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ORIGINAL RESEARCH REPORT

Ki-67 labeling index in glioblastoma; does it really matter?

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Abstract

Objective/Background: Glioblastoma (GB) is the most common primary malignant brain tumor in adults. Ki-67 is a nonhistone nuclear protein that is expressed by cells entering the mitotic cycle and is associated with the transcription of ribosomal RNA (rRNA). In gliomas, the extent of expression of Ki-67 is roughly proportional to the histologic grade. Over the years, association studies were conducted trying to link the poor outcome in different types of malignant tumors to the Ki-67 proliferative index. This study is designed to investigate the relationship between the proliferation marker, Ki-67, and the overall survival amongst glioblastoma patients diagnosed between 2006 and 2012 at a single institution in Riyadh, Saudi Arabia.

Methods: This is a retrospective cohort study which investigated the status of Ki-67 labeling index in glioblastoma patients diagnosed at King Abdulaziz Medical City, Riyadh, Saudi Arabia, between 2006 and 2012. The Kaplan–Meier survival analysis was used to assess the overall survival (OS) and the Mantel–Cox log-rank test was used to compare the survival curves. Multivariate analysis using Cox proportional-hazards model was used to investigate other factors that might influence the overall survival.

Results: A total of 44 glioblastoma patients were included in the study. The median age at diagnosis was 56 (1–91) years. The 12-month survival rate for all glioblastoma patients was 48%. The median survival for patients with Ki-67 labeling index of $\leq$27%, and >27% was 11 months and 14 months, respectively.

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Conclusion: The difference between the survival curves of patients with Ki-67 labeling index of ≤27%, and Ki-67 of >27% was statistically insignificant (p = .130). Therefore, Ki-67 labeling index alone cannot predict survival in glioblastoma patients.

Introduction

Glioblastoma (GB) is the most common primary malignant brain tumor in adults with a median survival of 1 year after the initial diagnosis despite multimodal therapy [1]. GB represents 60—70% of all diagnosed gliomas with an incidence rate of 3.2—5.3 per 100,000 people [2—4]. Only a small percentage of GB patients survive >3 years, and hence referred to as ''long-term survivors". The definition of long-term survivors remains controversial throughout the literature, and suggested periods include 18, 24, 36, 48 and 60 months [5—11].

Clinically, GB patients present with a variety of symptoms, depending on the tumor size, location, and peritumoral edema. Nonspecific neurological deficits include; headache, seizures, nausea, vomiting, hemiparesis, visual disturbance, lethargy, and personality changes [12].

Histopathologically, glioblastoma is extremely variable. However, areas of palisading necrosis and microvascular proliferation are usually characteristic [13]. The latter are considered essential for the diagnosis [14]. Based on the 2016 World Health Organization classification of central nervous system (CNS) tumors, there are three GB morphological variants: giant-cell, epithelioid, and gliosarcoma [4,14].

Ki-67 is a nonhistone nuclear protein that is expressed during the proliferative cell cycle and is associated with the transcription of ribosomal RNA (rRNA). Ki-67 is expressed throughout all active cell cycle phases, but not in the resting cell phase, G_0. In cancer cells, as expected, the percentage of Ki-67 expression is high [15—17]. Antibodies against the protein Ki-67 have been widely used to differentiate cells that are actively growing from nongrowing cells. However, Ki-67 prognostic value for glioblastoma remains controversial in the literature [18—20].

We aim to evaluate the relationship between Ki-67 index and outcome in a group of locally diagnosed and treated GB patients and thereby determining the usefulness of this proliferation marker as an independent prognostic parameter.

Materials and methods

Patients' eligibility and study setting

This was a retrospective cohort study investigating the Ki-67 labeling index amongst glioblastoma patients diagnosed between June 2006 and February 2012 at King Abdulaziz Medical City, Riyadh, Saudi Arabia. This organization is in the east part of Riyadh and was established in 1983 with a current bed capacity of 1501.

Sampling

Nonrandom, nonprobability sampling technique was used in the present study. All study participants were included using the consecutive sampling technique. The overall survival was calculated for GB patients diagnosed between June 2006 and February 2012. This period was chosen to allow for at least 5 years posttreatment follow up to enable the analysis of long-term survival.

