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ARTÍCULO ORIGINAL

Detection of genetic alterations via molecular cytogenetic analyses in a case series of pediatric glioma patients in Colombia: a pilot study

Detección de alteraciones genéticas mediante análisis citogenéticos moleculares, en una serie de casos de pacientes pediátricos con glioma en Colombia: un estudio piloto

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Resumen

Objetivo: determinar la presencia de alteraciones genéticas en vías clave de crecimiento y proliferación celular en pacientes pediátricos con gliomas en Colombia, mediante hibridación *in situ* fluorescente (*FISH*) e inmunohistoquímica.

Metodología: se realizó un estudio observacional descriptivo de una serie de casos, conformado por 50 pacientes pediátricos diagnosticados con glioma. Sobre tejido tumoral embebido en parafina remanente de los estudios diagnósticos, se emplearon microarreglos de tejido y *FISH* para detectar la fusión *KIAA1549::BRAF*, la deleción de *CDKN2A*, las amplificaciones de *EGFR* y *N-Myc*, y la codeleción 1p/19q. La expresión de H3K27me3 se determinó mediante inmunohistoquímica.

Resultados: la edad media al diagnóstico fue de 8,05 años, el 54 % eran niñas, el 44 % tenía gliomas de alto grado y el 56 % eran de bajo grado. El 18 % de los tumores presentaron alguna de las alteraciones genéticas, siendo la más frecuente la fusión *KIAA1549::BRAF* (12 %). Se detectaron alteraciones en la expresión de H3K27me3 (8 %), alteraciones en *EGFR* (4 %), deleción homocigota de *CDKN2A* (2 %) y pérdida heterocigota de 1p (2 %). No se evidenció amplificación de *N-Myc* en ningún caso.

Conclusiones: esta metodología resulta útil y de bajo costo para el estudio de gliomas pediátricos. Los hallazgos sugieren la presencia de un perfil genético, principalmente asociado a gliomas infantiles. Futuras investigaciones deberían explorar alteraciones características de gliomas de tipo adulto y paneles de estudio de esta naturaleza podrían incorporarse a la práctica de rutina en entornos de recursos limitados, con el fin de mejorar la clasificación de los gliomas pediátricos y su elección terapéutica.

Palabras clave: glioma; tumores pediátricos; hibridación *in situ*.

Abstract

Objective: To determine the presence of genetic alterations in key cell growth and proliferation pathways in Colombian pediatric glioma patients using fluorescent *in situ* hybridization (FISH) and immunohistochemistry.

Methodology: This study involved a descriptive observational analysis of a case series of 50 pediatric patients diagnosed with glioma. On paraffin-embedded tumor tissue remaining from diagnostic studies, tissue microarray techniques and FISH were employed to detect *KIAA1549::BRAF* fusion, *CDKN2A* deletion, *EGFR* amplification, *N-Myc* amplification, and 1p/19q codeletion. H3K27me3 expression was determined by immunohistochemistry.

Results: The mean age at diagnosis was 8.05 years; 54% of patients were female, 44% had high-grade gliomas, and 56% had low-grade gliomas. Eighteen percent of tumors presented at least one of the studied genetic alterations, the most frequent being the *KIAA1549::BRAF* fusion (12%). Alterations in H3K27me3 expression (8%), *EGFR* alterations (4%), homozygous deletion of *CDKN2A* (2%), and heterozygous loss of 1p (2%) were detected. *N-Myc* amplification was not observed in any case.

Conclusions: This methodology is useful and inexpensive for studying pediatric gliomas. These findings suggest the presence of a genetic profile primarily associated with childhood gliomas; future research should explore genetic alterations characteristic of adult-type gliomas. Study panels of this nature could be incorporated into routine practice in resource-limited settings to improve the classification of pediatric gliomas and their therapeutic choice.

Keywords: glioma; childhood cancer; *in situ* hybridization.

Introduction

Central nervous system (CNS) tumors are the most common solid neoplasms in children; among these, childhood gliomas stand out for their genetic features and clinical behaviors, which differ from those observed in adults (1, 2). The pediatric population is divided into three main groups (infants, children, and adolescents), each with specific epidemiological and pathophysiological characteristics regarding gliomas. Molecular analysis is a fundamental tool for classifying and selecting targeted therapies (3), although its implementation faces challenges in low- and middle-income countries. In addition to costs, other barriers include the availability of reagents and legislation (4).

The mitogen-activated protein kinase (MAPK), vascular endothelial growth factor (VEGF), and PI3K/AKT/mTOR pathways are involved in pediatric gliomagenesis (5). Relevant biomarkers include the *KIAA1549::BRAF* fusion, which activates the Ras/MAPK pathway due to the loss of the autoregulatory domain of *BRAF* and is predominant in pilocytic astrocytomas (6). *EGFR* and *N-Myc* amplifications have also been linked to the activation of the PI3K/AKT/mTOR pathway; these amplifications are typically present in midline gliomas and often associated with variants in histone 3 (7, 8). Another biomarker is the loss of *CDKN2A*, which is linked to the avoidance of cellular senescence, poor prognosis, and a reduced response to therapies (9).

Likewise, 1p/19q codeletion, a characteristic of oligodendrogliomas, confers sensitivity to alkylating agents and can be observed in adolescents (10). Finally, epigenetic changes contribute to the pathogenesis of high-grade gliomas. Several types of epigenetic modifications occur, including the loss of H3K27me3 (11).

The VEGF pathway, for its part, plays a fundamental role in angiogenesis, which is critical for tumor growth and metastasis. Elevated VEGF levels have been observed in gliomas, acting as tumor biomarkers. Consequently, this pathway has emerged as a target for immunotherapy to inhibit tumor growth and maintain the integrity and sustained immune function of dendritic cells within the tumor microenvironment (12, 13).

