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Ki-67 expression in astrocytomas

Arfa Nawazish[1], Huma Mushtaq[2], Muhammad Tahir[3], Sana Gul[4], Tehreem Atif [5]

[1] Specialty Doctor, Anatomical pathology, Australian Clinical labs, Perth, Australia.
[2] Associate Professor Histopathology, Islamabad Medical and Dental College, Islamabad
[3] Associate Professor Histopathology, Rawal Institute of Health Sciences, Islamabad.
[4] Medical officer, Federal Government Polyclinic Hospital.
[5]Speciality Doctor. Histopathology, Glan Clywd Hospital.Wales.UK

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Address of Correspondent
Dr. Huma Mushtaq
Associate Professor Histopathology, Islamabad Medical and Dental College, Islamabad
dr_kat18@hotmail.com

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ABSTRACT

Objective: To apply immunohistochemical marker Ki-67 and to check it's expression in various types of Astrocytomas according to their grades.

Methodology: The cross sectional study was conducted in Pathology department, Federal Government Services Hospital (Polyclinic), Islamabad from July to December 2015. All patients having diagnosis of astrocytomas grade II, III and IV on histopathology were included in the study. Microscopic examinations were carried out for accurate diagnosis and grading of astrocytomas. Immunohistochemical staining for Ki-67 was done and the percentage of cells with positive Ki-67 nuclear staining was determined. Quotients (positively stained tumor cells/totally counted tumor cells) were calculated as percentage and rounded to nearest integer.

Result: A total of 212 patients were included in the study. The mean age of the patients was 40.16 ± 12.763 years (Range 20 to 76 years). Majority of the patients (32%) were in age range of 30-40 years. In this study, 110 of 212 patients (51.9%) were males while 102 patients (48.1%) were females. Among the three types of Astrocytomas, Glioblastoma multiforme (WHO grade IV) was the most common variant. Overall Ki-67 staining was positive in 168 of 212 specimens (79.2%) and most commonly was in Glioblastoma multiforme WHO grade IV being 96.3%. Stratification of Ki-67 expression in tumors was also done according to age and gender of cases. P-value was found significant after stratification (P <0.05)

Conclusion: Ki-67 staining was positive in 79.2% cases of Astrocytomas and most commonly (96.3%) positivity was in Glioblastoma multiforme WHO grade IV. Increasing values of Ki-67 are associated with increasing grade of malignancy.

Key words: Astrocytoma, Ki-67, Glioblastoma.

Introduction

Astrocytic tumors constitute a wide range of neoplasms that differ in their location, age distribution, growth potential, extent of invasiveness, morphological features and tendency for progression and are the most common primary tumors of CNS.[1] Various studies have shown that tumor grade is the most statistically significant prognostic factor determining patient survival though histopathological features largely help in the determination of grade and prognosis. Histological differentiation may not be clear in some cases, especially when only small fragments of tissue are available. Studies have employed a wide range of parameters including proliferation indices for predicting clinical outcome and survival.[2]

In adults the most important types of astrocytomas are those that diffusely infiltrate the surrounding brain tissue making these diffuse astrocytomas resistant to surgical resection. An exception is the pilocytic astrocytoma (WHO grade 1), the most common paediatric glioma which is relatively well demarcated from the surrounding tissues and can be resected. Diffuse astrocytomas are categorized into low grade astrocytomas (WHO grade II), which usually demonstrate relatively slow growth and high grade gliomas (WHO grades III and IV) which grow more rapidly. The most malignant and the most common of the high grade gliomas is Glioblastoma multiforme(GBM).[3]

