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*Original Article*

# Differentiation between reactive gliosis and astrocytomas by MIB-1/ki67 immunostaining

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## Abstract

**BACKGROUND:** Astrocytic tumors are the most common primary tumors of the central nervous system. These tumors have an inherited tendency to progress and recurrence. The histopathological examination and grading do not always identify the subset of these tumors especially when the tumor sample is small or inadequate. This study was undertaken to answer the question whether MIB-1 expression could assist in discrimination between low grade and high-grade glioma and gliosis especially when the biopsy sample is small, such as in stereotactic brain biopsy.

**Methods:** This descriptive analytical study was performed on 114 glial and gliotic paraffin-embedded tissues. KI67 immunohistochemistry was also used on paraffin section using the monoclonal antibody MIB-1. The results were analyzed by ANOVA test.

**Results:** Based on light microscopic findings 89 (78.07%) were astrocytomas and 25 (21.9%) were reactive gliosis. The mean Ki67 labeling index (LI) was 25.2% (±30) for astrocytomas in general and 1.92 (±1.2) for gliosis. In other words, it was 1.8 (±1) for grade I, 14.5% (±4) grade II and 64.5 % (±19.3) for grade III/IV astrocytomas. The MIB-1 labeling index for astrocytic tumors was significantly higher than that for gliosis ($P<0.001$) and it increased with increasing tumor grade. However, MIB-1 labeling index was the same for pilocytic astrocytoma and gliosis so there was no meaningful difference between grade 1 astrocytoma and gliosis.

**CONCLUSIONS:** Given the conventional microscopic examination and KI67 (MIB-1) method for grading astrocytomas, MIB-1 is more reliable and a complementary method for definitive diagnosis.

**KEY WORDS:** Astrocytoma, gliosis, monoclonal antibody, MIB-1, proliferating index.

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In general, central nervous system (CNS) tumors are originated from neuroglia, neurons and meningeal membranes [1]. The most common CNS tumors are gliomas with different degrees of differentiation and tendencies for malignant progression [2]. Despite using different methods for grading gliomas, interobserver variability remains controversial particularly if the biopsy sample is small or inadequate for histological diagnosis. For example, although pilocytic astrocytomas usually have a typical histology but they may appear with different cellular densities and cell forms. Also, in low-grade astrocytomas increased cellularity and nuclear atypia may occur [3,4]. Therefore, differentiation of these tumors from anaplastic astrocytoma and glioblastoma multiforme is a challenge for the neuropathologist.

On the other hand, sometimes gliomas are indistinguishable from normal brain tissue and

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reactive gliosis, astrogliosis (characterized by an increased number of astrocytes in response to injury such as infection, arteriovenous malformation, demyelinating disease and metastatic tumor) [5]. Since precise differential diagnosis of above-mentioned pathological conditions is helpful for treatment planning and it is not possible to reach that goal with concurrent conventional microscopic examination, using cell proliferation marker (Ki67) is helpful for this purpose. One of the best and well known immunohistochemical methods for evaluating the proliferation rate is quantitation of the Ki67, which was developed and introduced by Gerdes in 1993 [6]; a technique which shows all phases of cell cycle except cells in G0 phase. Because the use of Ki67 antigen is restricted to frozen material, a new antibody (MIB-1) to an epitope of the Ki67 antigen is used on paraffin–embedded tissue [7,8]. Although this marker is helpful marker for evaluating cellular proliferation, previous studies that used it in grading gliomas have shown conflicting results. The aim of this study was to determine the value of MIB-1 in grading glioma and gliosis.

