10.1053.paor.2001.0335 available online at http://www.idealibrary.com on IDEAL

ARTICLE

# Clinicopathological Features, MIB-1 Labeling Index and Apoptotic Index in Recurrent Astrocytic Tumors

Angela Mercy RALTE, Mehar Chand SHARMA, Asis Kumar KARAK, Veer Singh MEHTA,* Chitra SARKAR

Departments of Pathology and *Neurosurgery, All India Institute of Medical Sciences, New Delhi

**This is a study of 64 cases of recurrent astrocytic tumors of all four WHO grades wherein a comparative evaluation of initial vs. recurrent tumor was done with respect to histological grading, MIB-1 labeling index (LI) and apoptotic index (AI). The aim was to identify factor/s that could influence interval to recurrence and/or malignant progression. Recurrence was noted in all grades and upon recurrence, 93.3% of grade II (low grade diffuse) astrocytomas and 63.6% of grade III anaplastic astrocytomas underwent malignant progression. However, none of the Grade I tumors showed evidence of malignant progression. Though interval to recurrence varied considerably, there was a correlation with histological grade of the initial tumor in that grade I and II tumors had a significantly longer mean interval to recurrence (43 months and 54.8 months respectively) as compared to grade III and IV (glioblastoma multiforme) tumors (17.6 and 12.8 months respectively). The interval to recurrence was also longer for grade II and III tumors which showed progression on recurrence (55.3 months for Grade II→Grade III; 54 months for Grade II→Grade IV and 20.6 months for Grade III→IV) as compared to tumors which recurred to the same grade (12.5 months for Grade III→Grade III and 12.8 months for Grade IV→Grade IV). A statistically significant inverse correlation of MIB-1 LI with interval to recurrence was noted. Higher the MIB-1 LI, shorter was the interval to recurrence. Further a cut off MIB-1 LI value of 2.8% could be proposed in predicting recurrence free survival. Interestingly, MIB-1 LI of grade II tumors, which had progressed to grade IV was significantly higher than MIB-1 LI of grade II tumors which had progressed to grade III. Thus, this study establishes the potential role of MIB-1 LI of the initial tumor in determining interval to recurrence. However, apoptotic index has no role in predicting either interval to recurrence or malignant progression.** (Pathology Oncology Research Vol 7, No 4, 267–278, 2001)

*Keywords:* astrocytic tumors, recurrent, MIB-1 labeling index, apoptotic index, interval to recurrence, progression

## *Introduction*

An important feature of astrocytomas is their inherent tendency to recur after surgical resection. Many WHO grade II (low grade diffuse) astrocytomas and nearly all high grade anaplastic astrocytomas and glioblastoma (WHO grades III and IV respectively) recur at some point[1] Another interesting feature is that upon recurrence, some tumors retain the same histological grade, whereas a significant proportion undergo malignant progression i.e. regrow into a more anaplastic form so that the grade of the recurrent tumor is higher than the original tumor.[28]

There are as yet no well defined parameters which can either predict the occurrence of recurrence/malignant progression or the interval to recurrence/malignant progression. Histological grading has not been found useful in this regard. Though generally recurrences in high grade tumors occur after shorter interval than in low grade tumors, this is not absolute and unpredictable for a given patient.[2,31]

Several workers have used proliferation index and p53 gene alterations for prediction of recurrence but results are controversial.[5,8,9,13,15,17,19,24,25,29] Similarly, the relationship between malignant progression and apoptosis is not clearly defined.[3,5,7,24,27] Nakamura et al[24] studied p53 protein expression, MIB-1 labeling index (MIB-1 LI) and apoptotic index (AI) in 46 recurrent anaplastic astrocytomas and reported that MIB-1 LI and AI were higher in the recurrent compared to the initial tumor. However, no correlation was noted with other parameters.

In this study, we report the clinicopathological features of 64 cases of recurrent astrocytic tumors and the

*Received:* Sept 25, 2001; *accepted:* Nov 19, 2001

*Correspondence:* Prof. Chitra SARKAR, Department of Pathology, All India Institute of Medical Sciences, New Delhi.-110029, India; Tel: 91-11-659 3371, fax: 91-11-686 2663;
e-mail: sarkarcs@hotmail.com

*Supported by Indian Council of Medical Research

 1219-4956/01/040267+12 $ 12.00/0

correlation of histological grade, MIB-1 LI and apoptotic index with interval to recurrence and malignant progression.

## *Materials and Methods*

The records of the Neuropathology Laboratory, Department of Pathology, AIIMS as well as those of the Department of Neurosurgery, AIIMS were reviewed over a period of 12 years (1989 to 2000) to identify cases of astrocytic tumors that had recurred after treatment for original tumor.

A total of 64 patients with astrocytic tumors were found to have experienced at least one surgically operated and histologically verified recurrence in this 12 year period. Of the 64 cases, paraffin blocks containing adequate tissues of both primary and recurrent tumor were available only in 56 cases. Staining for MIB-1 and apoptosis were done in these 56 cases.

### *Parameters evaluated and mode of evaluation*

#### *Clinical parameters*

Clinical data were obtained from the Departments of Pathology and Neurosurgery. These included age, sex, location and interval to recurrence.

#### *Histological evaluation*

The HE slides of the initial and recurrent tumors in each of the 64 cases were jointly reviewed and graded according to WHO criteria.[16]

#### *Definition of tumor groups*

The tumors were classified into following groups.

Group I: Recurrences showing the same grade as previous/initial tumor.

Group II: Recurrences with malignant progression i.e.

a) progression from grade II to grade III (II→III)
b) progression from grade II to grade IV (II→IV)
c) progression from grade III to grade IV (III→IV)

#### *Immunostaining method for MIB-1*

Immunohistochemical staining was done by the streptavidin–avidin–biotin immunoperoxidase technique (LSAB) using monoclonal antibody to MIB-1 (Immunotech, USA). The MIB-1 LI was then calculated. One or two representative blocks of formalin fixed paraffin embedded tissue was selected from the initial and recurrent tumor in each case. Serial 5 µ thick sections were cut from the block/s of initial and recurrent tumor thus chosen for each case and taken on poly-L-lysine coated slides (Sigma, USA). The tissue sections were deparaffinised and brought to water. They were then subjected to high temperature microwave antigen unmasking by heating the sections immersed in 10 mM citrated buffer pH 6.0 (prepared by dissolving 2.1gr anhydrous citric acid crystals in 1l distilled water and pH adjusted to 6.0) inside a 600 watt microwave oven in full power for 35 minutes. The slides were allowed to cool to room temperature and then washed briefly with 0.05 M Tris-HCl buffer, pH 7.4. To diminish nonspecific staining (endogenous peroxidase activity) each slide was treated with methanol containing 3 % hydrogen peroxide for 30 mins. (1 part 3% $H_2O_2$ + 4 parts absolute methanol). After brief rinsing the sections were placed in 0.05 M Tris-HCl buffer pH 7.4 for 10 mins. Excess buffer was tapped off and sections were then overlaid with adequate amount of appropriately diluted primary antibody followed by overnight incubation at 4°C in a humid chamber.