The current WHO classification of human astrocytomas has limitations in predicting prognosis and diagnosis and there is a need for additional factors.[4] In everyday practice, ki-67 immunohistochemical analysis is the most frequently used ancillary study to aid in diagnosis and grading.[5] Several studies have investigated the clinical value of proliferative activity in these tumors and show increasing values of ki67 with increasing grade of malignancy. In most studies positive correlations between MIB 1 labeling index and survival was found, though the proposed cut off values vary substantially between the reports. MIB 1 labeling index, however, cannot be used as a diagnostic factor alone but should be used in combination with established histologic criteria of malignancy. It may be especially useful in cases where histology reveals a low grade astrocytoma whereas other parameters indicate a more malignant neoplasm. Therefore, MIB 1 should be a part of the routine investigation in patients with astrocytic tumors.[6]

The ki-67 antigen is expressed during all active phases of the cell cycle (G1, S, G2 and M phases) but absent in the resting phase (G0). The MIB1 antibody recognizes the Ki-67 antigen in both formalin fixed and paraffin embedded tissue. This method was developed and introduced by Gerdes in 1993.[7] Since then, this has been increasingly used in assigning grading and prognosis to astrocytomas.

This study was directed towards the application of immunohistochemical marker Ki-67 on our local population diagnosed as having astrocytomas on histopathology to confirm their grades that allows determination of prognosis that correlates strongly with patient's survival.

Methodology

This cross sectional study was conducted in Pathology department, Federal Government Services Hospital (Polyclinic), Islamabad, from July to December 2015. Sample size was calculated using WHO sample size calculator and was found to be 212 cases with confidence level 95%, anticipated population 6.42% and absolute precision 3.3%. Non-probability, consecutive sampling technique was applied. All patients undergoing neurosurgical intervention for brain tumors ranging in age from 20 years to 80 years and having diagnosis of Astrocytomas grade II, III and IV on histopathology were included in the study.

Patients who have received chemotherapy or radiotherapy prior to surgery and having diagnosis of Pilocytic astrocytomas WHO grade I on histopathology were excluded from the study.

Inadequate samples with specimen insufficient for making histopathological diagnosis were also not included.

After approval from hospital ethical committee, data was collected from all patients undergoing neurosurgical intervention. The surgical specimens were examined grossly in the laboratory and the biopsy specimens were used for the preparation of histopathology slides. After receiving biopsy material, it was fixed in 10% formalin in the histopathology laboratory of FGPC PGMI, they were processed for paraffin sectioning using the tissue processor (SAKURA TISSUE TEK-VIP 5Jr). After paraffin embedding, 2-3µm thick sections were cut by rotator microtome. These slides were stained with eosin and hematoxylin stain for morphology. Microscopic examinations were carried out for accurate diagnosis and grading of astrocytoma.

For immunohistochemistry, 2-3 micron sections were cut from formalin fixed, paraffin embedded tumor block. A microwave oven or pressure cooker was used for antigen retrieval. The slides were incubated with normal horse serum for ten minutes at room temperature. After one hour incubation with primary antibody MiB1, sections were incubated with the secondary biothylated antibody and avidin-biotin peroxidase complexes for thirty minutes. Reaction products were revealed with diaminobenzidine as the chromogen and sections were counter stained with Harri'shematoxylin. The lab technician followed the same procedure for all slides. Ki-67 staining was done on separate slides. Positive and negative controls for Ki-67 were also applied. The entire section was screened to find the region with maximum positive nuclear staining of cells for Ki-67 .The percentage of positively stained cells were scored in the region using 40X objectives. The percentage of cells with positive Ki-67 nuclear staining was determined

independently in 100 tumor cells. Quotients (positively stained tumor cells/totally counted tumor cells) were calculated as percentage and rounded to nearest integer.

All the data was analyzed using SPSS version 17. Frequency and percentages were calculated for gender, tumor grade and Ki 67 expression. Mean ± SD was calculated for age of the patients. Effect modifiers like age, gender, grade of astrocytoma were controlled by stratification. Post stratification Chi-square was applied. P value ≤ 0.05 was significant.

