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# EVALUATION OF THE OPTIMAL KI-67 CUTOFF VALUE DETERMINING GRADES OF GLIOMA IN TURKISH POPULATION

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**Abstract:**

Gliomas are a heterogenous group of malignancies, occupying nearly 80% of central nervous system (CNS) primary malignant tumors and rank the first among brain tumors. The Ki-67 is a routinely assessed protein in the pathological study of a tumor. It's widely investigated as a proliferation marker throughout the literature for its prognostic, predictive and therapeutic roles. A local Turkish cohort of 102 patient in the Medical Park/ Goztepe, Bahcesehir university, were enrolled in this retrospective study, to identify a precise cut point of ki-67 protein level, separates between glioma WHO assigned grades, and to explore the possible predictive relationship between Ki-67 proliferation index and the preoperatively levels of differential white blood cells in series of archival adult malignant gliomas with grade II, III and IV lesions. The statistical tests revealed that ki-67 expression level of 9.5% is a significant cutoff value between grade IV Glioblastoma and grade II and III astrocytic and oligodendroglia gliomas. And according to the findings, Age and preoperatively measured Monocyte's count showed a statistical difference between patients with low (<10%) and high (≥10%) expression of ki-67. Thereby, result reinforces the statement that Ki-67 is in proportion to the glioma histologic malignancy degree and is a good tool for assigning grading between GBM and non-GBM gliomas. Hence, encouraging that ki-67 is a potent candidate in development of new classification system combining morphological, molecular and genetic features to better diagnose and treat glial malignant tumors.

**Keywords**: Ki-67, Grading, GBM, Glioma, Cutoff, Turkey.

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## Introduction:

Gliomas are highly invasive tumors, considered as the most frequent intracranial central nervous system neoplasms. (Bray F, 2018) Glioma patients can present a variety of neurological symptoms due to their infiltrative nature, such as epilepsy, motor deficits, or sensory and behavioral changes (Gerdes, 1984)

The immunohistochemical evaluated nuclear antigen ki-67 is found naturally in the normal proliferative cell and normally absent in the resting cells, but it is continuously and over-expressed and reflects the growth rate and proliferative activity of a mass of tumor cells (Byreddy VR, 2018)

The highly proliferative cell is one of the hallmarks of cancerous tumors, the score of ki-67 expression has been routinely used and widely documented among pathologists as a proliferation marker for various human tumors, including the brain malignancies (Wakimoto, 1996) .

Therefore, ki-67 standard measurement methods and clinically precise cutoff values are highly critical in predicting the biological behavior of CNS neoplasms.

This retrospective study was designed to investigate whether the over-expression of ki-67 (≥10%) proliferation index is a potent marker in determining the accurate glioma assessment protocol in Turkish Population. And assessing the statistical relationship between Glioblastoma, Astrocytic, oligodendrocytic differentiated glioma cases and patient or tumor characteristics.

## DATA AND METHODS

### Samples and data collection

A non-clinical, retrospective simple random approach have been chosen for patient selection fits with the purpose of this study and to reduce possible bias caused by the retrospective nature of the study. It should be noted that this study is archive-based, relied on histological reports done at the time of examining patients and did not include any recent chemical histological study.

A total number of 102 patients were considered in this study cohort were diagnosed for brain malignancies with different grades in the hospital of Bahcesehir University (Goztepe Medical Park), Istanbul, Turkey, in the period from July 2012 to January 2019.

All eligible patients were required to meet the following criteria to be enrolled in this single-institution retrospective study:

1. Eighteen years old or older.
2. Confirmed histopathological diagnosis grade II, III astrocytoma and oligodendroglioma and GBM grade IV
3. Availability of postoperative tested ki-67 expression value

Information was obtained included: patient history, biopsy report of the routinely stained sections after the surgery and blood test right before entering the operating room, for 102 local Turkish patients' cohort, diagnosed with oligodendroglioma and astrocytoma glial tumors grade II, III and IV, were included in this study.

