

# A Simple Panel of IDH1 and P53 in Differential Diagnosis Between Low-Grade Astrocytoma and Reactive Gliosis

Bitá Geramizadeh<sup>1,2</sup>, Mahsa Kohandel-Shirazi<sup>1</sup> and Ahmad Soltani<sup>3</sup>

<sup>1</sup>Department of Pathology, Medical School of Shiraz University, Shiraz University of Medical Sciences, Shiraz, Iran. <sup>2</sup>Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran. <sup>3</sup>Department of Neurosurgery, Shiraz University of Medical Sciences, Shiraz, Iran.

Clinical Pathology  
Volume 14: 1–4  
© The Author(s) 2021  
Article reuse guidelines:  
sagepub.com/journals-permissions  
DOI: 10.1177/2632010X20986168



## ABSTRACT

**BACKGROUND:** Reactive gliosis is a response of glial tissue to different types of injury such as brain abscess, trauma, hemorrhage, or even neoplastic process. In some circumstances, especially when the tissue biopsy is small, there may be difficulty to discriminate this reactive condition with low-grade diffuse astrocytoma (World Health Organization [WHO] grade II) by conventional hematoxylin and eosin (H&E) slides, so some immunohistochemical and molecular markers have been introduced for this differential diagnosis. One of the important aspects of updated WHO classification in 2016 has been dividing some of the glial tumor according to IDH1 (isocitrate dehydrogenase 1) mutation.

**OBJECTIVES:** In this study, we tried to evaluate IDH1 and P53 mutation by immunohistochemistry as a simple and highly specific and sensitive method to differentiate low-grade astrocytoma and reactive gliosis.

**MATERIAL AND METHODS:** For 5 years (2013-2018), 50 cases of clinically documented reactive gliosis and 50 cases of low-grade astrocytoma were evaluated for the presence or absence of IDH1 and P53 mutation by immunohistochemistry.

**RESULTS:** Isocitrate dehydrogenase 1 was positive in 92% and 4% of the astrocytoma and reactive gliosis cases and P53 was positive in 90% and 4% of the cases with the final diagnosis of astrocytoma and reactive gliosis, respectively.

**DISCUSSION AND CONCLUSION:** Combination of P53 and IDH1 as an immunohistochemical panel showed specificity of 96% and sensitivity of 91% for differential diagnosis of reactive gliosis and low-grade astrocytoma. These 2 markers can be extremely helpful for this differential diagnosis.

**KEYWORDS:** Reactive gliosis, low-grade astrocytoma, IDH1, P53

**RECEIVED:** February 10, 2020. **ACCEPTED:** December 9, 2020.

**TYPE:** Focus Section: Precision Medicine-Brief Report

**FUNDING:** The author(s) received no financial support for the research, authorship, and/or publication of this article: They received financial support from the deputy of research in Shiraz University of Medical Sciences.

**DECLARATION OF CONFLICTING INTERESTS:** The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**CORRESPONDING AUTHOR:** Bitá Geramizadeh, Department of Pathology, Medical School of Shiraz University, Shiraz University of Medical Sciences, Shiraz, Iran. PO BOX: 71359  
Email: geramib@gmail.com

## Introduction

Histologic differentiation of diffuse astrocytoma and reactive gliosis can be particularly challenging because histologic features of reactive glial proliferations show significant overlap with diffuse astrocytoma especially in small biopsies with limited tissue. Diseases such as stroke, central nervous system (CNS) inflammation, demyelination, or vasculitis cause reactive gliosis, ie, astrocytes increase in size and shape and show cellular atypia.<sup>1</sup> P53 mutation can be a good marker for differential diagnosis of reactive gliosis from low-grade astrocytoma; however, some controversies are still present and, in some studies, P53 mutation has been reported in reactive gliosis as well as in low-grade astrocytoma.<sup>2</sup> Recently, isocitrate dehydrogenase (IDH) has been introduced to be mutated in low-grade gliomas. The first introduction of IDH mutation has been reported by Parsons et al in 2008 in 12% of brain tumors with the diagnosis of glioblastoma multiforme. In that study,

the authors showed that all of these cases with IDH mutation also harbor P53 mutation.<sup>3</sup>

