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Research Paper

Immunohistochemical Expression of Alpha-Thalassemia/Mental Retardation Syndrome X-Linked Protein in Glioma And its Correlation with Age, Sex, Tumor Grade and Isocitrate Dehydrogenase Status

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ABSTRACT:

BACKGROUND:

Gliomas are tumors deriving from glia cells and cause significant cancer mortality globally. Alpha-thalassemia/mental retardation syndrome X-linked (ATRX) mutations are frequently distinguished in gliomas. There are increasing indications to study the ATRX protein immunohistochemically to enhance knowledge about diagnosis, therapy, and prognosis.

OBJECTIVE:

To determine the association between ATRX expression and different clinicopathological parameters in gliomas including age, sex, tumor grade and IDH status.

PATIENTS AND METHODS:

This cross-sectional retrospective study was carried out at the Department of Pathology and Forensic Medicine, Faculty of Medicine, University of Babylon, during the period from January 2023 through December 2023. Fifty formalin-fixed and paraffin-embedded tissue blocks of patients diagnosed as glioma have been obtained from the laboratory of histopathology in Al-Kafeel Specialized Hospital, Karbala, for the last five years (2019–2023), and different clinicopathological parameters have been correlated with ATRX expression.

RESULTS:

Loss of nuclear ATRX expression in tumor cells was observed in 37 cases (74%), while retained expression was observed in 13 cases (36%). Loss of ATRX was mostly observed in the age group of 31–40 years,while retained expression was mostly observed in age group of 61–70 years, and there was a significant association between ATRX expression and the age groups. Regarding sex, most of cases were males (54%), and there was no significant association between ATRX expression and sex. Concerning tumor grade, loss of ATRX expression was observed mostly (100%) in grade 2 tumors, and there was a significant association. There was a significant association between ATRX and IDH expression.

CONCLUSIONS:

Loss of nuclear ATRX expression is associated with younger patient age and a lower grade of glioma, and there is an association between IDH mutation (mutant type) and loss of ATRX expression.

KEYWORDS: ATRX; Glioma; IDH.

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INTRODUCTION:

Diffuse gliomas are characterized by a diffuse growth pattern and are glial (oligodendrocytes, ependymal cells, and astrocytes) in origin in the central nervous system. The tumor's infiltrative and diffuse nature provide significant challenges since they significantly hinder the ability to remove the entire tumor. Additional factors that are thought to contribute to treatment failure and tumor relapse include tumor diversity in terms of treatment responsiveness, mutations in genes, and subclones with varying susceptibility to chemotherapy and radiation therapy [1,2].

It has recently become clear that diffuse gliomas varied greatly in childhood compared to adults, despite the fact that it was previously believed that all cases of the disease were the same. Genes encoding isocitrate dehydrogenases (IDH) are frequently mutated in adult cases of glioma; in

children, these mutations are uncommon. Similar to this, histone 3 mutations are less common in adults and more common in children. These variations were acknowledged in the 2021 edition of the World Health Organization's (WHO) categorization of CNS (central nervous system) cancers [3,4].

Previously, tumors of the brain were mainly categorized based on histological features, while most recent versions added genetic characteristics (e.g., IDH mutation, 1p/19q codeletion, etc.) and methylation of the DNA patterns [5].

In twenty-first century, the World Health Organization established the fifth version of the classification of Central Nervous System tumors. The resulting classification takes into account developments in the understanding of brain tumor molecular genesis and histology to arrange brain tumors into biologically and molecularly more defined entities. Thus, better-described natural histories result in significantly improved tumor categorization. Gliomas are particularly affected by these modifications. Adult and pediatric gliomas are now diagnosed differently for the first time based on variations in molecular genesis and outcome. Additionally, the previous wide category of adult diffuse gliomas was recently organized into three types: astrocytoma IDH mutant type; oligodendroglioma IDH mutant and 1p/19q codeleted; and glioblastoma IDH wild type. All these changes are brought on by IDH alterations and involve the limitation of diagnosis of glioblastoma to IDH wild type tumors; the re- classification of tumors that were previously diagnosed as (glioblastomas IDH-mutant) into IDH mutated astrocytoma, grade 4; and a need for the presence of a mutation in IDH to classify as astrocytomas or oligodendrogliomas [6,7].