MIB-1 antibody was obtained from Immunotech, USA, and was used in a dilution of 1:50. The slides were then washed in three changes (5 mins each) of 0.05 M Tris–HCl buffer, pH 7.4, followed by incubation for 30 mins at room temperature after application of biotinylated secondary antibody of anti mouse immunoglobulins in phosphate buffered saline (PBS) containing carrier protein and 15 mM sodium azide (Large volume universal DAKO LSAB kit, Peroxidase, Dakopatts, Denmark). After 3 washes (5 mins each) in Tris HCl buffer, peroxidase conjugated streptavidin was applied to cover the sections and incubated at room temperature for 30 mins. Slides were rinsed with 3 changes of Tris HCl buffer for 5 mins each. Sections were then covered with substrate chromogen solution freshly prepared by dissolving 1 mg 3,3'-diaminobenzidine tetrahydrochloride or DAB (Sigma, St.Louis, USA) in 1 ml 0.05 M Tris-HCl buffer pH 7.4 containing 1 µl $H_2O_2$. The slides were incubated at room temperature for 5 to 10 mins under microscopic control till the optimum development of brown coloured peroxidase reaction product. After rinsing in distilled water, the sections were counter stained with Harris Hematoxylin followed by mounting with DPX as mounting media. During each batch of staining, appropriate positive and negative controls were used. Negative control was achieved by omitting the primary antibody. Sections of reactive lymphnode were used as positive control for MIB-1 staining. All incubations were done in humid chamber

#### *Estimation of MIB-1 labeling index (MIB-1 LI)*

The labeling index (LI) was calculated as a percentage of labeled nuclei per 1000 cells. This was done in initial and recurrent tumors of 56 cases on serial sections. One thousand tumor cells were counted in several areas of tissue in which positively stained nuclei were evenly distributed. But in those cases with uneven distribution of positive nuclei, the LI was generally calculated in the areas with highest density of positive nuclei by visual analysis. This modality was less prone to errors and pro-

vided evaluation of that part of the tumor with highest growth rate. The range and mean LI values were then calculated .

*Tunel technique (terminal nucleotidyl transferase d-UTP mediated nick end labeling)*

The TUNEL technique[3,5,7,23] was done using the TUNEL kit obtained from Boehringer–Mannheim, Germany. Briefly: five micron thick sections were deparaffinised and brought to water. Nuclei of the tissue sections were stripped from proteins by incubation with proteinase–K (20 µg/ml in 10 mM Tris–HCl buffer pH 7.6) for 30 mins at 37°C. The slides were then washed in phosphate buffered saline (PBS) 3 times. Endogenous peroxidase was blocked by covering the sections with 0.3% of hydrogen peroxide in methanol for 30 mins. at room temperature. The sections were rinsed with PBS 3 times and covered with 50 µl of TUNEL mixture and incubated in humid chamber at 37°C for 60 mins. Washing in PBS 3 times and the area around the sample dried. The sections were then covered with 50 µl of converter peroxidase mixture and incubated in humid chamber at 37°C for 60 mins. Washing with PBS x 3 times. Sections were then covered with substrate chromogen solution prepared freshly by dissolving 1 mg of 3,3'-diaminobenzidine tetra hydrochloride or DAB (M/s Sigma St. Louis, USA) in 1 ml 0.05 M Tris–HCl buffer pH 7.4 containing 1 l $H_2O_2$. The slides were incubated at room temperature for 5-10 min under microscopic control till the optimum development of brown colored peroxidase reaction product. After rinsing in distilled water the sections were counter stained with Harris hematoxylin followed by mounting with DPX as mounting media.

*Estimation of apoptotic index (AI)*

AI was determined by counting 1000 cells and expressing it as number of labeled cells per 1000 cells. Labeled cells adjacent to or within necrotic areas were excluded while counting the apoptotic index .The range and mean were then calculated.

*Statistical analysis*

Descriptive statistics i.e. mean, standard deviation, frequency distribution was calculated for continuous as well as categorical variables. Student 't' test (paired and unpaired), Wilcoxon's rank sum and Wilcoxon's sign rank test, one way analysis of variance (parametric and non-parametric) with post-hoc analysis and Pearson's correlation coefficient were performed wherever applicable. p-value of $\leq 0.05$ was considered statistically significant.

## *Results*

*Histological grading of primary tumors*

The histological grading of the 64 primary tumors (first surgical intervention) according to WHO criteria was as follows:

Grade I: Pilocytic astrocytoma (8 cases)
Grade II: Low grade diffuse astrocytoma (30 cases; 10/30 were gemistocytic astrocytomas)
Grade III: Anaplastic astrocytoma (11 cases)
Grade IV: Glioblastoma multiforme (15 cases)

*Age and sex distribution of primary tumors*

The grade I tumors mostly occurred in children and young adolescents with a mean age of 15.2 years. Grade II and III tumors were more common in adults with a mean age of 31 and 33.7 years respectively. The grade IV GBMS occurred in the elderly with a mean age of 45.1 years. There was a striking male preponderance in all grades of astrocytic tumors (*Table 1*).

*Location of primary tumors*

All 64 cases were supratentorial hemispheric in location except 4 cases of grade I (pilocytic astrocytoma) which occurred in the cerebellum. The various sites where the tumors were located are: frontal – 20, parietal –18, temporal – 16, frontoparietal – 2, optic nerve – 3, cerebellum – 4.

*Table 1.* **Age and sex distribution and histological grading of 64 astrocytic tumors**

| *Initial grade (No.)* | *Mean age in yrs. (range)* | *Sex (M:F)* | *No. recur* | *No. (%) Grade unchanged at recurrence* | *No. (%) Grade progression at recurrence* | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | *Gr II* | *Gr III* | *Gr IV* | *Total* |
| Grade I (8) | 15.2 (4-50) | 3:1 | 8 | 8 (100%) | 0 | 0 | 0 | 0 |
| Grade II (30) | 31 (16-46) | 2.7:1 | 30 | 2 (6.7%) | 0 | 12 (42.9%) | 16 (57.1%) | 28 (93.3%) |
| Grade III (11) | 33.7 (19.51) | 2.5:1 | 11 | 4 (36.4%) | 0 | 0 | 7 | 7 (63.6%) |
| Grade IV (15) | 45.1 (19.65) | 4.5:1 | 15 | 15 (100%) | 0 | 0 | 0 | 0 |
| Total | | | 64 | 29 (45.3%) | 0 | 12 (34.3%) | 23 (65.7%) | 35 (54.7%) |

*All 10 gemistocytic astrocytomas underwent malignant progression on recurrence – 3 progressed to Grade III and 7 to GBM.

*Treatment of initial and recurrent tumors*

All initial tumors were primarily treated by surgical resection which aimed at maximal tumor removal (macroscopic total resection >90%) without risking useful neurological functions. This usually implied frontal or temporal lobectomy for tumors situated in the polar regions and extensive removal for tumors in the central and parietal regions. All patients received conventional radiotherapy following surgery.

The recurrent tumors were also excised to variable extents. Subsequently, radiotherapy was given again. Information on chemotherapy was available in 23/26 patients with grade III and IV tumors. All received chemotherapy following the first surgery. In addition chemotherapy was given again following surgery for the recurrent tumor in 22 of these cases.

*Histological grading of recurrent tumors*

RECURRENT TUMORS WITH EVIDENCE OF MALIGNANT PROGRESSION (TABLE 1). This phenomenon was observed with grade II and III tumors. Thus, 28/30 (93.3%) of grade II astrocytomas underwent malignant progression on recurrence. Twelve (42.9%) progressed to grade III while 16 (57.1%) progressed to grade IV GBM (secondary GBM). Interestingly, all the 10 gemistocytic astrocytomas underwent malignant progression on recurrence-7 to GBM and 3 to grade III. Similarly, 7/11 (63.6%) of grade III anaplastic astrocytomas recurred as grade IV GBM (secondary GBM).

RECURRENT TUMORS WHEREIN HISTOLOGICAL GRADE WAS THE SAME AS THE ORIGINAL TUMOR (TABLE 1). All the 8 recurrent grade I pilocytic astrocytomas were of the same grade. There was no evidence of malignant progression in any of them. Similarly, all the 15 grade IV GBMS recurred to the same grade. However, of the 30 low-grade (grade II) astrocytomas, only 2 (6.7%) recurred to the same grade. Similarly, only 4 of the 11 (36.4%) grade III anaplastic astrocytomas underwent recurrence to the same grade.

PRIMARY VS. SECONDARY GBM. There were a total of 38 recurrent GBMs in this study. 23 of the 38 (60.5%) were secondary GBM having arisen by progression from grade II and III tumors. The remaining 15 were de novo (primary) GBMs and recurred to the same grade.