Isocitrate dehydrogenase is an enzyme with 2 isoforms (1 and 2). This enzyme catalyzes the conversion of isocitrate to ketoglutarate while reducing nicotinamide adenine dinucleotide phosphate (NADP<sup>+</sup>). Isocitrate dehydrogenase enzyme isoform 1 (IDH1) is located in peroxisomes, and the isoform 2 (IDH2) is present in mitochondria.<sup>4</sup> Majority of IDH1 mutations occur in exon 4 at codon 132, where a transition changes a single amino acid from arginine to histidine (R132H).<sup>5</sup> It has been found in the glial cells especially astrocytoma World Health Organization (WHO) grade II with a remarkably high frequency (around 75%), and IDH mutation has become definitional for infiltrating gliomas in adults.<sup>4,6</sup>

In this study, we are trying to evaluate the role of IDH1 mutation in combination with P53 mutation by immunohistochemistry (IHC) method to differentiate reactive gliosis from



low-grade astrocytoma (grade II). Our goal is to emphasize the benefits of this panel especially when there are limited resources of tests and inadequate tissue for evaluation.

### Patients and Methods

For 5 years (2013-2018), all the cases with definite final diagnosis of low-grade diffuse fibrillary astrocytoma (grade II) and reactive gliosis were retrieved from the archives of pathology department of affiliated hospitals of Shiraz University of Medical Sciences. During these 5 years, there were 50 cases in each group of low-grade fibrillary astrocytoma and reactive gliosis.

It is worthy to note that there were 67 cases in gliosis group and 72 cases in low-grade glioma group during this period of time; however, these 50 cases were selected because they have been documented and the final diagnosis has been proved by further surgery or other laboratory or imaging tests. The cases with low-grade astrocytoma have been proved after surgical removal of the tumor, and the cases with reactive gliosis have been documented with nontumoral lesions such as temporal sclerosis, infections, and brain abscess. Table 1 shows the underlying disease in these 50 documented cases.

The slides were reviewed and the best representative block was selected for IHC with mouse monoclonal anti-R132H-IDH1 antibody (H09, Dianova, dilution 1:50, citrate-EDTA for antigen retrieval) and P53 antibody (Dako, clone DO-7, dilution 1:100, citrate-EDTA [Ethylenediamine tetraacetic acid] for antigen retrieval). Cytoplasmic positivity for IDH1<sup>1,5</sup> and nuclear positivity for P53<sup>2</sup> were considered positive. We considered every positive case as positive and every negative P53 as negative.<sup>2</sup> The immunohistochemical positivity for IDH1 was considered strong with 3+ positivity and weak with 1-2+ positivity.<sup>5,7</sup> All the cases have been blindly examined by an expert neuropathologist and a general pathologist together. It means that there has been no information about the final diagnosis when the IHC slides were being reviewed by the pathologist.

### Results

For reactive gliosis, 50 cases were studied (mean age 30 years, ranging from 5 to 61 years). There were only 2 cases with weak staining for IDH1, both of which were negative for P53; 48 cases of reactive gliosis were P53 negative, and only 2 cases were reactive with P53. Both of these positive P53 cases were negative for IDH1. (Table 2) Therefore, in each marker (IDH1 and P53), 48 out of 50 cases were negative (96%; Figure 1A and B).

There were 50 cases with the final diagnosis of low-grade fibrillary astrocytoma (mean age 35 years, ranging from 5 months to 75 years), 14 (28%) cases showed weak and 32 (64%) cases showed strong staining for IDH1. It means that 46 out of 50 cases of low-grade astrocytoma were reactive with IDH1 (92%); 45 cases of this category showed positive P53 (90%; Figure 2A and B).

Table 3 shows the sensitivity and specificity of IDH1 and P53 alone and together.

### Discussion

Reactive gliosis is a nonspecific benign and reactive change that occurs in response to different types of brain injury. Distinction of this condition (reactive gliosis) from low-grade astrocytoma can be difficult especially in small biopsies when adequate tissue is not available.<sup>7,8</sup> There are some histologic characteristics that can help such as presence of uniformly distributed astrocytes with regular spacing and abundant processing in reactive gliosis versus presence of less uniformly distributed, and cell clusters of astrocytes with more variable degrees of perinuclear cytoplasm and satellitosis in low-grade astrocytes.<sup>8</sup> Although the above-mentioned histologic criteria can be helpful, however, in routine practice, there are many circumstances, in which ancillary studies are necessary for definite distinction of these 2 conditions.<sup>9</sup> Immunohistochemistry for GFAP (glial fibrillary acidic protein), and Ki-67 have been used in some studies to identify evenly distributed process in reactive astrocytes with low proliferative rate without atypia. However, both of these are nonspecific and cannot definitely discriminate reactive gliosis from low-grade diffuse fibrillary astrocytoma.<sup>10</sup>

