The Proliferative Potential of the Astrocytoma, the Relation between Ki-67 and Histopathologic Criterias

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Abstract

**Background:** Astrocytic tumors are the most common primary CNS tumors. The grading of the astrocytomas has been traditionally relied on histologic assessment, but sometimes its still a subject of debate. This study used MIB-1 monoclonal antibody, a proliferative marker that can be used in formalin fixed paraffin embedded tissue to evaluate its capability for differentiating between different grades of astrocytomas.

**Patients and Methods:** Ninety cases of low grade astrocytoma, anaplastic astrocytoma and glioblastoma (30 cases of each) were selected from total 236 cases of astrocytomas during a period of 1994 to 2003. Histologic grading of the tumors was performed based on WHO classification. The proliferative potential of the tumors was estimated by counting mitosis and using the MIB-1 LI performed on paraffin sections. Variety of analysis methods were used to evaluated differences in MIB-1 LI between the three groups.

**Results:** The mean MIB-1 LI was 10.13 (range: 1 to 63) in low grade astrocytomas 48.75 (range: 2 to 366) in anaplastic astrocytoma and 238 (range: 6 to 532) in glioblastoma. The mean of mitotic count was 0.133 (range: 0-3), 2.93 (range: 0-23) and 11.66 (range: 1 to 34) in 1000 counted nuclei in low grade astrocytomas, anaplastic astrocytoma, and glioblastoma respectively. Multivariate analysis showed that after omission of effect of age which was significantly higher in glioblastoma, there is a meaningful difference between mean of MIB-1 LI of the three groups (P<0.000).

**Conclusion:** This study suggests that MIB-1 LI can be used as an adjuvant to histopathologic grading for proper diagnosis and grading of astrocytomas especially in borderline cases and small biopsies. Due to high heterogeneity of this value, determination of cut off point is impractical.

**Keywords:** Astrocytoma, Proliferative index, Immunohistochemistry, Ki-67, Monoclonal antibody MIB-1.

Introduction

Astrocytic tumors are the most common primary CNS tumor. According to World Health Organization (WHO) classification protocol, low grade astrocytomas are defined as diffusely infiltrating tumors composed of well differentiated neoplastic astrocytes, while Anaplastic Astrocytoma (AA) is one with focal or diffuse...
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anaplasia and with the following histologic features: Increased cellularity, pleomorphism nuclear atypia and mitotic activity. Glioblastoma Multiformi (GBM), contains in addition to above findings, the presence of vascular endothelial cell proliferation and or necrosis.

A progressively poorer prognosis is associated with increasing grading of tumor along this range. All ages may be affected, but low grade astrocytoma typically occurs in third and fourth decades (mean 35 years), while anaplastic astrocytoma and glioblastoma multiformi tend to arise in higher age group.

Although, distinction between low grade astrocytoma, anaplastic astrocytoma and glioblastoma multiformi can often be made on histologic features, for some tumors, histologic differentiation is not clear especially when only small fragments of tissue from stereotactically guided needle biopsies are available. It is for this reason that more objective criteria for predicting the prognosis of brain tumors are required.

The proliferative index is a potent biologic marker that estimates the growth of neoplasms quantitatively and aids in identifying the prognosis of patients with neoplasms. A variety of methods has been used to estimate the proliferative index of central nervous system tumors. In recent years, the value of antibodies to proteins expressed in proliferating cells such as Ki-67 and PC10 has been investigated in astrocytic tumors. Ki-67 (MIB-1) monoclonal antibody recognizes non-histone, nuclear protein that is expressed throughout all phases of the cell cycle except G0 and early phase of G1 (172-191).

The aim of this study was to assess the value of Immunohistochemistry with MIB-1 and determining the relation between positivity of MIB-1 staining and different WHO grades of astrocytoma.

Materials and Methods

A total number of 236 cases of Astrocytic tumors were originally collected for this study. The patients had been operated in Shiraz University of Medical Sciences, affiliated hospitals between 1994 to 2003. All pathologic slides were reviewed and classified according to World Health Organization (WHO) criteria. (Table-1).