### Statistical analysis

Data was organized in an EXCEL datasheet (Microsoft Corporation, Redmond, WA, USA), and all analyses were performed with a statistical software SPSS version 22.0. Patient baseline characteristics were summarized using descriptive statistics. Data shown to have skewed distribution as the statistical analysis made known. So, non-parametric tests have been selected. Receiver operating characteristics (ROC) test was taken to define the optimal cutoff value of ki-67 expression, which could be used to separate Grade II, III from grade IV glioma cases.

Then cohort cases have been regrouped, two-tailed hypothesis tests were the basis for the analysis of categorical variables such as; chi-square, Mann–Whitney U tests, and Kruskal–Wallis tests. Variables were expressed by the median, to evaluate the relationship between ki-67 overexpression and other patients' or tumor variables. A P-value of lower than 0.05 was defined to point to a statistically significant divergence in the two-tailed hypothesis tests.

**Result**

Pathology records pertaining to these patients have been retrieved from archives and through departmental medical records of anatomic pathology division. Of them, 44 patients were grade IV glioblastoma multiforme (GBM), grade I, and ependyma glioma cases have been excluded from the study. Of the remaining 58 patients, 18 patients with grade II diagnosis, and 40 were patients in Grade III. Furthermore, we have retrieved the numerical data for preoperative blood cell count (total and differential white blood cell count) to find the most promising peripheral blood index for predicting ki-67 expression.

The WHO histopathological groups of the tested cohort and their assigned Ki-67 evaluated score at the time of patient's assessment (Table 1) have been analyzed by using of Kruskal Wallis Test (chi-square), concluding that ki-67 is a valid variable to differentiate between glioma grades, the difference of ki-67 value between grades was statistically significant **(p=0,000).**

***Table 1*** **Ki-67% correlation with histopathological glial grades**

| | | | Ki-67 Labeling index | | | | |
|---|---|---|---|---|---|---|---|
| *Glial Grade* | Frequency | percentage | Ki-67 mean | Standard Deviation | Median | Range | P* |
| *Grade II* | 18 | 17.65 % | 3.56 | 1.75 | 3.5 | 1-9 % | .000 |
| *Grade III* | 40 | 39.21 % | 16.9 | 16.71 | 13.5 | 1-80 % | |
| *Grade IV* | 44 | 43.14 % | 22.66 | 13.17 | 20.0 | 7-70 % | |

***Kruskal- Wallis test, p value <0.05 considered as statistically significant***

In (Figure 1) considering (grade) as the grouping variable, Ki-67 was not normally distributed between the two data subgroups (grade II, III) and (grade IV), so non-parametric tests were performed (Mann-Whitney u), which illustrated the significant positive difference between grades II, III and grade IV glioma group set patients (p=0.000) supporting the potential of using the ki-67 proliferative index to differentiate between high grade GBM and non-GBM lower histological glioma grades in Turkish population (Table 2).

***Table 2*** **Ki-67% correlation between grade II, III and grade IV**

| Grade subgroups | ki-67 | | | | | |
|---|---|---|---|---|---|---|
| | Number | Mean | Standard Deviation | Range | Median | *P** |
| Grade II –III | 58 | 12.76 | 15.19 | 1-80 % | 6.5 | .000 |
| Grade IV | 44 | 22.66 | 13.17 | 7-70 % | 20 | |