## Methods

The specimens consisted of 114 formalin fixed, paraffin-embedded archival materials of astrocytic neoplasms and glioses. The inclusion criteria were adequacy of specimen and histologic grading according to WHO classification, which included pilocytic (grade I), low grade (grade II) and high-grade (III/IV) astrocytomas. The processing to detect Ki67 protein was as the following: a four micron section was obtained from paraffinized block for hematoxylin-eosin and immunohistochemical staining; the sections were mounted by polylysine followed by deparaffinization in xylene and rehydration in alcohol for 5 minutes; then, the sections were socked in buffered citrate (pH = 6) for retrieval of antigens. The sections were also incubated with MIB-1 antibody (DAKO corporation M7240), biotinylated secondary antibody and peroxides–labeled strepavidin H-R-P complex, 10 minutes each. The mitotic figures were visualized using H202 as substrate and 3-3 aminobenzidine as chromogen and finally sections were counterstained with hematoxylin. MIB-1 labeling index (MIB-1 LI) was calculated by the ratio of the stained cells to the whole cells of the field (figure 1). Statistical analysis of the proliferating index was carried out by ANOVA and proved by spearman's test.

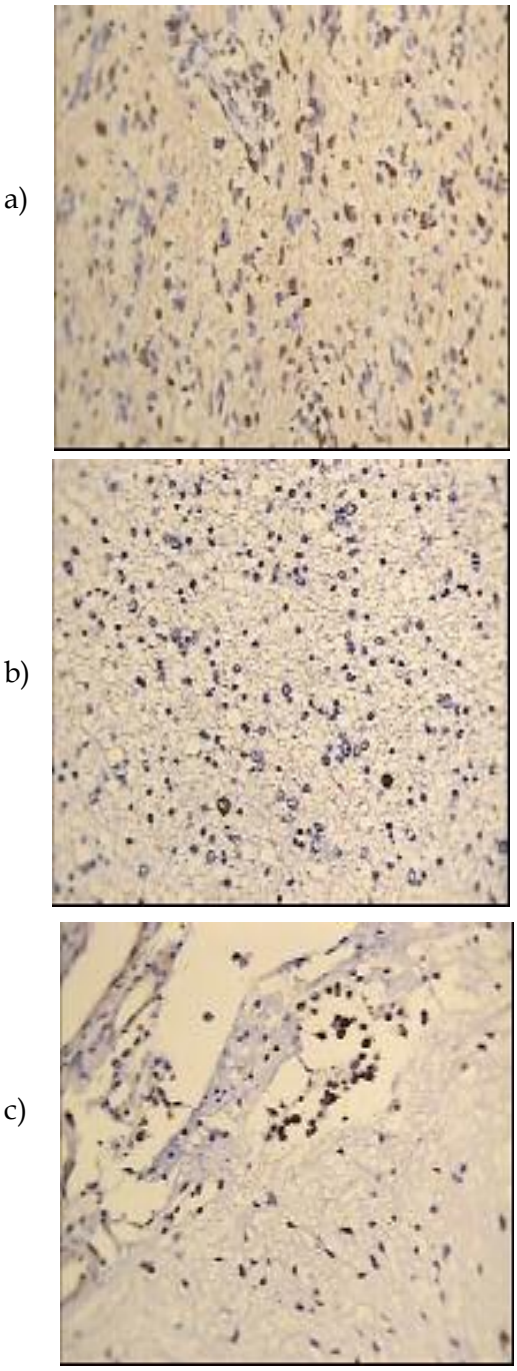


**Figure 1.** Shows Ki67 nuclear immunostaining in high-grade glioma (a), low-grade glioma (b) and gliosis around metastatic tumor (c).

## Results

Of the 114 patients, 46 were male and 56 were female. The mean age was 41 years (range 4-71 years). 89 (78.07%) were gliomas and 25 (21.9%) were glioses. The study included 38 (42.78%) grade I, 22 (24.71%) grade II and 29 (32.58%) grade III/IV. The mean proliferating index for astrocytic tumors was 25.2 ± 30 and for glioses was 1.92 ± 1.2. One-way analysis of variance showed that MIB-1 labeling index differs significantly in gliomas comparing with glioses (P<0.001). The mean labeling index in grade 1 was 1.8 ± 1, in grade II was 14.5 ± 4 and in grade III/IV was 64.5 ± 19.3. The ANOVA test showed that MIB-1 labeling index correlates significantly with the grading of the tumor; that is, the higher the grading of tumors, the higher the MIB-1 proliferating index. However, the comparison of MIB-1 LI in pilocytic astrocytoma and gliosis was not meaningful (P>0.05) (table I). The frequency distribution and range of age of the patients in different tumor grades were as follows: pilocytic tumors composed 20.4% of the total astrocytic neoplasms and was seen in patients younger than 20 years; grade II astrocytomas included 39.40% of the tumors and was observed in 21-40 year-old patients; anaplastic astrocytoma and glioblastomas involved 40.3% of the tumors and was seen in 41-60 year-old patients. There was no evidence of linear association between MIB-1 score and age in our series. The MIB-1 LI was 24.8 ± 28.8 for male and 22.5 ± 29.4 for female. Also, independent t-test showed that the proliferating index was not correlated with sex (P = 0.06).

