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Original Article

Poor diagnostic value of isocitrate dehydrogenase 1 R132H immunohistochemistry for determination of isocitrate dehydrogenase 1 status in patients with glioblastoma

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ABSTRACT

Background: The World Health Organization (WHO) classification of central nervous system (CNS) tumors is a major advance toward improving the diagnosis of adult brain tumors. Despite the promise of isocitrate dehydrogenase (IDH) mutations as an important biomarker for glioblastoma, not all institutions have ready access to mutation detection polymerase chain reaction (PCR) methods, and deoxyribonucleic acid (DNA) sequencing may be problematic in very small biopsies. However, a simultaneous evaluation of IDH1 status by DNA sequencing and immunohistochemistry (IHC) to determine the sensitivity and specificity of both methods, along with their predictive value, was unavailable.

Methods: This retrospective study included 33 patients who underwent surgical resection or biopsy, January 2016–December 2019. The diagnosis of glioblastoma was established. Surgically resected tumor tissues were fixated in 10%-formaldehyde preserved in paraffin-embedded blocks. Glioblastoma was classified according to the 2021 WHO classification of CNS tumors. The enrolled patients were followed up to obtain the overall survival rate (median follow-up time, 30 months).

Results: Thirty-three patients (14 male; 19 female), mean age of 44.74 ± 15.49 years. Eight had WHO Grade II, 2 with WHO Grade III, and 23 with WHO Grade IV. The sensitivity and specificity of IDH1 IHC were 81.82% (P = 0.0007), a positive predictive value of 90.00% (69.90-98.22%), and a negative predictive value of 69.23% (42.37-87.32%). The survival rate was significantly higher in IDH1 mutant than wild-type IDH1, whether based on IHC or PCR (P = 0.0014).

Conclusion: IDH1 status evaluation is crucial to predicting the survival rate and important for guiding the treatment decision for patients with glioblastoma. Despite the lesser sensitivity and specificity of IHC in comparison to DNA sequencing in this study, larger prospective studies are needed to validate our preliminary finding.

Keywords: Central nervous system tumor, Glioblastoma, Immunohistochemistry, Isocitrate dehydrogenase 1 IDH1 R132H, Polymerase chain reaction

INTRODUCTION

The new version of the World Health Organization (WHO) classification of central nervous system (CNS) tumors is a significant progress in improving the identification of adult brain tumors.[2] Previously, glioblastoma was identified based on histologic observations of microvascular proliferation and/or necrosis and contained both isocitrate dehydrogenase (IDH)-

mutated (10%) and IDH wild-type (90%) tumors with substantially different biological features and prognoses. In the most recent modification, glioblastoma will include solely IDH wild-type tumors.[2,23] This scheme encourages molecular testing for patients with glioblastoma. Despite the promise of IDH mutations as an important glioblastoma biomarker, not all sites have ready access to mutation detection methods, and deoxyribonucleic acid (DNA) extraction followed by sequencing may be problematic in very small biopsies.[16]

Wild-type IDH1 is an important metabolic enzymes that catalyze the oxidative decarboxylation of isocitrate to generate α-ketoglutarate (αKG) and carbon dioxide that play a role in malignancy. The common function of IDH1 active-site mutation is a neomorphic enzyme activity catalyzes the conversion of αKG to D-2-hydroxyglutarate (D2HG). Under physiological conditions, cellular D2HG accumulation is limited due to the actions of the endogenous D2HG dehydrogenase, which catalyzes the conversion of D2HG to αKG. However, the neomorphic activity of mutant IDH causes D2HG to accumulate to supraphysiological levels within cells. Elevated D2HG concentrations can be detected in the serum of patients with IDH-mutant gliomas in patients.[5]

IDH1 immunohistochemistry (IHC) can detect cancer cells with mutations by utilizing an antibody specific to the prevalent R132H mutant variant of IDH1.[3,6,9,15] The IHC method is a viable and less labor-intensive method for detecting IDH1 mutations; nevertheless, its sensitivity and specificity have not been tested by simultaneous sequencing and validation on clinically annotated samples. A recent study found that IDH1 mutation status in glioblastoma patients could be useful diagnostic and prognostic tools, as well as predict the response of glioblastoma management.[17] Here, we performed a simultaneous evaluation of IDH1 status in glioblastoma using both polymerase chain reaction (PCR) and IHC to determine the sensitivity and specificity of both methods. We chose IHC alongside PCR since, as a gold standard, somehow PCR is not always available in resource-limited settings such as IHC.