Alpha-thalassemia/mental retardation syndrome X-linked(ATRX) protein is involved in telomeres and is a crucial component of the chromatin remodeling complex. This protein is encoded with the ATRX gene, which is located at Xq21.1. Loss of ATRX protein can cause instability and lengthening of the telomere and may lead to genomic instability [8].

Physiologically, the ATRX protein is expressed universally in the nuclei, and alterations in its genes cause tumor cells to lose nuclear expression while non-tumor cells retain expression. In the clinical setting, loss of nuclear ATRX expression is regarded as a surrogate marker for ATRX mutation [9].

Isocitrate dehydrogenase(IDH) enzymes,which exist in three isoforms, are essential enzymes and involoved in variety of metabolic activities and the genes encode IDH are often changed in variety of human malignancies including gliomas.the majority of these mutations(90%) affect IDH1[10].

This study attempts to assess ATRX immunoexpression in gliomas and its correlation with different clinicopathological parameters, including age, sex, tumor grade, and IDH status.

PATIENTS AND METHODS:

This cross-section retrospective study was carried out from January 2023 through December 2023 at the University of Babylon's Faculty of Medicine, Department of Pathology and Forensic Medicine.

Specimen selection and data collection

Study group

Fifty tissue blocks that are paraffin embedded and fixed in formalin from archived tissue specimens' of patients who were diagnosed with glioma have been obtained from the laboratory of histopathology in Al-Kafeel Specialized Hospital, Karbala for the last five years (2019-2023).

The primary diagnosis was established by using slides stained with (Hematoxylin / Eosin). Furthermore, molecular subclassification was done by reexamination of the slides stained with immunohistochemistry for IDH and ATRX which were all extracted from the archive.

The Clinicopathological variables comprising age, sex, and histological grade were estimated.

Inclusion criteria

Diffuse glioma cases regardless of histological classification.

Exclusion criteria

Tumors with a non-diffuse growth pattern, which is considered a differential diagnosis for gioma and need further evaluation.

Immunohistochemical staining protocol

The immunostaining procedure used was the 3-step polymeric detection system. 5 µm-thickness histological sections were made from each of the paraffin-embedded blocks fixed on positively charged slides to be processed and stained to be subjected to IHC procedures to detect ATRX and IDH-1 expression.

Scoring system

The standards for IDH1-R312H staining are as follows:

(1) The strong cytoplasmic staining was rated positively.

(2) The diffuse weak reaction and staining of macrophages only were evaluated as negative[11,12].

Germany's Center for Cancer Research (DKFZ) standards for ATRX staining were as follows:

Tumor cells nuclear expression was rated as lost when the nuclei of cells were not stained and the nuclei of non-neoplastic cells such as endothelial cells, lymphocytes, and reactive astrocytes were stained. Two-grade grading systems were used to assess ATRX expression: 0 represented no staining or less than 10% of cells were positively stained, and 1 represented >10% of cells being positively stained [13].

Statistical analysis:

A statistical analysis study was done using version 26 of SPSS. Fisher's Exact Test and two-sided Chi-square test were used to calculate the relation between different. clinicopathologic parameters, IDH and ATRX expression in gliomas.Statistics were judged to be significant when P values were equal to or below 0.05.

RESULTS:

Fifty glioma cases have been studied, and the correlation between these cases and different clinical and pathological characters, including age, sex, tumor grade, and IDH status, has been evaluated.

Patient characteristics:

The distribution of patients by age ranged from 9 to 70 years. The overall (mean ±SD) patients' age was (43.08 ± 15.77) years old. Most of the patients were in the fourth decade (26%) 13 cases. Male to female ratio was 1:0.85, with 54% of the patients being male and 46% being female. Concerning the tumor grade, the predominant grade was grade 4, representing (58%) 29 cases, these features are listed in table 1.

Table 1: Clinicopathological parameters of patients with glioma.

Variables	Frequency
Age groups	
<=10	2(4%)
11-20	2(4%)
21-30	8(16%)
31-40	13(26%)
41-50	8(16%)
51-60	9(18%)
61-70	8(16%)
Sex	
Male	27(54%)
female	23(46%)
Tumor grade	
Grade2	9(18%)
Grade3	12(24%)
Grade4	29(58%)

The correlation between clinicopathological variables and ATRX expression

According to ATRX immunohistochemical staining, loss of nuclear staining of tumor cells was observed in 37 cases (74%), while retained nuclear staining was observed in 13 cases (36%).