***Mann-Whitney U, Wilcoxon W, Z tests, p value <0.05 considered as statistically significant**

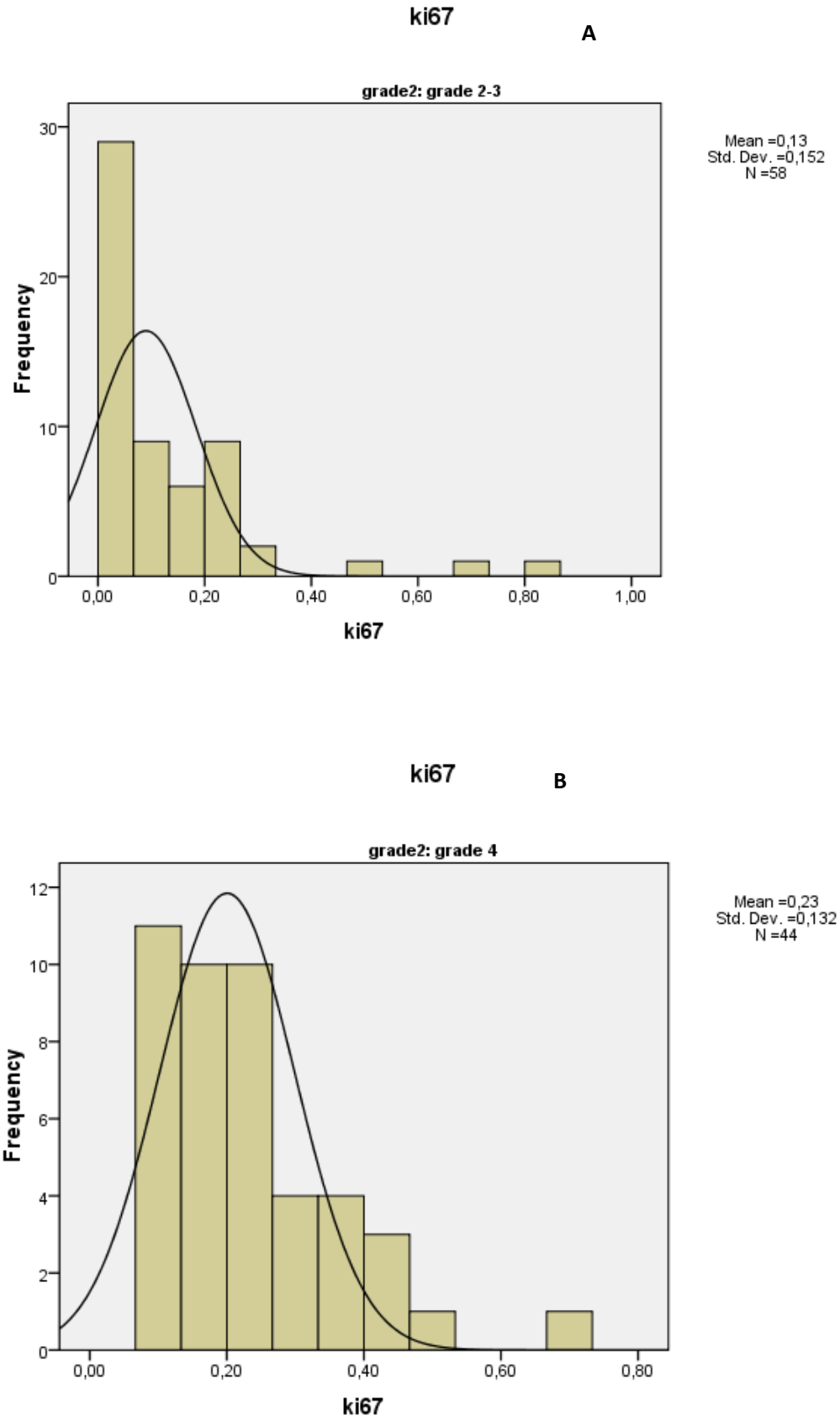


**Figure 1. Distribution of ki-67 percentage values among grade regarding subgroups, grade II and III (A) and grade IV (B)**

The cutoff value for the ki-67 index that could significantly separate patients in grade II and III from patients in grade IV was determined by receiver operating characteristics (ROC) curve analysis, which showed that when **ki-67 is equal to 9,5%,** the sensitivity is 93,2%, and specificity is 62,1% for separating glioma grade II, III from grade IV (Figure 2). By using Youden index method, obtained cutoff value had established the significant role of ki-67 labeling index value to separate Glioblastoma (GBM) from non-Glioblastoma cases in Turkish population.