In Colombia, 1,090 new pediatric cancer cases were diagnosed between 2022 and 2023. CNS tumors accounted for 12.2% of these diagnoses, and 12.78% of these tumors lacked a histological diagnosis due to access barriers (14). Furthermore, the biological characteristics of this group of tumors in Colombia are unknown. This study aims to describe and evaluate, using inexpensive techniques, some key genetic markers involved in the pathogenesis of childhood glioma in a group of pediatric patients. The findings from this research could enable the implementation of an affordable strategy to improve the classification of brain tumors, especially in low- or middle-income settings.

Methods

Population

From an initial sample of 133 medical records of patients diagnosed with glioma at a referral pediatric hospital between 2016 and 2020, only cases with available biological material (tumor pathology), complete clinical records, and diagnostic imaging were selected. Among these, 50 patients met the inclusion criteria. Their demographic and clinical data, tumor location, and general imaging features were collected. The histological and immunological characteristics were obtained from pathology reports. The biological material was independently reviewed by pediatric pathologists, who confirmed the diagnosis and selected representative tumor areas.

The 2016 and 2021 World Health Organization (WHO) classifications of tumors include molecular parameters for brain tumor taxonomy. Tumors in this study were classified for therapeutic purposes using histology, immunohistochemistry, clinical correlation, imaging, and, sometimes, available molecular parameters. Consequently, pathology reports of these patients were prepared according to the 2021 WHO classification of tumors, featuring an integrated diagnosis in layers, but rarely meeting all *essential and desirable* diagnostic criteria boxes for specific entities.

The biological material (paraffin blocks and slides) from the selected cases was reviewed independently by two pediatric pathologists, NO and EP. Areas with tumor cellularity exceeding 80% were identified for immunohistochemistry and molecular cytogenetic studies. As a quality control measure, the pathologists verified and confirmed the original diagnosis of each case.

Tissue microarray manufacture

The manual construction of paraffin tissue microarrays was performed following the manufacturer's (UNITMA®, Seoul, South Korea) recommendations (15). For each patient, two tumor cylinders with a diameter of 3 mm were taken from the sample blocks. From the microarrays, 4-µm sections were obtained on conventional plates for hematoxylin staining and loaded plates for immunohistochemistry and *in situ* hybridization.

Immunohistochemistry

H3K27me3 expression was evaluated by immunohistochemistry using an H3K27me3 antibody (Clone C36B11) (Vitro S.A., Seville, Spain) that detects the H3.1 isoform. Staining was performed following the manufacturer's instructions (16). Briefly, 3-µm sections were dewaxed and hydrated; endogenous peroxidase activity was quenched by immersing the slides in 3% hydrogen peroxide.

Antigen retrieval was achieved by immersing the slides in boiling citrate buffer (pH 6.0). Nonspecific labeling was inhibited, and the slides were incubated with the primary antibodies. Amplification was carried out using the Optiview system (Roche Basel, Switzerland), followed by staining with diaminobenzidine (DAB) and counterstaining with hematoxylin.

Fluorescent in situ hybridization on paraffin-embedded tissue

For the sections obtained on loaded plates, fluorescent *in situ* hybridization (FISH) was performed using probes from Cytocell (Cambridge, United Kingdom), following the manufacturer's protocols. The viewing was carried out using a Zeiss Axiophot microscope, and the analysis was conducted with the LUCIA Cytogenetics® software; at least 100 interphase nuclei per slide were evaluated, selected at random and without overlap (Table 1).

Table 1. Reagents used, commercial source specifications, and interpretation (45, 53–56)

Type	Name	Commercial house	Characteristics	Interpretation
FISH Probe	*BRAF/KIAA1549*	Cytocell	Dual Fusion	a) An orange signal and a green signal, separated by a distance greater than the diameter of two or three signals, along with a yellow signal. b) Presence of three signals of any color and location, observed in 20% or more nuclei.
FISH Probe	*N-MYC/LAF4*	Cytocell	Amplification	A ratio greater than 2, observed in 10% of the nuclei.
FISH Probe	*EGFR*/ Chromosome 7 centromere, D7Z1	Cytocell	Amplification	A ratio greater than 2, observed in 15% of the nuclei.
FISH Probe	*CDKN2A*/ Chromosome 9 centromere, D9Z3	Cytocell	Deletion	A ratio lower than 0.5, observed in 40% of the nuclei.
FISH Probe	*TP73/ABL2*	Cytocell	Deletion	A ratio lower than 0.5, observed in 10% of the nuclei.
FISH Probe	*MAN2B1/ GLTSCR1*	Cytocell	Deletion	A ratio lower than 0.5, observed in 10% of the nuclei.
Antibody	H3K27me3	Vitro S.A	Clone C36B11	At a dilution of 1:50: a) The antibody remains in at least ≥5% of nuclear staining in neoplastic cells. b) Loss of H3K27me3, with absent staining in >95% of neoplastic cells, while control tissue shows positive labeling.

Technical limitations

This study used archival paraffin-embedded tissue, which has inherent limitations in laboratory procedures, such as loss of material. Despite standardization, a certain proportion of tumor cores was inevitably insufficient, non-representative, or lost during the experimental process. Nevertheless, complete clinical histories and follow-up information were available for all patients, which is a strength of this study. As a result, although only 15 cases could be fully evaluated at the molecular level, 50 patients who met the selection criteria, including the availability of tumor pathology material, were ultimately included.