MATERIALS AND METHODS

Subjects

The Committee of Ethics of the Faculty of Medicine of Padjadjaran University provided ethical approval. This retrospective study comprised 33 patients who had surgical resection or biopsy from January 2016 to December 2019, and the diagnosis of primary glioblastoma was confirmed. Tissues from surgically excised tumors were fixed in 10% formaldehyde and implanted in paraffin blocks. Glioblastoma was classified and graded based on the 2021 WHO classification of CNS cancers.[2] All individuals were treated for gliomas using standard procedures. The recruited patients were followed up to determine their overall survival (OS) rate (median follow-up time: 30 months).

IDH1 IHC

Slides of tumor tissue with a thickness of four microns were deparaffinized and rehydrated. Antigen retrieval was carried out using a decloaking chamber (DC2008INTL; Biocare Medical, Pacheco, CA, USA) at 100°C for 20 min using an antigen retrieval solution (Tris ethylenediaminetetraacetic acid 10 mmol/L, pH 9.0). After cooling at room temperature, sections were washed 2× with phosphate-buffered saline (PBS) for 5 min each. Endogenous peroxidase activity was stopped by dipping sections in 3% hydrogen peroxide blocker (Boster Biological Technology, Pleasanton, CA, USA) for 10 min and rinsed in three changes of PBS. Following the initial processing step, sections were incubated at room temperature with primary antibodies anti-human IDH1 R132H mutant specific AB (GTX57185 Genetex, IHC 132, mouse monoclonal AB, at 1:200 dilution), followed by 30 min of incubation with the poly horseradish peroxidase (HRP) non-biotin detection system. Finally, the sections were counterstained with hematoxylin and eosin, then dehydrated and mounted. Positive results revealed strong cytoplasmic exclusively in tumor cells, while negative controls were produced concurrently for all 33 samples by replacing the primary AB with distilled water.[6,12] IDH1 status was determined by a pathologist independent of the PCR result.

Statistical analysis

We used GraphPad Prism v8.0 for the statistical analysis. $P < 0.05$ was considered as statistically significant.

RESULTS

Subjects characteristic

This study included 33 patients (14 male and 19 female), with a mean age of 44.74 ± 15.49 years old. According to the 2021 WHO categorization with PCR examination, the participants included eight patients with Grade II, two patients with Grade III, and 23 patients with Grade IV. Table 1 shows a summary of the subjects' characteristics.

The reliability of IHC

Direct DNA sequencing validated 23 IDH-wild type and 10 IDH1 gene mutant samples [Figure 1]. Nine samples had mutations in the IDH1 R132H site and one in the IDH1 R132G location. While in the IHC analysis, there were 13 samples of IDH1 R132H mutant and 20 samples of IDH-wild type [Figure 2]. Six samples of IHC results do not match those from DNA sequencing. Therefore, we discovered that

the sensitivity and specificity of IDH1 IHC were 81.82% (P = 0.0007), with a positive predictive value of 90.00% (69.90–98.22%) and a negative predictive value of 69.23% (42.37–87.32%), as shown in Table 2.

Survival rate

In this study, we discovered that patients with IDH1 mutants had a greater survival rate than patients with wild-type IDH1, whether using IHC or PCR [Figure 3], (P = 0.0014). There was no statistical difference in the survival rate between IHC and PCR-confirmed IDH1 mutant [Table 3], (P > 0.05).

DISCUSSION

Malignant gliomas are fundamentally a genetic disease that shares characteristics with nearly all human cancers.[13,14] They are derived from astrocyte-like neural stem cells that contain oncogenic alterations in the subventricular zone.[12] The genetic changes that cause gliomagenesis have been the topic of much research.[19] Notably, homozygous deletion or mutation of tumor suppressor genes cyclin-dependent kinase inhibitor (CDKN2A/B); phosphatase and tensin homolog (PTEN), loss of heterozygosity of chromosome 10q, and oncogenic amplification of epidermal growth factor receptor (EGFR) are identified in primary *de novo* glioblastoma, while mutations in the TP53 gene are typically detected in secondary glioblastoma.[7,11] However, the IDH1 gene is the most investigated in gliomas, as it is more prevalent in low-grade gliomas and is associated with a better prognosis. Thus, molecular detection of IDH1 has been added as a diagnostic criteria for glioblastoma in the most recent WHO CNS tumor classification.[2,21]