*Site of recurrence*

All the 64 tumors recurred at the same (original) site. Only one patient had a second recurrence.

*Interval to recurrence*

INTERVAL TO RECURRENCE AND HISTOLOGICAL GRADING OF INITIAL TUMOR. The interval to recurrence was defined by the time interval between the first operation for the initial/primary tumor and the operation for the first recurrence. The 64 tumors recurred 6 to 188 months after initial treatment (mean = 32 months). The range was astonishingly wide in all grades of astrocytomas (*Table 2*). For grade I tumors, the mean interval to recurrence was 43 months. Interestingly, the grade II tumors had a statistically significant longer mean interval to recurrence of 54.8 months as against 17.6 months for grade III and 12.8 months for grade IV tumors ($p < 0.05$). The difference between interval to recurrence of grade I vs. grade II and

*Table 2.* **Interval to recurrence yearly**

| *Grade* | *≤ 1 yr. (0-12 m)* | *1 – 2 (13-24 )* | *2 – 3 (25-36)* | *3 – 4 (37-48 )* | *4 – 5 (59-60)* | *5 – 10 (61-120)* | *> 10 (>120)* | *Range (mths)* | *Mean ± S.D. (mths)* |
|---|---|---|---|---|---|---|---|---|---|
| I → I (8) | 1 (12.5%) | 3 (37.5%) | 1 (12.5%) | 0 | 1 (12.5%) | 2 (25%) | 0 | 9 – 101 | 42.9 ± 35.0* |
| II → II (2) | 0 | 0 | 1 (50%) | 0 | 0 | 1 (50%) | 0 | 27 – 90 | 58.5 |
| II → III (12) | 1 (8.3%) | 1 (8.3%) | 3 (25%) | 2 (16.7%) | 3 (25%) | 1 (8.3%) | 1 (8.3%) | 6 – 185 | 55.3 ± 47.7 |
| II → IV (16) | 0 | 4 (25%) | 5 (31.25%) | 1 (6.25%) | 2 (12.5%) | 3 (18.75%) | 1 (6.25%) | 13 – 188 | 53.9 ± 48.30 |
| Total Gr II (30) | 1 | 5 | 9 | 3 | 5 | 5 | 2 | 6 – 188 | 54.8 ± 46.2** |
| III → III (4) | 2 (50%) | 2 (50%) | 0 | 0 | 0 | 0 | 0 | 6 – 22 | 12.5 ± 6.9 |
| III → IV (7) | 1 (14.3%) | 4 (57.1%) | 2 (28.6%) | 0 | 0 | 0 | 0 | 12 – 30 | 20.6 ± 6.4 |
| Total Gr.III (11) | 3 | 6 | 2 | 0 | 0 | 0 | 0 | 6 – 30 | 17.6 ± 7.5 |
| IV → IV (15) | 9 (60%) | 5 (33.3%) | 1 (6.7%) | 0 | 0 | 0 | 0 | 6 – 34 | 13.0 ± 7.8 |

Wilcoxon's rank sum test & Student 't' test; Gr. II → Gr. III vs. Gr. II → Gr IV; Not Significant; Gr. III → Gr. III vs. Gr. III → Gr. IV; Not Significant; One way Anova non-parametric analysis: *Gr. I vs Gr. III and Gr. I vs Gr. IV: Significant ($p < 0.05$); ** Gr. II vs. Gr. III and Gr. II vs. Gr. IV : Significant ($p < 0.05$); Gr. I vs. Gr. II and Gr. III vs. Gr. IV : Not Significant

*Table 3.* **Interval to recurrence of tumors with and without progression**

| *Grade initial recurrent* | *< 1 yr* | *1-2 yrs* | *2-3 yrs* | *3-4 yrs* | *4-5 yrs* | *5-10 yrs* | *>10yrs* | *Range (months)* | *Mean ± sd (months)* |
|---|---|---|---|---|---|---|---|---|---|
| III → III (4) | 2 (50%) | 2 (50%) | – | – | – | – | – | 6–22 | 12.5 ± 6.9 |
| II → III (12) | 1 (8.3%) | 1 (8.3%) | 3 (25%) | 2 (16.7%) | 3 (25%) | 1 (8.3%) | 1 (8.3%) | 6–185 | 55.3 ± 47.7[†] |
| IV → IV (15) De novo GBM | 9 (60%) | 5 (33.3%) | 1(6.7%) | – | – | – | – | 6–34 | 13.0 ± 7.8 |
| III → IV (7) | 1 (14.3%) | 4 (57.1%) | 2 (28.6%) | – | – | – | – | 12-30 | 20.6± 6.41[*] |
| II → IV (16) Secondary GBM | – | 4 (25%) | 5 (31.3%) | 1 (6.3%) | 2 (12.5%) | 3 (18.8%) | 11 (6.3%) | 13-188 | 53.9± 48.3[**] |

Wilcoxon's rank sum test and students 't' test: [†] $p<0.02$ (*significant*); * Grade III → IV vs Grade IV → IV: Significant ($p<0.03$); ** Grade II → IV vs Grade IV → IV: Significant ($p<0.05$); Grade II → IV vs Grade III → IV: Significant ($p<0.02$)

grade III vs. grade IV tumors was however not found to be statistically significant. Therefore, it appears that grade II tumors take a longer time to recur possibly because of their comparatively lower biologic malignant potential as well as because of the genetic mutations which they have to acquire to progress to higher grades. However, for a given patient, the course is unpredictable.

*Interval to recurrence in tumors with and without progression (Tables 2)*

GRADE I (PILOCYTIC ) ASTROCYTOMAS. The recurrence of grade I tumors was variable occurring over a wide range (9 months to 10 years) with a mean of 42.9 months. No special trend was observed.

GRADE II (LOW GRADE DIFFUSE ASTROCYTOMAS). *Without progression* (grade II to grade II): There were only 2 tumors in this group. 1 recurred at 27 months and the other at 90 months.

*With progression* (grade II to grade III/IV): The mean interval to recurrence of grade II tumors progressing to grade III was 55.3 months (range 6-185 months) while that of grade II progressing to grade IV was 54 months (range 13-188 months). There was statistically no difference in the mean interval to recurrence in the two groups. However, on dividing the interval to recurrence year-wise, an interesting pattern was observed. In the grade II to III progression group, 66.7% (8/12) recurred between 2-5 years. But, in the grade II to IV category, 56% (9/16) recurred between 1-3 years i.e there was a slight shift to the left.

GRADE III (ANAPLASTIC) ASTROCYTOMAS. *Without progression* (grade III to III): The mean interval to recurrence of the 4 tumors in this group was 12.5 months. All the 4 tumors recurred within the first two years.

*With progression* (grade III to IV): The mean interval to recurrence of grade III tumors which progressed to grade IV was 20.6 months. This was statistically not significant from the group without progression. An interesting difference observed was that 85% (6/7) of the grade III to grade IV progression tumors recurred in the 1-3 years period, unlike the grade III to grade III nonprogression tumors which recurred in the first two years i.e. there was a slight shift to the right in the progression category.

GRADE IV TUMORS (DE NOVO GBMs). 93.3% of the *de novo* GBMs which recurred to the same grade did so within the first two years, with 60% recurring within the first year. The mean interval to recurrence was 12.8 months.

INTERVAL TO RECURRENCE IN GRADE III TUMORS ARISING BY PROGRESSION VS. DE NOVO GRADE III TUMORS. There were a total of 16 recurrent grade III tumors, 4 arising from *de novo* grade III tumors and 12 by progression from grade II tumors. The time to recurrence of grade III tumors to the same grade was 12.5 months, with all 4 cases recurring within the first two years (*Table 2*). However, most grade III tumors arising by progression from grade II tumors recurred later (2-5 years). Also the mean interval to recurrence was significantly longer (55.3 months) (*Table 3*).