Statistical analysis

In the univariate analysis, measures of central tendency and dispersion were calculated based on whether the data followed a normal distribution. Data normality was assessed using measures of bias and kurtosis; in statistical tests like the Shapiro–Wilk test, 0.05 was the threshold for significance.

This research constitutes a pilot study, meeting the criteria for a small-scale evaluation of molecular cytogenetic and immunohistochemical methods. The main goal was to identify genetic glioma patients using low-cost techniques like FISH and immunohistochemistry while standardizing these methods for their future application in large-scale research.

Ethical considerations

This project complies with international regulations, including the Declaration of Helsinki, the ethical guidelines for biomedical research developed by the Council for International Organizations of Medical Sciences (CIOMS), and Resolution 8430 of 1993 issued by the Colombian Ministry of Health.

This study is classified as research without risk to patients or their families because it is a retrospective study, and no intervention or modification of the biological variables of participants occurs. This risk is identified according to Resolution 8430 of 1993 by the Ministry of Health. This study was approved by the Ethics Committee of the *Fundación Hospital de la Misericordia* (HOMI) under Minute No. 72 545-22R and received approval from the Ethics Committee of the Faculty of Medicine at the *Universidad Nacional de Colombia*.

Results

Study population

The mean age of the 50 patients in the study was 8.05 years (SD = 4.94), with 54% female; 68% were between 2 and 12 years old, 24% were adolescents, and 8% were under two years old.

Of the tumors, 70% were located in the supratentorial region, predominantly in the temporal lobe (18%), 24% were cerebellar, and 6% were in the medulla. Left laterality was more common (52%), and 46% of the patients had compromised midline structures.

Low-grade tumors accounted for 56% (42% grade 1), whereas 44% were high-grade tumors (30% grade 4). The primary histological diagnoses included pilocytic astrocytoma (30%) and grade 4 gliomas (28%); 2% of patients had type 1 neurofibromatosis, and another 2% had Van Asperen syndrome.

Complete resection was achieved in 22% of patients, partial resection (<100%) in 58%, and no surgery was performed in 20%. Additionally, 20% of patients received chemotherapy, 6% received radiotherapy, and 38% underwent combined treatments. The 3-year survival rate was 68%.

Immunohistochemistry and FISH

All alterations were analyzed in 15 patients. In the remaining cases, tumor tissue was exhausted or not evaluated in one or more experiments. Of the tumors, 18% (9) tested positive for at least one of the studied markers, except for *N-Myc* amplification, which was negative in all samples. Most positive cases involved patients aged 2 to 12 years (78%). Regarding histological grade, 67% (6) of the tumors were classified as grade I, and the remaining 33% (3) were classified as grade IV. A summary of the results is shown in Figure 1, and the impact of the evaluated biomarkers is illustrated in Figure 2.

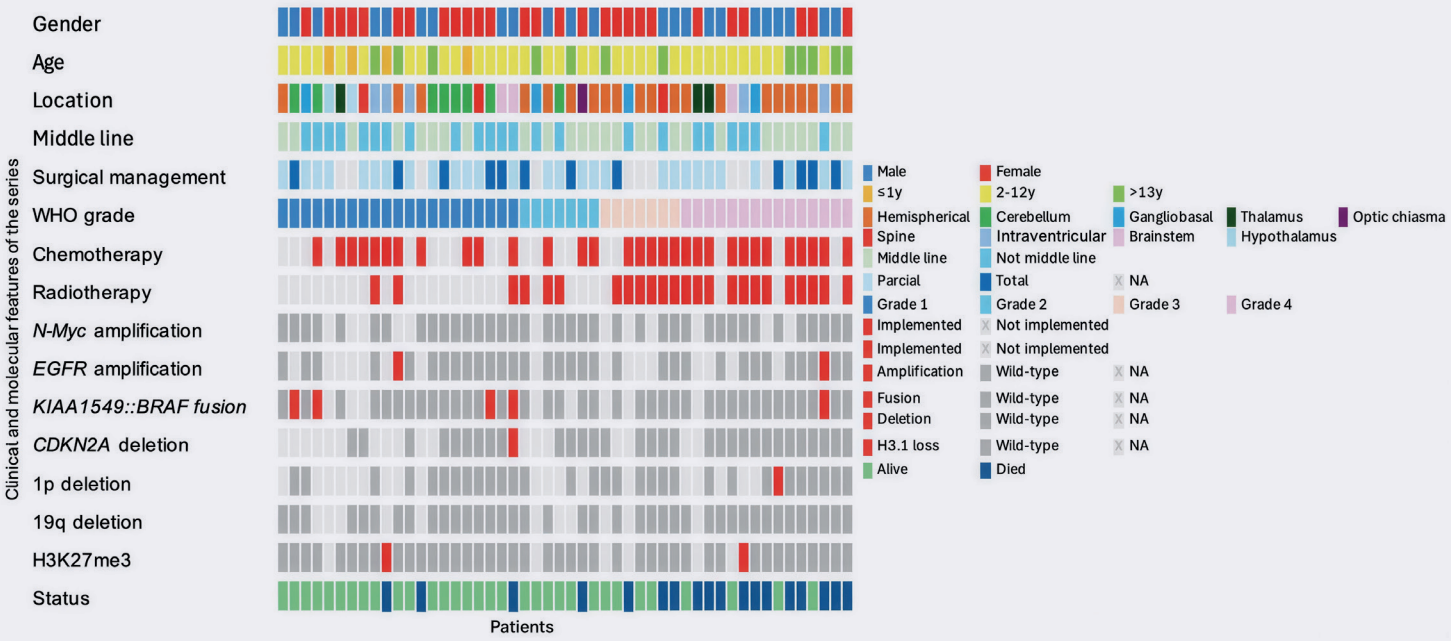

Figure 1. Summary of the key features of the 50 patients in this series, including imaging, clinical, and molecular findings, as well as treatment and outcomes

