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Evaluation of the Proliferation Marker Ki-67 in Glioblastoma and its Correlation with the Histopathological Findings

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Abstract: The aim of this study is to evaluate the expression of K-67 in glioblastoma and to establish the relationship of this marker with the clinical and histological findings of gliblastoma. This cross sectional study was conducted on 60 cases of glioblastoma admitted to Kasr El-Eini Hospital, Faculty of Medicine, Cairo University, Egypt. We determined immunohistochemically the expression of Ki-67 in each case. As regards Ki-67 Labeling indices (LI), 32 (53.3%) cases have low LI and 28 (46.7%) cases have high LI. The values ranged from 2 to 80 with mean value 15.22±13.4. Among the study group, there was no significant association between expression of Ki-67 and other clinicopathological variables. The proliferation index as measured by the Ki-67 LI is a potent biological marker that estimates the growth of neoplasms quantitatively and thus will aid in identifying the prognosis for patients with Glioblastoma. The average level of Ki-67 LI varies considerably in the different variants of Glioblastoma, as well as considerable overlap can be observed within the same variant. Ki-67 among cases of Glioblastoma is not dependent on factors like age, sex and tumor location.

Key words: Glioblastoma · Ki-67 · Immunohistochemistry

INTRODUCTION

Glioblastoma (GBM) is the most common and most lethal primary brain tumor [1] Tumor proliferation can be assessed immunohistochemically using number of markers including DNA polymerase alfa, p105, DNA topisomerase II-alfa, PCNA and ki-67. Of these, Ki-67 antibody is considered the most useful marker for evaluating cell proliferation [2]. Ki-67 is a labile, nonhistone nuclear protein that is tightly related to the cell cycle and is seen in all continuously cycling cells of mid-G1, S and G2 phase and in mitosis, but not in quiescent or resting cells in the G0 and early G1 phase [3]. Ki-67 can be used as a marker to assess the growth portion of a given cell population, as this protein is present in all proliferating cells (normal and tumor) [4]. The monoclonal antibody Ki-67/MIB-1 detects this antigen and the percentage of immunopositive cells is referred to as the Ki-67 labeling index (L1) [5]. The monoclonal antibody Ki-67/MIB-1 has proven prognostic and diagnostic power in astrocytic tumors [6]. It has been recorded that the average level of ki-67/MIB-1 LI is variable among different grades of astrocytomas, but considerable overlap can be observed between them [7]. Due to this variability among the various tumor grades, KI-67/MIB-1 LI cannot be used as a diagnostic factor alone but should be used in combination with established criteria of histological malignancy. It may be especially useful in cases where histology reveals a low-grade astrocytoma, whereas other parameters indicate a more malignant neoplasm [8].

MATERIAL AND METHODS

The material of this retrospective cross-sectional study consisted of 60 cases diagnosed as glioblastoma. The paraffin blocks were received from the Pathology department, Faculty of medicine, Cairo University, Kasr El-Eini Hospital over a two year period from January 2011 to December 2012. The personal data (age and sex), clinical details and pathological data pertaining to these patients were retrieved from the medical records department of Kasr El-Eini Medical College Hospital. Two sections were cut from each paraffin block by microtome at 5 microns thickness; One stained with hematoxylin and Eosin for routine histopathoogical examination, The other was mounted over positively charged slides for immunohistochemical staining of proliferating astrocytic cells by Ki-67.

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Immunohistochemical Staining for Ki-67: The Sections were deparaffinized and transferred into three changes of xylene, then slides were rehydrated through decreasing concentrations of alcohol. The slides were washed in phosphate buffer saline PBS for 5 minutes. The endogenous peroxidase activity was blocked using a 3% solution of hydrogen peroxide for 20 minutes at room temperature then slides were washed well in water. Sections were placed in an unsealed thermoresistent plastic jar filled with 10 ml of citrate buffer (pH 6) then microwaved on high power for three times 5 minutes each. The jar was removed and allowed to cool for 20-30 minutes, then slides were washed well with PBS for 5 minutes. Tissue sections were covered immediately with 2 drops of blocking agent (normal rabbit serum) on each slide and were left for 30 minutes. Each slide was incubated with one or two drops of 1/60 diluted Ki-67 mouse monoclonal antibody (Ki-67 Antibody (Code No. 61-0078-2 DAKO) in primary antibody diluents (Genemed). Slides were incubated horizontally in a humid room temperature for 120 minutes. Sections were washed three times in PBS and incubated with avidin-biotin-peroxidase system for 30 minutes. Brown color was developed using diaminobenzidine (DAB) which was applied for 4-5 minutes then slides were washed in buffer. Antigen binding sites were visualized then counterstaining with hematoxyline was done. Slides were washed in tape water then dehydrated in ascending grades of alcohol. Slides were then cleared in 2 changes of xylene. Slides were left to dry in air then a drop of Canada balsam was added and sections were covered by a glass cover. Controls: As negative controls, primary antibody was replaced by PBS and processed as above. As positive controls, immunoreactivity of normal blood vessels embedded in neoplastic sections was evaluated.

