Immunohistochemical expression of ATRX in gliomas

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Abstract: Glioma is one of the primary tumors of the central nervous system that occurs in the spinal cord or brain and the origin of the tumor is from glial cell cells. The most common site of glioma tumors is the brain. Glioma accounts for 30% of all central nervous system tumors and 80% of malignant brain tumors. Alpha-thalassemia/mental retardation syndrome X-linked (ATRX) mutations are frequently distinguished in gliomas. Current research is an attempt to assess ATRX immunoeexpression in different types of gliomas diagnosed, in Erbil-Iraq, and to evaluate its association with patient’s age, gender, tumor location, grade and type. From January 2015 to January 2017, we reviewed and analyzed 97 cases of glioma. Immunohistochemical staining, for ATRX, was performed using an automated immunostainer technique. According to the WHO grading system for brain tumors, 16 (16.5%) cases were grade I gliomas, 27 (27.8%) were grade II, 10 (10.3%) were anaplastic gliomas (grade III), and 44 (45.3%) cases were glioblastomas WHO (grade IV). Positive ATRX immunoeexpression was demonstrated in 27 (27.8%) cases. The highest rates of ATRX expression (55.6%) were among 30-39 years’ age group, supratentorial (34.2%), and among grade II and III tumors (40.7% and 30% respectively). A significant association was observed between ATRX expression and patient’s age, tumor location, type and grade (p-values 0.010, 0.004, 0.004, and 0.037 respectively). No significant association was found between ATRX expression and patient’s gender (p-value 0.097). It was found that ATRX is frequently expressed in grade II and III astrocytomas and was significantly related to the patient’s age, tumor location, type and grade, so it can be used as a good diagnostic and prognostic indicator for glioma.

Key words: Glioma; ATRX; Immunohistochemistry.

Introduction

Glioma is a tumor that develops in a person’s brain and spinal cord. Glioma usually begins in the supporting cells of the throat (glial cells) that surround the nerve cells and help them function. Three types of glial cells can cause tumors. Glioma disease is classified according to the glial cell involved and the genetic characteristics of the tumor and according to these cases can predict the status of this tumor in the future. In addition, identifying these cases can greatly help in the treatment of glioma. As with other primary brain tumors, the underlying cause of glioma is unknown. But some factors can increase the risk of developing this brain tumor (1-5).

The choice of treatment for glioma will depend on the type, size, extent and location of the tumor, age, health, and even personal preferences. In addition to the steps taken to remove the tumor, in the treatment of glioma, a person may need to use medications to reduce the signs and symptoms of the tumor. Your doctor may prescribe steroids to reduce inflammation and pressure in the affected area of the brain. Antiepileptic drugs may also be used to control seizures (4, 5).

Glioma, depending on the type (astrocytomas, oligodendrogliomas, and ependymomas), location and rate of growth, can affect brain function and even endanger a person’s life. Glioma is one of the most common types of brain tumors. Diagnosis of glioma is one of the most important cases that can help in the correct diagnosis and treatment of this tumor. Of course, in general, glioma treatment options include surgery, radiotherapy, chemotherapy, and targeted therapy (4, 5).

Based on clinical-pathological criteria, the WHO has classified gliomas into grades I to IV (1,2). Therefore, grade I gliomas grow slowly and are usually cured after complete surgical resection (2). Grade II is invasive and may progress to higher-grade lesions with a poor prognosis. Grade III lesions are more invasive and have a poor prognosis. Glioblastoma (GBM), a WHO grade IV tumor, is the most common biologically most aggressive astrocytoma with a poor prognosis (3-5).

ATRX protein is an important part of the chromatin remodeling complex and plays a role in telomeres. This protein is encoded with ATRX (Alpha-thalassemia/mental retardation syndrome X-linked) gene that has been located at Xq13.

Because it is on the X chromosome, there is only one copy in males and only one allele inactivation in females, so that a single inactivating mutation on the active allele is sufficient to lose ATRX function. Loss of ATRX can cause telomere instability and lengthening and may lead to genetic instability (6,7).