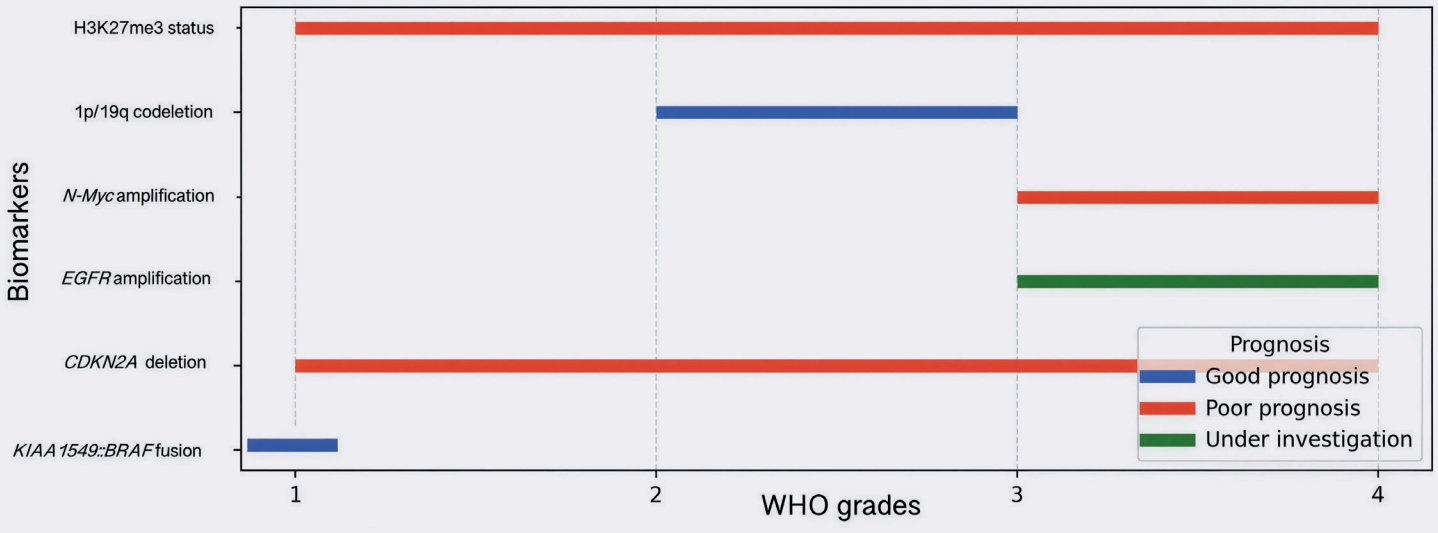

Figure 2. Association between the identified biomarkers, their tumor histological grade (according to the 2021 WHO classification), and prognostic impact

H3K27me3

H3K27me3 expression was assessed using immunohisto-chemistry in 88% (44) of the samples, revealing three patterns: loss of H3K27me3 expression in 5% (2) of the samples, weak H3K27me3 expression in 5% (2), and retained H3K27me3 expression in 90% (40) (Figure 3).

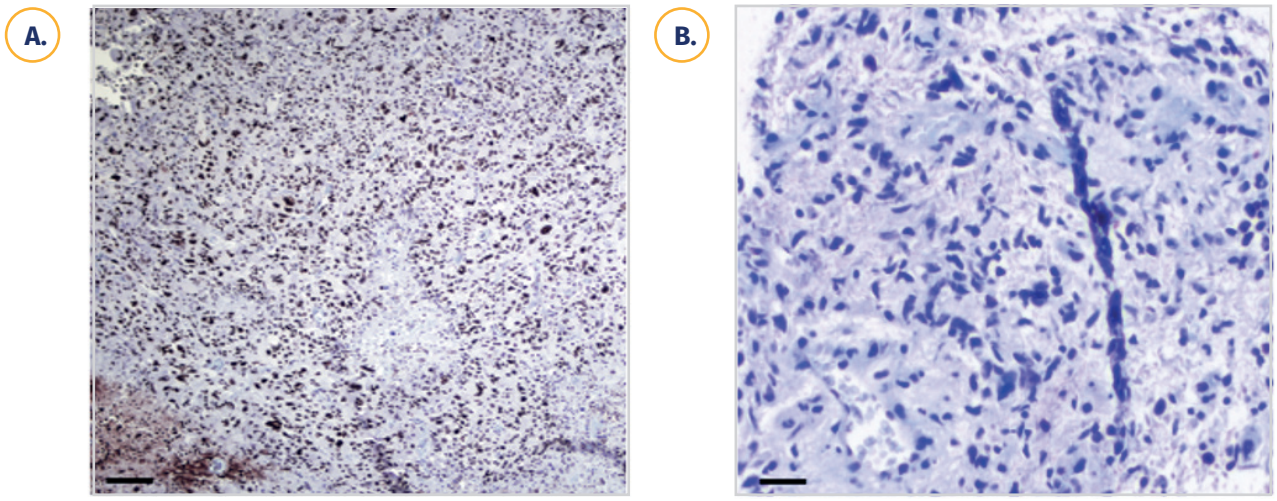

Figure 3. A) 10X: Positive control. All nuclei in this lesion are positive for H3K27me3, with the diaminobenzidine precipitate appearing brown. **B)** 20X: High-grade glioma with loss of H3K27me3. All nuclei appear blue.

N-Myc

N-Myc amplification was examined in 76% (38/50) of the tumors, and it was not detected in any tumor. There were no alterations in the *LAF4* control region or additional clonal lines.

