Asian Journal of Medical Research Original Article

Role of IDH1 Immunostain In Differentiating Between Diffuse Astrocytoma and Reactive Astrocytosis

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Abstract

Background: The distinction of diffuse astrocytoma from astrocytosis is a major challenge in neuropathology. Reactive astrocytosis can occur in a variety of non neoplastic CNS lesions and can be seen in some CNS tumors. This can be particularly challenging with small biopsies considering the marked differences in prognosis and therapy after a pathologic diagnosis. Somatic IDH1 mutations are present in the vast majority of low-grade diffuse astrocytoma (WHO grade II). **Methods:** In the present study we investigated the specificity of anti-IDH1 immunostaining in differentiating diffuse low grade astrocytomas from reactive CNS lesions. **Results:** The study included 24 WHO grade II diffuse astrocytomas and 22 reactive conditions with astrocytosis (8 infarcts, 10 gliosis, and 4 traumatic brain injury). All cases were stained by IDH1 immunostain. Positive granular cytoplasmic staining of tumor cells for IDH1 was found in 13 out of 24 (54.2%) WHO grade II astrocytomas, but was entirely absent in all 22 reactive samples . Tumor cells in astrocytoma cases demonstrated staining both in the densely cellular areas of the tumor, as well as in the less cellular infiltrating tumor edges. While reactive astrocytes at the tumor edges were IDH1 negative. **Conclusion:** This study showed that IDH1 is a useful immunohistochemical marker to differentiate reactive gliosis from low-grade astrocytoma. IDH1 has potential as an independent marker to diagnose neoplastic conditions especially in small surgical samples with high specificity.

Keywords: IDH1, immunohistochemistery, glioma,gliosis

INTRODUCTION

Gliomas are the most common cancers of the nervous system. They are classified as grade I to grade IV on the basis of histopathologic and clinical criteria established by the World Health Organization (WHO). Gliomas comprise 3 common histologic subtypes: astrocytomas, oligodendrogliomas, and ependymomas, which are based on their morphological similarities to their normal cellular counterparts.[1]

A major challenge in the routine practice of surgical neuropathology is the distinction between reactive astrocytosis and a low-grade infiltrating diffuse astrocytoma [World Health Organization (WHO) grade II]. Reactive astrocytosis can occur in a variety of non neoplastic CNS lesions including stroke, hemorrhage, metastatsis, CNS inflammation, demyelination, or vasculitis. Also reactive astrocytes can be seen in some CNS tumors. This can be particularly challenging with small biopsies considering the marked differences in prognosis and therapy after a pathologic diagnosis. Diffuse astrocytomas are characterized by an extensive diffuse infiltration of the central nervous system (CNS). Histologic features of reactive glial proliferations as seen in many nonneoplastic overlap with those of diffusely infiltrating gliomas.[2]

As a response to pathologic changes reactive astrocytes typically increase in number and size and may display pleomorphic nuclei. Reactive astrocytes proliferate, sometimes reaching proliferation indices of up to 5% and may even display mitotic figures. Irradiated brain may also become highly gliotic and sometimes considerable radiation-induced atypia in nonneoplastic cells is observed. The differentiation of reactive glia from posttherapy glioma may be challenging in such a situation.[3]

The most commonly used markers to differentiate astrocytoma from astrocytosis are immunohistochemical stains for glial fibrillary acid protein (GFAP), proliferation markers (e.g. Ki-67) and p53. However, these markers do not differentiate between reactive and neoplastic glia cells, because they are also highly expressed in gliomas. GFAP is used to highlight astrocyte distribution, the architecture, and the type of processes involved. Astrocytosis should feature evenly spaced astrocytes with multiple, thin, long, radiating glial processes. In contrast, diffuse astrocytoma involves clustered astrocytic cells with shorter, thicker processes. However, these stains are not always diagnostic because reactive astrocytosis is often seen at the infiltrating edges of astrocytomas.[4]

Previous studies of Ki67 proliferation index value in glioma and gliosis proved that the rates of astrocytic proliferation in reactive conditions are similar to those seen in low-grade glioma and concluded that this marker is not reliable for definitive diagnosis.[5] Immunohistochemical staining of p53 is the most widely used marker in neuropathology practice to differentiate reactive and neoplastic glial cells. However, p53 is not a sensitive stain because it is expressed in a subset of reactive astrocytes and is only detected at moderate frequency in low-grade gliomas.[6] New molecular markers have emerged in recent decades and have been shown to be useful as predictive, prognostic, and, in some cases, diagnostic markers.[7]

The first mutations discovered in the genes encoding isocitrate dehydrogenases (IDHs; including IDH1 and IDH2) were identified in metastatic colon cancer.[8] In 2008, the genes encoding IDH1, and to a lesser extent IDH2, were found to be mutated in low-grade gliomas and a subset of secondary glioblastomas.[9]

