvascular proliferation	Not seen	Not seen	Not seen	26

[Table/Fig-3]: Comparison of microscopic features in different grades of astrocytoma.
AA: Anaplastic astrocytoma; GBM: Glioblastoma multiforme; PA: Pilocytic astrocytoma; DA: Diffuse astrocytoma

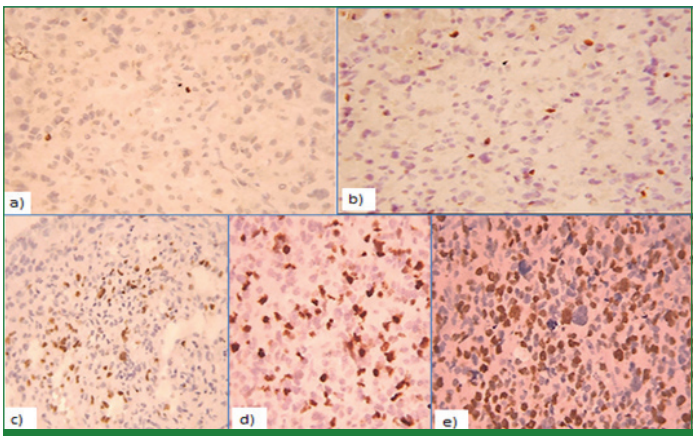


[Table/Fig-4]: a) Pilocytic astrocytoma showing microcystic changes H&E (400X); b) Diffuse Fibrillary Astrocytoma showing cytological atypia H&E (400X); c) Anaplastic astrocytoma with increased cellularity, marked cytological atypia, increased mitosis H&E (400X); d) GBM showing microvascular proliferation and necrosis H&E (400X); e) GBM showing pseudopalisadin necrosis and microvascular proliferation H&E (400X).

The Ki-67 labelling index and grade of astrocytoma: The immunoreactivity for Ki-67 was strong, distinct and easy to count. The immunoreactive nuclei were identified. Positive nuclei showed granular staining pattern [Table/Fig-5,6]. An increasing mean Ki-67/MIB-1 LI was found across the range of astrocytomas from low grade astrocytoma, AA and GBM. Low grade astrocytomas showed low counts of Ki-67 positively labelled tumour cells whereas high grade astrocytoma contained numerous labelled cells. The comparison is made among different groups which came out to be significant in many groups [Table/Fig-7].

Tumour grade	Mean±SD (%)	Median (%)	Range	95% confidence intervals
Low grade (n=13)	2.64±1.42	2.2	0.8-5.5	-5.89-11.18
PA (n=5)	2.63±1.33	3	0.8-4	
DA (n=8)	2.65±1.57	2.6	0.9-5.5	
High grade (n=37)	27.1±17.7	20	5-70	22.08-32.19
AA (n=10)	18.85±12.35	16.5	5-45	10.01-27.69
GBM (n=27)	30.20±18.50	26	8-70	22.88-37.52

[Table/Fig-5]: Comparison of Ki-67/MIB-1 LI in different grades of astrocytoma.
AA: Anaplastic astrocytoma; GBM: Glioblastoma Multiforme; PA: Pilocytic astrocytoma; DA: Diffuse astrocytoma; SD: Standard deviation



[Table/Fig-6]: a)Pilocytic astrocytoma showing MIB-1 L1 0.8% (400X); b) Diffuse Fibrillary astrocytoma showing MIB-1 L1 2%(400X); c) Anaplastic astrocytoma showing MIB-1 L1 8% (400X); d) Anaplastic astrocytoma showing MIB-1 L1 55% (400X); e) GBM showing MIB-1 L1 70% (400X).
ki 67/ mib li; (a,b,c,e- ihc DAB; d- haematoxylin)

Comparison group	Mean±SD (%)	p-values
Low grade astrocytoma	2.64±1.42	<0.0001
High grade astrocytoma	27.1±17.7	
Low grade astrocytoma	2.64±1.42	0.0024
AA	18.85±12.35	
Low grade astrocytoma	2.64±1.42	0.0001
GBM	30.20±18.50	
PA	2.63±1.33	0.002
AA	18.85±12.35	
PA	2.63±1.33	<0.001
GBM	30.20±18.50	
PA	2.63±1.33	0.98
DA	2.65±1.57	
DA	2.65±1.57	0.002
AA	18.85±12.35	
DA	2.65±1.57	0.001
GBM	30.20±18.50	
AA	18.85±12.35	0.042
GBM	30.20±18.50	

[Table/Fig-7]: Comparison of Ki-67 labelling index between different grades.
For comparison of the mean Ki-67 LI values in low grade astrocytoma, anaplastic astrocytoma and glioblastoma multiforme, unpaired "t" test was applied and p-value calculated.
AA: Anaplastic astrocytoma; GBM: Glioblastoma multiforme; PA: Pilocytic astrocytoma; DA: Diffuse astrocytoma; SD: Standard deviation

It was seen that as age increased Ki-67 LI also increases. There was a significant correlation between the age of cases studied and Ki-67 LI (p=0.02). Increase of Ki-67 with age signifies that greater grade tumour is common in older age and lower grade tumours are common in young age. The difference in the mean MIB-1 LI between male and female was not significant statistically (p=0.760).