Evaluation of Ki-67 Immunostaining: Each Ki-67 slide was evaluated through 10 random high power fields (40X), which included counting the number of positive and negative nuclei. Based on the average number of nuclei evaluated in each microscopic field, we estimated that at least 1000 invasive tumor nuclei were counted for each case. Every brown stained nucleus is considered positive irrespective for intensity. The estimated number of positive nuclei was divided by the total estimated number of counted nuclei, which resulted in the proliferative or Ki-67 index for each case. Necrotic or thick areas and severely overlapping tumor cells were avoided during evaluation the tumor were divided into those with low labeling index LI (=10% stained nuclei) and high LI (>10% of stained nuclei) [8].

Statistical Analysis: Data management and analysis were performed using Statistical Package for Social Sciences (SPSS) vs. 21. Comparisons between two groups with respect to normally distributed numeric variables were done using the t-test. Non-normally distributed numeric variables were compared by Mann-Whitney test. For categorical variables, differences were analyzed with 2 (Chi square) test and Fisher's exact test when appropriate. All p-values are two-sided. P-values < 0.05 were considered significant.

RESULTS

This retrospective study was conducted on sixty cases diagnosed as glioblastoma in Kasr El-Eini Hospital, Faculty of Medicine, Cairo University, Egypt over a two year period from January 2011 to December 2012. As regards the histological variants of glioblastoma among the studied cases, 49 (82%) cases were classified as classical variant of glioblastoma (Fig. 1), 5 (8%) cases were classified as gliosarcoma (Fig. 2), 4 (7%) cases were classified as glioblastoma with oligodendroglioma component (Fig. 3) and 2 (3%) cases as pediatric high grade glioma. Among the classical variant 34 (69.4%) cases were denovo cases and 15 (30.6%) cases had history of previous excision with local recurrence; 12 (24.5%) cases were previously diagnosed as glioblastoma (primary GBM) while 3 (6.1%) cases were previously diagnosed as astrocytomas with lower grade of malignancy (Secondary GBM). The age of patients in this study ranged from 6 year to 82 years with mean age (48.73±11.75 years). Median age was 50.5 years. Statistical analysis showed that the two peak incidences of GBM were found in the fifth and sixth decade of life. Male preponderance (42 male and 18 female patients) was noted with a female to male ratio of 1:2.33. As regards the location of the lesions, the parietal lobe was the commonest site involved. It was involved alone in 17 cases representing 28.3% of the cases and it was involved together with other brain lobes in 9 cases representing 15% of cases with total parietal lobe involvement in 26 cases representing 43.3% of cases. The cerebral hemispheres were involved in 55 (91.7%) cases; 46 of which involved single lobe (76.67%) and 9 (15%) of which involved more than one lobe. 4 (6.7%) cases involved deeper brain structures and one (1.6%) case involved the cerebellum. As regards the Ki 67 LI, 32 (53.3%) cases have low LI (Fig. 4) and 28 (46.7%) cases have high LI (Fig. 5). The values ranged from 2 to 80 with mean value 15.22±13.4. The median was 10. Correlation between the clinico-pathological variables and expression of Ki-67

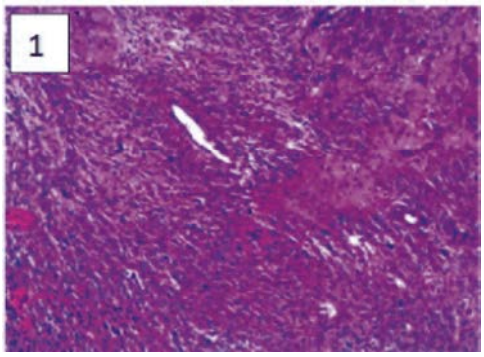

Fig. 1: Glioblastoma, classic variant (H&E, 100x).

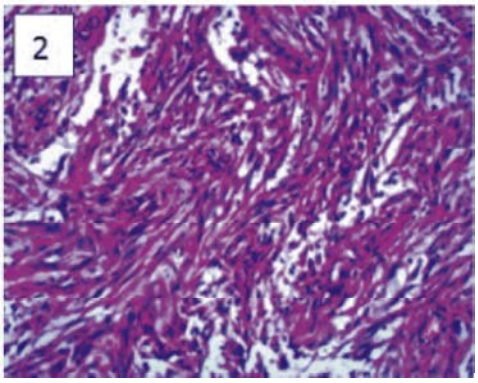

Fig. 2: Gliosarcoma (H&E, 200x).