ATRX mutations were first detected in neuroendo-
cine tumors, and then in pediatric and adult gliomas. Mutations are truncated mutations (3/4) or missense mutations mainly located in the highly conserved region in the helicase domain (1/3), and these changes are highly correlated with the negative staining of immunohistochemistry (6). Physiologically, ATRX protein is universally expressed in the nucleus. Mutations in its genes result in the loss of nuclear protein expression in tumor cells, while expression is retained in non-tumor cells (for example, endothelial cells and pre-existing glial cells), thus becoming a positive internal control (8).

In clinical practice, negative staining by IHC for ATRX is used as a surrogate marker for ATRX mutations (9). The analysis of ATRX mutations in a series of adult glioma cases showed that in contrast to the lower incidence of astrocytoma, oligoastrocytoma, and glioblastoma, this change in diffuse astrocytoma The incidence is higher (10, 11).

The prognostic value of ATRX mutations in different types of tumors is still controversial: in pancreatic neuroendocrine tumors, changes in ATRX expression are related to more aggressive behaviors (12).

In childhood acute myeloid leukemia with FLT3 mutations, higher ATRX expression is associated with a better prognosis (13). In gliomas, in retrospective cohorts of grade II, III, and IV gliomas, ATRX mutations were associated with better prognosis (10).

This study was an attempt to assess ATRX immunohistochemical expression in different types of gliomas and its association with several clinical and pathological parameters including age, gender, tumor type, grade, and site.

Materials and Methods

From January 2015 to January 2017, 97 reported glioma samples were collected, reviewed, and tested for ATRX immune expression. Samples were retrieved from the Department of Histopathology of Rizgary Teaching Hospital in Erbil City. Clinical data including age, gender, and site of the tumor were recorded. Paraformaldehyde-embedded blocks were cut to a thickness of 3 µm and stained with Hematoxylin and Eosin. Then according to the 2007-WHO classification and classification of CNS tumors (1). Ethical approval was obtained from the Ethics Committee of the author’s University.

Immunohistochemical technique

Another 3 µthick slide from the tumor was dewaxed and rehydrated. The antigen recovery was performed by autoclaving at 97°C for 20 min using an antigen recovery solution (citrate buffer 10 mmol/L, pH 6.0). The sections were allowed to cool at room temperature and then washed 3 times in phosphate-buffered saline (PBS) for 3 minutes each. After this initial processing step, the sections were incubated with a 1:200 dilution of primary anti-human ATRX (U.S., mouse monoclonal) antibody overnight at room temperature. Then incubate for 35 minutes with the ultra-sensitive non-biotin HRP detection system. Finally, the sections were counter-stained with hematoxylin, dehydrated and fixed.

The cut-off value was 10%; i.e., ATRX loss (positive mutation): staining loss in >10% of tumor cells, and ATRX retained (negative): staining loss in <10% of tumor cells, accompanied with endothelial cell-positive staining (14, 15, 16).

Statistical analysis

Data were interpreted in terms of frequencies and percentages. A Chi-square test and Fisher exact test were used to correlate ATRX status and the different study variables. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 20. A p-value of less than 0.05 was considered significant.

Results

Ninety-seven cases of different types of gliomas were included in this study. Patients’ age ranged from 3 months to 84 years with a mean of 37.08±20 years and a median of 38 years. Fifty-nine patients were males and 38 were females with a male to female ratio of 1: 0.64.

Positive (loss of) ATRX staining was observed in 27 (27.8%) cases while negative (retained) staining was observed in the remaining 70 (72.2%) cases. As shown in Figure 1, the highest frequency of ATRX immunoregistration was found in the age group 30-39 years (55.6%) giving a statistically significant difference (p-value 0.010).

Out of 38 females and 59 males, 7 (18.4%) and 20 (33.9%) were positive for ATRX respectively. The association was statistically not significant (p-value 0.097).

Twenty-seven out of 79 supratentorial gliomas were positive, while all infratentorial (n= 18) were negative for ATRX. This gave a statistically significant (p-value 0.004) association of ATRX expression with the supratentorial location.

Regarding tumor type, ATRX expression was statistically high among AA (75), followed by DA (55.6%) and GBM was 30.2% (p-value 0.004). All PA and ependymomas were negative for ATRX expression and the association of ATRX expression with tumor type was statistically significant (p-value 0.004) (Table 1).