DISCUSSION

Histological grading criteria of astrocytomas must be sufficiently objective and defined to minimise variation among observers and to maximise reproducibility [14]. It had been demonstrated that tumour proliferative index derived from Ki-67 immunostaining was useful in differentiating between low grade and AA wherever morphologic criteria are not accurate [15]. But, it was seen that there was a considerable overlap of Ki-67 LI among tumour grades. Therefore, low Ki-67 LI alone is neither specific nor predictive of low grade astrocytoma. So Ki-67 LI cannot be used alone diagnostically to exclude a high grade astrocytoma. All the cases were studied and graded using the World Health Organisation criteria published in 2007 and 2016 [1,2]. The findings of present study were similar to many studies, as shown in [Table/Fig-8] [8,15-25]. The age of patients in the present study ranged from 2-75 years with a mean of 43.8±17.7 years which was similar to study by Neder L et al., [19]. PA was predominately a childhood tumour with mean age of 12±9.9 years. This was in keeping with findings of studies done by Ambroise MM et al., Tihan T et al., and Ralte AM et al., [8,16,17]. More malignant astrocytomas or high grade astrocytomas (Grade III and IV) occurred at older age. The present study included 31 males and 19 females with a male to female ratio of 1.6:1. Preponderance of men matched the findings of some previous studies as shown in following table [Table/Fig-8].

Study	No of cases	Mean age (Years)	M:F ratio	PA	AII	AA	GBM
Tihan T et al., 2000 [16]	50	-	1:1	11	8	15	16
Ralte AM et al., 2001 [17]	64	-	-	8	30	11	15
Torp SH, 2002 [18]	41	-		-	22	10	9
Neder L et al., 2004 [19]	40	42.7	2.1:1	-	10	5	25
Rathi KR et al., 2007 [15]	90	-	-	-	30	30	30
Arshad H et al., 2010 [20]	28	-	-	3	1	2	22
Ambroise MM et al., 2011 [8]	145	-	-	23	34	21	67
Chaloob MK et al., 2012 [21]	51	35.9	1.1:1	7	22	6	16
Abd El Atti RM et al., 2013 [22]	111	48.5	2:1	-	28	38	45
Shivaprasad NV et al., 2016 [23]	30	48.8	1.7:1	1	7	6	16
Stoyanov GS et al., 2017 [24]	47	-	-	-	2	4	41
Das B et al., 2018 [25]	40	-	2:1	3	13	9	15
Present study	50	43.8	1.6:1	5	8	10	27

[Table/Fig-8]: Comparison of case distribution, age, male:female (M:F) ratio of astrocytic tumours in various studies [8,15-25].
AA: Anaplastic astrocytoma; GBM: Glioblastoma multiforme; PA: Pilocytic astrocytoma; DA: Diffuse astrocytoma

Seizure was seen in 26 (52%) in the present study. Ranjan M et al., found seizure as the most common symptom, seen in 62% of their patients [26] whereas Das B et al., had observed headache as most common symptom (50% cases) [25]. The macroscopic findings were replaced by radiological observations. Location-supratentorial, infratentorial or intra-ventricular, pattern of growth-infiltrative or circumscribed, pattern of enhancement-enhancing or non enhancing, presence or absence of calcification, edema and necrosis should all be integrated with histological findings in reaching the correct diagnosis [27]. Contrast enhancement in MRI imaging was one of the criteria that are significantly associated with tumour grade. In the present study, enhancement was seen in a single case of DA, six cases of AA and all the cases of GBM. It was seen contrast enhancement in MRI was associated with high tumour grade [28]. The use of proliferative markers such as Ki-67/MIB-1 LI was an objective measure of proliferative activity and growth potential and was capable of distinguishing tumours of borderline cases especially between grade II and III astrocytoma [2]. Mean Ki-67 LI was significantly lower in low grade astrocytoma (PA+DA) than in the high grade astrocytoma (AA+GBM). Therefore, the association between high Ki-67 LI and higher tumour grade suggested that Ki-67 LI was useful in detecting aggressive astrocytoma, lacking defining histopathological features [6].

