ARTÍCULO ORIGINAL

# Ki-67 Proliferation Index Correlates to IDH-1 Mutation Status in High-Grade Gliomas

# Correlación del índice de proliferación Ki-67 con el estado de mutación IDH-1 en gliomas de alto grado

# Índice de proliferação Ki-67 se correlaciona com o status de mutação IDH-1 em gliomas de alto grau

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## Abstract:

**Objectives:** Molecular diagnosis is essential to establish the behavior of the tumor and the prognosis of patients with HGG. The IDH1 mutation status represents the cornerstone for the current diagnosis of these tumors and is associated with prognosis. We hypothesized that Ki-67 proliferation index may be related to IDH

mutation status and this relationship may be used to better categorize HGG in emerging economies.

**Methods:** An institutional database was reviewed for high-grade gliomas treated in the period from January 2020 to December 2021. Patients with a HGG that the pathology report had information regarding IDH-1 mutation status, Ki-67 proliferation index and p53 overexpression were included. The statistical analysis was performed using the software SPSS® (IBM) version 25.

**Results:** A total of 25 patients were included in the study. We found mutation for IDH1 in 8 patients, while 17 patients retained the wild-type variant. Nine patients had a Ki-67 of 20% or less while the remaining 16 had a proliferation index above 20%. Statistical analysis showed a positive correlation between Ki-67 index and IDH1 status.

**Conclusion:** Our findings suggest that there is a positive relationship between a high Ki-67 proliferation index and the lack of IDH1 mutation in HGG.

**Key Words:** High-grade Glioma, Glioblastoma, Ki-67, IDH-1, Correlation

## Resumen:

**Objetivos:** El diagnóstico molecular es esencial para establecer el comportamiento del tumor y el pronóstico de los pacientes con gliomas de alto grado (HGG). El estado de la mutación IDH1 representa la piedra angular para el diagnóstico actual de estos tumores y está asociado con el pronóstico. Hipotetizamos que el índice de proliferación Ki-67 puede estar relacionado con el estado de la mutación IDH y que esta relación puede usarse para categorizar mejor los HGG en economías emergentes.

**Métodos:** Se revisó una base de datos institucional para gliomas de alto grado tratados en el período de enero de 2020 a diciembre de 2021. Se incluyeron pacientes con un HGG cuyo informe de patología tenía información sobre el estado de la mutación IDH-1, el índice de proliferación Ki-67 y la sobreexpresión de p53. El análisis estadístico se realizó utilizando el software SPSS® (IBM) versión 25.

**Resultados:** Se incluyó un total de 25 pacientes en el estudio. Encontramos mutación para IDH1 en 8 pacientes, mientras que 17 pacientes retuvieron la variante de tipo salvaje. Nueve pacientes tenían un Ki-67 del 20% o menos, mientras que los 16 restantes tenían un índice de proliferación superior al 20%. El análisis estadístico mostró una correlación positiva entre el índice Ki-67 y el estado de IDH1.

**Conclusión:** Nuestros hallazgos sugieren que existe una relación positiva entre un alto índice de proliferación Ki-67 y la falta de mutación IDH1 en los HGG.
**Palabras clave:** Glioma de alto grado, Glioblastoma, Ki-67, IDH-1, Correlación

## Resumo:

**Objetivos:** O diagnóstico molecular é essencial para estabelecer o comportamento do tumor e o prognóstico dos pacientes com gliomas de alto grau (HGG). O status da mutação IDH1 representa a pedra angular para o diagnóstico atual desses tumores e está associado ao prognóstico. Nossa hipótese é que o índice de proliferação Ki-67 pode estar relacionado ao status da mutação IDH e que essa relação pode ser usada para categorizar melhor os HGG em economias emergentes.

**Métodos:** Um banco de dados institucional foi revisado para gliomas de alto grau tratados no período de janeiro de 2020 a dezembro de 2021. Foram incluídos

pacientes com HGG cujo relatório de patologia continha informações sobre o status da mutação IDH-1, índice de proliferação Ki-67 e superexpressão de p53. A análise estatística foi realizada utilizando o software SPSS® (IBM) versão 25.

**Resultados:** Um total de 25 pacientes foi incluído no estudo. Encontramos mutação para IDH1 em 8 pacientes, enquanto 17 pacientes mantiveram a variante do tipo selvagem. Nove pacientes tinham um Ki-67 de 20% ou menos, enquanto os 16 restantes tinham um índice de proliferação acima de 20%. A análise estatística mostrou uma correlação positiva entre o índice Ki-67 e o status de IDH1.

**Conclusão:** Nossos achados sugerem que existe uma relação positiva entre um alto índice de proliferação Ki-67 e a ausência de mutação IDH1 em HGG.

**Palavras-chave:** Glioma de alto grau, Glioblastoma, Ki-67, IDH-1, Correlação

## Introduction:

Intracranial Gliomas as a whole have an estimated annual incidence of 6.6/100,000 individuals in the United States [1], and High-grade gliomas (HGG) account for about 76% of all gliomas [2].