Concerning the tumor grade, the predominant grade was grade IV representing 44 cases followed by grade II; 27 cases then grade I; 16 cases and lastly grade III; only 10 cases. The highest ATRX expression was among grade II tumors forming (40.7%) of the positive cases, followed by grade III (30.0%) then grade IV (29.5%), while all grade I tumors were negative. The association between ATRX expression and grade of gliomas was statistically significant (p-value 0.037) (Figure 2).

Representative pictures of ATRX immunoregistration in different grades of astrocytoma (DA, AA, and GBM)
Immunohistochemical expression of ATRX in gliomas.

A brain tumor is an abnormal mass in the brain that, depending on the nature of the cells that make it up, can be benign or malignant. The tumor may originate in the brain tissue or spread to another location in the brain or so-called metastasis. A brain tumor is a type of hard, solid intracranial neoplasm, or tumor (abnormal cell growth), inside the brain or central spinal canal (17).

Brain tumors include all intracranial tumors or tumors within the central spinal canal. These tumors are caused by uncontrolled and abnormal cell division and are usually found in the brain itself, including neurons, glial cells (astrocytes, oligodendrocytes, ependymal cells, myelin sheath cells), lymphatic tissue, or blood vessels. In the cranial nerves, the meninges, skull, pituitary, and pineal gland are formed. These tumors can also be the result of the spread of malignancies that have primarily involved other organs, in which case they are called metastatic or metastatic tumors. In the world, brain tumors are considered to be one of the most serious and deadly cancers (17).

Glioma is a type of tumor that develops in the brain and spinal cord of an infected person. Glioma usually first involves the throat support cells (glial cells) that surround the nerve cells and help them function. Three types of glial cells can cause tumors. The choice of treatment for glioma will depend on the type, size, extent and location of the tumor, age, health, and even personal preferences.

A variety of genetic events have been identified during the development of brain tumors, and these events involve several defects in signaling pathways with multiple genes. Among these genes, ATRX is located on the chromosome of Xq13. ATRX mutations are related to the loss of nuclear ATRX protein expression detectable by commercially available antibodies, thus making ATRX a promising prognostic biomarker in routine neuropathology practice (8).

As per our knowledge, this study is the first of its kind in our region and Iraq to detect ATRX immunoeexpression in gliomas by using antibody specific for ATRX.

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Positive no. (%)</th>
<th>Negative No. (%)</th>
<th>Total no. (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pilocytic astrocytoma</td>
<td>0 (0.0%)</td>
<td>15 (100.0%)</td>
<td>15 (15.5%)</td>
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<tr>
<td>Subependymal giant cell astrocytoma</td>
<td>0 (0.0%)</td>
<td>1 (100.0%)</td>
<td>1 (1%)</td>
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<tr>
<td>Diffuse astrocytoma</td>
<td>10 (55.6%)</td>
<td>8 (44.4%)</td>
<td>18 (18.6%)</td>
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<tr>
<td>Oligodendroglioma</td>
<td>1 (50.0%)</td>
<td>1 (50.0%)</td>
<td>2 (2.1%)</td>
<td></td>
</tr>
<tr>
<td>Oligoastrocytoma</td>
<td>0 (0.0%)</td>
<td>1 (100.0%)</td>
<td>1 (1%)</td>
<td></td>
</tr>
<tr>
<td>Pleomorphic xanthoastrocytoma</td>
<td>0 (0.0%)</td>
<td>2 (100.0%)</td>
<td>2 (2.1%)</td>
<td></td>
</tr>
<tr>
<td>Ependymoma</td>
<td>0 (0.0%)</td>
<td>4 (100.0%)</td>
<td>4 (4.1%)</td>
<td></td>
</tr>
<tr>
<td>Anaplastic astrocytoma</td>
<td>3 (75.0%)</td>
<td>1 (25.0%)</td>
<td>4 (4.1%)</td>
<td></td>
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<tr>
<td>Anaplastic oligodendroglioma</td>
<td>0 (0.0%)</td>
<td>2 (100.0%)</td>
<td>2 (2.1%)</td>
<td></td>
</tr>
<tr>
<td>Anaplastic ependymoma</td>
<td>0 (0.0%)</td>
<td>4 (100.0%)</td>
<td>4 (4.1%)</td>
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</tr>
<tr>
<td>Glioblastoma multiforme</td>
<td>13 (30.2%)</td>
<td>30 (69.8%)</td>
<td>43 (44.3%)</td>
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<tr>
<td>Gliosarcoma</td>
<td>0 (0.0%)</td>
<td>1 (100.0%)</td>
<td>1 (1%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>27 (27.8%)</td>
<td>70 (72.2%)</td>
<td>97 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. ATRX expression with tumor type.