Immunohistochemistry for P53 mutation is a marker which is seen in diffuse astrocytoma and can be useful for the differential diagnosis.<sup>11</sup> In our study, we detected P53 mutation in

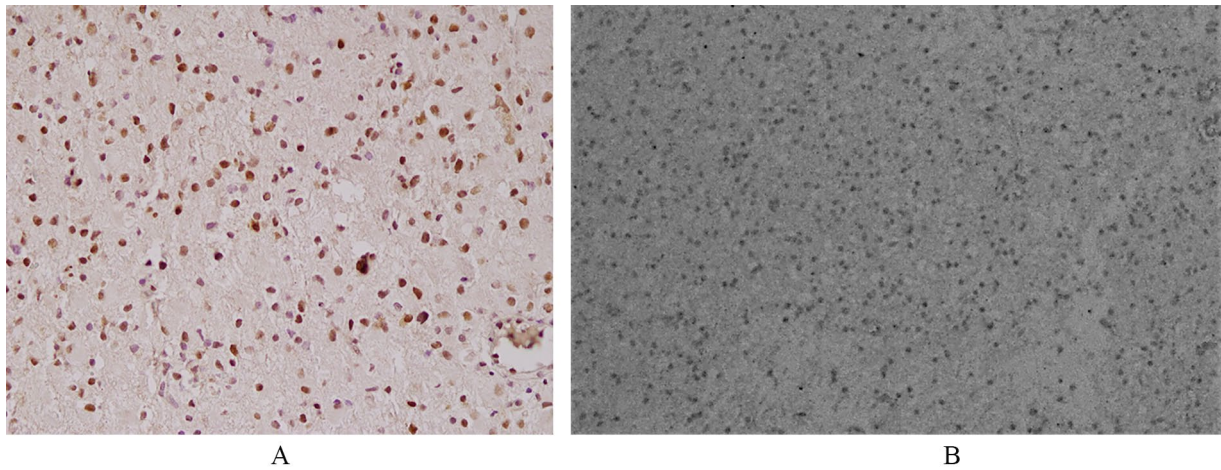
**Table 1.** Underlying diseases in 50 cases of reactive gliosis.

UNDERLYING DISEASES	NUMBER OF CASES
Temporal sclerosis	4
Infection	32
Brain abscess	11
Trauma	3
Total	50

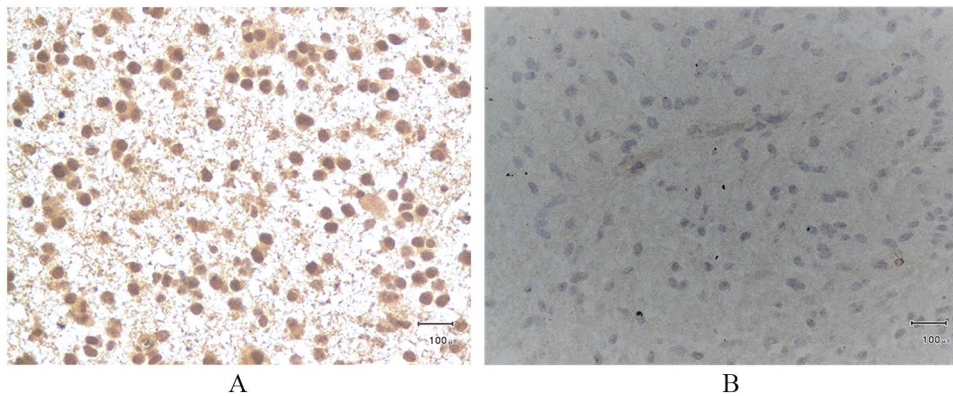
**Table 2.** Immunohistochemical results of IDH1 (R132H) and P53 in reactive gliosis vs diffuse astrocytoma.

FINAL DIAGNOSIS	NUMBER OF CASES	POSITIVE IDH1 (WEAK)	POSITIVE IDH1 (STRONG)	POSITIVE P53
Reactive gliosis	50	2 (4%)	0	2 (4%)
Astrocytoma grade II	50	14 (28%)	32 (64%)	45 (90%)
Total	100	16 (16%)	32 (32%)	47 (47%)

Abbreviation: IDH1, isocitrate dehydrogenase 1.



**Figure 1.** (A) Nuclear positivity of P53 in a case of low-grade astrocytoma. (B) Negative P53 (wildtype).



**Figure 2.** (A) Positive IDH1 in the cytoplasm of astrocytes in a case of low-grade astrocytoma. (B) Negative IDH1. IDH1 indicates isocitrate dehydrogenase 1.