The associatin between ATRX expression and the age groups:

Loss of ARX expression was observed mostly (92.3%)in the age group of 31–40 years, while retained expression was observed mostly (75%) in the age group of 61–70 years. There is a significant association between ARX expression and age groups (p value 0.005), as in figure1.

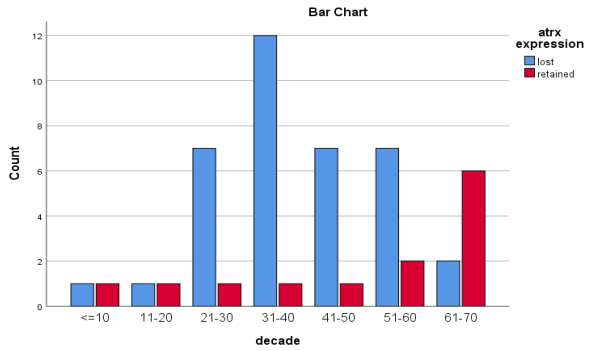

Figure 1: The correlation between age groups and ATRX expression.

The correlation between sex and ATRX expression

The association was statistically not significant (p-value 0.208). as shown in table 2.

Table 2: The association between ATRX expression and the sex.

Sex	ATRX expression	Frequency	Percent	P value
Male	Lost	18	66.7	0.208
	Retained	9	33.3	
	Total	27	100.0	
Female	Lost	19	82.6	
	Retained	4	17.4	
	Total	23	100.0	

The correlation between tumor grade and ATRX expression

Regarding grade 2 gliomas, all the nine cases(100%) showed loss of ARX expression. In grade 3 tumors, 9 cases(75%) lost ARX expression and 3 cases (75%) retained expression. In grade 4 gliomas, 19 cases (65.5%) lost ARX expression and 10 cases (34.5%) retained expression. The correlation between ARX expression and the grade of gliomas was statistically significant (p-value 0.047), as in Figure 2.

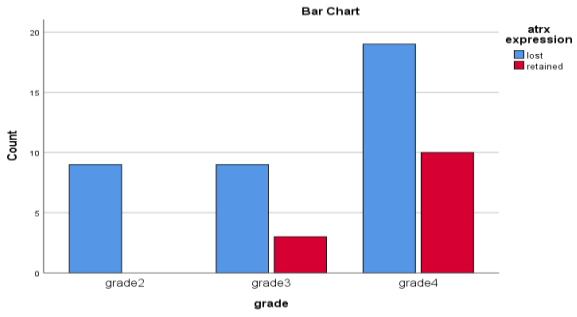

Figure 2: The correlation between tumor grade and ATRX expression

The correlation between ATRX and IDH expression

Regarding IDH expression, 47 cases (94%) were positive (mutant) and 3 cases (6%) were negative (wild type). From the IDH-positive (mutant) cases, 37 cases (78.7%) lost ATRX expression, and 10 cases (21.3%) retained ATRX expression. While IDH-negative (wild type), all three cases retained ATRX expression. There is a significant relationship between ATRX and IDH expression (p value 0.02) as shown in figure 3.

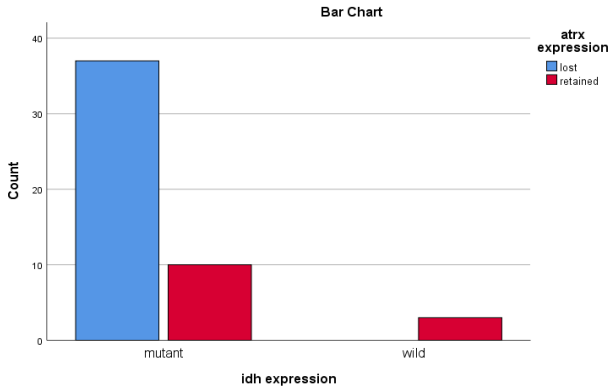

Figure 3: The correlation between IDH expression and ATRX.