Data collection

The pathology reports for GB cases diagnosed during the specified period were electronically retrieved from the archives of anatomic pathology division. A total of 44 cases were identified and their paraffin-embedded blocks were used to generate the hematoxylin—eosin slides and perform immunohistochemistry using the antibody clone MIB-1 against the protein Ki-67.

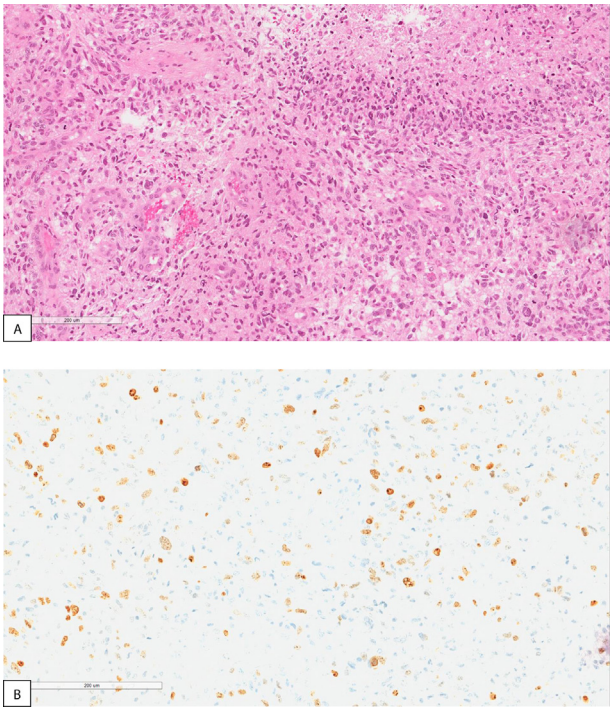

Fig. 1 (A) Hematoxylin and eosin-stained section from a viable portion of one of the studied glioblastoma cases; there is marked cellularity, microvascular proliferation (lower left), and palisading necrosis (upper right); (B) the Ki-67 (MIB-1) labelling index is 28% based on manual calculation of the most labeled high-power field.