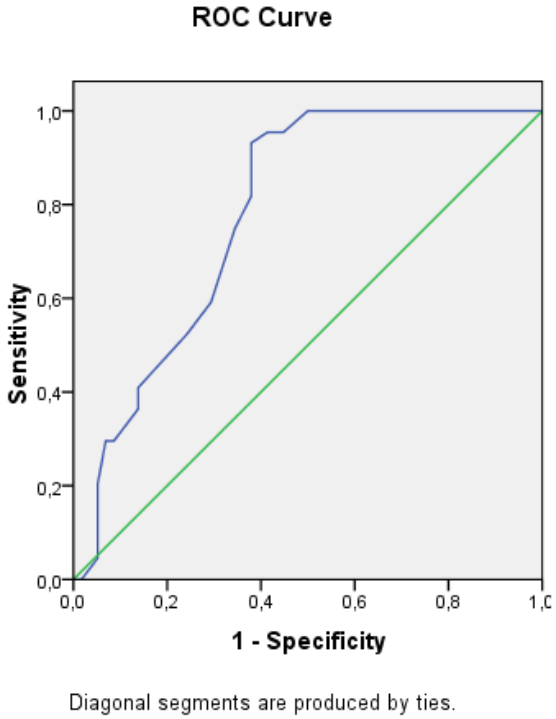


**Figure 2: Receiver operating characteristics (ROC) curve showed that the optimal cutoff value of ki-67 for separating grade II, III from group IV patient subset to be 9.5% with 93.2% sensitivity and 62.1% specificity.**

Based on the reported ki-67 cutoff value previously (9.5%), we considered 10% to be a threshold to describe a glioma tumor cell to be overexpressing ki-67 or not.

The statistical analysis of the cases regarding the Ki-67 index was divided dichotomously into two categories regarding patient and tumor parameters analysis, normal (<10% ki-67) and overexpressed (≥10% ki-67) categories, represented by 57% and 43%, among the whole population sample, respectively. 100% of grade II cases showed <10% ki-67 proliferative index, increasing with grade; to reach 93% of the total grade IV (GBM) cases were overexpressing ki-67 protein. This highlights that higher expression of ki-67 is linked with higher malignant CNS tumors.

As (Table 3) shows, there is also a notable statistically significant difference between grades after sub-grouping of data set. by performing Chi-Square tests, there was a statistical difference in grades of normal and over-expressed ki-67 groups **(p=0,000)** which proves the validity of Ki-67 to be categorizing marker between glioma grades.

***Table 3.*** **Correlation of glioma malignancy grade and Ki-67 subgroups**

| | Number | Ki-67 <10% | Ki-67 ≥10% | *P** |
|---|---|---|---|---|
| **Grade II** | 18 (102) | 100 % | 0% | .000 |
| **Grade III** | 40 (102) | 45 % | 55 % | |
| **Grade IV** | 44 (102) | 6.8 % | 93.2% | |

***Ci-Square test, p value <0.05 considered as statistically significant**

The statistically significant variation between ki-67 categorial groups regarding age (p= 0,000), suggest that older age could be a predictor of ki-67 over-expression, and vice versa (Table 4). This result is consistent with the study of (McKeever, 2001) who concluded that a low level of ki-67 proliferative index expression in younger glioblastoma patients linked with longer survival time and thereby better prognosis.

This archive-based study was limited by the availability of data, parameters such as: tumor laterality and intracranial location, molecular markers (GFAP, P53 and IDH-1) were analyzed to show no significant difference between Ki-67 expression groups.

