Original Article

Study of ATRX and p53 Mutation in Astrocytomas and Its Role in Tumor Grading

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Abstract

Background: Astrocytomas are the most common primary brain malignancies. They come under a large group of clinically, pathologically and genetically diverse glial neoplasms designated as diffuse gliomas. The majority of IDH mutant astrocytomas also harbour ATRX and p53 mutations. The aim of the present study is to analyse ATRX and p53 mutation in astrocytomas by immunohistochemistry and its role in tumor classification and grading.
Methods: The cross-sectional study was conducted in the department of neuropathology, Madras Medical College for a period of 18 months from December 2022 to May 2024. A total of 54 cases with clinical suspicion and radiological evidence of astrocytoma were included in the study. Immunohistochemical analysis of ATRX and p53 were done and expressions were evaluated.
Results: Among 54 cases, 44.5% of them are grade 2 followed by 25.9% in grade 4, 18.5% in grade 3 and 11.1% in grade 1. Correlation with age, gender and anatomical location were done. Loss of ATRX expression was significantly associated with astrocytomas ($p < 0.001$), p53 mutation was lost in 9.3% of cases ($p = 0.41$).
Conclusion: ATRX is an important immunohistochemical marker in astrocytomas, the loss of which is associated with higher grades, hence this can be used as a prognostic marker in astrocytomas. P53 mutation in astrocytomas may enable tumor cell survival in setting of ATRX loss.

Keywords: Astrocytomas; immunohistochemistry; ATRX; p53

Introduction

Tumours of the central nervous system are rare compared to breast, gastrointestinal tract or lung cancers not only in India but throughout the world. In our country the estimated incidence rate of nervous system cancer for the year 2022 was 1 in 341 for males and 1 in 546 for females [1] based on data obtained from the National Cancer Registry Programme. No recent data was available for the exact burden of astrocytomas in the programme. But a review of different studies had showed that astrocytomas (38.7%) were the most common primary CNS tumours with the majority being high-grade gliomas (59.5%) [2].

WHO Classification and grading: WHO Classification of Tumours CNS 5th edition, 2021 had made some significant changes for gliomas compared to CNS 4th edition, 2016. Diffuse gliomas are now divided into those occurring primarily in adults (adult-type) or in children (pediatric type). Adult-type diffuse gliomas now constitute only 3 categories namely astrocytoma IDH-mutant, oligodendroglioma, IDH-mutant and 1p/19-codeleted and glioblastoma, IDH-wild type. So astrocytic tumors are divided into with and without IDH mutations. Those without IDH mutations (wild type) are termed glioblastomas IDH-wild type. Grade 4 designation is achieved by any of the following features: tumour necrosis, microvascular proliferation, or homozygous loss of CDKN2A and/or CDKN2B. Thus, even in the setting of a low-grade appearing

IDH-mutant astrocytoma without significant mitotic activity, homozygous deletion of CDKN2A and/or CDKN2B would result in a grade 4 designation. Testing for CDKN2A/B status is therefore necessary on all IDH-mutant astrocytomas [3].

ATRX Gene (Alpha Thalassemia/Mental Retardation Syndrome-X Linked gene): In humans, the ATRX gene lies on the long arm of the X chromosome in position 21.1. ATRX is a chromatin remodeler gene that encodes a protein containing an ATPase/helicase domain which belongs to the SWI/SNF family of chromatin remodeling proteins. Recently, ATRX gene status has become a critical marker that defines molecular WHO classification of tumours of the central nervous system, such as glioma [4, 5, 6, 7].

P53 gene: The p53 gene located in chromosome 17 is the most common tumor suppressor gene. In about 30–40% of astrocytoma cases loss of function in the p53 tumor suppressor gene due to mutation occurs early in tumorigenesis. Genetic instability due to the impaired ability of p53 to mediate DNA damage repair further facilitates the acquisition of new genetic abnormalities, leading to malignant progression of an astrocytoma. This results in 60 to 70% of such cases having p53 mutations [8].

Both ATRX and p53 mutations had been found to be associated with astrocytomas and their detection at the time of presentation as a routine procedure can help in prognosis of the patients. So far no standard guidelines had been provided for such routine detection. Hence we planned to conduct this study for detecting ATRX and P53 mutations by immunohistochemistry among patients admitted with clinical astrocytomas in our tertiary care hospital [9, 10, 11, 12, 13, 14].

Objectives: To study ATRX and p53 mutations in astrocytomas. To classify different types of astrocytomas and its grading by ATRX and p53 immunohistochemistry.