INTERVAL TO RECURRENCE IN SECONDARY VS. DE NOVO GBMs. In the present study, there were a total of 38 recurrent GBMs, of which 15 were de novo and the remaining 23 were secondary GBMs. The mean interval to recurrence of de novo GBMs was very short (12.8 mths) with 93.3 % recurring within the first two years and 60% within the first year (Table 2 and 3). Interestingly, the mean interval to recurrence of secondary GBMs was significantly longer than that of *de novo* GBMs- 20.6 months for grade III to grade IV progression and 53.9 months for

*Table 4A.* **MIB-1 LI in initial tumors**

| *Tumor grade* | *Initial tumor LI* | | |
|---|---|---|---|
| | *No.* | *Range* | *Mean ± sd* |
| Grade I | 8 | 0.1–2.3 | 0.44 ± 0.76* |
| Grade II | 24 | 0.2–11.8 | 3.73 ± 3.70** |
| Grade III | 10 | 0.5–19.6 | 9.65 ± 7.22 |
| Grade IV | 14 | 0.4–23.5 | 10.33 ± 7.98 |

Kruskal Wallis' test non-parametric one way ANOVA with multiple range test: ** Gr II vs Gr III : Significant ($p<0.01$); Gr II vs GrIV : Significant ($p<0.01$); *GrI vs other Grds : Significant ($p<0.01$); Gr III vs GrIV; Not significant

grade II to grade IV progression. Also, most secondary GBMs recurred 1-3 years after initial tumor surgery. Further, even the difference between grade III to IV progression was statistically significantly shorter (20.6 months) than grade II to IV tumor progression which was much longer (53.9 months) (*Table 3*).

Hence, there seems to be a correlation of interval to recurrence both with initial grade of tumor and progression. Progression requires longer time especially with grade II tumors and possibly because of the time required for acquiring genetic mutations.

*MIB-1 labeling index (MIB-1 LI)*

MIB-1 LI OF PRIMARY TUMORS. The MIB-1 labeling index (LI) of initial tumors showed a positive correlation with histologic grade (*Table 4A* and *Figure 1A,B*) with the MIB-1 LI increasing with increase in grade. This difference was found to be statistically significant except between grades III and IV. The median MIB-1 LI for 56 cases was 2.8%. Gemistocytic astrocytomas showed a relatively higher MIB-1 LI (mean 4.2) as compared to other grade II astrocytomas (mean 3.8).

*MIB-1 LI in recurrent tumors*

WITH PROGRESSION. Parallel with increase in anaplasia, the recurrent tumors with malignant progression showed a higher LI than their initial tumors (*Table 4B and Figure 2*). The mean initial LI of grade II astrocytomas was 1.8% whereas it increased to 8.64% when it recurred as grade III anaplastic astrocytoma. Similarly the initial LI of grade II tumors which progressed to grade IV was 5.35% which increased to 12.16% in the recurrent tumors. This difference in LI between initial and recurrent grade II tumors with progression was found to be statistically significant (*Table 4B and Figure 2*). The recurrent tumors of grade III with progression also had higher labeling index than the initial tumors. However, this variable did not quite reach statistical significance which may be attributed to the relatively small population of patients with such tumors that were studied (*Table 4B and Figure 2*).

WITHOUT PROGRESSION. The MIB1-LI of the recurrent tumors which were of the same grade as their initial tumor [II (n=8), IIII (n-=1), IIIIII (n=4) and IVIV (n=14)] was slightly more, but the difference was not statistically significant (*Table 4B and Figure 2*). An interesting observation was that when we compared the initial MIB-1 LI in grade II tumors which had progressed to grade III vis a vis grade II tumors which had progressed to grade IV, there was a statistically significant difference. No similar significant difference was however found between grade III tumors which had progressed to grade IV and which had not (*Table 4B*). This was again attributed to the small number of cases of this grade in each subgroup.

MIB-1 LI IN RECURRENT GRADE III TUMORS ARISING BY PROGRESSION VS RECURRENT DE NOVO GRADE III TUMORS. No difference was observed in MIB-1 LI between the two groups *(Table 4B*). Thus, the MIB-1 LI of grade III tumors

*Table 4B.* **MIB-LI in initial and recurrent tumors**

| *Tumor grade* $1 \rightarrow r$ | No. | *Initial tumor* | | *Recurrent tumor* | | *p value (Paired 'T' Test)* |
|---|---|---|---|---|---|---|
| | | *Range* | *Mean ± sd* | *Range* | *Mean ± sd* | |
| Gr. I → I | 8 | 0.1–2.3 | 0.44 ± 0.8 | 0.1–3.8 | 0.56 ± 1.3 | 0.82 (NS) |
| Gr. II → II | 1 | 0.4 | 0.4 | 3.1 | 3.1 | |
| Gr. II → III | 10 | 0.2–7.6 | 1.8 ± 2.15 | 0.2–33.1 | 8.64 ± 10.7 | 0.02 (S) |
| Gr. II → IV | 13 | 0.4–11.8 | 5.35 ± 4.03* | 2.1–30.1 | 12.16 ± 8.48 | 0.001 (S) |
| Gr. III → III | 4 | 3.2–19.6 | 11.15 ± 8.5 | 4.2–29.6 | 15.4 ± 12.3 | 0.2 (NS) |
| Gr. II → IIV | 6 | 0.5–18.8 | 8.65 ± 6.90 | 1.5–19.7 | 9.95 ± 7.96 | 0.53 (NS) |
| Gr. IV → IV | 14 | 0.4–23.5 | 10.33 ± 7.98 | 0.4–30.2 | 13.8 ± 9.40 | 0.79 (NS) |

Wilcoxon rank sum test: * Initial tumor grade II → grade III vs grade II → grade IV: Significant ($p<0.02$); Initial tumor grade III → grade III vs grade III → grade IV: Not Significant ($p=0.5$).

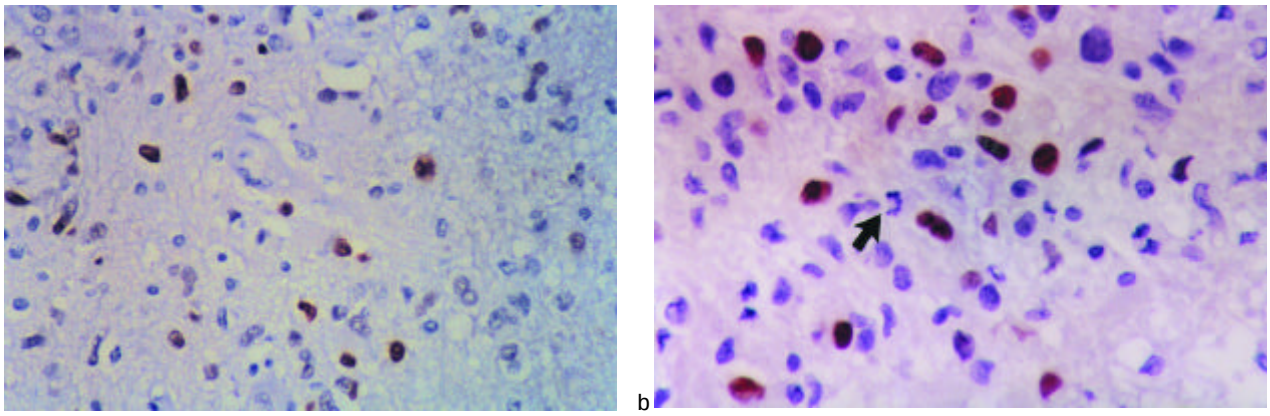

*Figure 1. a) Photomicrograph showing low MIB-1 labeling in a grade II astrocytoma (MIB-1 IHC counterstained with haematoxylin X 400). b) Photomicrograph showing large number of MIB-1 labeled cells in a case of anaplastic astrocytoma. Note the unlabeled mitotic figure. (MIB-1 IHC counterstained with haematoxylin X 400).*

which had arisen from Grade II was 8.64±10.7 while those of grade III which had recurred without progression was 15.4±12.3. This difference was not statistically significant (One way ANOVA non-parameteric).