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<tr>
<th>Table-1: The Histopathologic Grading of Diffuse Astrocytoma According to WHO Classification.</th>
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<tr>
<td>Cytologic Atypia</td>
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<td>Mitosis</td>
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<td>Endothelial Proliferation</td>
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<td>Tumor Necrosis</td>
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A tumor with only atypia is classified as low grade. Tumor with atypia, mitosis and foci of necrosis is classified as anaplastic astrocytoma. Presence of three or four malignant histopathologic criteria (Table-1) especially necrosis and vascular endothelial proliferation places tumor in glioblastoma multiformi group.

Ninety cases were selected including 30 cases of low grade astrocytoma, 30 anaplastic astrocytoma and 30 glioblastoma multiform. No pigicystic astrocytomas were included in these series.

For Immunohistochemical detection of Ki-67 antigen, formalin fixed, paraffin-embedded materials were used. The sections were selected for histologic diagnosis by Hematoxylin-Eosin staining.

Immunostaining was performed as the following:

The sections were pretreated in an autoclave to enhance immunoreactivities according to method of Shin et al. with some modifications. After deparaffinization in xylene and rehydration in ethanol, 4 μm section were immersed in Phosphate Buffer Saline (PBS), H2O2, buffered citrate with pH6 and then washed with distilled water and again PBS. The MIB-1 monoclonal antibody (Monoclonal Mouse-Anti-human, M 7240, Dako) was used as the primary antibody for Ki-67. Immunohistochemical detection was carried out using labeled streptavidin – biotin method with the LSAB kit (K0673, Dako). Sections were incubated with the primary antibody overnight and then secondary antibody...
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(Biotinylated linked anti mouse and anti rabbit, K 0673, Dako) for 30 minutes and finally reacted with peroxidase-labeled streptavidin for 30 minutes. The sections were developed with Diaminobenzidine and stained with Hematoxylin-Eosin.

Ki-67 LI values were determined by counting at least 1000 nuclei, at high power fields (x400), in those areas expressing the highest positivity. Vascular cells and hematogenous cells, when recognized were excluded from the counting. Positive control was tonsil with reactive follicular hyperplasia which shows positivity in germinal centers and basal cells of lining epithelium. Negative control was the same as positive control but without adding primary antibody.

Differences between the mean of MIB-1 LI values for histologic grades, mitotic count and age were compared using analysis by Chi-square test, Pearson’s correlation and analysis of Variance (ANOVA).

Results
For each type of astrocytic tumors, the mean of patients age was 19.7 (3 to 58) years, 36.9 (17 to 68) years and 46 (12 to 68) years for low grade astrocytoma, anaplastic astrocytoma and glioblastoma respectively. (Table-2).

From the overall 90 cases, 63.3% (n=57) were male and 36.7% (n=33) were female. The glioblastoma group was consisted of 56.7% male and 43.3% female. (Table-2).

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<th>Table-2: Age and Sex Characteristics</th>
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<td>Tumor Groups</td>
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<td>Age</td>
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<td>Male / Female</td>
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There was a meaningful differences for age, between the three groups (P<0.05) with tendency to increase within higher histologic groups. Satisfactory staining was obtained in all three grades of astrocytomas.

The MIB-1 reactivity was strong and easy to count. The MIB-1 immunoreactivity showed clear nuclear staining with nucleolar prominence. Positive nuclei were easily detectable in most of the tumors. High magnification was sometimes helpful for counting fainting stained cells. The mean MIB-1 LI found in low grade astrocytoma was 10.13±13.45. The MIB-1 LI of anaplastic astrocytoma and glioblastoma were (48.75±79.26) and (238±174.02) respectively. (Table-3).

The mean of MIB-1 LI found in grade II astrocytoma was significantly lower than the indices of grade III to IV astrocytoma (P<0.000). There was also meaningful difference between indices of anaplastic astrocytoma and glioblastoma (P<0.000).

Rare MIB-1 positivity was seen in a few cases of low grade astrocytoma (Fig. 1), but it could be seen with higher count in anaplastic astrocytoma (Fig-2) and the highest count was seen in glioblastoma (Fig. 3).

Few mitotic counts were seen only in a few cases of low grade astrocytomas, but it could be seen frequency especially in glioblastomas (1 to 34/1000 cells).