Results

A total of 212 patients with mean age of 40.16±12.763 (Range 20 to 76 years) were included in the study. Majority of the patients (32%) were in age range of 30-40 years. In this study, 110 of 212 patients (51.9%) were males while 102 patients (48.1%) were females. Among the three types of Astrocytomas, Glioblastoma multiforme (WHO grade IV) was the most common variant. There were 165 cases (77.8%) of Glioblastoma multiforme (WHO grade IV), followed by Anaplastic astrocytoma (WHO grade III) with 24 cases (11.3%) and Diffuse fibrillary astrocytoma (WHO grade II), of which 23 cases (10.8%) were found (Table 1).

Ki-67 staining was negative in all cases of Diffuse fibrillary Astrocytoma. In 9 cases of Anaplastic astrocytoma (WHO grade III) it was positive while it was negative in 14 cases. In Glioblastoma multiforme (WHO grade IV), it was positive in 159 cases while negative in 6 cases (Figure 1). Overall Ki-67 staining was positive in 168 of 212 specimens (79.2%) and most commonly was in Glioblastoma multiforme WHO grade IV being 96.3%. Stratification of Ki-67 expression in tumors was done according to age and gender as shown in table 2 and 3.

P-value was found significant after stratification (P <0.05).

Table 1: Distribution of cases according to WHO grade of the tumor

Type of tumor	Number of cases	Percentage
Diffuse fibrillary astrocytoma WHO grade II	23	10.8
Anaplastic astrocytoma WHO grade III	24	11.3
Glioblastoma multiforme WHO grade IV	165	77.8

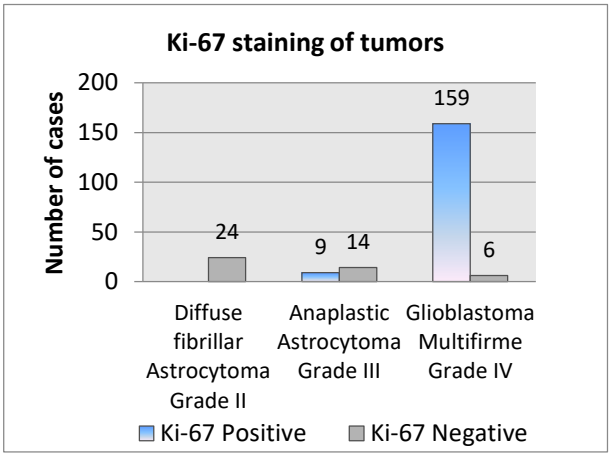

Figure 1: Ki-67 staining of tumors in the study.

Table 2: Stratification of Ki-67 staining with respect to gender

Gender	Ki-Staining		Total
	Positive	Negative	
Male	96	14	110
Female	72	30	102
Total	168	44	212

Table 3: Stratification of Ki-67 staining with respect to age

Gender	Ki-Staining		Total
	Positive	Negative	
20-30 Years	41	13	54
31-40 Years	54	14	68
41-50 Years	42	8	50
51-60 Years	23	4	27
61-70 Years	6	3	9
71-80 Years	2	2	4
Total	168	44	212

Discussion

Astrocytomas are the most common and aggressive primary intracerebral tumors. They arise from astrocytes, glial progenitor cells, or cancer stem cells. Cerebral gliomas are important and the most common primary brain tumors. The diagnosis and grading of astrocytoma is important. MR imaging plays a critical role in the preoperative assessment and grading of gliomas. The classification and grading of gliomas on conventional MR imaging are sometimes unreliable. The sensitivity, specificity, PPV, and NPV for identifying high-grade gliomas on conventional MR imaging are 72.5%, 65.0%, 86.1%, and 44.1%, respectively.[8] Physicians must perform a biopsy or surgical resection to make a

pathologic diagnosis and evaluate the need for postoperative chemoradiotherapy. However, lesions for which the risks of biopsy are high cannot be accurately diagnosed and graded. The noninvasive evaluation of gliomas results in a more precise assessment for selecting the surgical approach or chemoradiotherapy.[9]

Astrocytoma is generally considered to be predominant among males. In this study, 110 of 212 patients (51.9%) were males while 102 patients (48.1%) were females. Johnston DL et al. found in their study that astrocytoma was present in 56% of male and 44% of female patients.[10] Jung KW and colleagues conducted a large study on brain tumors of 4721 patients. They found that male patients were 55.9% of the total patients while female patients were 44.1%.[11]

