ORIGINAL ARTICLE

COMPARISON OF KI-67/MIB-1 LABELLING INDEX AND HISTOPATHOLOGICAL GRADING OF ASTROCYTOMA

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Background: Astrocytomas are common malignant brain tumours. Ki-67/MIB-1 index serves as an important supplementary tool in the diagnosis and prognosis of human astrocytoma. **Methods:** Forty (40) cases of various histopathological (WHO) grades of astrocytoma (Pilocytic, diffuse, Anaplastic and Glioblastoma multiforme) were included in this study. Ki-67/MIB-1 labelling index of these tumours was estimated by immunohistochemistry, performed on paraffin sections. Histopathological grading and Ki-67/MIB-1 labelling index were compared. The results were analyzed by one way ANOVA. **Results:** Out of 40 cases two were diagnosed as pilocytic astrocytoma (WHO grade-1) with a mean Ki-67/MIB-1 labelling index of 0.7±1.4 (range 0.6–0.8%). Thirteen cases were of diffuse astrocytoma (WHO grade II), with a mean Ki-67/MIB-1 labelling index of 3.07±3.7 (range 1–15%). Nine cases were diagnosed as Anaplastic astrocytoma (WHO grade III) with a mean Ki-67/MIB-1 labelling Index of 11.55±7.8 (range 2–35%). Sixteen cases were diagnosed as Glioblastoma multiforme with a mean Ki-67/MIB-1 labelling Index of 14.1±8.9 (range (5–36%). ANOVA showed a significant differences between four compared groups (p=0.003). **Conclusion:** Immunohistochemical analysis of Ki-67/MIB-1 labelling index increases significantly with increasing grade of malignancy. The Ki-67/MIB-1 labelling index can be used as an adjuvant to histopathological grading for proper diagnosis and grading of astrocytoma especially in borderline cases and in small biopsies.

Keywords: astrocytoma, immunohistochemical grading, histopathological grading, Ki-67

INTRODUCTION

Primary brain tumours are approximately 2% of all the body tumours.[1] The annual incidence of tumours of CNS ranges from 10–17/100,000 for intracranial and 1–2/100,000 for intraspinal tumours.[2] Primary CNS tumours are the most common cancers in infants and children accounting for 20% all childhood cancers under the age of 15 years, exceeded in frequency only by leukemia.[3,4] Astrocytoma being the commonest, accounts for approximately 80% of all the adult primary brain tumours occurring in 4th to 6th decade of life. These are usually located in the cerebral hemispheres. Less common sites include cerebellum, brain stem and spinal cord.[2] In children cerebellar astrocytoma rank second only to medulloblastoma in frequency of occurrence.[5,6] According to WHO classification astrocytomas are graded I-IV. Pilocytic astrocytoma (grade-1) and diffuse astrocytoma (grade-II) are considered low grade astrocytoma. While anaplastic astrocytoma (grade-III) and Glioblastoma multiforme (grade-IV) are considered high grade or malignant astrocytoma. The WHO grading scheme is based on the appearance of certain characteristics, i.e., atypia, mitosis, endothelial proliferation and necrosis.[7]

Although low grade astrocytomas have tendency to grow slowly, they may progress to a higher grade as anaplastic or Glioblastoma multiforme. Since the WHO classification of astrocytoma has some limitations in predicting the clinical outcome and survival. So there is need for additional diagnostic and prognostic measures.[8] Astrocytomas have an inherent tendency to progress and are common malignant brain tumors.[9] The knowledge of their proliferative potential may prove vital in histological grade and stage.[10] Many studies have focused on the value of Ki-67/MIB-1 labelling index in these tumours. The proliferative index (Labelling index) is a potent biological marker that estimates the growth of a neoplasm quantitatively and helps in grading of a neoplasm.[11] Ki-67 immunostaining serves as an important supplementary tool in the diagnosis and prognostic evaluation of human astrocytoma.[9,12,13]

Proliferative index is a good parameter to assess the disease outcome in astrocytic gliomas.[14] The aim of this study was to evaluate the immuno-histochemical analysis of Ki-67/MIB-1, labelling index in various WHO grades of astrocytomas.

MATERIAL AND METHODS

This study was conducted in the Department of Pathology, Postgraduate Medical Institute (PGMI) Lahore, from March 2010 to July 2016. Paraffin blocks of forty cases of astrocytomas were included in this study. They were having different grades of astrocytoma including pilocytic, diffuse, anaplastic and Glioblastoma multiforme. Histopathological grading

of these tumours was done according to WHO classification.