***Table 4.*** **Correlation between Age and Ki-67 subgroups**

| | Number | Mean | Standard deviation | *P** |
|---|---|---|---|---|
| Ki-67 <10% | 39 | 40,67 | 10.628 | .000 |
| Ki-67 ≥10% | 63 | 51.89 | 13.931 | |

***Mann-Whitney U, Wilcoxon W, Z tests, p value <0.05 considered as statistically significant**

The second hypothesis helps in the early and preoperatively assessment of glioma, is evaluating the relationship between Ki-67 index and preoperative blood cell count in a population of Turkish local adult patients diagnosed with glial tumors from different grades II, III, IV.

The second endpoint of this study was to examine the potential of using preoperative differential white blood cell count for the patient to predict Ki-67 index score, some trend in the values was notable, the percentage of patients with high abnormal were 82%, 92% 62.7% for WBC, Neutrophil and Monocyte, respectively. On the other hand, 50%, 19.6%, was the population of patients with a low abnormal reading of Lymphocyte and Monocyte, respectively. The mean of WBC, Neutrophil, between the two ki-67 categories of <10% and ≥10%, were in the range of high abnormal, whereas Lymphocyte mean value was in the low abnormal range for both ki-67 categories.

The reported result below (Table 5) shows that there is a statistically significant difference in Monocytes' count between ki-67 expression <10% and over-expressed ki-67 group (≥10%) (p= 0.040).

High levels of monocytes may indicate the presence of cancer or other medical conditions. In our study population, Monocyte average count was in the normal range for both ki-67 categories, but higher Monocyte in the group of higher expression of ki-67 indicates the active immune response inside the body is proportional with the level of proliferation and malignancy of the tumor.

***Table 5.*** **Correlation between ki-67 level of expression and preoperative white blood cells count**

| | Ki-67 <10% | | Ki-67 ≥10% | | P* |
|---|---|---|---|---|---|
| Number | 39 | | 63 | | |
| | Mean | Standard Deviation | Mean | Standard Deviation | |
| WBC | 14.1669 | 4.71783 | 14.2483 | 5.23304 | ,959 |
| Neutrophil | 12.0454 | 4.62339 | 12.1700 | 4.69166 | ,959 |
| Lymphocyte | 1.2826 | 0.66003 | 1.3365 | 0.76619 | ,893 |
| Monocyte | 0.5477 | 0.53241 | 0.7100 | 0.59346 | **,040** |
| Eosinophil | 0.0077 | 0.01739 | 0.0127 | 0.02548 | ,291 |
| Basophil | 0.0228 | 0.02127 | 0.0214 | 0.01777 | ,923 |

***Mann-Whitney U, Wilcoxon W, Z tests, p value <0.05 considered as statistically significant**

## DISCUSSION

### *Clinical significance of Ki-67 protein evaluation*

Ki-67 protein expression has been established as an indicator for prognosis and prediction in the evaluation of cancer patients' biopsies. It is recorded that aggressive high graded tumors express increased levels of ki-67 expression, concluding that tumors with high expression of Ki-67 are more likely to be malignant (Nielsen LAG, 2018)

In the majority of studies, the rate of cellular proliferation, measured by the antibody against the ki-67 nuclear protein, is proven to be a prognostic marker. Most of these studies point to a significant increase in the levels of Ki-67 proliferative index between astrocytomas of high grade when compared to those of low grade (Hsu DW, 1997) (Saha R, 2014). However, there are articles that mention the importance of anti-Ki67 as a method of gliomas classification, especially (Khalid H, 1997), who set the ki-67 values above 10% seem to be a cutoff point for differentiating the astrocytomas in benign and malignant tumors. (Zhu L, 2015) underscored the significant positive correlation between histological different glioma grades and ki-67 proliferative index (P<0.01). (AL, 2006) mentioned that increased expression of Ki-67 has been shown to be positively correlated with the higher degree

of malignancy and worst prognosis in gliomas patients, and it is not enough to use it alone as a diagnostic indicator and must be combined with other prognostic parameters. In his work, (Skjulsvik AJ, 2014) highlighted the statistically significant difference in the values of a ki-67 index between grade II and IV glioma patients.