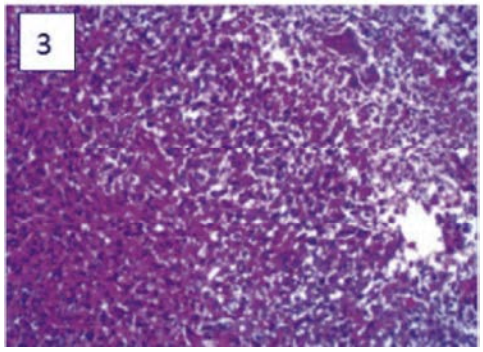

Fig. 3: Glioblastoma with oligodendroglioma component (H&E, 200 x).

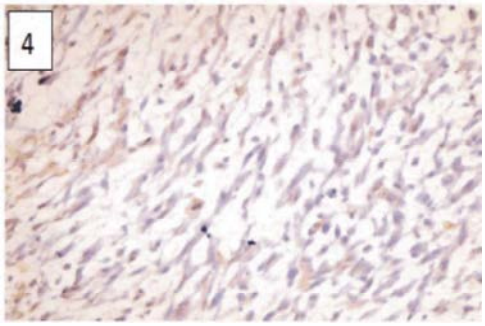

Fig. 4: A case of GBM, classical variant with low Ki 67 LI (200x).

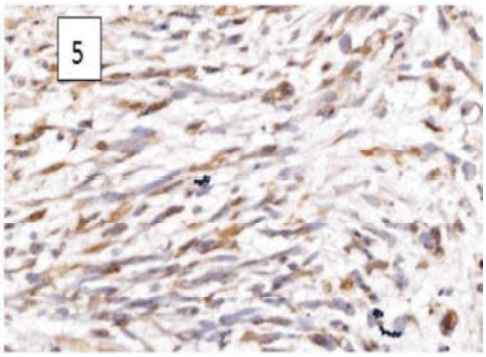

Fig. 5: A case of GBM, pediatric HGG variant with high Ki 67 LI (200x).

Table 1: Relationship between Ki-67 and clinicopathological variables.

	Ki-67 LI			
Factors	Low LI (n=32)	High LI (n=28)	P value	Significant
Age (years)				
Mean ±SD	49.7±15.7	47.7±14.3	0.936	Insignificant
Sex				
Male	22	20	0.481	Insignificant
Female	10	8		
Tumor location				
Occipital	2	1		
Parietal	8	9		
Frontal	7	8		
Temporal	8	3		
Temporo-parietal	2	4	0.442	Insignificant
Fronto-parietal	1	1		
Occipito-parietal	0	1		
Cerebellum	1	0		
Others	3	1		
Histological variant				
Classical	27	22		
GS	1	4		
GBMO	3	1	0.141	Insignificant
Pediatric HGG	1	1		

among the study group (Table 1) showed a non statistically significant correlation however the gliosarcoma variant showed the highest Ki 67 LI with mean value (27.2±14.7) and the glioblastoma with oligodendroglioma component variant showed the lowest mean value (7.5±3.3).

DISCUSSION

As regards the Ki 67 LI, 32 (53.3%) cases have low LI and 28 (46.7%) cases have high LI. The values ranged from 2 to 80% with mean value 15.22 ±13.4. The median was 10. These findings were consistent with findings

