Original Research Article

Clinicohistopathological study of astrocytomas along with Ki-67 proliferative index

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ABSTRACT

Background: Astrocytomas form the largest group of gliomas (>75%) and diffusely infiltrating accounting for more than 60% of all the primary brain tumors. The ki67 proliferative index is a potent biologic marker that estimates the growth of neoplasms quantitatively and thus will aid in identifying the prognosis for patients with neoplasms. The aim of the research work was to study various histopathological and clinical features of Astrocytomas in detail, to evaluate Ki-67 proliferative index in patients of Astrocytomas and to compare the results of Immunohistochemistry with histological grade of Astrocytomas.

Methods: A total number of 40 cases of Astrocytomas were included in the study. Ki-67 immunostaining was done on all cases and compared with WHO histological grading of astrocytomas.

Results: The mean Ki- 67 LI in Grade I astrocytomas was 4.66, range 4-5 , in Grade II astrocytomas mean was 8.07, range 5-12 ,in Grade III astrocytomas mean was 13.5 , range 8-20, in Grade IV astrocytomas mean was 22.93, range 15-50. There was a highly significant correlation between the histopathological grade of astrocytomas and Ki-67 LI (p<0.05).

Conclusions: The monoclonal antibody Ki-67 has proven its prognostic and diagnostic power in astrocytic tumors. Ki-67 LI is the simplest and the most reliable method for evaluating cell proliferation. Ki-67 LI increased with histological grade and the difference between low grade (I and II astrocytomas) and high grade (grade III and IV) is significant. In the present study Ki-67 LI is not dependent on factors like age and sex and is solely dependent on histological grade.

INTRODUCTION

Astrocytic tumors are the most common primary tumors of the central nervous system.[1] Several grading systems are used to grade astrocytomas. The most widely used system is the World Health Organization (WHO) classification (1979, 1993, 2000, and 2007) that grades astrocytomas (I-IV) based on cytological atypia, mitotic activity, vascular proliferation, and necrosis: pilocytic astrocytoma (grade I), diffuse astrocytoma (grade II), anaplastic astrocytoma (grade III), and glioblastoma (grade IV).[2]

Though histopathological features largely help in the determination of prognosis, histological differentiation may not be clear in some cases, especially when only small fragments of tissue biopsies are available. Studies have employed a wide range of parameters from tumor suppressor genes to proliferation indices for predicting clinical outcome and survival.[3]

A variety of methods have been employed to estimate the proliferative index of central nervous system tumors. Of these, one of the most potent methods is the Ki- 67 labeling index (Ki- 67 LI). The value of Ki- 67 LI in the

assessment of cell proliferating activity has been widely documented for various human tumors, including the brain neoplasm.[4] Ki-67 is a nuclear antigen expressed in the G1, S, G2, and M phases of the cell cycle but absent in the resting phase.[5]

The purpose of the present study is to establish the mean and range of Ki-67 labeling index in Astrocytomas and its utility in differentiating Astrocytomas of varying grades, also to assess the Ki-67 LI in correlation with the World Health Organization (WHO) histological grades of astrocytomas in order to predict the biological behavior, which may give a direction for using Ki-67 LI as an adjunct to the routine histology in grading astrocytomas precisely, thus will aid in identifying the prognosis for patients with astrocytomas and their survival.

METHODS

A total number of 40 cases of Astrocytomas for a period of 2years from August 2014 to July 2016 were included in the study. All the relevant clinical details were obtained. Gross features like size, shape, color, consistency, cystic and necrotic changes were noted. Tissue was subjected to routine processing and sections were stained with hematoxylin and eosin (H&E). The histopathological sections were diagnosed basing on WHO classification of central nervous system tumours.

Ki- 67 immunostaining was done on all the 40 cases. 3-4 micron sections were taken from paraffin embedded tissue blocks for IHC (Immunohistochemistry). Sections were taken on poly L lysine coated slides and slides are baked at 600C for 1hour, followed by deparaffinization of sections with 3 changes in xylene and alcohol. Antigen retrieval performed in Tris buffer at pH 6.0 to 6.8 using microwave oven. Ki-67 primary antibody was applied for 30min followed by secondary antibody. Diamino Benzidine (DAB) chromogen was applied for 5 minutes. Sections were Counter stained with Mayers Hamatoxylin solution and Mounted with D.P.X mountant.

Positive and negative controls were run with each batch. One case each of Burkitt's lymphoma and high grade breast carcinoma which showed high degree of immunostaining with Ki 67 were taken as internal positive control; the negative control was performed on the same tissue without primary antibody. Areas showing more extensive staining were chosen under low magnification. cells showing diffuse or granular brown nuclear stain were taken as Ki67 positive A total of 1000 cells were counted (200 cells each in 5 fields) in high power magnification (400x).