IDH1, IDH2, and IDH3 are enzymes involved in the citric acid cycle and catalyze the oxidative decarboxylation of isocitrate

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to a-ketoglutarate while reducing NADP+ to NADPH (NAD+ to NADH in the case of IDH3) . IDH1 is found within the cytoplasm and peroxisomes, whereas IDH2 and IDH3 are localized solely in the mitochondria.[10] Later, multiple studies corroborated these? ndings and additionally revealed that somatic IDH1 mutations are present in the vast majority of low-grade diffuse astrocytoma (WHO grade II). Mutations in IDH2 have also been identified in gliomas, although they are much less common and are mutually exclusive with mutations in IDH1.[11,12]

Notably, nearly all IDH1 mutations are the same, with CGT–CAT transition causing a specific amino acid change from arginine to histidine at codon 132 (R132H). As a result, the detection of IDH1 mutations may be a specific means to aid in differentiating between glioma and gliosis.[6] Immunohistochemical detection of IDH1 mutation is a rapid and practical method of screening mutated IDH1 gene in gliomas.[13,14]

In the present study we investigated the specificity of anti-IDH1 R132 immunostaining in differentiating gliomas from reactive CNS lesions. We analyzed reactive gliosis of various etiologies for binding of IDH1 and cases of astrocytomas.

MATERIALS AND METHODS

Tissue samples

A retrospective study including 46 formalin-fixed paraffin-embedded tissue blocks from patients diagnosed within the Pathology Department, Tanta University Hospital from January 2010 to 2015. We analyzed 24 WHO grade II diffuse astrocytomas and 22 reactive conditions with astrocytosis (8 infarcts, 10 gliosis, and 4 traumatic brain injury). Approval to perform this work was obtained from the Institutional Research Ethics Committee. The clinical data of the investigated cases were obtained from patients clinical files.

Immunohistochemistry

Sections of 4 mm thick of the selected paraffin blocks were de-paraffinized in xylene for 20 min, rehydrated in graded alcohol and incubated in 0.5% hydrogen peroxide/methanol for 10 min to block endogenous peroxidase activity. The antigens were retrieved by boiling for 10 min in 10 mM citrate buffer, pH 6.0, using a microwave, followed by cooling to room temperature for 20 min. After washing in phosphate buffered saline (PBS), different sections were incubated with ready to use rabbit IDH1 Polyclonal Antibody PA5-28206 (Thermo Scientific) for overnight at 4 _C. Next day, the sections were washed in PBS before incubation with secondary antibody for 10 min at room temperature. The sections were then washed in PBS, incubated with streptavidin for 10 min at room temperature, washed and exposed to 3,30-diaminobenzidine tetrahydrochloride solution (DAB) to yield an insoluble brown deposit. Finally, the sections were counterstained with hematoxylin, washed in running water, dehydrated in graded alcohol and mounted as usual. Replacement of the primary antibodies with PBS worked as negative controls for the immunohistochemistry process.

Evaluation of IDH1 immunostaining

The expression of IDH1 was determined by semiquantitatively assessing the proportion of positively stained tumor cells. IDH1 immunoreactivity was positive when tumor cells showed strong cytoplasmic staining. The percentage of positive cells was rated as follows: cases with 10% cells were rated as positive, and cases with < 10% cells were rated as negative

RESULTS

Among the 24 studied cases of diffuse astrocytoma (79.2%) were males and the remaining 5 (20.8%) were females. The mean age of the patients was 45.4 years. The 22 cases with reactive gliosis were obtained as follows 8 infarcts, 10 gliosis and 4 traumatic brain injury. The mean patient age for low-grade astrocytomas and reactive cases was 43.4 and 32.5 years, respectively.

Histological findings

All included cases were stained by routine H&E to confirm the diagnosis. The included 24 cases of diffuse astrocytoma showed moderate increase in cellularity with low mitoses. No or minimal atypia and no necrotic lesions were seen Figure 1. Reactive astrocytes seen adjacent to areas of heamorrhage or infarction showed evenly distributed hypercellularity with no atypia or mitosis.