The assessment of IDH1 status is critical for diagnosis and developing an effective treatment strategy. This can be performed either through DNA sequencing or IHC.[2,21,22] IDH1 mutations were identified using PCR and direct sequencing of amplified complementary DNA isolated from fresh frozen or formalin-fixed paraffin-embedded tissue. IHC is commonly conducted as part of the histological investigation.[26] The use of a particular antibody that binds the mutant IDH1-R132H proteins enables the identification of mutant tumor cells by IHC in most situations, and this technology has a sensitivity that may exceed normal sequencing.[1]

In a study comparing IHC to genetic testing, Sporikova *et al*., discovered that IHC was 100% sensitive and specific for detecting IDH1-R132H mutations, demonstrating that anti-IDH1-R132H immunostaining is a trustworthy technique for

Table 1: Subject's Characteristics.

Characteristics	Value
Age (Mean ± Standard deviation)	44.74±15.49
Sex	
Male	14
Female	19
WHO Grade (2021)	
Grade II	8
Grade III	2
Grade IV	23

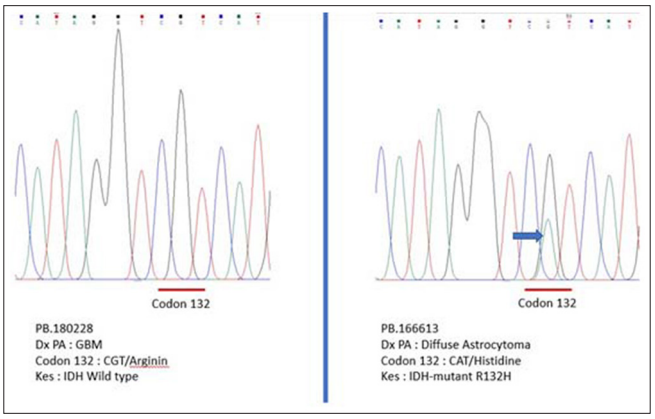

Figure 1: Sequencing results of IDH1 mutations and wild-type samples; (left) wild-type and (right) IDH1 R132H. IDH1: Isocitrate dehydrogenase 1. (Right, in blue arrow): IDH1 mutation at R132H

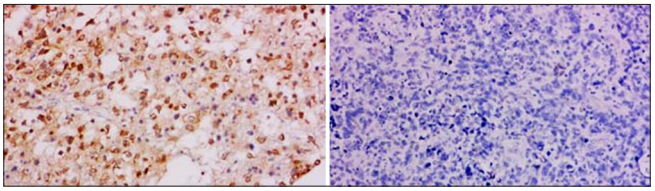

Figure 2: IHC results of IDH R132H mutation; (left) positive and (right) negative. IHC: Immunohistochemistry, IDH1: Isocitrate dehydrogenase 1. Magnification: 100x

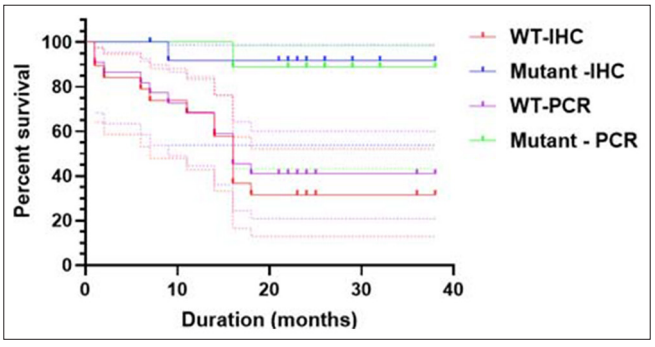

Figure 3: The survival rate of patients with IDH1 status. Patients with IDH1 mutant have a higher survival rate than patients with gliomas with wild-type IDH1 status, whether based on the immunohistochemistry or PCR (Log rank mantle cox, P = 0.0014). IDH1: Isocitrate dehydrogenase 1, PCR: Polymerase chain reaction. WT: Wild type, WT-IHC :Wild type-immunohistochemistry.

Table 2: Result of IDH1 mutation by IHC analysis and direct sequencing method (*n*=33).

	WT-PCR	Mutant-PCR	*P*	Sensitivity (95% Confidence interval)	Specificity (95% Confidence interval)	PPV (95% Confidence interval)	NPV (95% Confidence interval)
WT-IHC	18	2	0.0007	81.82% (61.48-92.69%)	81.82% (52.30-96.77%)	90.00% (69.90-98.22%)	69.23% (42.37-87.32%)
Mutant-IHC	4	9					

IDH: Isocitrate dehydrogenase, PCR: Polymerase chain reaction, PPV: Positive predictive value.,NPV: Negative predictive value, IHC: Immunohistochemistry, WT: Wild type

Table 3: Comparison of survival rate at 24 months between immunohistochemistry and PCR-confirmed IDH1 mutant.