MIB-1 LI IN SECONDARY GBM VS RECURRENT DE NOVO GBM. Again no statistically significant difference was observed between MIB-1 LI of recurrent *de novo* tumors vs. recurrent tumors of the same grade which had arisen by malignant progression (One way ANOVA non-parameteric) (*Table 4B*). The mean MIB-1 LI of the de novo GBMs was 13.8±9.4 while that of all secondary GBMs (13 arising from II-IV progression and 6 arising by III-IV progression) was 11.53±8.4.

MIB-I LI AND INTERVAL TO RECURRENCE. When comparing MIB-1 LI with interval to recurrence it was found that the interval was inversely proportional to the MIB-1 LI (*Table 5, Figure 3A*). Thus interval to recurrence was ≤ 1 year when the mean MIB-1 LI was the highest (9.74±8.97), while the interval was longest (5-10 years) when mean MIB-1 LI was lowest (1.26±0.45) (*Figure 3A*). This correlation was found to be statistically significant by Pearson's correlation coefficient (*Table 5A*). When this comparison was done according to grades, the same trend was observed. There was decrease in the MIB-1 LI with increasing interval to recurrence in each grade. But no statistical analysis could be done due to the small numbers in each group *(Figure 3B, Table 5B).* Another observation showed that MIB-1 LI correlated with interval to recurrence. We took the median MIB-1 LI of 2.8% of 56 cases as the cut off point and then compared the mean and median interval to recurrence of those cases which had an LI of ≤ 2.8% vs. those with an LI of > 2.8%. It was found that the mean and median interval to recurrence of those cases which had an LI lower than 2.8% was significantly higher than those of LI more than 2.8%. In the 28 cases, which had MIB-1 LI of ≤ 2.8%, the mean interval to recurrence was 42 months and the median was 32 months. In contrast, the 28 cases which had MIB-1 LI of >28% had a

*Table 5A.* **MIB-1 LI and interval to recurrence**

| *Interval to recurrence* | *No.* | *MIB-1 LI* | | |
|---|---|---|---|---|
| | | *Range* | *Mean ± sd* | *Median* |
| ≤ 1 yr (0-12 mths) | 14 | 0.1–23.5 | 9.74 ± 8.97 | 8.55 |
| 1–2 yrs. (13–24 mths) | 17 | 0.1–17.3 | 5.30 ± 5.07 | 4.15 |
| 2–3 yrs. (25-36 mths) | 11 | 0.1–11.8 | 4.69 ± 4.26 | 3.75 |
| 3–5 yrs. (37-60 mths) | 8 | 0.3–10.5 | 2.5 ± 1.9 | 1.5 |
| 5–10 yrs. (61–120 mths) | 6 | 0.1–4.4 | 1.26 ± 0.45 | 0.7 |

Pearson's correlation coefficient; Significant (r= –0.36; p<0.01).

*Table 5B.* **MIB-1 LI and interval to recurrence in each histological grade**

| | *Grade I (8)* | | *Grade II (24)* | | *Grade III (10)* | | *Grade IV (15)* | |
|---|---|---|---|---|---|---|---|---|
| | *No.* | *Mean LI* | *No.* | *Mean LI* | *No.* | *Mean LI* | *No.* | *Mean LI* |
| ≤ 1 yr. | 1 | 0.1 | 1 | 7.8 | 3 | 13.9 | 9 | 10.4 |
| 1–2 yrs. | 3 | 0.9 | 4 | 5.8 | 6 | 8.2 | 4 | 5.7 |
| 2–3 yrs. | 1 | 0.2 | 8 | 5.21 | 1 | 5.6 | 1 | 0.1 |
| 3–5 yrs. | 1 | 0.3 | 7 | 2.81 | – | – | – | – |
| 5–10 yrs. | 2 | 0.3 | 4 | 1.81 | – | – | – | – |

mean interval to recurrence to 23 months and a median of 16 months. This difference was statistically significant by the log rank test ($p < 0.02$).

MIB-1 LI AND MALIGNANT PROGRESSION. We again used the median LI of 2.8% as the cut off point and compared whether there was any relationship to malignant progression. There was no statistical difference between the two groups. Thus, of the 28 cases with MIB-1 LI of ≤2.8, 14 (50%) of cases did not undergo progression while the remaining 14 (50%) underwent progression in both LI groups. Similarly, in the 28 cases with MIB-1 LI >2.8, 16 cases (57.1%) showed malignant progression while the remaining 12 (42.9%) did not show progression. The difference was not statistically significant by the chi-square test.

*Apoptotic index*

Apoptosis was low in both the initial and recurrent tumors in all grades (*Table 6 and Figure 4*). Though recurrent tumors had a slightly higher AI than their initial counterparts, this was not statistically significant. There was no correlation between AI with interval to recurrence and malignant progression.

## *Discussion*

One of the important features of astrocytic tumors is their inherent tendency to recur. Another interesting feature is that this recurrence is often associated with the transition to a higher grade of malignancy. For all practical purposes, recurrence appears to be inevitable, irrespective of the therapeutic modality employed for the treatment of the primary tumors. It is this high recurrence rate which is one of the main reasons for the poor prognostic outcome in astrocytic tumors.

The present series is a comparative study of 64 cases of recurrent astrocytic tumors of all 4 grades, diagnosed and treated in the same institution. To the best of our knowledge, this is one of the largest series of recurrent astrocytic tumors in the English literature wherein markers of proliferation and apoptosis have been simultaneously evaluated.

In the present study, recurrence was associated with malignant progression in 93.3% of low grade diffuse (Grade II) astrocytomas. Interestingly, all the 10 gemistocytic astrocytomas were associated with progression on recurrence – 3 to Grade III and 7 to GBM. This is in keeping with the reports in literature that gemistocytic astrocytomas have a poorer outcome as compared to other types of low grade astrocytomas.[36] 64% of the anaplastic astrocytomas alone underwent progression on recurrence. Interestingly, none of the 8 pilocytic astrocytomas showed progression. All of them recurred to the same grade

The present study revealed that the interval to recurrence was primarily dependent on the initial grade of the tumor. Though unpredictable in a given case and displaying a wide range within each tumor grade, a statistically significant trend was noted. Thus the mean interval to recurrence of Grade I tumors was 43 months and grade II