In 2016, the WHO established a new classification for glial tumors that integrated molecular and histological characteristics. Isocitrate dehydrogenase (IDH) mutations represent an important variable in this classification as it dichotomizes gliomas into two groups and also represents an important prognostic factor for glial tumors [2]. As important as it is to establish the IDH mutation status, this technology is not readily available everywhere in the world.

The Ki-67 protein is a cellular marker associated with ribosomal RNA transcription and thus cell proliferation. The protein is identified regularly through staining during histopathologic analysis of specimens from many types of cancer including HGG [3]. There have been conflicting evidence on the relationship of Ki-67 staining and the prognosis in patients with HGG, but many studies have found that a value over 20% is associated with worst prognosis [4-7].

Given the known relationship between IDH mutation status and the prognosis of patients with HGG, we hypothesized that Ki-67 proliferation index may be related to IDH mutation status and this relationship may be used to better categorize HGG in emerging economies.

## Methods

An institutional database was reviewed for high-grade gliomas treated in the period from January 2020 to December 2021. We reviewed the clinical records of the patients and collected clinical and epidemiological data (age, sex), and tumor data (histological diagnosis, tumor grade, IDH1 mutation status, Ki-67 proliferation index and p53 expression status).
We included all patients with a HGG with a pathology report that had information regarding IDH-1 mutation status, Ki-67 proliferation index and p53 overexpression.
Patients with pilocytic astrocytomas and midline gliomas were excluded from the final analysis since the genetic profile of these tumors is very different from supratentorial adult gliomas.

The histological diagnosis was verified via a pathology report generated by our institution.
The diagnostic testing for IDH1 was performed by immunostaining with an antibody to the IDH1-R132H protein. The proliferation index was established via immunohistochemistry using staining for Ki-67, according to the information that several studies reported [6], a cut-off value equal or greater than 21% was determined to be high in this study.
The p53 expression status was also established via immunohistochemistry.

The statistical analysis was performed using the software SPSS® (IBM) version 25.
A comparative Fisher's test was performed to compare the dichotomous Ki-67 proliferation index groups (Low VS High) with the IDH mutation status (mutated VS Wild type) and another group comparing the IDH and P53. A value of $p < 0.05$ was considered to have statistical significance.

## Results

A total of 25 patients were included in the study, 17 male patients and 8 female patients met the inclusion criteria. The median age of the population was 50 years (ranging from 21 to 74 years). The most frequent histological diagnosis according to

the WHO 2016 guidelines was Glioblastoma in 17 patients (68%), followed by anaplastic astrocytoma in 5 patients (20%) and anaplastic oligodendroglioma in 3 patients (12%). We found mutation for IDH1 R132H in 8 patients, while 17 patients retained the wild-type variant (32% VS 68%). Regarding the Ki-67 proliferation index, the mean percentage was 35% ranging from 5% to 80%, after dichotomizing this variable 9 patients had a Ki-67 of 20% or less while the remaining 16 had a proliferation index above 20%. The p53 gene was overexpressed in 19 patients (77.3%).

Table 1 summarizes the clinical and pathological variables.

**Table 1. Clinical and demographic variables of the study population**

| | **N=25 (%)** |
|---|---|
| Age | 50.6 ± 13.9(21-74) |
| Sex (%)<br>Female<br>Male | <br>8(32)<br>17(68) |
| Tumor type (%)<br>Glioblastoma<br>Anaplastic Astrocytoma<br>Anaplastic Oligodendroglioma | <br>17(68)<br>5(20)<br>3(12) |
| IDH<br>Mutant<br>Wildtype | <br>8(32)<br>17(68) |
| Ki67 % | 35.6% ± 21.9(5-80) |
| Ki67<br>Low <%<br>High >% | <br>9(36)<br>16(64) |
| P53<br>Overexpression<br>None | <br>19(76)<br>6(24) |
| IDH: isocitrate dehydrogenase<br>Mean Values are presented as ±SD | |

A comparative Fisher test was performed between the variables dichotomic Ki-67 proliferation index (low VS high) and the IHD1 mutation status (mutated VS wild-type), and also between IDH1 mutation status and p53 expression status (overexpressed VS not).

When addressing the relationship between Ki-67 proliferation index and IDH1 mutation status, we found that there is a significant relationship between having a low Ki-67 and having a mutated IDH1 HGG (p=0.04). On the other hand, there is no

positive relationship between IDH1 mutation status and p53 expression status (p=0.585).
A Spearman test is also performed between the IDH1 mutaion status and Ki-67 dichotomous variables, which shows a bilateral Rho coefficient value of 071 with a value of p=0.001. The same Spearman test is performed between the IDH1 mutaion status and p53 expression status variables, however with a Rho coefficient of -0.18 and a value of p=0.20, the correlation was non-significant. There was no reletionship between p53 expression and the Ki-67 proliferation index (Table 2).