In the present study, statistically significant differences in mean Ki-67 LI was seen between low grade (PA and DA) and high grade astrocytoma (AA and GBM) and also grade II and III; grade II and IV and grade III and IV as shown in [Table/Fig-8]. Most of the studies showed statistically significant differences in Ki-67 LI between high grade (grade III and IV) and low-grade (grade I and II) astrocytoma [8,15-21,23]. Studies identified significant differences in mean Ki-67 LI when comparing grade II with grade III and grade II with grade IV, but not when comparing the differences between grade III and IV [17-19]. In contrast, other reports indicated statistically significant differences in Ki-67 LI between grade III and IV tumours as well [15]. The present cases of PA showed low proliferative activity as assessed by Ki-67 LI with Ki-67 LI <5%. The difference in the mean Ki-67 LI of PA and DA was not significant statistically (p=0.98). However, the mean Ki-67 LI of PA was significantly lower when compared to that of AA (p=0.002) and GBMs (p<0.001). Relatively few studies have analysed Ki-67 LI in PA compared to other three grades (DA, AA and GBM) [8,16,17,21]. Studies have shown significant difference in the mean Ki-67/MIB-1 LI between PA and DA [8,17]. In the present study, only a few scattered Ki-67 positive nuclei were noted in most of the low grade astrocytoma, corresponding to low Ki-67LI values (<5% in most of the cases). In a single case of DA, MIB-1 LI was 5.5%. Higher values of MIB-1 LI in grade II astrocytoma have also been reported in study of Stoyanov GS et al., [24]. In the present study there was significant correlation between the age and the Ki-67 LI (p<0.05). This result is supported by Chaloob MK et al., [21]. However, there was no significant correlation between gender and Ki-67 LI (p>0.05). This result was in agreement with Ambroise MM et al., [8]. The problem in the diagnostic value of Ki-67 LI was that the range of Ki-67 LI overlapped considerably among tumour grades as seen in present study as well as in other studies [Table/Fig-9] [8,15-19,21-25,29]. In the present study, mean Ki-67 LI ranged

Studies	Mean (range) (in years)			
	PA	DA	AA	GBM
Tihan T et al., 2000 [16]	1.83±2.1	3.7±3.9	11.4±15.0	20.2±14.5
Nagamachi S et al., 2001 [29]	-	6 (0-1.8)	5 (0.9-30)	13 (3-37)
Ralte AM et al., 2001 [17]	0.44 (0.1-2.3)	3.73 (0.2-11.8)	9.65 (0.5-19.6)	10.33 (0.4-23.5)
Torp SH, 2002 [18]	-	Median 2.7	Median 13.9	Median 12.1
Neder L et al., 2004 [19]	-	2.35±3 (0-7.4)	6.44±2.7 (3.1-9.8)	12.28±8.2 (5.7-42.7)
Rathi KR et al., 2007 [15]	-	1.75(0.1-7.2)	8.74(2.5-26)	20.54(5-45.2)
Ambroise MM et al., 2011 [8]	3.78±4.03 (0.3-18.2)	2.76±2.17 (0.2-9)	7.45±6.3 (0.5-22)	13.85-12.6 (1.2-59)
Chaloob MK et al., 2012 [21]	1.51	6.42	23.13	38.45

Abd El Atti RM et al., 2013 [22]	-	4.26±2.43	13.54±2.82	26.43±5.18
Shivaprasad NV et al., 2016 [23]	0.02	0.81	9.14	17.81
Stoyanov GS et al., 2017 [24]	-	25	4±2.16	17.34±10.79
Das B et al., 2018 [25]	4.66 (4-5)	8.07 (5-12)	13.5 (8-20)	22.93 (15-50)
Present study	2.63±1.33 (0.8-4)	2.65±1.57 (0.9-5.5)	18.85±12.35 (5-45)	30.2±18.5 (8-70)

[Table/Fig-9]: Mean (range) of Ki-67/MIB-1 LI in astrocytomas of varying grades in various studies [8,15-19,21-25,29].

from 0.8-4% in grade I, 0.9-5.5% in grade II, 5-45% in grade III and 8-70% in grade IV. Therefore, low Ki-67 LI are neither specific nor predictive of low grade astrocytoma and so, could not be used diagnostically to exclude a high grade astrocytoma. A low Ki-67 LI value in high grade astrocytoma could also result from faulty tissue sampling and tumour heterogeneity [8]. Absolute values of Ki-67 LI cannot be used between different laboratories. To use Ki-67 LI in diagnostic histopathology standardisation of staining protocols and counting procedures in one's own laboratory as well as in depth study of variability of MIB-1 LI among astrocytoma of different grades were required.

Limitation(s)

Lesser number of cases was one of the main limitation of the study, along with short follow-up and partly retrospective nature of the study as another limitations.

CONCLUSION(S)

Conventional histological assessment of astrocytoma was, is and most probably will be the basis of grading and classifying majority of cases. The use of proliferative markers such as Ki-67/MIB-1 is an objective measure of proliferative activity and growth potential and is capable of distinguishing tumours of borderline cases, especially between grade II and III astrocytoma. It is of importance when histopathology reveals a low grade astrocytoma and other factors including clinical status, neuroimaging indicate a more malignant neoplasm. Thus, both histological grading and Ki-67 LI are subject to heterogeneity induced diagnostic accuracy. Fewer grading errors occur when using both methods together than when using either method alone. Therefore, MIB-1 LI should constitute a part of routine investigations in patient with astrocytomas.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Sep 15, 2020
- Manual Googling: Oct 14, 2020
- iThenticate Software: Dec 28, 2020 (16%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Sep 12, 2020**
Date of Peer Review: **Oct 01, 2020**
Date of Acceptance: **Oct 16, 2020**
Date of Publishing: **Oct 01, 2021**