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CORRELATION OF HISTOLOGICAL GRADING OF GLIAL TUMOR WITH Ki-67 PROLIFERATIVE MARKER

Pathology

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ABSTRACT

Context: Gliomas are prone to spreading and recurrence due to an inherent propensity. The proper classification of gliomas is crucial for patient care and survival/outcome. The Ki-67 immunostaining-derived labelling index has been shown to be useful in identifying different degrees of malignancy. **Aim:** To correlate histopathological grades of gliomas with Ki-67 immunohistochemical proliferative marker. **Settings And Designs:** Hospital Based Retrospective Analytical Study. **Materials And Methods:** A total of 50 cases with confirmed diagnosis of glioma were retrieved from the departmental records. Cases from July 2015 to June 2021 were included in the study. Slides and wax blocks were retrieved. All the cases were reviewed and graded according to WHO 2016 classification the and for each case immunohistochemical stain Ki67. **Statistical Analysis:** To compare Ki-67 expression with different WHO grades, the data was examined using the Analysis of Variance (ANOVA) test. The relationship between histologic grade and Ki 67 proliferation marker expression was determined using Spearman's rank correlation coefficient. **Results:** There was significant difference in Ki 67 labelling index (Ki 67 LI) between grade I and IV($p<0.05$) and significant difference between grade II and grade IV ($p<0.05$). There was no significant difference in Ki 67 labelling index between grade I and grade II. The study shows strong positive correlation between WHO grades and Ki 67 Labelling index. **Conclusion:** Ki-67 labelling index correlates well with histopathological grades of glioma.

KEYWORDS

Ki 67 Immunohistochemical Markers And Glioma

INTRODUCTION-

Gliomas are the largest group of primary CNS tumors forming 35%–50% of the CNS tumors.[1] Gliomas are neuroepithelial tumors originating from the supporting glial cells of central nervous system. Glial cells consist of astrocytic, oligodendroglial, ependymal cells, which forms astrocytomas, oligodendrogliomas, or ependymal tumors, respectively.[2]

Astrocytomas are the major group of gliomas (>75%) and among that diffusely infiltrating astrocytomas account for more than 60% of all the primary brain tumors.[3] The distribution of astrocytomas differs by anatomic site in adults and children.[4] Despite multimodal therapy, glioblastoma (GB) is the most frequent primary malignant brain tumour in adults, with a median survival of one year following diagnosis.[5]

Gliomas are categorised I-IV based on histological criteria such as cytological atypia, mitotic activity, cellularity, microvascular proliferation, and/or necrosis, according to WHO recommendations.[6] Grade II and grade III gliomas are distinguished by mitotic activity, whereas high-grade gliomas such as anaplastic oligodendroglioma and glioblastoma are identified by microvascular growth and/or necrosis.

Advances in imaging investigations have helped tremendously. These modalities are not confirming, but they can help with the diagnosis. Immunohistochemistry is an important clinical research method in today's medical science. Ki-67 is a proliferation marker that has been proposed as an ancillary marker in the grading of gliomas[6,7.] The Ki-67 protein is found in the nucleus of the cell and can be detected during the active phases of the cell cycle, but not during the G0 quiescent phase.[8] The so-called Ki-67 labelling index (LI) is defined as the percentage of ki 67 positive tumor nuclei of all tumor nuclei and appears to correlate with tumor grade.[9,10]

The purpose of this study is to compare the Ki 67 LI to the World Health Organization (WHO) histological grades of gliomas in order to predict biological behaviour, which could lead to the use of Ki 67 LI as a supplement to routine histology in accurately grading gliomas

MATERIALS AND METHODS-

This Retrospective Analytical Study was conducted in Department of Pathology, Gandhi Medical College, Bhopal, Madhya Pradesh. The study was approved by Institutional Ethics Committee of Gandhi Medical College, Bhopal (M.P.). A total of 50 cases with confirmed diagnosis of glioma were retrieved from the departmental records. Cases from July 2015 to June 2021 were included in the study. Slides and wax blocks were retrieved. Wax Blocks were cut using microtome and Hematoxylin and eosin staining was done wherever necessary. All the cases were reviewed by two pathologists, graded according to WHO 2016 classification the and for each case, a representative block was selected for immunohistochemical stain Ki67. 3-4 micron thick deparaffinized tissue section mounted on polylysine coated slides were stained with Ki 67 antibody using Pathinsitu kits for immunohistochemistry. IHC Sections were examined by two pathologists and Ki 67 scoring was done. Distinct nuclear staining (brown colour) was taken as positive. Tonsil tissue was taken as the positive control. Maximum positivity staining area were examined under 40 X magnification. The Ki-67 Labelling Index is calculated as the percentage of nuclei stained positive in total 1000 cells in high power field. Statistical Analysis was carried out. Data was analysed using Analysis of Variance (ANOVA) test to compare the Ki-67 expression with different WHO grades. Spearman's rank correlation coefficient, was used to determine the relationship between histologic grade and Ki 67 proliferation marker expression. Appropriate software was used for analysis.