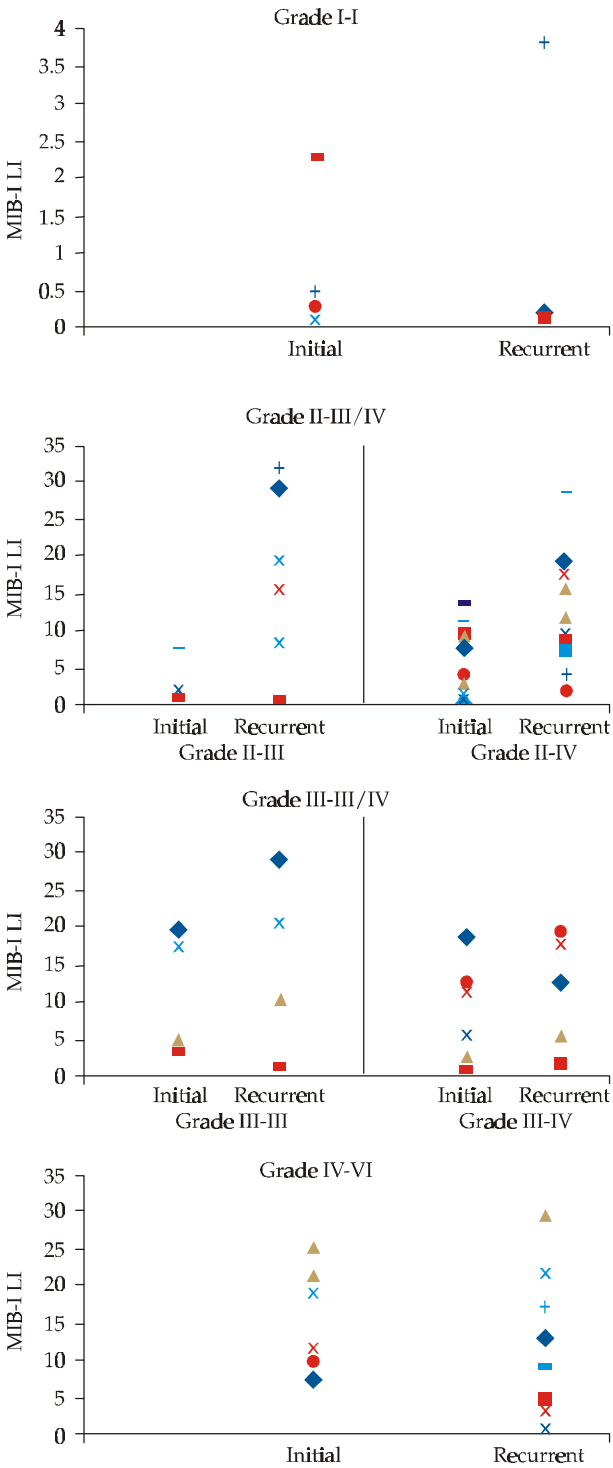


*Figure 2. Scattergram showing distribution of MIB-1 LI in initial and recurrent tumors of all grades of astrocytoma.*

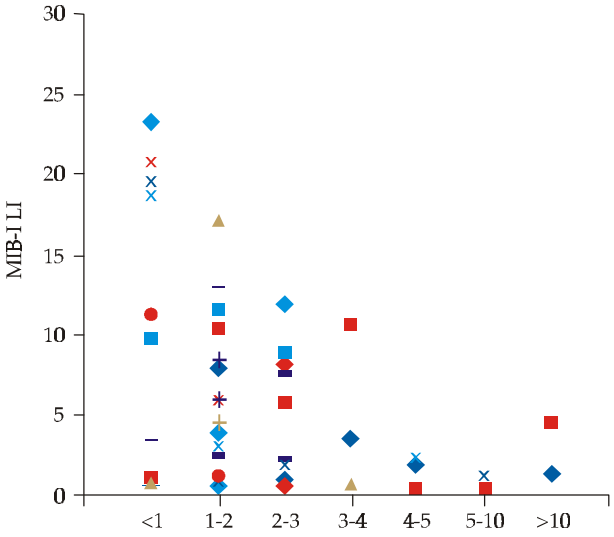


*Figure 3. a) Scattergram showing correlation of MIB-1 LI with interval to recurrence.*

tumors 54.8 months. This was in contrast to grade III and IV tumors where the interval to recurrence was very short – 17.6 months for grade III and 12.9 months for grade IV tumors respectively.

Another interesting observation in the present study was that the interval to recurrence showed a correlation with malignant progression. Thus, mean interval to recurrence of grade III and Grade IV astrocytomas recurring to the same grade was very short (12.5 months and 12.8 months respectively). Further, all such recurrences occurred within the first 2 years, with 50-60% within the first year.

In contrast, the mean interval to recurrence of grade II and grade III astrocytomas with progression to GBM was relatively longer – 20.6 months for Gr III→IV, 55.3 months for Gr II→III and 53.9 months for Gr II→IV. Further, most of the recurrences with progression (33/35 or 94%) occurred after the first year (shift to the right). Only 1 out of 12 Gr II→III progression and 1 out of 6 Grade III→IV progression occurred within the first year.

Interestingly, recurrence to the Gr II→III/IV conversion took significantly longer (53.3 & 53.9 months respectively) than the Gr III→IV conversion (20.6 months). All the above observations suggest that recurrence with progression possibly takes longer than recurrence to the same grade. This can possibly be attributed to the time required to acquire genetic mutations for progression. Recurrence to the same grade may possibly be shorter due to re-growth of residual tumor, without the acquisition of further genetic alterations.

The incidence and timing of the conversion of a diffuse cerebral astrocytoma with a low degree of malignancy into an anaplastic astrocytoma or a GBM, while to be anticipated are impossible to predict in an individual case. However, if a tumor recurred more than 2 years after initial

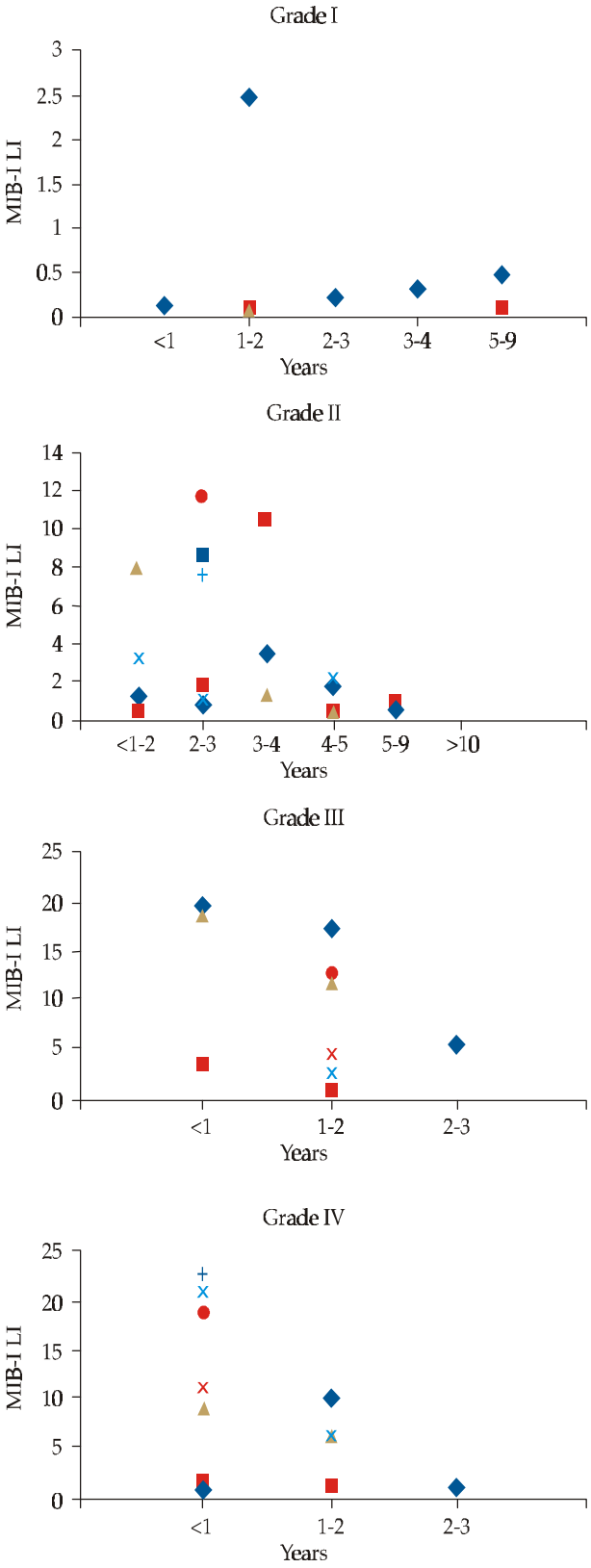


*Figure 3. b) Scattergrams showing correlation of MIB-1 LI with interval to recurrence in the various grades of astrocytic tumors.*

*Table 6.* **Apoptotic index**

| *Tumor grade* | *Initial tumor* | | *Recurrent tumor* | |
|---|---|---|---|---|
| | *Range* | *Mean ± sd* | *Range* | *Mean ± sd* |
| Grade I | 0.01–0.1 | 0.07 ± 0.04 | 0.01–0.09 | 0.05 ± 0.04 |
| Grade II | 0.01–0.6 | 0.14 ± 0.18 | 0.01–3.3 | 1.2 ± 0.7 |
| Grade III | 0.05–0.13 | 0.07 ± 0.05 | 0.02–0.9 | 0.32 ± 0.2 |
| Grade IV | 0.01–0.76 | 0.17 ± 0.19 | 0.02–10.7 | 1.8 ± 1.3 |

surgery, the chances of it having undergone malignant progression was very high.