CDKN2A

Loss of *CDKN2A* was assessed in 64% (32/50) of the tumors. One patient with low-grade glioma exhibited a deletion of two *CDKN2A* signals in 100% of the cells studied, with no alterations in the CEP9 control locus; this patient also presented with the fusion *KIAA1549::BRAF* (Figure 4).

EGFR

EGFR amplification was evaluated in 68% (34/50) of the tumors. One tumor showed amplification in 20% of the cells, with five *EGFR* signals and two from the CEP7 control locus.

1p/19q codeletion

Loss of 1p was assessed in 62% (31/50) of the tumors. 1p/19qcodeletion was not detected in any of these tumors. One patient exhibited loss of a TP73 signal in 31% of the cells examined, without alterations in the ABL2 control region. Conversely, deletion of 19q was evaluated in 74% (38/50) of the tumors, but no deletions were reported.

KIAA1549::BRAF fusion

The *KIAA1549::BRAF* fusion was examined in 84% (42/50) of the tumors; it was detected in 12% of the evaluated tumors. Four of the five tumors with *KIAA1549::BRAF* fusion were grade 1, and one was a grade 4 tumor. The fusion was identified as three signals in the nuclei analyzed. In high-grade gliomas, 150 nuclei were evaluated, while 100 nuclei were assessed in other tumors. Eighteen percent of patients with this rearrangement were male, with a mean age of 6.6 years. A significant association was observed between fusion and infratentorial tumor location ($p = 0.01038$).

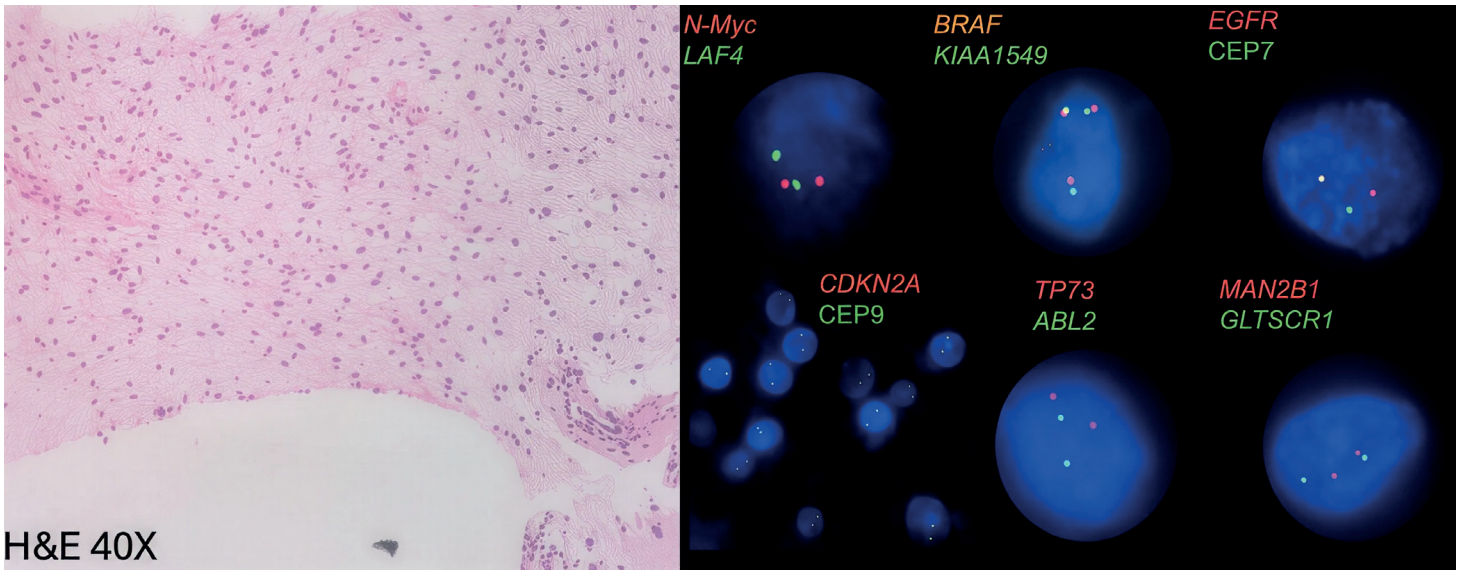

Figure 4. Molecular evaluation of a low-grade glioma using FISH. **A)** 40X: Microcystic and hypercellular proliferation with uniform cells, lacking atypia, necrosis, or mitosis. Cytogenetic-molecular evaluation by FISH. **B)** Negative N-Myc amplification. **C)** Positive KIAA1549::BRAF fusion, indicated by the presence of three fluorescence signals. **D)** Negative EGFR amplification. **E)** CDKN2A deletion, showing the loss of both red signals. **F)** Negative 1p deletion. **G)** Negative 19q deletion.

Discussion

Brain tumors are among the leading causes of cancer-related deaths in Colombian children. According to a Colombian health registry, 133 new cases of nervous system tumors were reported in children during 2023 (14). For successful treatment, having access to a complete diagnosis is recommended (17). Molecular tools, moreover, enable classification based on specific criteria and the optimal use of available treatments (18). Therefore, although studying all cases would be ideal, the results obtained are valuable. Even though the number of cases may seem low for drawing significant conclusions, pediatric tumors, if individual diagnoses are considered, are rare diseases.

Two similar studies were conducted in South America, one in Brazil and the other in Argentina. The Brazilian series involved next-generation sequencing (NGS) on a series of 95 pediatric gliomas. The Argentinian series aimed to identify the most common alterations in 102 glioma cases using FISH, immunohistochemistry, and Sanger sequencing (19, 20).