Immunohistochemistry was performed on paraffin sections and Ki-67/MIB-1 Labelling index was estimated. A known case of Glioblastoma with a Ki-67/MIB-1 labelling index of about 43±2.1 was used as positive control, while the normal brain tissue was used as negative control.

Labelling index (Ki-67/MIB-I LI or LI) was calculated as:

$$L.I = \frac{No.\ of\ positive\ stained\ Cell}{Total\ No.\ of\ cells\ conunted} \times 100$$

Ki-67/MIB-1 labelling index values were determined by counting at least 1000 nuclei at high power magnification of ×100 in oil immersion in those areas expressing the highest positivity. The slides were examined and findings were recorded. Recorded values were compared with different grades of Astrocytoma, and $p \leq 0.05$ was considered as statistically significant.

RESULTS

The results were analyzed by one way ANOVA. Out of 40 cases two were diagnosed as pilocytic astrocytoma (WHO grade-1) with a Ki-67/MIB-1 mean labelling index of 0.7±1.4 (range 0.6–0.8%). Thirteen cases were of diffuse astrocytoma (WHO grade-II), with a mean Ki-67/MIB-1 labelling index of 3.07±3.7 (range 1–15%). Nine cases were diagnosed as Anaplastic astrocytoma (WHO grade-III) with a mean Ki-67/MIB-1 labelling Index of 11.55±7.8 (range 2–35%). Sixteen cases were diagnosed as Glioblastoma multiforme with a mean Ki-67/MIB-1 labelling Index of 14.1±8.9 (range (5–36%). ANOVA showed a significant differences between four compared groups ($p=0.003$). Post hoc Tukey b showed a statistically significant difference between group 2 and 4 ($p=0.003$). Games Howell test showed a statistically significant difference between group 1 and 4 ($p=0.000$) and between group 2 and 4 ($p=0.001$).

Table-1: Comparison of Ki-67/MIB-I labelling index with histopathological grading in cases of astrocytoma

Group	Types of Astrocytoma	Histolopathogical Grade	Ki-67/MIB-1 (Mean±SD)
1	Pilocytic Astrocytoma (n=2)	I	0.7±1.4
2*	Diffuse Astrocytoma (n=13)	II	3.07±3.7
3	Anaplastic Astrocytoma (n=9)	III	11.55±7.8
4**	Glioblastoma Multiforme (n=16)	IV	14.1±8.9

ANOVA: $p=0.003$; *Post hoc Tukey b: $p=0.003$ compared with group 4; **Games Howel test: $p=0.000$ compared with group 1 and $p=0.001$ compared with group 2

DISCUSSION

The quantitative analysis of astrocytic proliferation increases our knowledge about the biological behaviour of the tumour and will help us in predicting the outcome of the patients suffering from these tumours. In the treatment of malignant tumours, the clinical decision making is founded on evidence based facts and personal experience. The current WHO classification of astrocytoma has some limitations in predicting the clinical outcome and survival. So there is a need for additional diagnostic and prognostic measures. The aim of this study was to compare the proliferative activity of Ki-67/MIB-1 labelling index in various WHO grades of astrocytoma.

Walkimoto et al[15], Khalid et al[16] Hsu et al[17] Johannessen and Torp[18] performed Ki-67 immunochemistry on astrocytomas and showed that Ki-67/MIB-1 labelling index increases with increasing grade of astrocytomas although their absolute values of LI differed a little. In these studies the Ki-67/MIB-1 LI varies considerably. This variation may be due to several factors including tissue processing, immunohistochemical procedures and interpretation of immunostaining. Other reasons may be tumour heterogenecity and tissue sampling techniques. However Ki-67/MIB-1 LI increases with increasing grade of malignancy. A labelling index of more than 10% seems to indicate a highly malignant astrocytic tumour. Our findings were similar to the findings of these workers as there was increasing Ki-67/MIB-1 labelling index with the increasing histopathological grading of astrocytoma.

CONCLUSION

Immunohistochemical analysis of Ki-67/MIB-1 labelling index determines cells' proliferative activity in astrocytoma and increases significantly with increasing grade of malignancy. Ki-67/MIB-1 labelling index can be used as an adjuvant to histopathological grading for proper diagnosis and grading of astrocytoma especially in border line cases and in small biopsies.

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