**Table 2. Correlation between IDH, Ki-67 and p53**

| **Variable** | Chi square | P value | Rho coefficient score | P value |
|---|---|---|---|---|
| **IDH and p53** | 1.8 | 0.263 | -0.18 | 0.20 |
| **P53 and Ki67** | 8.3 | 0.472 | -0.13 | 0.37 |
| **Ki67 and IDH** | 23.7 | 0.001 | 0.71 | 0.001 |

## Discussion

When assessing a patient with a glial tumor, histological classification was for many years the 'gold standard' for diagnostics, but is associated with considerable interobserver variability, particularly in the context of diffusely infiltrating gliomas [8]. Several advances in the understanding of the molecular biology of gliomas concluded that the molecular classification has a better correlation with the clinical outcome of these patients than the histological classification [9-11].

In 2016, the WHO established a new classification for glial tumors that aside from the histological characteristics included molecular genetic alterations that help to better capture the biologic behavior of these tumors [12].
A major improvement in the 2016 WHO classification of gliomas, compared with the preceding 2007 classification, is the distinction of different glioma entities according to isocitrate dehydrogenase 1 or 2 (IDH)-mutation status [12, 13].
IDH mutant gliomas acquire a neomorphic enzymatic activity that results in the conversion of a-ketoglutarate to 2-hydroxyglutarate, which in turn inhibits a-KG dependent dioxygenases, such as ten-eleven translocation (TET) family 5-methylcytosine hydroxylases and the Jumonji-C-domain-containing histone-lysine demethylases [13, 14].

As a consequence, IDH mutation causes aberrant DNA and histone methylation, eventually leading to wide spread hypermethylation of CpG islands [13, 15].
It is now recognized that IDH mutated gliomas behave differently from their wild-type counterpart and tend to have better prognosis, therefore these tumors should be treated as a different entity with impact on the adjuvant treatment and follow-up [16-18]. Unfortunally access to best clinical practices is not always possible, especially in developing countries, this affects the diagnosis and treatment of patients with glial tumors, and includes access to different immunohistochemical techniques to achieve the current diagnosis of gliomas that must include the IDH mutation status [19, 20].
Ki-67 is a marker of cell proliferation, and its index correlates with the clinical course of several cancer types. Moreover, the Ki-67 proliferation index is one of the most widely used since its low or high expression levels are directly associated with grade II-III or grade IV gliomas, and it's readily available in most centers, even in developing countries [6, 21].
Since both IDH mutation status and Ki-67 proliferation index are related to the prognosis of patients with glial tumors, we hypothesized that the two of them could be related in some way.
Most studies have found that a value over 20% of Ki-67 proliferation index represent a variable associated with worst prognosis in patients with HGG [4, 6, 7], although this is not universally accepted, as some studies have found no relationship between the proliferation index and the survival of these patients [5]. We decided, based on previous studies to establish a high Ki-67 percentage as 21% or above, and the statistical analysis shows that there is a statistically significant relationship between the IDH mutation status and the dichotomized Ki-67 proliferation index (p=0.001).

Cai et al published a retrospective analysis of 47 patients, and found a strong association between ATRX loss and IDH1-R132H, as well as a Ki-67 high expression restricted in the tumors with IDH1-R132H negative [21]. In this paper a Ki-67 high expression was considered to be >10% of cells positively stained, also this study included grade II and III astrocytomas, as well as GBM, which we believe should be addressed separately.
In a similar study Yan published a study that included 118 patients with primary GBM, in this study the Ki-67 status was classified in 3 categories according to the staining intensity, as appreciated by different pathologists. They found, among other results, that there was a positive relationship between IDH1 mutated tumors and a low expression of Ki-67 [22].
We believe what makes our study valuable is the inclusion of only HGG and the establishment of a cut-off value from witch an estimate can be made from the Ki-67 proliferation index to predict the IDH1 mutation status of these tumors.

Our results show that most tumors that are histologically classified as Glioblastoma (GBM) have a proliferation index of over 21%, and most of them are negative for IDH mutation. We did find one case in which the histological diagnosis was a GBM but the Ki-67% was low and the IDH mutation status was positive, this means that the proliferation index alone could guide the diagnosis of these tumors to a secondary GBM, or a grade IV astrocytoma as it is classified according to the 2021 WHO classification [23].
On the other hand, another patient had a tumor that was initially classified according to the histological characteristics as a grade III astrocytoma, however the immunohistochemical analysis showed a low Ki-67 proliferation index and a negative IDH-1 mutation status. This means that this relationship is not a 100% reliable, and appropriate measures should be taken to adequately classify these tumors whenever possible.

This study has limitations, first of all the limited number of patients that we were able to include in this study could give rise to incorrect results, and so we encourage other institutions to attempt to confirm of contradict our findings.
Second, immunohistochemical staining for IDH1-R132H is not the ideal way to determine the IDH1 mutation status of a glial tumor, and our results could vary if a PCR analysis was performed in these tumors, so this is something that should be taken into account when interpreting our findings, and something that maybe could be addressed in future studies.

## Conclusion

Our findings suggest that there is a positive relationship between a low Ki-67 proliferation index (20% or less) and the presence of IDH1-R132H positive staining via immunohistochemistry.
This relationship could help to better categorize HGG in centers in which the lack of resources limit the possibility to determine the IDH1 mutation status of HGG.

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