The primary conclusion from analyzing these three efforts is that molecular analysis is essential for a personalized clinical approach. Since resources are limited in low- or middle-income countries, using molecular cytogenetics and immunohistochemistry helps cut costs and is recommended.

The demographic distribution in our study, however, slightly differs from the previously mentioned series. For instance, the percentage of female patients (54%) was somewhat higher than that of males (46%), unlike other studies, such as Yang *et al.*, where men predominated (21). This difference could be due to our small sample size or environmental or genetic factors unique to our population, like potential barriers to healthcare access. The mean age at diagnosis was 8.05 years, younger than the reported age in the Latin American case series of Colli *et al.* (19).

Of the tumors, 70% were supratentorial, 24% were infratentorial, and 6% were spinal. Low-grade tumors accounted for 56% of all tumors, with pilocytic astrocytoma being the most common histological diagnosis, present in 30% of the patients. These results are similar to those reported by Ramirez *et al.* (22). On the other hand, 44% of patients had high-grade histology, with grade 4 being the most prevalent (28%), consistent with other series (23).

Clinical perspectives

The critical role of specific biomarkers in the diagnosis, molecular classification, prognosis, and therapeutic decision-making in pediatric gliomas warrants a detailed review of some key findings from this series. The next section will explore the clinical and biological implications of each biomarker, comparing our results with established international literature. This discussion aims to evaluate the practical applicability of these markers and refine diagnostic protocols for our local context (Table 2).

Table 2. Overview of the biomarkers evaluated in this study, detailing their histopathological and clinical importance in pediatric gliomas (57–63)

Biomarker	Histopathological feature	Clinical significance
KIAA1549::BRAF fusion	The *KIAA1549::BRAF* fusion is a diagnostic criterion for pilocytic astrocytoma, as established by the 2021 WHO classification. It can be detected using molecular biology and cytogenetic techniques.	This fusion has been associated with a better prognosis and enhanced therapeutic response, likely because of MAPK pathway activation that happens independently of Ras, promoting cellular senescence.
CDKN2A deletion	*CDKN2A* deletion is a diagnostic marker for high-grade astrocytomas with pilocytic features. It can be evaluated through immunohistochemistry, molecular biology, and cytogenetic methods.	Loss of *CDKN2A* plays a key role in cell cycle dysregulation and senescence. It is linked to poor prognosis and has been reported in patients with prior radiation exposure. The prognostic impact seems to be independent of whether the deletion is heterozygous or homozygous.
EGFR amplification	*EGFR* amplification serves as a biomarker in pediatric, adolescent, and young adult tumors. Its assessment is often linked to other molecular alterations, requiring concurrent evaluation of H3 and *IDH1/2* status. Its detection is possible via cytogenetic and molecular biology techniques.	This amplification is associated with the expression of the constitutively active EGFRvIII protein, which lacks proper negative regulation and enhances PI3K/Akt/mTOR pathway signaling, thereby promoting proliferation. It is considered an unfavorable biomarker and correlates with reduced overall survival. *EGFR* loss, on the other hand, may activate the mTORC2 pathway, contributing to resistance to *EGFR*-targeted therapies.
N-Myc amplification	*N-Myc* amplification has been reported in patients with Li-Fraumeni syndrome and in those with somatic H3G34 mutations. It can be assessed using cytogenetic and molecular methods.	*N-Myc* amplification is associated with poor prognosis and more aggressive tumor behavior. It promotes tumor initiation, progression, recurrence, and maintenance by impairing apoptotic pathways. This alteration is often linked to chromosomal instability.
1p/19q codeletion	The 1p/19q codeletion is used as a diagnostic marker for oligodendrogliomas and oligoastrocytomas. It is detectable using cytogenetic and molecular biology techniques.	This codeletion is linked to metabolic changes that directly influence the tumor microenvironment and immune response. In the literature, it has been associated with improved response to alkylating agents and radiotherapy.
H3K27me3 status	Evaluation of H3K27me3 is crucial for diagnosing diffuse midline gliomas. Immunohistochemistry is recommended to assess epigenetic marks such as H3K27me3, H3K27M, and H3K27ac, particularly those involving histone 3 alterations at codon 27. It can be evaluated through immunohistochemistry and molecular biology methods.	Loss of H3K27me3 has been extensively studied due to its relevance in determining therapeutic strategies. It affects both the metabolic and immune phenotypes of tumors and is generally linked to poor outcomes. However, the prognostic significance varies depending on the affected histone isoform: H3.3 (more common, worse prognosis) or H3.1 (linked to better outcomes).

H3K27me3

Loss of H3K27me3 is the hallmark of altered H3K27 midline diffuse glioma, a high-grade diffuse pediatric glioma according to the 2021 WHO classification, regardless of histological grade. This tumor is associated with a poor prognosis. Analyzing point variants in H3p.K27M, H3p.K27I, and/or *EGFR*, along with methylation profiling, is necessary for diagnostic confirmation of this glioma (24, 25).

In two tumors (5%), the loss of nuclear expression of the H3K27me3 isoform H3.1 was observed. This is similar to what has been reported in other series, where changes in H3K27 were more common in the H3.3 isoform, highlighting the importance of evaluating both or conducting additional studies (26, 27).

One of our patients with altered H3K27me3 H3.1 had a low-grade histological tumor, but the patient survived for 17 months. Alterations in H3.1 are associated with a better prognosis than alterations in H3.3 (27). This fact, linked to more well-circumscribed tumors, may have facilitated successful surgical resection, ultimately improving the patient's overall prognosis (28). We believe it is necessary to evaluate H3K27me3 and H3K27M in all midline tumors, regardless of histological grade.