observed by Facoetti *et al.* [9] in their study on 10 cases of GBM with Ki-67 LI mean value = 15.04%±6.9. Johannessen and Torp [8] analyzed 16 studies comprising a total value of KI-67/MIB-1 LI of 915 patients of astrocyomas and found an average value of KI-67/MIB-1 LI for glioblastomas to be approximately 16. With 67 patients of GBM in Ambroise *et al.* [7] study, The Ki-67 LI ranged from 1.2 to 59, with mean value 13.85 ± 12.26 and the median was 9.8. Hsu *et al.* [10] in their study on 80 cases of astrocytomas found that the Ki-67 LI of GBM cases ranged from 0% to 29.83%, with mean value 9.12%. Similar findings were observed by Ralte *et al.* [11] in their study on 64 cases of astrocytomas in which the Ki-67 LI of GBM cases ranged from 0.4% to 23.5%, with mean value 10.33. Giannini *et al.* [12] in their study on 271 cases of astrocytomas found that the Ki-67 LI of GBM cases ranged from 0.3% to 36.2%, with mean value 9.1. Deb *et al.* [13] on their study found that the proliferation index as reflected by KI-67/MIB-1LI, ranged between 6-20% with a mean of 12%. Mastronardi *et al.* [14] correlated the Ki-67 LI in 26 glioblastoma cases with survival in patients operated on for a malignant glioma and treated postoperatively with tamoxifen. They found that the Ki-67 LI ranged from 2.3% to 62% (mean 24.1%, median 20.5%). Using the whole tissue section (WTS), Chiesa-Vottero *et al.* [15] found that the Ki-67 LI ranged between 3.0% and 76.4% (mean, 20%; SD, 15.3%; median, 14.8%). Burton *et al*. [16] on their study on 41 cases of GBM found that the Ki-67 LI ranged from 3.4% to 85.2%, with mean value 22.36% and median of 15.7%. Nearly similar findings were observed by Tihan *et al.* [17] in their study on 50 cases of astrocytomas in which the mean value of Ki-67 LI of GBM cases was 20.2. Ribeiro *et al.* [18] on their study on 30 patients of GBM in which the Ki 67 Li ranged from 13.9% to 33.4, with mean value 25.1%. Rathi *et al.* [19] in their study on 90 cases of astrocytomas found that the Ki-67 LI of GBM cases ranged from 5% to 45.2%, with mean value 20.54. While, Giangaspero *et al.* [20] in their study on 6 cases of GBM found that the Ki-67 LI ranged from 10% to 40%, with mean value 28% ± 9.8. With much higher values, Wakimoto et al. [21] in their study on 72 cases of astrocytomas found that the Ki-67 LI mean value of GBM cases was 31.6. *Krishna et al*. [22] in their study on 105 cases of astrocytomas found that the Ki-67 LI of GBM cases ranged from 20% to 52%, with mean value 38.7±7.19, while Rodriguez-pereira *et al*. [23] in their study on 137 cases of astrocytomas found that the Ki-67 LI of GBM cases ranged from 3.8% to 63%, with mean value 46. Many factors are responsible for such a variation in KI-67/MIB-1 LI between studies. The KI-67/MIB-1 LI can be influenced by the fixative used; immunohistochemical procedures, especially antigen retrieval; and interpretation of the immunostaining. A low KI-67/MIB-1 LI value in high-grade astrocytoma could also result from faulty tissue sampling and tumor heterogeneity [24]. Selection of a block of "representative" tumor for immunostaining does not necessarily guarantee that the most proliferative area of the neoplasm is present in that block [25]. The distribution of Ki-67–positive nuclei was variable in different sections of solid GBM tissue [26]. Dalrymple *et al.* (1994) [27] confirmed these findings in both in vitro and in vivo studies by demonstrating spatial variability in proliferative activity. They observed a higher proportion of proliferating cell nuclear antigen–positive glioma cells at the periphery of the solid tissue component and relatively little reactivity within tumor cells located in the surrounding infiltrated parenchyma. In our study, no statistical significant correlation was detected between Ki-67 LI and tumor location, age and sex. The same was found in study made by Dziecioł *et al.* [28] on 24 patients of glioblasoma. Ambroise *et al.* [7] in their study, Ki-67/MIB-1 LI did not vary significantly with respect to age and sex as we stated in our study. In our study, as regards the gliosarcoma variant, The Ki-67 LI ranged from 10 to 50%, with mean value 14.7%. Lower range was observed by Horiguchi *et al.* [29] in which the Ki-67 LI ranged from 7.7 to 36.1% but much higher range was observed by Buhl *et al.* [30] in which the Ki-67 LI ranged from 20 to 70%. Sengupta *et al.* [31] and Suri *et al.* [32] observed that the mean value of MIB1 proliferation index among pediatric HGG was 28.8% and 38.6 respectively. In contrast to our results, the mean value of Ki-67 LI was 16.

CONCLUSION

The proliferation index as measured by the Ki-67 LI is a potent biological marker that estimates the growth of neoplasms quantitatively and thus will aid in identifying the prognosis for patients with Glioblastoma. The average level of Ki-67 LI varies considerably in the different variants of Glioblastoma, as well as considerable overlap can be observed within the same variant, Researchers and pathologists therefore should clearly delineate between different variants of Glioblastoma due to the divergent expression patterns in immunohistochemistry among different variants of GBM and within the same tumor tissue. Our study emphasizes that Ki-67 LIs among cases of Glioblastoma are not dependent on factors like age, sex and tumor location. Clear cut-off levels for Ki-67 LI are needed to guarantee interlaboratory compatibility.

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