Because of the need for a prognostic marker that might help clinicians to direct therapy and predict tumor behavior and prognosis, ki-67 has been heavily investigated to examine the relationship between the overall survival of the patients with gliomas and ki-67 protein expression. In the systematic review with meta-analysis done by (Chen WJ, 2015), he showed that an increased level of ki-67 expression is a statistically significant biomarker for the worst prognosis of patients with glioma (Yoshida Y, 2010) (Preusser M, 2012). Later in his prospective study (Saha R, 2014) underscored, using the Kaplan-Meier curve, that astrocytomas patients with the level of ki-67 expression equal and greater than 14.3% showed lower survival time. Similarly, (Montine TJ, 1994) concluded that patients with ki-67 < 3% showed higher survival time than patients with a ki-67 index ≥3.

The Ki-67 level of expression has the potential to be a postoperative biomarker of poor prognosis in astrocytomas recurrences cases also. (Uehara K, 2012) concluded that ki-67 has a predictive ability beside its previously mentioned prognostic role, he found that GBM patients with a higher rate of recurrence had a higher proliferative rate. Furthermore, in his study, (Gzell C, 2016)categorized the patients in the IHC examination of recurrence into two groups, above and under 10% level of ki-67 expression, to conclude that there is an inverse correlation between ki-67 proliferative index and survival time of the patient.

On the contrary, other studies in the literature mentioned that the higher expression of ki-67, the longer the survival (Bredel, 2002). This raised the hypothesis of (Wong, 2019) in glioblastoma and (Fasching, 2011) in breast cancer, which claimed that the higher susceptibility to adjuvant therapy agents could be achieved by patients with higher proliferative index. Otherwise, (Dirven CM, 1998)showed that there is no prognostic relationship with the ki-67 index, and recently (Alkhaibary, 2019) used the median of Ki-67 expression as a cutoff, to concluded that there is no established correlation between the Ki-67 expression and overall survival. Yet now, findings regard the association between time of survival and proliferative activity of glioma tumors is conflicting.

By reviewing the literature, it's clear that the cell proliferation rate in glioma tissues, as evaluated by ki-67 immuno-reactivity, has been marked as an informative indicator and independent marker for predicting the length of survival, tumor grade, and clinical outcome for glioma patients.

The reported studies about prognostication of glioma patients showed very varied cutoff values of Ki-67 expression in survival curves. These notable differences may be responsible for the problems in determining a standard limit in daily practice. Some used the average value of the ki-67 index (Fisher B. J., 2002)(Kanyilmaz, 2018), some authors have used median as the arbitrary value, whereas others proposed a variety of cutoff values in their articles such as: 1.5% (Hsu DW L. D.-W., 1997), 5 % (Jaros, 1992) to 15.3% (Sallinen PK, 1994). While (Schiffer D C. P., 1997) and (Di X N. T., 1997) employed 8% as the cutoff in their work (Enestrom S V. L., 1998) suggested value of 10% level of expression as the arbitrary value.

(Karamitopoulou E1 P. E., 1994) is one of the first literature documents highlighted the significant difference of the Ki-67 proliferative index between low-grade and high-grade gliomas (p= 0.004).

This study proved the findings of the latest published work by(Krishnan, 2019), who concluded that there is a marked statistical divergence in the expression of ki-67 between grade III and grade IV with ($P$ = 0.00025) and (0.04) respectively. There was a significantly prominent existence of grade IV patients, with a ki-67 index value above a cutoff of 10% compared to grade III tumors ($P$ < 0.0001). without consideration of the histological grade, he concluded that a low ki-67 index (≤10) to be correlated with longer survival among high-grade gliomas.