Discussion

A brain tumor is an abnormal mass in the brain that, depending on the nature of the cells that make it up, can be benign or malignant. The tumor may originate in the brain tissue or spread to another location in the brain or so-called metastasis. A brain tumor is a type of hard, solid intracranial neoplasm, or tumor (abnormal cell growth), inside the brain or central spinal canal (17).

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ATRX mutation.

In the current study, ATRX was expressed in 27 (27.8%) cases of gliomas, this figure was within the range that has been reported in the literature as shown in Table 2.

The highest frequency of ATRX expression was found in patients between 30-40 years (55.6%); similar findings were reported by many other studies (10,15,17,18). This finding suggests that, like Isocitrate dehydrogenase 1, ATRX mutation occurs as an early event in gliomagenesis specifically in the 3rd and 4th decades (22, 23).

Although statistically not significant, ATRX expression was observed more among males than females. A finding that is parallel to has been observed among other studies (10, 18, 24). It is well known that the incidence of gliomas is 30-50% higher in males than in females and the male predominance increases with age (25).

All of the ATRX positive tumors were supratentorial; a finding which was observed in several studies (15, 18, 21). This could be explained by the fact that all cases of DA and AA which showed ATRX expression were supratentorial in location.

Histologically, the highest frequency of ATRX expression among AA (75.0%) and DA (55.6%) are in agreement with many other studies done in different countries (6,9, 10,15, 17, 18, 21, 24, 26, 27).

Concerning GBM, ATRX expression was positive in 13 (30.2%) cases which are comparable to what has been reported by others (6, 9, 15, 18, 24). One explanation of lower ATRX expression in GBM in comparison to DA and AA is that some of our cases probably represent primary GBM in which ATRX expression is minimal rather than secondary GBM which are well known to show high ATRX expression. Also in our study, there was a significant association between ATRX expression and tumor type (p-value 0.004) which was in concordance with another study (17).

Regarding tumor grade, grade II gliomas showed the highest ATRX expression (40.7%) which is lower than others that reported a higher frequency of ATRX expression in grade II gliomas ranging from (54.3%-87.0%), in the same line; in grade III gliomas ATRX expression was found in (30%) which is also lower than other studies that their expression was ranging from 55.7%-100% (9,21,24) and this probably due to limited sample size in our study, in addition among the grade II cases, there were 4 ependymomas, 2 pleomorphic xanthoastrocytomas and among the grade III gliomas there were 4 anaplastic ependymomas and 2 anaplastic oligodendrogliomas all of which are well known to be negative for ATRX expression as ATRX expression usually associated with astrocytic tumor lineage (9,15, 17). In Grade IV gliomas 29.5% of the cases were positive and our results fall within the range that has been reported by others (6,9,15,18,24), regarding the association of ATRX with tumor grade; it was statistically significant (p-value 0.037) which was in agreement with other studies (15, 18, 24).

Another observation in our study, we failed to demonstrate ATRX expression among all 15 pilocytic astrocytomas (grade I), all 4 grade II ependymoma and all 4 anaplastic ependymoma cases (grade III); a result which is similar to other studies (10,15,21) confirming that these tumors are genetically distinct from the conventional astrocytomas, perhaps arising through different mechanisms and may be derived from a different type of glial progenitor cells and expressing other markers like Fibroblast growth factor receptor 1 (FGFR 1) and fibroblast growth factor receptor 3 (FGFR 3) (28).

Our study revealed that ATRX is frequently expressed in different astrocytomas, particularly in grades II – III and it’s significantly associated with younger patient’s age, supratentorial location, tumor type and grade, thus it may act as a good diagnostic and prognostic marker for gliomas.

Conflict of interest
The authors declare no conflict of interest.

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