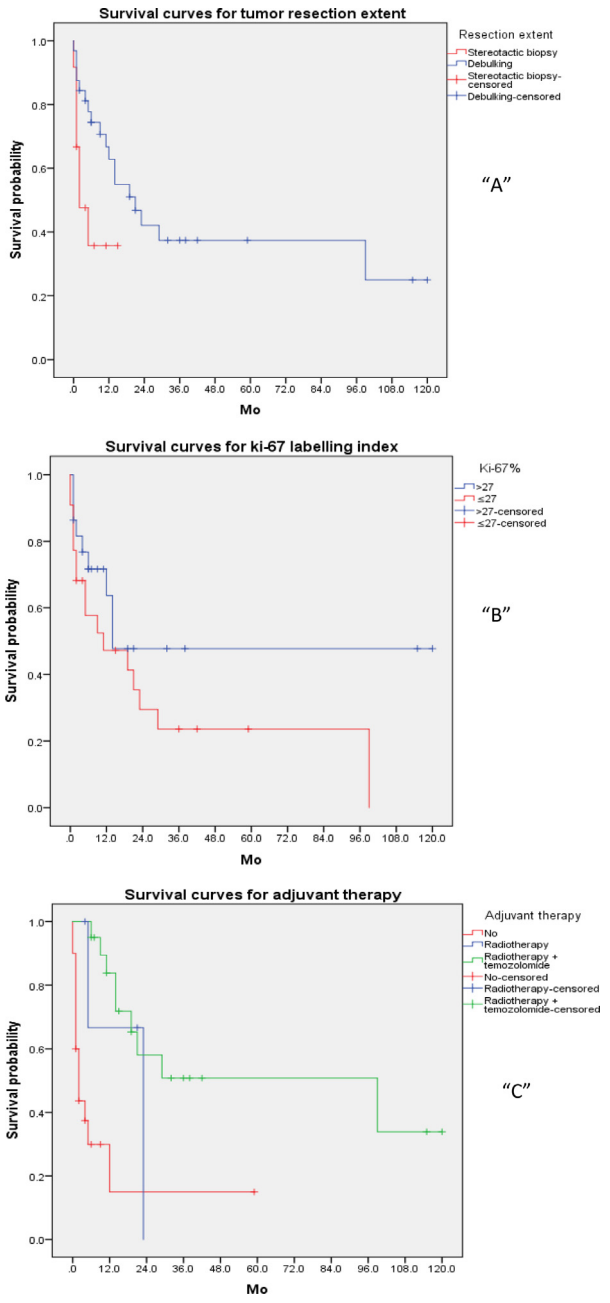

Fig. 2 ''A" Comparison between the survival curves for patients who have had a stereotactic biopsy and debulking surgery; ''B" comparison between survival curves for Ki-67 labeling index; ''C" comparison between survival curves for adjuvant therapy.

The diagnosis in each case was microscopically confirmed independently by two Canadian-board certified neuropathologists (A.H.A. and F.A.). The Ki-67 labeling index (Dako, Produktionsvej, 2600 Glostrup Denmark) was calculated by selecting the most labeled tumor zone at high power (40× objective lens, Zeiss microscope AX10, Carl Zeiss Microscopy Ltd, Jena, university city, Germany) in each case and through the attached microscope camera (Zeiss AxioCam MRc5, Carl Zeiss Microscopy Ltd, Jena, university city, Germany) snap shots from those high-power fields were collected and printed in colors by a laser printer (Fig. 1).

Once the photomicrographs from all cases were obtained in hard copies, the calculation of the index was performed manually by one of the neuropathologists (A.H.A.) to ensure consistency and accuracy of the results. The resultant indices were tabulated against the clinical outcomes (see Fig. 2).

The overall survival (OS) was calculated from the date of diagnosis to either the date of death, or the last date of follow-up visit for those who are still alive. Patients' care at King Abdulaziz Medical City, Ministry of National Guard Health Affairs, Riyadh, Saudi Arabia relevant clinical information were obtained from the hospital information system including demographics, extent of resection, and adjuvant chemotherapy. The details of radiotherapy when given were obtained directly from the archives of the department of neuro-oncology at King Abdullah Specialist Children's Hospital, Riyadh, Saudi Arabia.

Data management and analysis plan

Data were coded and entered into the software program IBM® SPSS® Statistics (version 23), IBM Corportation, Armonk, New York, United States for statistical analysis. Descriptive statistics were used to present the prevalence related data, such as, gender, Ki-67 labelling index, original tumor location, cerebral lobe affected, presenting symptoms, extent of resection, and status of adjuvant therapy. These were presented as frequencies and percentages. Means and standard deviations were calculated for numerical variables such as the overall survival in months, age, and size of the tumor.

Kaplan–Meier survival analysis was used to investigate the overall survival (OS). The Mantel–Cox log-rank test was used to compare the survival curves between different Ki-67 labeling indices. A multivariate analysis using Cox proportional-hazards model was performed to simultaneously investigate other covariates that might influence the overall survival. These included: tumor size, extent of resection, adjuvant therapy, age, and gender. Lastly, all tests with $p < .05$ were considered statistically significant.

Ethical considerations

This study has been approved by the Institutional Review Board (IRB) at King Abdullah International Medical Research Center (KAIMRC), National Guard Health Affairs (NGHA), Riyadh, Saudi Arabia. Data were collected from the hospital information system (BestCare) and patients' identities were kept concealed.

Results

A total of 44 patients were included in the present study. The median age of diagnosis was 56 years. Patients' ages ranged from 1 year to 91 years. Female patients are slightly outnumbered by males ($n = 25$; 56.81%).

Most patients ($n = 32$; 72.72%) had a debulking surgery. Around half of the patients received adjuvant therapy, that

Table 1 Glioblastoma patients' characteristics.

Variable	*N*	%
Median age, range		
56 (1–91)	–	–
Gender		
Male	25	56.81
Female	19	43.18
Tumor resection extent		
Debulking	32	72.72
Stereotactic biopsy	12	27.27
Adjuvant therapy		
None	20	45.45
Radiotherapy	4	9.09
Radiotherapy + Temozolomide	20	45.45

Note. Mo = month.

Table 2 Presenting symptom for glioblastoma patients.