Although, in some cases with numerous MIB-1 positivity, only a few mitosis were counted, there was strong correlation between number of mitosis of MIB-1 positivity (Pearson correlation = 0.836).

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<th>Table-3: MIB-1 LI and Mitotic Count</th>
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<td>Tumor Groups</td>
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<td>MIB-1 LI</td>
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<td>Mean MIB-1 LI</td>
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<td>Mean Mitotic Count</td>
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<td>Mean Mitotic Count</td>
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*MIB-1 Labeling Index (LI) was expressed at the total positive cases per 1000 nuclei

**Mitosis was counted in 1000 cells

SD: Standard Deviation
LI: Labeling Index

It is noteworthy that due to increasing age in higher histologic groups, we exclude the effect of age on MIB-1 LI. As mentioned previously it became meaningful after this exclusion. Also, we tried to reach to cut-off point value in MIB-1 LI between different groups, but due to great
heterogeneity of this value in each group we could not reach to this purpose.

**Discussion**

Although the classification of astrocytomas into the prognostic categories of pilocytic astrocytoma (WHO grade-I), low grade astrocytoma (WHO grade-II), anaplastic astrocytoma (WHO grade-III) and Glioblastoma (WHO grade-IV) is well known and accepted, morphologic criteria are not always accurate prognostic indicators of individual cases, especially in small biopsies. Regarding borderline cases and also in some cases of low grade (WHO grade-II) astrocytomas and anaplastic types (WHO grade-III) the morphologic criteria are not always helpful. So, the clinical outcome of patients with anaplastic astrocytoma can be excellent or dismal. However the distinction of these tumors is of high importance, because it is critical to patients managements. It has been suggested that the proliferative potential of gliomas may be strong predictor of prognosis. Bromodeoxyuridine labeling studies and cytomeric determination of S phase fraction have been shown to be of value, where immunostaining for Proliferating Cell Nuclear Antigen (PCNA) and Ki-67 (MIB-1) molecules expressed in cycling cells are readily applicable tests of proliferative potential.

Burger et al. (1986) were the first to show an increasing Ki-67 labeling index (Ki-67 LI) along with increasing histopathologic grade in astrocytomas. They found that Ki-67 (MIB-1) staining was particularly useful for distinguishing anaplastic admixture of tumor cells, especially in better differentiated tumor regions. Subsequently, a number of studies have demonstrated a close correlation of Ki-67 LI with astrocytoma grades, and suggested that the method has potential clinical value in assisting the diagnostic evaluation of the tumors. The results of the most recent studies are as following:

Ellison, et al (1996), worked on 123 patients with cerebral astrocytoma and showed that the mean of Ki-67 LIs differed significantly (P<0.0001) between different grades of astrocytoma and tumors with Ki-67 LI less than 2% had a significantly (P<0.0001) better prognosis.

Walkimoto and his colleagues (1996) performed Ki-67 immunochemistry on paraffin sections of 72 supratentorial astrocytoma using MIB-1 monoclonal antibody. The mean Ki-67 LI was 3.8% (±2.7% SD) in grade II, 18.4% (±9.7% SD) in grade III and 31.6% (±12.9% SD) in grade IV astrocytomas. They concluded that Ki-67 LI obtained using MIB-1 monoclonal antibody, is an important and practical tool for estimating biologic behavior of gliomas as well as for predicting survival.

Cunningham, et al. (1997), studied on 105 cases of astrocytomas for expressing of Ki-67 (MIB-1) and proliferating cell nuclear antigen (PCNA). In his study the MIB-1 and PCNA labeling indices increased with increasing tumor grading but showed no association with other clinicopathological parameters.

Khalid and colleagues (1997), performed Immunohistochemical analysis of progesterone receptor and Ki-67 LI over 86 astrocytic tumors and concluded that the mean of Ki-67 LI was significantly higher in the high grade (III and IV) astrocytomas compared with low grade (I and II) astrocytomas (P<0.0001) and also suggested that progesterone receptors, correlate with histologic grading and may participate in the growth of these tumors and tumor angiogenesis.