RESULTS-

Mean age of the study population was found to be 34 with minimum age of 4 years and maximum of 68 years old. Out of the 50 cases of gliomas, 25 cases (50%) were of males and 25 cases (50%) were of females. The study found that glioblastoma is the most common histopathological diagnosis (32%), followed by diffuse astrocytoma (24%) and then oligodendroglioma (18%). frontal lobe was the most frequented location (24%) of gliomas followed by left temporal lobe (16%). 52% (n=26) of the participants were graded into WHO grade II followed by grade IV ,32%(n=16).

Table 1: Shows Different Gliomas In This Study.

	Frequency	Percent
Pilocytic astrocytoma I	3	6.0
Diffuse astrocytoma II	13	26.0
Anaplastic astrocytoma III	2	4.0
Glioblastoma IV	16	32.0
Oligodendroglioma II	9	18.0
Myxopapillary ependymoma I	3	6.0
Ependymoma II	4	8.0
Total	50	100.0

The present study found that Ki67 LI in glioblastoma and anaplastic astrocytoma being significantly higher than that of the other gliomas as

shown in table 2.

Table 2: Shows Mean Ki 67 LI Of Different Gliomas In This Study

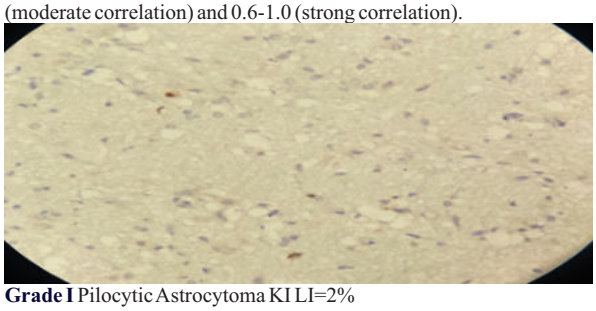
Glioma	Mean Ki 67 LI
Pilocytic astrocytoma	3.5667%
Diffuse astrocytoma	2.6769%
Anaplastic astrocytoma	12.6500%
Glioblastoma	25.6750%
Oligodendroglioma	3.9333%
Myxopapillary ependymoma	1.3333%
Ependymoma	7.0250%

The mean Ki67 index scores were higher in WHO grade IV (25.675) followed by grade III (12.65). The Ki 67 scores were lower in grades I and II. And the mean Ki67 LI increases as the grade increased. This result obtained was statistically significant also($p<0.05$).

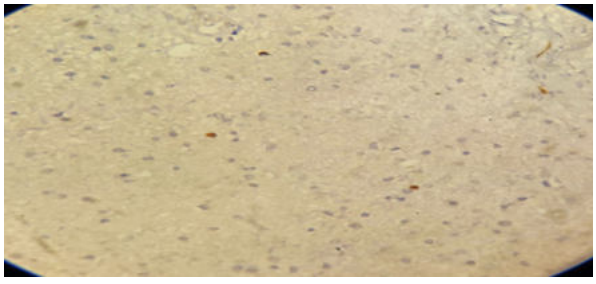
Table 3: Shows Mean Ki 67 LI Of Different WHO Grades of Gliomas

WHO GRADE	MEAN Ki67 LI
I	2.4500%
II	3.7808%
III	12.6500%
IV	25.6750%

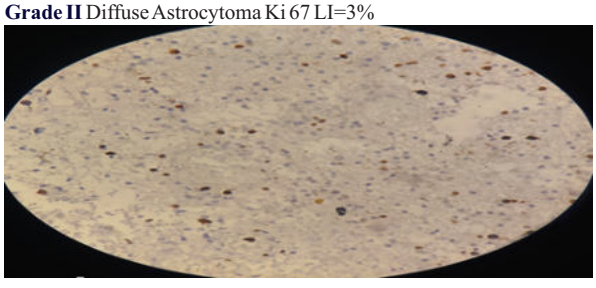
By Spearman correlation its seen that WHO grades have positive correlation with Ki67 labelling index. Correlation co-efficient of 0.775 indicates a strong positive correlation, which means Ki67 labelling index increases as the WHO grade increases. The ranges for Spearman's rank correlation are 0.2-0.39 (weak correlation), 0.4-0.59 (moderate correlation) and 0.6-1.0 (strong correlation).