IDH1 immunostaining

Positive granular cytoplasmic staining of tumor cells for IDH1 was found in 13 out of 24 (54.2%) WHO grade II astrocytomas, but was entirely absent in all 22 reactive samples . Tumor cells in astrocytoma cases demonstrated staining both in the densely cellular areas of the tumor, as well as in the less cellular infiltrating tumor edges. While reactive astrocytes at the tumor edges were IDH1 negative. [Figure 2,3,4]

DISCUSSION

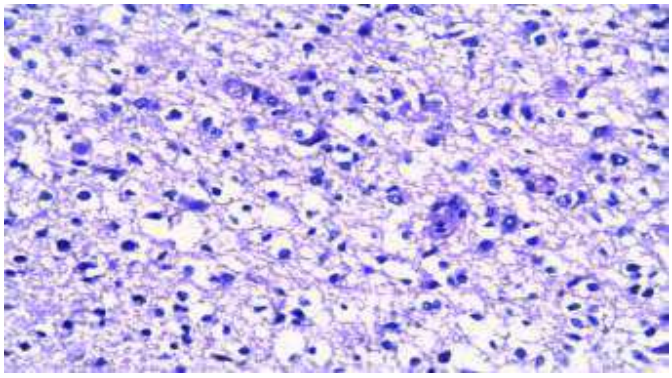

Figure 1: A case of diffuse astrocytoma showing minimal atypia and mild increase in cellularity (H&E x 200)

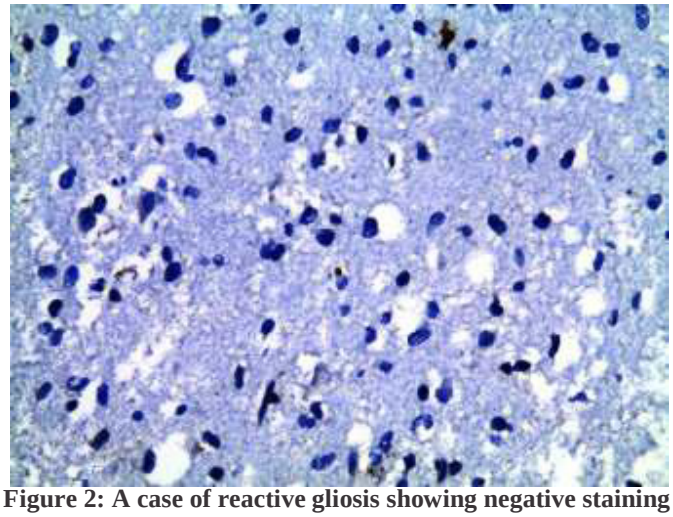

Figure 2: A case of reactive gliosis showing negative staining for IDH1 (immunoperoxidase x 400)

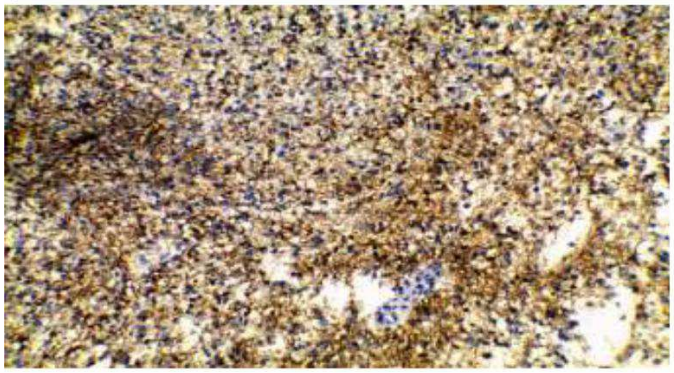

Figure 3: Representive case of diffuse astrocytoma showing positive cytoplasmic IDH1 immunostaining (immunoperoxidase x 100)

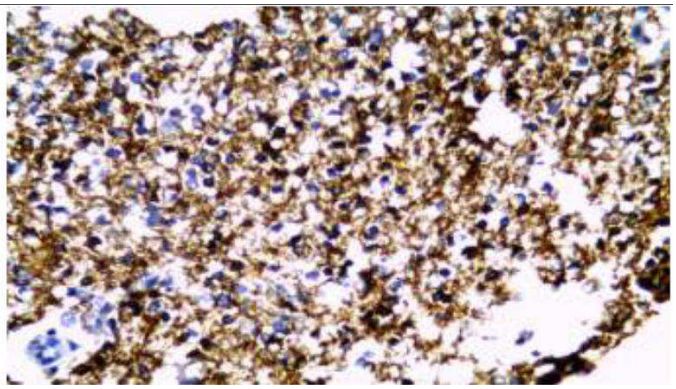

Figure 4: Positive cytoplasmic IDH1 immnuostaining in diffuse astrocytoma (immunoperoxidse x 400)

Correct interpretation of central nervous system (CNS) resection specimens can be challenging in current practice in which pathologists receive limited tissue samples from stereotactic image guided biopsies. One of the most challenging decisions is the differentiation between low grade astrocytoma and reactive astrocytosis. Misdiagnosis of reactive and neoplastic astrocytes can occur at the infiltrative tumor margins. Atypical reactive gliosis is a non-neoplastic process that will have markedly different prognosis and therapies.[2] Further molecular understanding of specific tissue markers can reduce the problems of histological assessment and small tissue sampling.[12]