This result is similar to work done by (Rathi KR R. B., 2007)and (Wakimoto H. , 1996), who used the previous and current antibody, respectively, to show a strong relationship between the ki-67 index and WHO tumor grading. And after a while, (Hu, 2014) defined the correlation of different molecular markers in 152 glioma patients to conclude that the increase in the expression of EGFR and Ki-67 significantly correlate with higher graded glioma tumors. Nevertheless, there is no novel identified molecular marker with precise cut point value to work out better diagnosis and thereby better therapy plans.

It is hard to determine a standard threshold for ki-67 to be used in routine clinical practice, and this variation in ki-67 cutoff values is obvious in the meta-analysis conducted by (WJ, 2015). This statistical study had concluded that 9.5 -10% to be the most accurate threshold range to describe overexpression of ki-67, consensus the claim of (Yuan, 2013) who considered (ki-67 ≥10%) as the overexpression cut point in his study with grade II WHO glioma patient set. And generally, there is a proportional relationship that ki-67 increases definitely with increased heterogenicity and malignancy of glial tumor mass.

This further support the notion that most malignant tumor (GBM) with high ability to infiltrate and a higher probability for metastasis and thereby shortest survival time amongst glioma malignancies, is probably reflected on the over-expression of ki-67 which means more capable cells to continue to proliferate after resection of the tumor.

***Molecular categorizing of Glioma***

ki-67 can be a reliable marker to categorize glioma malignancies and be a part of a new combined grading system that might help to guide the physicians to better predict tumor behavior and prognosis, more precise diagnosis and hence, best treatment plans.

The World Health Organization (WHO) stratification and diagnosis system of glioma considered to be not optimal, because it depends on subjective and personal perspective the evaluation of the histopathological criteria. Because the routine histological assessment of tumor mass is limited in predicting tumor behavior. (Kim BH B. Y., 2013) introduced Ki-67 as an excellent representative for growth rate and tumor behavior, empowering it its potential to improve the information the grading system provides and thereby, ki-67 might be used in routine grading of cancer (Klöppel G P. A., 2004)(Inwald EC K. M., 2013).

A systematic review of molecular and genetic predictors of survival of gliomas in 14678 patients (Thuy, 2014) concluded that, beside IDH1/2, 06- MGMT and LOH 10/10q, Ki-67 is a main informative biomarker in active highly proliferative gliomas.

Nevertheless, ki-67 cannot on its own be used as a cornerstone for the classification of glial tumors, we join the voices of many authors, who raised the attention toward the need for supplementary molecular and immunohistochemical diagnostic parameters to be employed in the assessment and diagnosis of astrocytomas. The value of Ki-67 for molecular staging of glioma also needs to be confirmed in controlled trials involving a larger number of patients before any definitive conclusions can be made.

It is not surprising to found the ki-67 proliferative index involved in glioma classification suggested models, independent of WHO grades. Many studies in the recent literature tried to reclassify gliomas into distinct subgroups combing ki-67 with other prognostic factors in glioma. In his work, (Zeng *et al.*, 2015) found that IDH-1/2 mutated cases survived longer, as well as cases with low expression of ki-67, then he linked the prognostic value of using IDH-1/2 mutation status combined with ki-67 level of expression, to classify astrocytomas into five biologically different prognostic groups, the worst prognosis groups were defined with IDH-1/2 wildtype and moderate to the high level of ki-67 (≥10%). They were suggesting a potentially clinically applicable molecular approach to better treatment options.

(Yan, 2012) showed that low level of ki-67 expression is linked to IDH-1 mutation in GBM patients, which further confirmed by(Cai, 2014) findings, who set a molecular classification of astrocytic tumors into three groups regardless of WHO grades, combing ki-67 expression and IDH-1/2 with ATRX mRNA expression. He concluded that patients who had the shortest survival time were those who carry IDH-1/2 wildtype and high ki-67 proliferative index percentage (>10%).