N-Myc

Bjerke *et al*. reported *N-Myc* amplifications in high-grade pediatric gliomas with H3G34 variants, suggesting a potential link between H3G34 and alternative mechanisms that drive the increase in transcription of this proto-oncogene (29). *N-Myc* amplifications have also been observed in tumors with wild-type histone 3 and IDH, which are characterized by their low frequency and association with a lower overall survival time, close to 14 months (30).

In this case series, no amplification of *N-Myc* was detected, likely because these subtypes are rare, as shown by the cohort studies of Mackay *et al*. (23). In conclusion, we believe that testing for this marker is unnecessary in a basic molecular biology panel.

EGFR

EGFR amplification has been reported in high-grade gliomas (31). In this case series, an adolescent had amplified *EGFR* in a low-grade glioma with few anaplastic cells. Despite resection and chemoradiotherapy, the tumor progressed; however, the patient is still alive after 36 months.

The possibility of a high-grade glioma with intermediate features between pediatric and adult subtypes is suggested (32). This hypothesis aligns with the findings of Korshunov *et al*. (33), who described a wild-type tumor subtype for H3 and *IDH1/2*, with a methylation profile associated with receptor tyrosine kinase (RTK) involvement through *EGFR* gene amplification. This subtype is characterized by supratentorial tumors of high biological grade, with a median survival of 44 months. As established by the WHO in 2021, evaluating variants in *EGFR*, *IDH1/2*, and *TERT* is recommended in brain gliomas (17). Therefore, we believe it is advisable to analyze these markers in pediatric high-grade gliomas or those featuring unusual behavior.

1p/19q codeletion

Codeletion of 1p/19q is a hallmark of oligodendroglioma and is associated with better survival (10). We did not find any oligodendroglioma in this series, which is expected since it has been found that tumors with the classical histologic appearance of oligodendroglioma in the pediatric population do not always harbor 1p19q codeletions (34). Conversely, the isolated loss of 1p, whether from its 1p36 region or the entire short arm, has rarely been described in high-grade gliomas in adults or children. In pediatric case series, including this one, the loss of 1p has been seen more often than the 19q deletion or the 1p/19q codeletion (35).

A tumor with loss of heterozygosity of 1p, without a 19q deletion, was identified in a school-aged male with a high-grade glioma, confirmed both histologically and radiologically. We believe it would be interesting to briefly describe this case. The child underwent total tumor resection, did not require adjuvant treatment, and was still alive at 25 months. His immunophenotype was positive for *IDH* and *BRAF V600E*.

Variants in *IDH1/2* are found in up to 20% of adolescents and young adults, and their presence in school-aged children, such as this patient, is rare (36). These variants have been observed in astrocytomas and oligodendrogliomas, mostly in the cerebral hemispheres, which makes surgical resection easier and improves the prognosis (37).

The *BRAF V600E* mutation is also one of the more common genetic changes in gliomas. Typically, its occurrence is around 7% in children and 4% in adults. This variant has been found in 6 to 15% of pediatric high-grade gliomas, and its prevalence decreases with age (38). It should be mandatory to assess this mutation in children because it offers therapeutic possibilities.

In this case, the tumor could be an adult-type glioma. Molecular studies are recommended since *IDH1/2* and *BRAF V600E* are usually exclusive to adult gliomas unless they are noncanonical variants (39). In addition, analysis of other genes, such as *TERT*, *CDKN2A/B*, *ATRX*, and *TP53*, is important due to their impact on the prognosis and evolution of these tumors (17). We do not recommend the analysis of 1p/19q in most cases.

KIAA1549::BRAF fusion

The *KIAA1549::BRAF* fusion and/or amplification is a driver rearrangement for pilocytic astrocytomas (40). In this series, 12% of the tumors exhibited this alteration; of these, 80% showed histological characteristics of low-grade gliomas, and 60% met the 2021 WHO criteria for classic pilocytic astrocytoma. This finding is consistent with reports where this fusion was identified in 60% of low-grade gliomas and 75% of pilocytic astrocytomas (41). Sixty percent of the patients with the fusion showed no other alterations. This group had the longest survival in the entire series (67.6 months), representing the longest survival reported in this study. This aligns with studies demonstrating that the positive regulation of the MAPK pathway associated with the *KIAA1549::BRAF* fusion can lead to spontaneous arrest of some low-grade gliomas (42).

Other rearrangements associated with the *KIAA1549::BRAF* fusion protein have been described in the literature (41). In this case series, two patients showed, in addition to this fusion, other positive biomarkers; unlike patients with only the *KIAA1549::BRAF* fusion, these patients experienced shorter survival times. The presence of additional variants contributes to increased intra-tumor heterogeneity, which, as reported in the literature, is known to negatively affect the efficacy of therapeutic measures (43).

In this case series, the frequency of fusion in histological pilocytic astrocytomas was 27%, similar to what was reported in the Moroccan series (44). This variability can be attributed to factors such as the number of patients, tumor heterogeneity, or differences in morphological characterization.

The design of the probe used, together with the established cut-off point, should also be considered a possible cause of variation in the results (45). In particular, tumors with clonal lines that are not representative of the probe cut-off point were observed in this case series. Therefore, reevaluating and potentially lowering the cut-off point established in the literature for this test is advisable. In such cases, complementary studies with *break-apart* probes for *BRAF* and/or molecular biology techniques, such as reverse transcription-polymerase chain reaction (RT–PCR), are recommended to confirm or rule out the presence of this rearrangement. We strongly recommend analyzing *KIIA1549::BRAF* in pediatric glioma.