Pollack, et al. (1997) investigated the relationship between MIB-1 LI and its outcome over 29 malignant gliomas and concluded that there is striking difference in outcome between tumors with MIB-1 LI less than 12 and those with indices more than 12. Median progression free survival was more than 48 months for the low MIB-1 group compared with only six months for the high MIB-1 group.

Hsu et al. (1997) studied the value of MIB-1 (Ki-67) LI in the differentiation between grade II and grade III gliomas by WHO grading system and St. Anne-Mayo grading scale. They indicated a significant difference in mean LIs between grade II and III and grade II and IV (P<0.0001), but not between grade III and IV.
Univariate analysis showed that MIB-1 LI with cut-off point at 1.5% was a significant prognostic factor (P<0.0005)7.

HO et al. (1998), studied 101 pediatric patients with low grade astrocytoma, anaplastic astrocytoma and glioblastoma multiform and their proliferative potential was estimated using MIB-1 LI. The mean of MIB-1 LI was as follow: low grade astrocytoma (3.9±4.3 SD), anaplastic astrocytoma (24.3±15.6 SD) and glioblastoma (35.9±16.4SD). They concluded that histopathologic grading can predict the outcome of patients with astrocytomas and glioblastomas, where MIB-1 LI can separate better and worse prognostic groups in patients with anaplastic astrocytoma6.

Ricco and colleagues (2000) performed Mitotic Index (MI) and ki-67 immunostaining on specimen from 42 cases of glioblastomas, 17 cases of anaplastic astrocytomas and 14 cases of low grade astrocytomas. A positive trend from low grade to glioblastoma was found for Ki-67 LI and MI. They concluded that effective separation of different grades of astrocytoma can be made with Ki-67 LI and mitotic index5.

In the present study, we have demonstrated that MIB-1 (Ki-67) LI is useful in distinguishing between three groups of astrocytomas and showed meaningful differences in univariate analysis. The mean of MIB-1 LI in three groups of astrocytomas were as follow: 10.13 in low grade astrocytoma (WHO Grade II), 48.75 in anaplastic astrocytoma (WHO Grade III) and 238.40 in Glioblastoma (WHO Grade IV) (P<0.000). Its differences present after omitting the effect of age. We also tried to determine the best cut-off point of MIB-1 LI, especially in anaplastic astrocytomas and glioblastomas by using mean SD, but it was so wide and impractical. In only one study Hsu et al. calculated a cut-off point of 1.5% for MIB-1 LI that gave the most significant statistical value between low grade and anaplastic astrocytomas4,20,21.

The association between high MIB-1 LI and higher tumor grading suggests that MIB-1 LI is useful in detecting aggressive astrocytomas lacking definitive histopathologic features. Our results are in concordance with most other studies, which all demonstrated the significance of differences between means of MIB-1 LI in different grades of astrocytoma1,2,10-15,18,22,23.

Mitotic count is exceptional in low grade astrocytomas and easily found in glioblastomas. In our study, a significant correlation have been found between number of mitoses and MIB-1 LI and grading of astrocytomas (P<0.000). Among various methods for measuring tumor growth potential, Ki-67 (MIB-1) Immunostaining is superior to others such as Bromodeoxyuridine incorporation and flow cytometry, for its
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noninvasive nature and its ease of usage. It is a preferred immunohistochemical marker for cell proliferation because of its short half-life (2-5 minutes), which makes it less likely to label cells that have exited from the cycling phase\(^2\). Considering the selection of the antibody for cell proliferation estimation, the wider coverage of proliferating cells by the MIB-1 (Ki-67) method, when compared with the PCNA method, provided a better statistical distinction between the cell proliferation activity and different grades of astrocytomas\(^2\).

We concluded that MIB-1 (Ki-67) immunohistochemistry, determines cell proliferation activity in astrocytoma and increases significantly differences along with the histopathological malignancy. The significant differences in MIB-1 proliferation activity between different astrocytoma malignancy categories, indicates that estimation of cell proliferation can help pathologists in the diagnostic determination of astrocytomas, especially in cases of borderline grades. So, MIB-1 LI may play an important role in supplementing traditional classification schemes to determine the prognosis of patients with astrocytoma.

References