24 months	WT-IHC	WT-PCR	*p*	OR (95% Confidence interval)
Died	13	13	0.5362	1.50 (0.42-4.88)
Survived	6	9		
24 months	**Mutant IHC**	**Mutant-PCR**	***p***	**OR**
Died	1	1	0.8456	0.75 (0.04 – 15.70)
Survived	12	9		

IDH: Isocitrate dehydrogenase, PCR: Polymerase chain reaction, IHC: Immunohistochemistry, WT: Wild type, OR: Odds ratio

assessing the status of IDH1 gene mutations.[24] Furthermore, one study found that the concordance rate between IHC and sequencing ranged from 88% to 99%. In five of eight trials, the number of mutations discovered by IHC exceeded that detected by sequencing. This can be explained by the fact that IHC can identify the mutation even if there is just a limited number of IDH1-R132H mutation-positive tumor cells in the sample.[1] However, in our cohort, IHC identification of the IDH1-R132H mutation failed in 6 out of 33 samples (18.2%), with a sensitivity and specificity of 81.82% ($P = 0.0007$). This failure could be caused by laboratory errors or poor tissue processing. As a result, the molecular-histological definition of gliomas necessitates the practical application of IDH1 genomic sequencing.

Either based on IHC or PCR, we discovered that the survival rate was significantly higher ($P = 0.0014$) in patients with IDH1 mutant than in patients with IDH1 wild-type [Figure 3]. Furthermore, these findings are consistent with those of Howard *et al*., who reported that the presence of IDH1 R132H mutation in tumor tissue serves as a positive prognostic factor for glioblastoma patients in terms of progression-free survival (PFS) and OS.[8] Similarly, Tabei *et al*., and Tateishi and Yamamoto showed that IDH1 mutant glioblastoma patients had a threefold longer survival rate than those with IDH1 wild type.[25,27] Iurlaro *et al*., and Wen and Packer observed an increased median OS in IDH mutant patients. Those with IDH-mutated disease had a median OS of 65 months, whereas patients with IDH wild-type disease had a median OS of 20 months.[10,28] In addition, Iurlaro *et al*., and Chen *et al*., reported that the PFS of patients with IDH mutant glioblastoma was improved.[4,10] In contrast, Zou *et al*., found no statistically significant difference in OS based on IDH1 mutation status.[29]

In our cohort, we found no difference in survival rates between the IHC and sequencing-detected IDH1 mutants; this impact could be related to the small number of samples. Despite its reduced sensitivity and specificity compared to DNA sequencing, IHC is beneficial for detecting the IDH1 R132H mutation, is less expensive, and is more easily available in countries with limited resources. However, the correct identification of mutation status in diffuse gliomas is crucial for identifying appropriate tailored therapies and adhering to the latest 2021 WHO system, which is based on the presence of validated biomarkers, including IDH mutations.

IHC is an affordable and simple procedure that can be performed with few resources, a powerful technique to study localization and presence/absence of a target at the tissue and cellular level, paraffin embedded and frozen tissue samples can be stored and accessed when required, and stained tissue sections can be stored and referred to whenever required. However, the specificity of antibodies can be variable and needs to be thoroughly checked using appropriate controls. The method is semi-quantitative, and the absolute abundance of the target cannot be reliably determined; the tissue is highly processed and may lead to a loss of information about the natural state. IHC is a multi-step procedure, and variability can be introduced at any stage, leading to poor reproducibility of results.[20] Somehow, in countries with limited resources, allocating the available resources to patient care is a crucial component of healthcare policy. Correct diagnosis tools could improve the quality of life of a patient and increase cost-effectiveness.[18]

Study limitation

Limitations of this study should be considered, such as the small sample size and lack of control for confounders, may be issued to the retrospective design study.

CONCLUSION

Assessing IDH1 status is critical for predicting survival rates and directing treatment decisions for patients with gliomas. Despite the lesser sensitivity and specificity of IHC in comparison to DNA sequencing in this study, larger prospective studies are needed to validate our preliminary finding.

Ethical approval: The research/study was approved by the Institutional Review Board at the Faculty of Medicine, Universitas Padjadjaran, Bandung, number 34/UN6.KEP/EC/2022, dated November 20, 2022.
Declaration of patient consent: The authors certify that they have obtained all appropriate patient consent.
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