Of the 79 cases of recurrent low grade tumors reported by Laws et al,[18] about half had converted to a higher grade of malignancy (grades III or IV). In their survey of 137 examples, Muller et al[22] concluded that an increase of malignancy was to be expected in approximately two thirds of the patients. Since their series was based largely on biopsy material, it is possible that their estimate minimizes the frequency of such an occurrence. Moreover, as brought to light by the clinicopathologic analysis of Muller et al,[22] there was no statistically significant relationship between the time interval preceding clinical recurrence and the demonstration of increased malignancy on subsequent histologic examination.

In the study of Soffieti et al,[30] recurrences were histologically proved in 24 out of 85 adults (28%). Of these, 19 had progressed to grade III or IV, usually from 1-5 years after the initial diagnosis. A similar 5 year interval was seen by Vertosik et al[32] with 7 out of 25 patients (28%) showing malignant progression. Thus, the mean interval to recurrence of the low grade astrocytomas which had undergone malignant progression was longer than those which recurred to the same grade.

In a study by Watanabe et al,[36] they showed that the time interval for progression of anaplastic astrocytoma to GBM varies considerably with a mean of 2 years. Recht et al[36] reported that 50% of astrocytomas undergo malignant change within 6 years. Winger et al[37] noted that 10% of high grade gliomas had a clinically apparent low grade phase.

Tandon et al[31] from AIIMS showed that out of 200 patients of supratentorial gliomas treated by radical surgical excision and radiotherapy, 100 cases (50%) showed CT confirmed recurrence, of which 44 were histologically confirmed. Interestingly, 15 of the 20 recurrent grade II astrocytomas had undergone malignant progression. Recurrence was generally earlier in patients with anaplastic astrocytoma and GBM. However, in a given case, the timing of recurrence was unpredictable; as short as 1 year in one pilocytic astrocytoma and as long as 6 years in one case of GBM. Afra et al[2] also noted a variable period of recurrence the shortest being 4 months and longest 89 months.

Analysis of a wide range of astrocytic tumors showed a gross correlation of proliferation with clinical outcome.[4,10,11,12] Several studies have given a cut off value for MIB-labeling index beyond which prognosis was poor. Montine et al[21] reported that a MIB-LI >7.5% was associated with higher histological grade and poorer survival. Jaros et al[14] found a LI of >5% to be the threshold value for predictive shorter survival. McKeever et al[20] in their analysis of 50 cases of grade II astrocytomas, used an LI of 2% as cut off value and found that 82% of patients who had a MIB-1 LI of > 2% died within the 10 year follow up period while only 23% of patients who had a MIB-1 LI of ≤ 2% died within the same period. Similarly, Enestrom et al[6] studied MIB-1 LI in 22 cases of recurrent astrocytomas. They found that dividing MIB-1 LI in < or = 10% vs > 10% significantly separated parameters such as the time interval for recurrence as well as cumulative proportion of survival. Schiffer et al[29] with 8% as cut off value, divided the MIB-I LI in two groups with significantly different survival and concluded that MIB LI is an important prognostic indicator. Hoshi et al[9] in their study of 47 low grade astrocytomas found a correlation between MIB-1 LI and recurrence. Nakamura et al[24] studied 46 recurrent astrocytomas and found that the clinical outcome of patients was associated with the proliferative activity of the initial tumor particularly in anaplastic astrocytomas.

Thus, several studies suggest that the proliferative potential correlates inversely with survival and time interval to recurrence. However, this finding is inconsistent in individual cases as shown by Kaluza et al[15] who analyzed the MIB-LI in 37 recurrent tumors and did not find any correlation with interval to recurrence or malignant progression. Similarly, Litofsky et al[19] studied the MIB-LI in 27 recurrent cases of astrocytomas and also found no such correlation. Hilton et al[8] also reported that survival in patients with fibrillary astrocytoma did not correlate with Ki-67 expression.

It was observed in the present study that the MIB-I LI correlated with the grade of the initial tumor. There was an inverse correlation of MIB-1 with interval to recurrence in

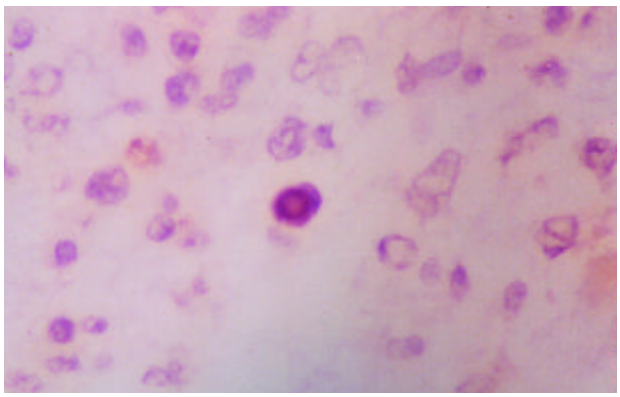

*Figure 4. GBM showing Tunel positive apoptotic cell (x400).*

that tumors with higher LI were found to have a shorter interval to recurrence and this was statistically significant. On taking the median LI of 2.8% as cut off value, we found that tumors with LI <2.8% had statistically significant longer interval to recurrence. We did not find a similar statistical correlation with malignant progression at recurrence although the tumors at recurrence showed a higher LI than the initial tumors. However, there was a statistical difference between the LI of grade II tumors which had progressed to grade III vis a vis grade II which progressed grade IV, the latter LI being significantly higher.

In this study, gemistocytic astrocytomas seem to have a sinister prognostic outcome because of their high propensity for malignant progression at recurrence and higher MIB-1 LI (mean 4.2), similar to that reported by Watanabe et al.[35] Grade I pilocytic astrocytomas appear to be biologically different from grades II, III & IV astrocytic tumors which formed a continuous spectrum of progression. Thus none of the Grade I tumors progressed to higher grades at recurrence. These findings are similar to that reported by von Deimling et al.[33,34]

The relationship between malignant potential and apoptosis in astrocytic tumors is not clearly defined. Ellison et al,[5] Heesters et al[7] and Carrol et al[3] found no correlation between apoptotic rate and histopathological grades. Nakamura et al[24] observed that AI was higher in the recurrent compared to the initial tumors. In the present study, we did not observe any correlation between AI and interval to recurrence or malignant progression.

Thus, this study establishes that though interval to recurrence varies considerably, there is a correlation with the following three parameters:

*a)* histological grade of primary tumor. Thus grade II tumor show longer interval to recurrence than grades III and IV tumors;

*b)* MIB-1 LI of the primary tumor at diagnosis – higher MIB-1 LI is associated with shorter interval to recurrence;

*c)* malignant progression at recurrence presumably because of accumulation of genetic mutations. Malignant progression is associated with longer interval to recurrence. Thus, a primary grade II tumor with low initial MIB-1 LI of <2.8 and which undergoes progression to Grade III/IV at recurrence will predictably have a relatively longer interval to recurrence than a Grade IV tumor with high MIB-1 LI recurring to same grade.

Another important observation in this study was that malignant progression at recurrence did not show an absolute correlation with MIB-1 LI or AI. Apoptotic index had no role as a parameter for predicting either interval to recurrence or malignant progression.

Therefore, it can be inferred that MIB-1 proliferation index can be used as a prognostic marker in predicting recurrence free survival in astrocytic tumors.