Vascular components, inflammatory cells, necrotic, degenerated and poorly preserved areas were excluded. Ki67 labeling index (LI)/ proliferative index (PI) was ascertained by the percentage of positive labeled nuclei. The mean of Ki67 LI were tabulated. P value was calculated for individual variable using Chi-Square test and results were tabulated.

RESULTS

In the present study the age groups ranged from 5 years – 72 years and the most common age was 4th decade (27.5%). The male to female ratio is 2.07:1, showing male preponderance. Majority of the Astrocytomas in the present study belong to grade IV (GBM), 15 cases, followed by grade II Astrocytomas (Figure 1, 2) in 13 cases (Table 1).

Table 1: Histopathological grades of Astrocytomas (total cases=40).

Histological grade	No. of cases (n=40)	Percentage
Grade I	3	7.5
Grade II	13	32.5
Grade III	9	22.5
Grade IV (GBM)	15	37.5

There were 4 cases of recurrent Astrocytomas in which two were histopathologically diagnosed as grade IV (GBM) (Figure 3, 4).
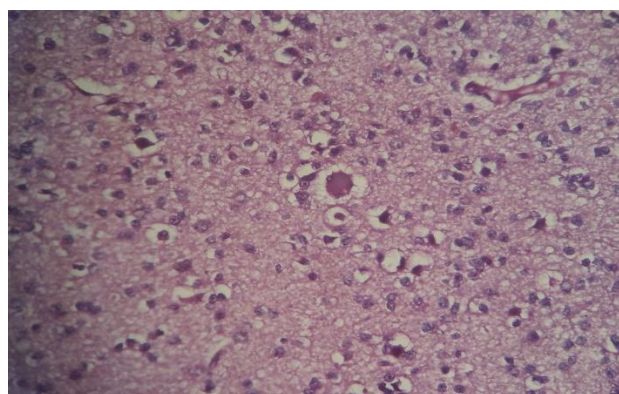

Figure 1: Grade II astrocytoma showing increased cellularity and well differentiated astrocytes (H&E, 100X).
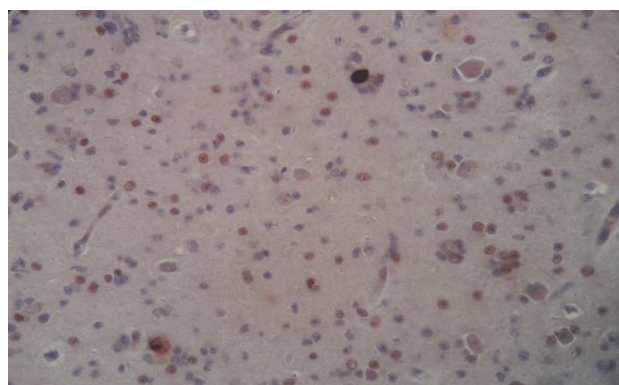

Figure 2: Ki-67 expression (nuclear positivity) in Grade II Astrocytoma (IHC, 100X).
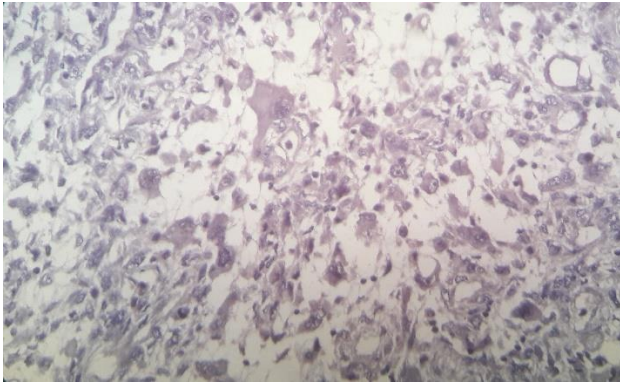
Figure 3: Grade IV astrocytoma showing poorly differentiated pleomorphic cells with nuclear atypia (H&E, 100X).
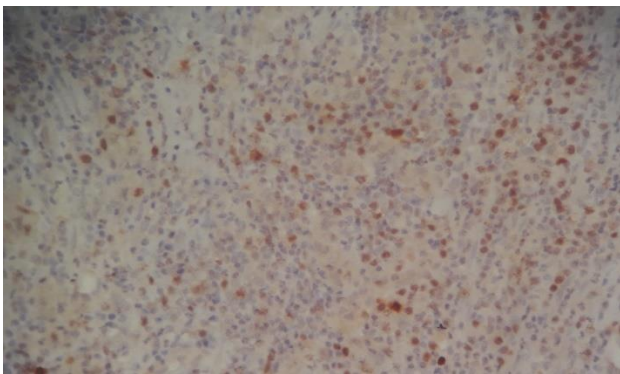

Figure 4: Ki-67 expression (nuclear positivity) in Grade IV Astrocytoma (IHC, 100X)

Majority of the pilocytic astrocytomas belongs of to the 1st decade and most of the grade IV Astrocytomas (GBM), fall above the age of 30 years. Age wise distribution in various astrocytomas is depicted in Table 2.