**Table 3.** Sensitivity and specificity of IDH1 and P53 and their combination in the diagnosis of low-grade astrocytoma (grade II) vs low-grade glioma.

PARAMETER	IDH1	P53	IDH1 + P53
Specificity	96%	96%	96%
Sensitivity	90%	90%	91%

Abbreviation: IDH1, isocitrate dehydrogenase 1.

90% of the cases with the diagnosis of low-grade astrocytoma and 4% of the reactive astrocytosis.

Another genetic change common in low-grade astrocytomas is IDH1 gene mutations which have not been reported in reactive gliosis, so it seems specific for astrocytoma. Majority of IDH1 mutations involve substitution of arginine by histidine at codon 132 (R132H). This amino acid change can be detected by IHC with a monoclonal antibody.<sup>12,13</sup> According to 2016 WHO classification, each grade II or III diffuse astrocytoma is now classified as IDH-mutant, IDH-wildtype, and NOS (not otherwise specified) categories. If IHC for mutant R132H IDH1 protein and sequencing for IDH1 codon 132

and IDH2 codon 172 gene mutations are both negative, or if sequencing for IDH1 codon 132 and IDH2 codon 172 gene mutations alone is negative, then the lesion can be diagnosed as IDH wildtype. It is important to note that diffuse astrocytoma, IDH wildtype, is an uncommon diagnosis and that such cases need to be carefully evaluated to avoid misdiagnosis of lower grade lesions.<sup>12,13</sup> The study by Camelo-Piragua et al<sup>10</sup> showed that the combination of P53 and mutant IDH1 by IHC provides a sensitivity of 71.4% which is significantly higher than either test alone (47.8%). IDH1 mutation has been reported in 64.6% and 45.4% of low- and high-grade gliomas of adult patients.<sup>11</sup> There are controversial reports in the literature and there are reports indicating that in the cases with suspicion to low-grade glioma, IDH1 IHC testing in nondiagnostic biopsies can be false negative because of sampling error when there is scant tissue present.<sup>14</sup> There are also reports indicating that IDH1 can be a useful marker for small biopsy specimens.<sup>12</sup>

In our experience in this study, more than 90% of low-grade astrocytomas have shown mutant IDH1 (R132H) and the IHC for IDH1 was positive; this marker was only positive in 4% of reactive cases.

TP53 is considered as one of the most frequent mutated genes in cancers, such as astrocytoma. According to IHC and protein expression, more tumors express P53 immunoreactivity in the absence of TP53 mutations.<sup>2</sup> In our cases, all of the negative IDH1 cases of astrocytoma were positive for P53 and vice versa. Cases with reactive gliosis which were positive for IDH1 showed weak IDH1 staining and also their P53 was negative. Therefore, an immunohistochemical panel of IDH1 and P53 can be helpful for differential diagnosis of glioma vs gliosis.

Isocitrate dehydrogenase 1 can be a helpful immunohistochemical marker not only for prognostic evaluation of glial tumors. Isocitrate dehydrogenase gene mutation assessment has been reported as highly specific marker for low-grade diffuse glioma and is recommended as an additional test not only for classification and prognosis<sup>16</sup> but also for differentiating of these tumors from their mimics. P53 has also introduced as a biomarker for classification in 2016, which can be helpful in differential diagnosis of glial tumors and nonneoplastic process in the brain.<sup>15</sup>

Using IDH1 and P53 as a panel for differential diagnosis of glioma and gliosis have been rarely used,<sup>12</sup> and majority of the previous studies have used each of them not together. Also there has been no study from Iran so far to confirm their utilization when resources are limited.<sup>8-17</sup>

This panel is a simple immunohistochemical panel which is feasible in the majority of surgical pathology laboratories, and also it does not need so much tissue and can be performed in small stereotactic biopsies.

The best practice is to separate pediatric patients from adult and to confirm the immunohistochemical results with molecular methods.<sup>3</sup> These 2 parts are the limitations of our study.

Another limitation in this study is about the equivocal cases, which have not been included in this study, because we were trying to test the immunohistochemical panel and find out the sensitivity and specificity of the panel, and so we just included the documented cases. We did not perform mutation analysis by molecular tests to know about the 2 false-positive cases which were not glioma.