**Table I.** Summary of KI67 (MIB-1 labeling index) in gliomas and glioses.

| Group | Number of patients | MIB-1 LI (%) (mean ± SD) |
|---|---|---|
| Pilocytic astrocytoma (PA) | 38 | 1.8 ± 1 |
| Astrocytoma (A) | 22 | 14.5 ± 4 |
| Anaplastic astrocytoma and glioblastoma multiforme (AA+GB) | 29 | 64.5 ± 19.3 |
| Gliosis | 25 | 1.92 ± 1.2 |

P values:
PA versus (AA + GB) <0.001
A versus (AA + GB) <0.001
Gliosis versus (AA + GB) <0.001
PA versus gliosis >0.05

## Discussion

The quantitative analysis of astrocytic proliferation increases our knowledge about the biological behavior of gliomas and will help predict the outcome of patients suffering from these tumors. MIB-1 is one of the most useful markers for evaluating cellular proliferation in various human neoplasms including intracranial tumors [9-11]. However, previous studies using proliferation markers have shown conflicting results. In some studies, MIB-1 has correlated significantly with the grade of astrocytomas while in others no significant correlation has been found [12,13]. The goal of the present study was to determine the value of MIB-1 in grading glioma and gliosis. In our study, reactive gliosis and pilocytic astrocytoma had the lowest MIB-1 labeling index and expression of MIB-1 was not significantly higher in pilocytic astrocytoma compared with gliosis. However, the mean MIB-1 labeling index differed significantly between the low and the high-grade gliomas. Colodner in a recent study demonstrated that there is modest amount of astrocytic proliferation in non-neoplastic conditions and average labeling index could change from zero in longstanding gliosis to 5.8 ± 2 in progressive multifocal leukoencephalopathy.

The results proved that the rates of astrocytic proliferation in reactive conditions are similar to those seen in low-grade gliomas [5,14,15]. Although in pilocytic astrocytoma mitotic activity is generally nil or difficult to demonstrate, in some pilocytic astrocytomas MIB-1 labeling index is similar to that of grade II gliomas [16,17]. Matsumoto et al found that MIB-1 labeling index in both pilocytic astrocytoma and other low-grade astrocytomas were significantly lower than that in high-grade gliomas but in 2 of 18 pilocytic astrocytomas and 6 of 14 astrocytomas, immunoreactivity for MIB-1 was compatible with anaplastic astrocytoma and glioblastoma multiformis. He concluded that this marker alone is not useful for differentiation of these tumors [4]. Klein et al demonstrated that proliferation rates in astrocytomas not only reflect proliferation of tumor cells but also that of microglial, 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. However, because the proliferation rate does not solely reflect the proliferation of tumor cells, authors stressed on a double labeling study [using the antibodies MIB-1 (Ki-67) as proliferation marker and Ki-M1P (CD68) as microglia marker] for final diagnosis [18]. The close correlation of MIB-1 labeling index and histologic grade was detected by Cunningham, Scott, Pollack and Thomas [19-22]. Sallinen also mentioned that MIB-1 is helpful for differentiating grade II from grade III astrocytoma and stressed on capability of this marker for grading diffusely infiltrating astrocytomas [23]. In general, cell proliferation index correlates with tumor grade but broad range of proliferation index in pilocytic gliomas should be considered in grading system.

## Conclusions

The immunohistochemical analysis of astrocytic tumors may facilitate tumor grading and help us predict prognosis. But, using this marker to differentiate pilocytic astrocytomas from low-grade gliomas need more studies including a double labeling study.

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