Table 2: Age distribution in astrocytomas (n=40).

Age group(yrs)	No. of cases (total no. of cases=40)	Percentage
0-10	2	5
11-20	2	5
21-30	7	17.5
31-40	11	27.5
41-50	3	7.5
51-60	8	20
61-70	5	12.5
>70	2	5

Most common clinical presentation in this study is headache (20 out of 40), followed by seizures. Most common site of astrocytomas in the present study is frontal lobe (27.5%). As the grade of astrocytoma increased, the mean Ki-67 LI also increased. The mean Ki67 LI and range in different grades of astrocytomas is depicted in Table 3.

Table 3: Mean Ki-67 LI and range of Ki-67 LI for various grades of astrocytomas.

Grade	Mean Ki-67 LI	Range of Ki-67 LI
Astrocytoma Grade I	4.66	4-5
Astrocytoma Grade II	8.07	5-12
Astrocytoma Grade III	13.5	8-20
Astrocytoma Grade IV (GBM)	22.93	15-50

There was a highly significant correlation between the histopathological grade of astrocytomas and Ki-67 LI ($p<0.05$).

DISCUSSION

Astrocytic tumors constitute a wide range of neoplasms that differ in their growth potential, extent of invasiveness, morphological features and tendency for progression. Histopathological grading is considered to be strong prognostic factor. Monoclonal antibody Ki-67 quantification which was developed by Gerdes in 1993 was known to be the best method for evaluating proliferation rate of astrocytic tumors.[6]

In the present study there was male preponderance as observed with studies done by Ganju et al, Thotakura M et al.[7,8] Majority of cases belonged to fourth decade (27.5%) followed by 20% in sixth decade. Pilocytic astrocytoma (grade 1) occurred in first decade, which interrelated with other studies.[8,9] In this study Anaplastic astrocytomas (grade III) mostly occurred in 4th decade which correlated with study by Katsetos CD et al, Glioblastoma multiformae mostly occurred in 3rd to 6th decade, which correlated with the study by Thotakura M et al and Katsetos CD et al Strong influence of patient age on survival has been explained by the typical occurrence of aggressively behaving high grade astrocytomas at a somewhat later age and by decreased host resistance with age.[8-10]

There was a highly significant correlation between the histopathological grade of astrocytomas and Ki-67 LI ($p<0.01$) This result agrees with the studies done by Ambroise et al, Thotakura M et al, Wakimoto et al (72 cases) (Table 4).[4,8,11]

In a study by Montine et al using the previous monoclonal antibody and frozen sections, the Ki-67 LI was found to be a significant prognostic indicator for the entire group of astrocytomas and was more significantly related to survival than histological grade.[12] But studies by Wakimoto et al. and Rathi et al. demonstrated significant relation between tumor grade and Ki-67 LI.[4,13]

Klein and Roggendorf demonstrated that proliferation rates in astrocytomas not only reflect proliferation of tumor cells but also that of microglial cells, especially in pilocytic astrocytomas. So, using this marker to

differentiate pilocytic astrocytoma from gliosis should be done with caution and this marker is not reliable for definitive diagnosis.[14] Similar observations were made by Ambroise et al.[11]

Table 4: Comparison of mean (range)/Ki67 LI in various grades of astrocytic tumors according to different studies.

Study series	Grade I tumor	Grade II tumor	Grade III tumor	Grade IV tumor
Ambroise et al	3.78 (0.3-18.2)	2.76 (0.2-9.0)	7.45 (0.5-22)	13.85 (1.2-59)
Thotakura M et al	3.36 (0.4-16)	7.05 (0.4-18.8)	28.24 (16-34.8)	38.7 (20-52)
Wakimoto et al (72 cases)	-	3.8	18.4	31.6
Present study (40 cases)	4.66 (4 to 5)	8.07 (5 to 12)	13.5 (8 to 20)	22.93 (15 to 50)

Similar to the present study, some studies showed a very low LI for pilocytic astrocytomas and found significant difference in the distribution of Ki-67 LI between pilocytic and diffuse infiltrating astrocytomas.[15,16] This study also did not reveal any significant prognostic role for Ki-67 labeling index in pilocytic astrocytomas as it is for other studies like Thotakura M et al and Ambroise et al.[8,11]