Ki-67/MIB-1 immunostaining to distinguish gliosis and low-grade gliomas should be interpreted with caution. Normally, reactive astrocytes do not exhibit proliferative activity, but in some non-neoplastic conditions reactive astrocytes may have a proliferation rate of 1-5%.[12] In such cases, immunohistochemical analyses for mutated p53 and isocitrate dehydrogenase (IDH) proteins can be useful, though p53 immunoreactivity may occur in both settings, and there are gliomas without IDH mutation.[13]

The procedure for Ki-67/MIB-1 immunostaining is not standardized and has various analytical and clinical elements of uncertainty. Nevertheless, the method is regarded as being robust, which is also in accordance with our experience during several years with both clinical and experimental use. The recommended fixative is buffered formalin. The storage time, delay in fixation and fixation time does not seem to substantially affect the staining results.[14] Loss of immunoreactivity has been described if cut sections are exposed to room air for some months. A prerequisite for satisfactory immunostaining is adequate antigen retrieval. Various antibodies against the Ki-67 antigen are commercially available, but MIB-1 is the predominant antibody.[15] Counting procedures vary across studies. Usually counting is performed in areas with the highest immunoreactivity ("hot spots"), and approximately 1000 cells are counted using the 40× objective. The PI is calculated as the percentage of labeled tumor cell nuclei to the total number of tumor cells. As the expression of the Ki-67 antigen changes during the cell cycle, the intensity of nuclear staining will vary; principally, all types of staining should be regarded as positive. Counting can be done manually or by digitalized image analysis systems, but manual counting has turned out to be applicable for most diagnostic purposes.[16] Defining a cut-off value is also a topic of interest due to its impact on the determination of patients classified as "high Ki-67", which is indicative of a poorer outcome. Generally, these patients will receive more aggressive treatment. However, the definition of threshold value is not straight forward mostly due to inter-/intra-observer variability and counting procedures. Accordingly, extrapolating values from other laboratories can be deceptive; thus, Ki-67/MIB-1 immunostaining should be interpreted in the context of one's own practice. Each pathology department should regularly adjust its Ki-67/MIB-1 PIs by tumor grade and survival and develop its own in-house policy. Such a work-up will constitute an important part of a department's quality assurance and accreditation programs.[17] For astrocytomas, a cut-off of approximately 10% has appeared clinically feasible. However, the predictive value of Ki-67/MIB-1 is ambiguous.[18]

In this study, there were 165 cases (77.8%) of Glioblastoma multiforme WHO grade IV, followed by Anaplastic astrocytoma WHO grade III with 24 cases (11.3%) and Diffuse fibrillary astrocytoma WHO grade II , of which 23 cases (10.8%) were found. Lakhtakiaet al. found that the most common subtype among astrocytoma was Glioblastoma multiforme WHO grade IV, followed by diffuse fibrillary astrocytoma WHO grade II. They also found that grade 1 tumor was the least common subtype.[19] Similarly Thotakura et al. found that among all the subtypes of astrocytoma, most common was Glioblastoma multiforme WHO grade IV (39.95%). In their study, second most common subtype was Diffuse fibrillary astrocytoma WHO grade II in 36.2% of the cases.[20] In our study the most common subtype among Astrocytomas was also Glioblastoma multiforme.

Conclusion

Ki-67 staining was positive in 79.2% cases of Astrocytomas and most commonly (96.3%) positivity was in Glioblastoma multiforme WHO grade IV. Increasing values of Ki-67 are associated with increasing grade of malignancy. Therefore, Ki-67 should be a part of the routine investigation in patients with astrocytic tumors, especially in cases where histology reveals a low grade astrocytoma whereas other parameters indicate a more malignant neoplasm. So we recommend further trials to reveal other aspects of Ki-67 staining of astrocytoma.

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