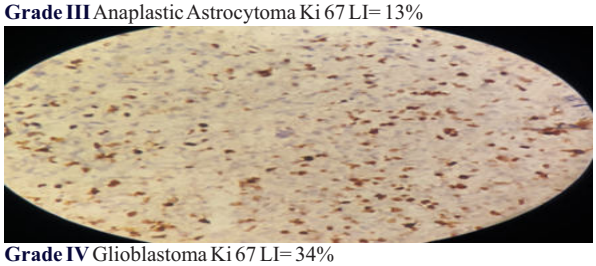
Grade I Pilocytic Astrocytoma KI LI=2%



Grade II Diffuse Astrocytoma Ki 67 LI=3%



Grade III Anaplastic Astrocytoma Ki 67 LI= 13%



Grade IV Glioblastoma Ki 67 LI= 34%

Fig I-Showing Ki67 IHC Staining Among Different Grades Of Gliomas

DISCUSSION-

The art of making a diagnosis in oncology has progressed from microscopic examination of H and E-stained slides to the contemporary era with the use of supplementary methods such as immunohistochemistry and molecular biology procedures. These adjuvant techniques have not only aided in a better knowledge of malignancy biology, but they have also paved the way for the creation of specialized targeted therapies. Ki-67 LI is a valuable marker for assessing cellular proliferation in a variety of human malignancies, including intracranial tumors. In this study a total of 50 glioma cases were included. Out of the fifty cases of gliomas in this study, 25 cases (50%) were of males and 25 cases (50%) were females with male to female ratio (sex ratio) of 1:1. No gender preponderance was seen in this study. This was against the study conducted by Atef et al[11] in 2021 which showed slight male predominance with 25 males and 19 females. Belghali et al. (2017) [12] also showed a slight male preponderance with a sex ratio of 1.13:1in a study conducted on 32 cases. This slight difference in gender wise distribution of gliomas can be due to small sample size in all the mentioned study. Mean age of glioma in the present study is 34 years which is comparable to the study Arshad et al (2010)[13] and Theresia et al (2020) [14] in which mean age was 35 years and 38 years respectively. Gliomas show higher incidence in frontal lobe (24%) in the present study. This result is consistent with Larjavaara S et al (2007)[15] study in which 40 % cases are seen in frontal lobe in the study on 331 glioma cases. On analysing the distribution of gliomas as per WHO grade, maximum number of gliomas (52%) were WHO grade II in the present study. This is comparable to the studies by Bharadwaj A et al (2021)[16], Xu G et al(2021)[17] and Byreddy et al (2018)[18] as WHO grade II gliomas are the most common gliomas in these studies. In the present study mean Ki 67 LI is maximum in Glioblastoma (25.6 %) followed by Anaplastic Astrocytoma (12.6%). Similar pattern of higher mean Ki 67 LI in glioblastoma and anaplastic astrocytoma were described in the studies by Arshad *et al (*2010)[13] and Jham et al. (2017)[19]

Table 4: Shows Study Comparison Of Expression Of Ki67 Labelling Index And WHO Grades

	MEAN Ki67 LI			
STUDY	GRADE I	GRADE II	GRADE III	GRADE IV
Giannini et al(1999)20	4.8%	5.2%	32%	40%
Tihan et al(2001)21	1.83%	3.7%	11.4%	20.2%
Thotakura et al (2014)22	4.5%	7%	28%	38.7%
Theresia et al (2020) 14	1.24%	3.96%	23.02%	23.88%
PRESENT STUDY (2021)	2.45%	3.78%	12.65%	25.67%

In the present study the mean Ki 67 LI is higher in Grade IV (25.67%) followed by Grade III (12.65%) and the ki67 LI were lower in grade I and grade II. Thus indices for high-grade gliomas (grade III and grade IV) were higher than that of low-grade (grade I/II) tumors. And we noticed an increase in mean Ki67 LI as the WHO grade increased. These findings were comparable with other studies mentioned in the table 4.

In our study spearman's rank correlation coefficient, was used to determine the correlation between WHO grade and Ki 67 LI. And the correlation coefficient was 0.775 that indicates strong positive correlation between Ki67 LI and WHO grade. This finding was comparable with the study by Bharadwaj A et al (2021)[16] in which the correlation coefficient was 0.79 and with the study by Arshad *et al (*2010)[13] which showed a correlation coefficient of 0.78, both suggest strong positive correlation between Ki 67LI and WHO grade.

The present study suggests that Ki-67 LI is not dependent on factors like age and sex but only on the histological grade of glioma. And Ki-67 LI shows a strong positive correlation with histological grade.

AUTHOR CONTRIBUTION:

Prasad Krishna: Study conception, Data Collection, Data Analysis, Writing

Pahwa Sagarika: Study conception, Data Collection, Data Analysis, Writing
Balani Sharda: Data Analysis, Interpretation
Malik Reeni: Data Analysis, Interpretation

Key Message-

In the evaluation of glial lesions, Ki 67 immunohistochemistry is essential. Ki-67 isn't a stand-alone prognostic factor, but it can be combined with others. Because of new concepts in genetic changes, introducing molecular markers will be crucial in glioma patients' targeted treatment and personalized care.

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