This is powerfully suggesting that the best patient outcome patients' group in the combined stratification system for glioma should include a low rate of proliferation accompanying with IDH-1 mutation. This raises questions about the efficiency of these biomarkers as an informative candidate for new glioma classification models combining histological and genetic aspects to grade diffuse astrocytoma cases.

These documented data must be justified by further prospective studies to reach greater knowledge for the physicians about the disease course. Given the recent breakthrough discoveries have highlighted the intermolecular associations and to further support the molecular categorization

of glioma tumors, we encourage the incorporation of Ki-67 protein expression with the other molecular prognostic markers into the classification model.

***Preoperatively prediction of ki-67 proliferative index***

The ki-67 percentage is one of the crucial parameters obtained postoperatively by biopsies in the pathology laboratories. Therefore, exploring the area of noninvasive ki-67 preoperatively prediction parameters could be clinically useful.

Whereas (Peng M P. F., 2015) found an equation to predict ki-67 preoperatively in GGO nodules pathological assessment using 3D CT images parameter analysis, we sought to identify peripheral blood cell indices associated with ki-67 proliferative index amongst patients diagnosed with different grades of glial malignancies. (Wang, 2019) proved in his work that the Neutrophil-to-Lymphocyte ratio (NLR) <3.2 statistically corresponds with poor prognosis glioma patients, and vice versa. In general, he concluded that the count of preoperative Neutrophil, Eosinophil, and Lymphocyte was strongly associated with glioma grades. However, parameters such as white blood cells count, to a certain extent, manifesting the body's immune attack against the highly proliferative dividing cancerous cells. and this correlation is biological and pathophysiological more than just statistical and mathematical.

Based on the reported results in this study, a preoperatively differential white blood count test cannot be used to estimate the level of Ki-67 expression in glioma patients, except for Monocyte because the difference in median of Monocyte count between the two groups of the low and high ki-67 proliferative index was statistically significant ($P < 0.004$).

Although team was keen to consider the very preoperative blood test to ensure the patient was in the best health condition qualify him to enter surgery; we could not make sure about the exact patient manifestations may affect the white blood cell counts, as well as, for defining such a multivariant correlation relationship between different blood parameters and two categories of ki-67 expression, our sample considered to be small, this makes this result vulnerable to bias. However, Ki-67 remains a valuable IHC pathological tool, and further efforts to explore markers that allow us to predict ki-67 preoperatively are warranted.

Taking into account that this study has some limitations regarding it is a single-institution cohort, our heterogenous population number of patients. Besides, 50% of the cases included in this study have been evaluated according to WHO classification of central nervous system tumors 2007 which needed to be reclassified after the updating in 2016, the retrospective feature of the study was the major limitations of the study. We should mention here that ki-67 evaluated values varied amongst the literature studies due to using different protocols and techniques, ki-67 level heterogenicity, and the personnel subjectivity. (Raghavan R S. P., 1991)

Irrespective of the potential ki-67 shows, there is no enough solid shreds of evidence yet to definitely consider ki-67 proliferative index as a diagnostic or predictor of prognosis in gliomas due to the lack of published prospective studies validating the preliminary findings of multiple authors who have conducted single-center retrospective studies.

**CONCLUSION**

In a nutshell, the primary finding of the present retrospective study was to determine the value of ki-67 is a priority in predicting the biological behavior of grade II, III (astrocytoma and oligodendroglioma) and IV (glioblastoma) patients. Most notably this work has identified that ki-67, besides its prognostic and therapeutic attribution in glioma assessments, it has the validity to be used in molecular grading of glioma. Furthermore, this study confirms that the ki-67 cutoff point of (9.5%) index level separates between high-grade GBM and non-GBM glioma cases. Overall impression that Age and Monocyte are a reliable predictor of higher expression of ki-67 in glioma tumor, which already known to be linked to higher malignances and poorer prognosis.

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