Immunohistochemical detection of p53 is the most widely used marker for this differentiation; However, p53 is not an entirely accurate marker since it may show light staining of non-neoplastic cells and strong positivity in some reactive conditions.[15] Another marker of neoplastic cells is the epidermal growth factor receptor (EGFR) protein. Unfortunately, this marker is not of diagnostic utility in differentiating gliomas from gliosis since EGFR is primarily expressed in glioblastomas rather than lower-grade astrocytomas.[16] A mutation in isocitrate dehydrogenase 1 (IDH1), the enzyme involved in lipid and glucose metabolism, has been identified in a variety of diffuse gliomas. Recently, isocitrate dehydrogenase 1 (IDH1) and IDH2 mutations have been demonstrated in a variety of diffuse gliomas, with IDH1 mutations occurring commonly in lower-grade gliomas.[17]

In attempts to differentiate between reactive and neoplastic astrocytes, multiple molecular genetic methods have been used, including in situ hybridization, to detect chromosomal aberrations absent in non-neoplastic tissues. However, they are expensive, and cannot replace immunostaining.[18]

In this study; isocitrate dehydrogenase 1 (IDH1) expression in cases of diffuse astrocytoma and reactive astrocytosis was evaluated by immunohistochemistry. Positive cytoplasmic IDH1 staining was observed in 54.2% of studied cases of diffuse astrocytoma while non of the reactive cases showed IDH1 positivity. Our positivity rate for diffuse astrocytoma was similar to the 42.5 % reported in previous study which screened a total of 195 gliomas for isocitrate dehydrogenase (IDH1) mutations using a tissue microarray (TMA) - based approach and assessed the role of immunohistochemical expression of IDH1 protein in these tumors.[13]

These results were also in agreement with Piao et al who concluded that IDH1 tends to express preferentially in low-grade gliomas, and it thus may serve as a valuable marker in distinguishing low grade gliomas from gliosis.[19] These results were also close to previous study of Capper et al.[3] used IDH1 antibody to differentiate diffuse gliomas from non-neoplastic CNS lesions and therapy induced changes. They examined 120 reactive gliosis specimens using anti IDH1 antibody. They also investigated staining for WT1 and p53 proteins. All of their gliosis samples were IDH1-negative, 17 % were positive for WTI, and 63 % were positive for p53. These results were also in line with Camelo-Piragua et al.[6] who compared 20 non-neoplastic lesions with 21 grade II diffuse astrocytomas, found that while 42.9 % of the gliomas expressed IDH1, non of the 20 reactive gliosis samples showed IDH1 expression. Both groups concluded that IDH1-specific immunohistochemistry is useful for the differentiation between diffuse astrocytoma and non-neoplastic astrocytosis. Makino et al analyzed the expression of mutant IDH1 in 10 non-neoplastic and 52 neoplastic lesions and stated that IDH1 was negative in all non neoplastic samples. The study concluded that IDH1 does not generate false-positive results in non-neoplastic tissues, which may mimic glioma, and it facilitates the detection of mutations even in biopsy samples that yield equivocal histological results.[4]

In this study tumor cells in astrocytoma cases demonstrated staining both in the densely cellular areas of the tumor, as well as in the less cellular infiltrating tumor edges. While reactive astrocytes at the tumor edges were IDH1 negative. This finding was in agreement with previous studies whom reported that reactive astrocytes in the infiltration zone of gliomas are not bound by IDH1 and by the finding of Camelo-Piragua et al,[6] who investigated 20 specimens of reactive gliosis with IDH1 and also reported no staining. In line with our results, Horbinski et al studied IDH1 gene mutational analysis in gliomas and reported that IDH1 mutations are not found in DNA from reactive CNS lesions. The study concluded that the IDH1 fulfills the criteria of a tumor-specific marker.[20]

To argue the sensitivity of IDH1 immunostaining; Camelo-Piragua et al. evaluated a combination of immunohistochemical and molecular techniques to detect TP53 and IDH1/2 mutations as well as copy number gain in chromosome 7. They reported that the combination of p53 and IDH1 immunohistochemistry resulted in higher sensitivity (71.4 %) than either test alone (47.8 %) and that the best total sensitivity (95 %) was achieved when FISH for chromosome 7 gain was added to the p53 and mutant IDH1 immunohistochemical panels
[6]. Also a study by Makino et al investigated the specificity of IDH1 and FAS antibody staining for differentiating diffuse gliomas from non-neoplastic lesions and recommended a practical combination of immunohistochemical panels with these metabolic-related molecules to increase diagnostic specificity.[4]

CONCLUSION

In conclusion, this study showed that IDH1 is a useful immunohistochemical marker to differentiate reactive gliosis from low-grade astrocytoma. IDH1 has potential as an independent marker to diagnose neoplastic conditions especially in small surgical samples with high specificity.

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