Like the present study, most of the studies have found significant differences in Ki-67 LI between high- and low-grade diffuse astrocytomas like Wakimoto H et al, Rathi et al. Giannini C et al.[4,13,15,17] Though some studies have found significant difference in Ki-67 LI between anaplastic astrocytomas and glioblastomas others could not find a significant difference between them.[4,13,17,22]

A problem in the diagnostic value of Ki-67 LI is considerable overlap across tumor grades. This was evident in this study also. It was observed that all diffuse astrocytomas had Ki-67 LI less than 10%, except for one case which had LI of 12%. In the study by Ambroise et al reveals that all diffuse astrocytomas had an LI less than 10%, which was comparable to present study.

However, it is evident from our study and other earlier studies that a low Ki-67 LI does not rule out high-grade astrocytomas. A recent study on cellular proliferation on pilocytic astrocytomas and diffuse astrocytomas points to the value of Ki-67 LI as a predictor of survival and its ability to better differentiate between Grade II and Grade III tumors than does the presence of mitotic figures.[11]

Gemistocytic astrocytoma though classified as a grade II tumor generally shows an aggressive behavior and is characterized by a low proliferative activity with a mean Ki-67 LI of 3.7%.[18] In the present study 2 cases out of 40 cases were gemistocytic astrocytomas with KI-67 LI of 5 and 6, and although most studies conclude with statistically significant differences between low- (II) and high-grade (III-IV) astrocytomas, the average level of Ki-67 LI in the different tumor groups varies considerably. This dilemma may partly be illustrated by comparing the study of Hsu et al and that of Eneström et al who found mean levels of Ki-67 LI in glioblastomas of 9.12% and

24.3%, respectively.[18,19] In the present study the mean Ki-67 LI for glioblastoma is 22.93 with Ki-67 LI range of 15 to 50. In the study by Thotakura M et al the mean Ki-67 LI was 12.5 with Ki-67 LI range of 0 to 76.4. This did not correlate with the present study.[8] In present study mean Ki-67 LI of grade III astrocytomas is 13.5, with Ki-67 LI range of 8 to 20.

Regarding prognostic indicators for astrocytomas, the histopathological grade still appears to be the best guide to prognosis. Age to a certain extent also affects prognosis which also correlated with the present study. The study by Ambroise et al reveals that survival of patients with non-pilocytic astrocytomas showing a Ki-67 labeling index of more than 5% was lesser than that of patients with KI-67 labeling index of less than 5%.[11] However, the significance of this was lost when adjusted for other factors. Though some studies claim that increased Ki-67 LI is an independent prognostic variable, others find it significant only in univariate analysis.[15,20-24]

Studies have also differed on identifying a cut-off value for distinguishing between astrocytomas with good and poor prognosis. In the study by Jaros et al found that a value of 5% is useful for this purpose, whereas Ellison et al have suggested a value of 2%.[20,24] Another study found that 3% is useful as a cut-off point in differentiating with regard to the survival times this variation could be due to inter-laboratory variation in Ki-67 estimation.[21]

Studies which investigated the role of immunohistochemical markers in glioblastomas did not reveal any prognostic role for MIB-1/Ki-67 labeling index.[25,26] Another extensive study on glioblastomas also proved that MIB-1/Ki-67 estimation does not provide additional independent information for predicting survival or response to radiation.[27]

Many factors are responsible for such a variation in Ki-67 LI between studies. The Ki-67 LI can be influenced by the fixative used, immunohistochemical procedures, especially antigen retrieval, and interpretation of the immunostaining.[4,26] A low Ki-67 LI value in high-grade astrocytoma could also result from faulty tissue sampling and tumor heterogeneity. Antigen retrieval can be better

with hydrated autoclave treatment than with microwave treatment and can result in a higher Ki-67 LI.[4] This could possibly result from more successful denaturation of formalin fixed antigens. Computer-assisted methods for quantitation of LI seem to underestimate LI (up to 30%) compared to manual methods.[15,28] Inter-observer variability can also affect the Ki-67 LI estimation.[29]

CONCLUSION

To conclude it was observed that there was male predominance of astrocytomas with majority occurring in 4th decade and most common histopathological grade encountered is grade IV astrocytoma. Ki-67 LI did not depend on factors like age and sex but there was significant correlation with histopathological grading. So Ki 67 LI can be used as an adjunct to the histopathological diagnosis where there is lack of correlation between clinical parameters, histological diagnoses and grade of the tumour.

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