Variable	*N*	%
Presenting symptom		
Headache	13	29.54
Hemiparesis	10	22.72
Altered mental status	10	27.27
Seizures	7	15.90
Nausea & vomiting	7	15.90
Slurred speech	4	9.09
Visual disturbance	3	6.81

Table 3 Glioblastoma tumor characteristics.

Variable	*N*	%
Glioblastoma variant		
Conventional	43	97.73
Gliosarcoma	1	2.27
Ki-67 labeling index: 27 (2–76)%		
≤27%	21	47.72
>27%	23	52.27
Laterality		
Left	21	47.72
Right	17	38.63
Bilateral	6	13.63
Average tumor size, range		
4.76 ± 1.78 (1.7–10.1) cm		
Tumor site		
Multiple	25	56.81
Temporal	8	18.18
Frontal	6	13.63
Parietal	4	9.09
Occipital	1	2.27

is, radiotherapy and chemotherapy. A subset of patients (n = 20; 45.45%) received both radiotherapy and chemotherapy (Temozolomide). Around onetenth of patients (n = 4; 9.09%) received radiotherapy alone. Details of patients' characteristics are listed in Table 1.

Patients presented to the hospital with a variety of signs and symptoms. Headache was the most common complaint in GB patients (n = 13; 29.54%), followed by hemiparesis and altered mental status (n = 10; 22.72%). Details of signs and symptoms at presentation are listed in Table 2.

As far as histomorphology is concerned, the vast majority of GBs (n = 43; 97.73%) were classical/conventional. Gliosarcoma variant was identified in one patient. The median expression of Ki-67 labeling index was 27% with a range of 2–76%. Using the median as the cut-off point, GB cases were separated into two groups either ≤27% or >27% Ki-67 labeling index.

The average tumor size was 4.76 ± 1.78 cm. The smallest tumor size was 1.7 cm, whereas the largest tumor size measured 10.1 cm at its greatest diameter. Most tumors (n = 25; 56.81%) involve more than one cerebral lobe. Details of the tumor characteristics are listed in Table 3.

Patients were divided into two categories; those with a ≤27% Ki-67 labeling index against those with >27% and comparison between the two groups survival curves was made (Fig. 2).

The 12-month survival rate for all GB patients is 48%. However, the median survival in months for patients who underwent a debulking surgery ''subtotal resection" was 21 months versus 2 months only for those who only underwent a biopsy procedure. Those who received combined adjuvant chemoradiotherapy had the best 12-month survival rate at 72% compared to 15% rate only in those who had no adjuvant therapy. The median survival in months for patients who received adjuvant radiotherapy alone and chemoradiotherapy is 23 months and 99 months, respectively. Details of median survival in months and 12- and 36-month survival rates are listed in Table 4.

Lastly, a multivariate analysis was performed using the Cox-proportional-hazards model to identify the hazard ratios of age, gender, original tumor location, lobe, resection extent, and the adjuvant therapy on survival. The hazard ratio (HR) for an increase of 1 year of age was 1.035 (95% confidence interval [CI] = 1.007–1.064). The HR for receiving radiotherapy alone was 3.122 (95% CI 0.402–24.084). Table 5 lists the HRs of the variables along with their corresponding p values.

Discussion

The present study investigated Ki-67 labeling index and its association with the overall survival (OS) amongst glioblastoma patients. Using the median of Ki-67 expression as a cut-off, the difference between survival curves (≤27% vs. >27%) amongst patients diagnosed with glioblastoma was statistically insignificant. Consequently, no correlation between the expression of Ki-67 and overall survival could be established.

Ki-67 labeling index determines the growth fraction of tumors in percentages [21]. According to Hu et al., [22] a significant increase in Ki-67 labeling index correlates with tumors of higher grades, concluding that tumors with high expression of Ki-67 are more likely to be malignant. Ki-67, therefore, can be utilized to differentiate between benign and malignant tumors [18]. This is especially true when it comes to distinguishing Grade 2 from Grade 3 diffuse astro-

Table 4 Median survival in months, 12/36-month survival for glioblastoma patients.