## Conclusion

A simple immunohistochemical panel consisting of IDH1 and P53 can be extremely sensitive and also specific for discrimination of reactive gliosis from low-grade astrocytoma. We recommend routine using of these 2 immunohistochemical markers for the brain lesions with the impression of reactive gliosis vs low-grade glioma. It is especially useful when there is limited

sample for further studies and also where molecular studies are not feasible.

## Author Contributions

BG: Idea of the project, Evaluation of the pathology slides and writing the paper. MKS: Evaluation of the slides with BG and case analysis. AS: Surgery of the cases and helping to find the documented cases.

## ORCID iD

Bitra Geramizadeh  <https://orcid.org/0000-0003-1009-0049>

## REFERENCES

- Capper D, Sahm F, Hartmann C, Meyermann R, von Deimling A, Schittenhelm J. Application of mutant IDH1 antibody to differentiate diffuse glioma from nonneoplastic central nervous system lesions and therapy-induced changes. *Am J Surg Pathol*. 2010;34:1199-1204.
- Kurtkaya-Yapicier O, Scheithauer BW, Hebrink D, James CD. P53 in nonneoplastic central nervous system lesions: an immunohistochemical and genetic sequencing study. *Neurosurgery*. 2002;51:1246-54.
- Parsons W, Jones S, Zhang Z, et al. An integrated genomic analysis of human glioblastoma multiforme. *Science*. 2008;321:1807-1821.
- Balss J, Meyer J, Mueller W, Korshunov A, Hatmann C, von Deimling A. Analysis of the IDH1 codon 132 mutation in brain tumors. *Acta Neuropathol*. 2008;116:597-602.
- Ali A, Bell S, Bilsland A, et al. Investigating various thresholds as immunohistochemistry cutoffs for observer agreement. *Appl Immunohistochem Mol Morphol*. 2017;25:599-608.
- Horbinski C, Kofler J, Kelly LM, Murdoch GH, Marina N, Nikiforova MN. Diagnostic use of IDH1/2 mutation analysis in routine clinical testing of formalin-fixed, paraffin-embedded glioma tissues. *J Neuropathol Exp Neurol*. 2009;68:1319-1325.
- Lin F, Chen Z. Standardization of diagnostic immunohistochemistry literature review and geisinger experience. *Arch Pathol Lab Med*. 2014;138:1564-1577.
- Rivera-Zengotita M, Tachnis AT. Gliosis versus glioma? do not grade until you know. *Adv Anat Pathol*. 2012;19:239-249.
- Paulus W. GFAP, Ki67 and IDH1: perhaps the golden triad of glioma immunohistochemistry. *Acta Neuropathol*. 2009;118:603-604.
- Villaamil VM, Garcia AA, Gallego GA, et al. Tissue array analysis for the differentiation of gliosis from gliomas. *Mol Med Rep*. 2011;4:451-457.
- Yaziji H, Massarani-Wafai R, Gujrati M, Kuhns J, Martin A, Parker JC. Role of p53 immunohistochemistry in differentiating reactive gliosis from malignant astrocytic lesions. *Am J Surg Pathol*. 1996;20:1086-1090.
- Camelo-Piragua S, Jansen M, Ganguly A, et al. A sensitive and specific diagnostic panel to distinguish diffuse astrocytoma from astrocytosis: chromosome 7 gain with mutant isocitrate dehydrogenase 1 and p53. *J Neuropathol Exp Neurol*. 2011;70:110-115.
- Narasimhaiah D, Miquel C, Verhamme E, Desclee P, Cosnard G, Godfraind C. IDH1 mutation, a genetic alteration associated with adult gliomatosis cerebri. *Neuropathology*. 2012;32:30-37.
- Anderson MD, Abel T, Moots PL. Re-evaluation of nondiagnostic biopsies of suspected low-grade glioma using isocitrate dehydrogenase 1 mutation immunohistochemistry. *Neuro Oncol*. 2013;15:811-813.
- Choi J, Lee EY, Shin KJ, Minn YK, Kim J, Kim SH. IDH1 mutation analysis in low cellularity specimen: a limitation of diagnostic accuracy and a proposal for diagnostic procedure. *Pathol Res Pract*. 2013;209:284-290.
- Cahill DP, Sloan AE, Nahed BV, et al. The role of neuropathology in the management of patients with diffuse low-grade glioma. A systematic review and evidence-based clinical practice guideline. *J Neurooncol*. 2015;125:531-549.
- Capper D, Weibert S, Balss J, et al. Characterization of R132H mutation-specific IDH1 antibody binding in brain tumors. *Brain Pathol*. 2010;20:245-254.