***Acknowledgement***

The technical help received from Mrs. Kiran Rani, Mr. Rajeshwar Khadia, Mr. Anil and Mr. Gajender is gratefully acknowledged. We also thank Mr. Kamal for secretarial assistance and typing of the manuscript. The work was supported by funds provided by Indian Council of Medical Research.

***References***

1. *Afra D, Muller W:* Recurrent low grade gliomas. Dedifferentiation and prospects of re-operation. In: Glioma. [Edt. Karim and Laws]. Springer Verlag, Berlin, Heidelberg. pp 189-203, 1991.
2. *Afra D, Muller W, Benoist G, et al:* Supratentorial recurrences of gliomas : Results of re-operation on astrocytomas and oligodendrogliomas. Acta Neurochir (Wien) 43:217-227, 1978.
3. *Carrol RS, Zhang J, Chauncey BW, et al:* Apoptosis in astrocytic neoplasms. Acta Neurochir (Wien) 139:845-850, 1997.
4. *Ellison DW, Steart PV, Bateman AC, et al:* Prognostic indicators in a range of astrocytic tumors: An immunohistochemical study with Ki-67 and p53 antibodies. J Neurol Neuro Surg Psychiatry 59:413-419, 1995.
5. *Ellison DW, Steart PV, Gatter KC, et al:* Apoptosis in cerebral astrocytic tumor and its relationship to expression of the Bcl-2 and p53 proteins. Neuropathol 21:352-361, 1995.
6. *Enestrom S, Vavruch L, Franlund B, et al:* Ki-67 antigen expression as a prognostic factor in primary and recurrent astrocytomas. Neurochir 44:25-30, 1998.
7. *Heesters MA, Koudstaal J, Go KG, et al:* Analysis of proliferation and apoptosis in brain gliomas : prognostic and clinical value. J Neurooncol 44:255-266, 1999.
8. *Hilton DA, Love S, Barber R, et al:* Accumulation of p53 and Ki67 expression do not predict survival in patients with fibrillary astrocytomas or the response of these tumors to radiotherapy. Neurosurgery 42:724-729, 1998.
9. *Hoshi M, Yoshida K, Shimazaki K:* Correlation between MIB-1 staining indices and recurrence in low grade astrocytomas. Brain Tumor Pathol.14:47-51, 1997.
10. *Hoshino T, Ahn D, Prados MD, et al:* Prognostic significance of the proliferative potential of intracranial gliomas measured by Bromodeoxyuridine labeling. Int J Cancer 53:550-555, 1993.
11. *Hoshino T, Rodriguez LA, Cho KG, et al:* Prognostic implications of the proliferative potential of low grade astrocytomas. J Neuro Surg 69:839-842, 1988.
12. *Ito S, Chandler KL, Prados MD, et al:* Proliferative potential and prognostic evaluation of low grade astrocytomas. J Neuro Oncol 19:1-9, 1994.
13. *Iuzzolino PO, Ghimenton C, Nicolato A, et al:* p53 in low grade astrocytomas : a study with long-term follow-up. Br J Cancer 69:586-591, 1994.
14. *Jaros E, Perry RH, Adam L, et al:* Prognostic implication of p53 protein, EGFR and Ki67 labeling in brain tumors. Br J Cancer 66:373-380, 1992.
15. *Kaluza J, Adamek D, Pyrich M:* Ki-67 as a marker of proliferation activity in tumor progression of recurrent gliomas of supratentorial localization. Immunocytochemical quantitative studies. Pol J Pathol 48:31-36, 1997.
16. *Cavenee WK, Bigner DD, Newcomb EW, et al:* Diffuse astrocytomas. In : Pathology and genetics tumors of the nervous system. (Eds. Kleihues P and Cavenee WK). International Agency for Research on Cancer, Lyon, 1997, pp 2-9.
17. *Krans JA, Bolln C, Wolf HK, et al:* TP53 alterations and clinical outcome in low grade astrocytoma. Genes Chromosomes 10:143-149, 1994.

18.[2]*Laws ER Jr, Taylor WG, Clinjton MB, et al:* Neurosurgical management of low grade astrocytoma of the cerebral hemispheres. J Neurosug 61:665-73, 1984.
19.[2]*Litofsky NS, Mix TC, Baker SP, et al:* Ki-67 proliferation index in recurrent glial neoplasms : no prognostic significance. Surg Neurol 50:579-585, 1998.
20.[2]*McKeever PE, Strawderman MS, Yamini B, et al:* MIB-1 proliferation index predicts survival among patients with grade II astrocytoma. J Neuropathol Exp Neurol 57:931-936, 1998.
21.[2]*Montine TJ, Vandersteenhoven JJ, Aguzzi A, et al:* Prognostic significance of Ki 67 proliferation index in supratentorial fibrillary astrocytic neoplasms. Neurosurgery 34:674-678, 1994.
22.[2]*Muller W, Afra D, Schroeder R:* Supratentorial recurrences of gliomas. Morphological studies in relation to time intervals with astrocytomas. Acta Neurochir 37:75-91, 1977.
23.[2]*Mustonen M, Rainio H, Paakko P, et al:* The extent of apoptosis is inversely associated with bcl-2 expression in pre-malignant and malignant breast lesions. Histopathology 31:347-354, 1997.
24.[2]*Nakamura M, Konishi N, Tsunoda S, et al:* Retinoblastoma protein expression and MIB-1 correlate with survival of patients with malignant astrocytomas. Cancer 80:242-249, 1997.
25.[2]*Rasheed BKA, McLendon RK, Herndon JD, et al:* Alterations of the TP53 gene in human gliomas. Cancer Res 54:1324-1330, 1994.
26.[2]*Recht LD, Law R, Smith TW:* Suspected low grade glioima : Is deferring treatment safe? Ann Neurol 31:431-436, 1992.
27.[2]*Rieger J, Ohgaki H, Kleihues P, et al:* Human astrocytic brain tumors express Apo2L/Trail. Acta Neuropathol (Berl) 97:1-4, 1999.
28.[2]*Reifenberger J, Ring GU, Gies U, et al:* Analysis of p53 mutation and epidermal growth factor receptor amplification in recurrent gliomas with malignant progression. J Neuropathol Exp Neurol 55:822-831, 1996.
29.[2]*Schiffer D, Chio A, Giordano MT, et al:* Prognostic value of histologic features in adult cerebral astrocytomas. Cancer 61:1386-1393, 1988.
30.[2]*Soffietti R, Chio A, Giordano MT, et al:* Prognostic factors in well differentiated cerebral astrocytomas in the adult. Neurosurgery 24:686-692, 1989.
31.[2]*Tandon PN, Mahapatra AK, Sarkar C, et al*: Recurrence of supratentorial gliomas. Neurol India 45:141-149, 1997.
32.[2]*Vertosik FT, Selker RG, Arena VC:* Survival of patients with well differentiated astrocytomas diagnosed in the era of computed tomography. Neurosurgery 28:496-501, 1991.
33.[2]*von Deimling A, Eibl RH, Ohgaki H, et al:* p53 mutations are associated with 17p allelic loss in grade II and grade III astrocytomas. Cancer Res 52:2987-2990, 1992.
34.[2]*von Deimling A, Louis DN, Menon AG, et al:* Allelicloss on the long arm of chromosome 17 in pilocytic astrocytoma. Acta Neuropathol 76:81, 1993.
35.[2]*Watanabe K, Peraud A, Gratas C, et al:* p53 and PTEN mutations in gennistocytic astrocytomas. Acta Neuropathol (Berl) 95:559-560, 1998.
36.[2]*Watanabe K, Tachibana O, Yonekawa, et al:* Role of gemistocytes in astrocytoma progression. Lab Invest 76:277-284, 1997.
37.[2]*Winger MJ, Macdonald DR, Cairncross JG:* Supratentorial anaplastic gliomas in adults. The prognostic important of extent of resection and prior low grade glioma. J Neurosurg 71:787-793, 1989.