Variable	Median survival (mo)	Survival rate, % (12-mo)	Survival Rate, % (36-mo)
Ki-67 labelling index, %			
≤27	11	48	24
>27	14	48	48
Resection extent			
Stereotactic biopsy	2	36	0
Debulking	21	55	37
Adjuvant therapy			
No	2	15	15
Radiotherapy only	23	66	0
Radiotherapy + temozolomide	99	72	51

Note. Mo = month.

Table 5 Cox-proportional-hazards model analysis for glioblastoma patients.

Variable	HR	95% CI	*p*
Tumor size, cm	0.891	0.666–1.191	0.435
Age	1.035	1.007–1.064	0.015
Gender			
Males	Reference	–	–
Females	1.420	0.401–5.036	0.587
Resection extent			
Debulking	Reference	–	–
Stereotactic biopsy	1.008	0.200–5.036	0.992
Adjuvant therapy			
Radiotherapy + temozolomide	Reference	–	–
Radiotherapy	3.122	0.402–24.084	0.277
No	22.36	4.605–108.523	0.000116

Note. CI = confidence interval; HR = hazard ratio.

cytomas and cut-off values have been suggested in this regard [23].

The value of Ki-67 as an independent prognostic factor for glioblastomas is not established yet in the literature. Studies correlating between Ki-67 index and outcome in glioblastoma patients showed conflicting results. However, two later studies confirmed a positive correlation between Ki-67 labeling index and overall survival in GB patients. In these two studies the authors concluded that the higher the Ki-67 index, the longer the survival [24,25]. Wong et al [25] proposed that tumors with higher proliferation index may be more susceptible to adjuvant therapy and this hypothesis is substantiated by the results of similar studies related to lung and breast cancers [26,27].

However, Ho et al [28] found that the means of MIB-1 and topoisomerase II alpha labeling indices were lower in the 34 GB patients who lived >2 years compared to the other 34 GB control patients who lived <2 years after diagnosis. This was reproduced in other studies such as Yoshida et al. [29] who found in a cohort of 38 GBs the 1-year survival rate was 77% and 32% for Ki-67 index ≤ 20% and ≥20% respectively and the difference in survival curves were statistically significant (p = .02) [30].

Other studies found no relationship between the clinical outcome and the proliferation index attributing that to the wide variation in mitotic activity between different tumor areas [19,20]. In the present study, the difference in survival of GB patients with ≤27% vs. >27% Ki-67 indices is statistically insignificant (p = .130) by the log–rank test (Mantel–Cox) which further support the notion that the Ki-67 index on its own cannot predict the outcome in GB patients.

According to McKeever et al. [31], younger patients diagnosed with glioblastoma have a better prognosis and longer survival, due to lower levels of expression of Ki-67 labeling index. They have concluded that younger age at diagnosis can be a significant predictor of low expression of Ki-67 [31,32]. In the present study the median survival in months for patients aged <60 and >60 is 14 months and 6 months, respectively but this was statistically insignificant (p = .205) by the log-rank test.

In the present data, only seven patients lived more than 36 months, i.e., long-term survivors. Four of whom had a Ki-67 of ≤27%, and one of whom was >60 years of age. The elderly patient was diagnosed with the gliosarcoma variant. Gender does not influence the survival as well as the original tumor location and lobe involved. Factors which best predicted survival were the age at diagnosis and the usage of combined adjuvant chemoradiotherapy. GB patients who did not receive adjuvant therapy had a hazard ratio of 23.36 compared to those who received adjuvant chemoradiotherapy (p = .000116).

GB patients who did not receive adjuvant therapy had a median survival of 2 months, whereas patients who received

radiotherapy alone had a median survival of 23 months. Furthermore, GB patients who received both radiotherapy and chemotherapy had a median survival of 99 months reflecting the significant positive impact of the combined adjuvant therapy on the overall survival leading to a 12-month survival rate of 72% compared to 15% for those with no adjuvant therapy.

Limitations

This study represented a single-center experience, and therefore, it would have been more conclusive if other national institutes were involved to obtain a larger sample size.

Conclusion

The difference between the survival curves of patients with Ki-67 labeling index of $\leq$27% versus >27% was statistically insignificant ($p = .130$). Therefore, Ki-67 labeling index alone cannot predict the survival in GB patients arguing against its prognostic importance as an independent factor.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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