CDKN2A

A case of an infratentorial glioma classified as pilocytic astrocytoma, presenting a *KIAA1549::BRAF* fusion and a homozygous deletion of *CDKN2A*, was reported in a school-aged boy. Survival was only one month after diagnosis. The patient had an ependymoma years before the pilocytic astrocytoma, which was successfully treated with radiotherapy. In this case, we consider that this tumor could be a radiation-induced second tumor (46).

The deletion of *CDKN2A* has been associated with poor prognosis in patients with secondary gliomas (9). In primary low-grade tumors, a statistically significant relationship between this deletion and overall survival has not been found (47). This finding could indicate that *CDKN2A* deletion does not act as an independent factor for poor prognosis (48).

This case may indicate a high-grade astrocytoma with piloid characteristics according to the 2021 WHO criteria. It would be advisable to verify the deletion of *CDKN2A* using molecular

biology techniques. Furthermore, it would be relevant to analyze deletions of *CDKN2B*, amplifications of *CDK4*, or variations in *ATRX* (17). Finally, methylation profile analysis is required to confirm high-grade astrocytomas with piloid characteristics. We would recommend implementing this marker in the panel and using it in secondary gliomas, which are increasingly common.

EGFR

A case of malignant astrocytoma grade IV was identified in a school-aged child. The tumor tested positive for *BRAF V600E* in immunostaining, and a *KIAA1549::BRAF* fusion was detected. Two clonal lines were observed: one with a heterozygous deletion of *EGFR* and another with a homozygous deletion of the same gene (12% and 15% of the cells studied, respectively).

The *KIAA1549::BRAF* fusion is rare in high-grade gliomas. Antonelli *et al*. (49) associated this finding with longer-than-expected survival. The observed alterations compromise chromosome 7, which is frequently seen in adult-type gliomas (50). Giunti *et al*. (51) analyzed the complete genome of pediatric high-grade gliomas and reported that genomic imbalance was associated with tumor progression. In this series, one patient presented alterations similar to those described by these researchers. These findings suggest that this glioblastoma could be a high-grade adult-type glioma. Therefore, as mentioned before, studying *EGFR*, *IDH1/2*, and alterations in *ATRX* and *TP53* is recommended (52).

The small size of the series and the limited choice of markers do not allow for inferring a comprehensive pattern of genetic damage. However, we believe that, in general, these results show a similar pattern to what has been reported worldwide, in the sense that most pediatric gliomas are low-grade tumors, with pilocytic astrocytoma being the most common, with the *KIAA1549::BRAF* alteration being the most frequent genetic change. Additionally, we believe that finding alterations typical of adult gliomas requires evaluating the most important adult glioma markers in pediatric panels, such as *IDH1/2*, *ATRX*, *EGFR*, *TP53*, and *CDKN2A*.

Finally, when analyzing some individual cases from this series, we can explain certain clinical facts based on the results. We recognize that our hypothesis about particular cases can only be verified through molecular analysis.

Limitations of the study

It is important to acknowledge several limitations of this study. First, patient recruitment was limited to a single institution, which may affect the generalizability of our conclusions and findings. The selection of cases based on the availability of archival tumor material could have introduced selection bias, and confounding factors related to tumor heterogeneity and pre-analytical tissue processing could not be fully controlled.

These limitations are inherent to studies on solid tumors, and paraffin-embedded tissue should also be considered when interpreting the results. The small sample size limits the generalizability of our conclusions, and variability in the quality of paraffin wax created challenges during the processing of certain samples.

Data validation remains a significant challenge due to the lack of comprehensive, integrated regional databases and standardized molecular characterization for these tumors. Although initiatives such as the Latin American and Caribbean Biobank Network (REBLAC) and various national institutions like the Mexican *Instituto Nacional de Cancerología* and the *Banco Nacional de Tumores Terry Fox* in Colombia exist, their fragmented nature hinders effective interregional validation and direct comparison of results. Consequently, our findings must be interpreted within these limitations, highlighting the urgent need for collaborative efforts to strengthen and standardize regional tumor registries and biobanks.

Based on these findings, future research on pediatric gliomas in Colombia should adopt an integrated approach that combines clinical, molecular, and advanced imaging data, moving beyond simply using techniques like next-generation sequencing and methylation profiling. Prospective, multicenter studies with long-term follow-up are essential to strengthen the links between biomarkers and outcomes, and to identify subgroups most likely to benefit from targeted therapies.

Another promising direction is the detailed characterization of pediatric tumors that exhibit markers typical of adult gliomas, which could improve our understanding of their biology and guide personalized treatments. Lastly, establishing a national registry that consolidates epidemiological, clinical, and molecular data would support the development of cost-effective diagnostic strategies suitable for resource-limited settings.

Conclusion

The combination of tissue microarrays, FISH, and immunohistochemistry is effective and inexpensive and could be used in the diagnosis of pediatric gliomas. However, careful selection of the markers to analyze is essential.

In this series, low-grade gliomas were the most common, with the *KIAA1549::BRAF* fusion being the predominant alteration. This finding emphasizes the importance of analyzing this fusion in pediatric pathology. Some tumors exhibited adult-type tumor markers, highlighting the need to include routine analysis of markers such as *IDH1/2*, *ATRX*, *TERT*, *CDKN2*, and *TP53* in children. Although frequent midline involvement was observed clinically and radiologically, alteration of H3.1 was rare, likely because only this isoform was evaluated. We recommend a comprehensive analysis of all isoforms.

These findings underscore the need for a comprehensive evaluation that combines morphological and molecular techniques. Furthermore, this approach paves the way for developing targeted therapies and identifying new prognostic biomarkers in our country.

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