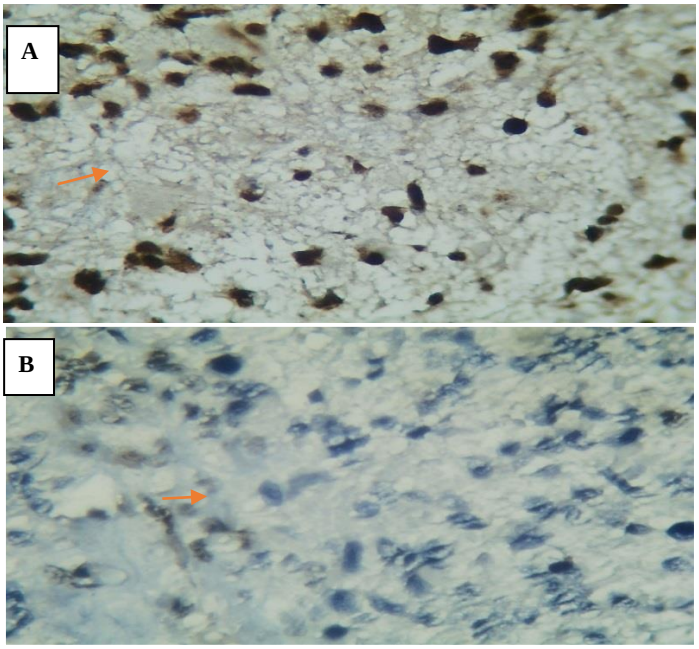

Figure 4: ATRX stained slides of glioma (400X) :
A: slide shows retained nuclear ATRX staining in tumor cells
B: slide shows loss of nuclear ATRX expression in glioma cells with retained expression in endothelial cells, which considered as a positive controls (arrows).

DISCUSSION:

Gliomas are brain tumors that develop in the brain or spinal cord and begin within glial cells, which surround and support nerve cells. Glioma tumors are classified by the involvement of glia cells and the tumor's genetics, which can predict its prognosis. Furthermore, the detection of these cases can greatly help in the management of gliomas [14].

Multiple genetic events involved in the evolution of brain tumors have been found, and these involve various abnormalities in signaling networks involving multiple genes. ATRX is one of these genes, and it is found on chromosome Xq21.1. ATRX mutations are linked to a decrease in nucleus ATRX protein expression, which can be detected using commercially available antibodies [15].

In current study, ATRX loss in glioma cells was found in 37 cases (74%), while retained nuclear staining was observed in 13 cases (36%). Loss of

nuclear ATRX staining was observed in the highest frequency (12 cases) (92.3%) in the age in glioma pathogenesis, especially in the 3rd and 4th decades [18].

The correlation between expression of ATRX and sex was not significant statistically, and these Regarding tumor grade, loss of ATRX expression mostly seen in grade 2 gliomas (100%) followed by grade 3 (75%). while in grade 4 gliomas, 19 cases (65.5%) lost ATRX expression and 10 cases(21.3%) retained expression. These findings are comparable to other studies that demonstrated that loss of ATRX expression is more common in low-grade gliomas [16,18]. In grade 4 gliomas, Previous research on ATRX expression seems to vary. Liu and colleagues found that ATRX was lost in secondary glioblastomas and more in younger patients [20]. in addition, Cai and colleagues found that ATRX expression was lower in primary GBM and anaplastic gliomas than in grade 2 gliomas [21]. Multiple studies indicate that higher-grade gliomas involve different mechanisms of carcinogenesis and that the ATRX role is not specific to this type of tumor [22]. ATRX loss was associated with IDH mution. From the 47 cases with mutant IDH expression, 37 cases (78.7%) had lost ATRX expression, and 10 cases (21.3%) retained expression. While all 3 cases with IDH wild-type had retained ATRX expression. Our findings showed a strong association between IDH mutations and ATRX loss, and this result has been accepted by many studies, as recent researches have demonstrated that IDH mutations and ATRX loss coexist in some diffuse glioma subtypes, and loss of ATRX rarely occurred in IDH wild-type gliomas [23,24].

CONCLUSION:

1. Loss of ATRX expression is associated with good prognostic factors, including a lower grade of glioma (grades 2 and 3) and a younger patient age than retained expression.

2. Cases with a loss of ATRX expression show a significantly high percentage of IDH mutation. (75%) in the age of 61–70 years. Similar results were published by many other studies [16,17]. This result implies that, like IDH-1, the ATRX mutations arise as an early of 31–40 years, while retained expression was observed in the highest frequency (6 cases)

event

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