Materials and Methods

A cross-sectional study was conducted in the department of neuropathology, Madras Medical College for a period of 18 months from December 2022 to May 2024.

Inclusion criteria: Patients with clinical suspicion and radiological evidence of astrocytoma.

Exclusion criteria: Patients with diagnosis or suspicion of CNS tumors other than gliomas.

The study was initiated after obtaining approval from Institutional Ethical Committee, Madras Medical College. A total of 54 cases with clinical and radiological evidence of astrocytoma were included in the study. Collection of paraffin blocks of these 54 histopathological specimens were done. Slides are prepared and tissue morphology studied. Immunohistochemistry analysis for ATRX and p53 done. Loss of ATRX nuclear staining in tumor cells is considered as positive for ATRX mutation. Nuclear positivity of p53 in tumor cells is considered as positive for p53 mutation.

Data was entered in MS Excel and analyzed using statistical software SPSS 21.0. All qualitative variables were expressed in proportions. Fisher exact test was used to test significant association between two qualitative variables and a p-value of less than 0.05 was considered statistically significant. All descriptive data were expressed in tables and charts.

Results

Among study participants 42.5% belong to age group 21-40 followed by 29.7% in age group 41-60, 18.5% in age group 1-20 and 9.3% in age group 61-80. The mean age of the study participants was 37.8 (SD-15.3) while the age range of the study participants was between 4-75. Half of them were from the left side of the brain and 38.8% of them were from the right side of the brain. Specimens from intramedullary and intraventricular areas were 3.8% each while specimens from insular area and CP angle were 1.8% each. Frontal lobe was the most common involved lobe (25.9%) followed by the temporal lobe (18.5%). Around 14.8% specimens were from the frontotemporal region and another 12.9% were from the parietal lobe. Final histopathological diagnosis showed that 44.5% of them were grade 2 which includes astrocytoma, oligodendroglioma and glioneuronal tumor followed by 25.9% in grade 4, 18.5% in grade 3 and 11.1% in grade 1. Based on Atrx expression around three-fourths of the study specimens had lost Atrx expression (72.2%) while the remaining 27.8% retained Atrx expression. 90.7% were positive for p53 expression while the remaining 9.3% were negative. Based on Ki67 immunohistochemistry among study specimens we found that 79.6% of specimens had 1-20% expression followed by 16.6% specimens having 21-50% expression. Only 3.8% had more than 50% expression. Comparing the histological grading and Atrx expression among study specimens (clinical and radiological evidence of astrocytomas) it was observed that as the grading increases Atrx expression was lost. Grade 3 and grade 4 specimens had 100% loss of Atrx expressions while in Grade 2 specimens 54.2% had lost Atrx expression and in Grade 1 specimens only 33.3% had lost. This difference in Atrx expression among different histopathological grades was also statistically significant ($p < 0.05$). No statistically significant difference was observed between different histopathological grading and P53 expression.

Table 1: Distribution of Study Specimens based on Histopathological Grading (n=54)

Histopathological Grading	Frequency (%)
Grade 1 (Pilocytic astrocytoma)	6 (11.1)
Grade 2 (Astrocytoma, oligodendroglioma, Glioneuronal tumor)	24 (44.5)
Grade 3 (Astrocytoma)	10 (18.5)
Grade 4 (Astrocytoma)	14 (25.9)
Total	**54 (100)**

Table 2: Association between Histopathological Grading and Atrx Expression among Study Specimens (n=54)

Histopathological Grading	Atrx Expression (Retained)	Atrx Expression (Lost)	Total	p value*
Grade 1	4 (66.7)	2 (33.3)	6 (100)	
Grade 2	11 (45.8)	13 (54.2)	24 (100)	< 0.001
Grade 3	0 (0)	10 (100)	10 (100)	
Grade 4	0 (0)	14 (100)	14 (100)	
Total	**15 (27.8)**	**39 (72.2)**	**54 (100)**	

*Fisher exact test

Table 3: Association between Histopathological Grading and P53 Expression among Study Specimens (n=54)

Histopathological Grading	P53 Expression (Positive)	P53 Expression (Negative)	Total	p value*
Grade 1	6 (100)	0 (0)	6 (100)	
Grade 2	20 (83.3)	4 (16.7)	24 (100)	0.41
Grade 3	9 (90)	1 (10)	10 (100)	
Grade 4	14 (100)	0 (0)	14 (100)	
Total	**49 (90.7)**	**5 (9.3)**	**54 (100)**	

*Fisher exact test

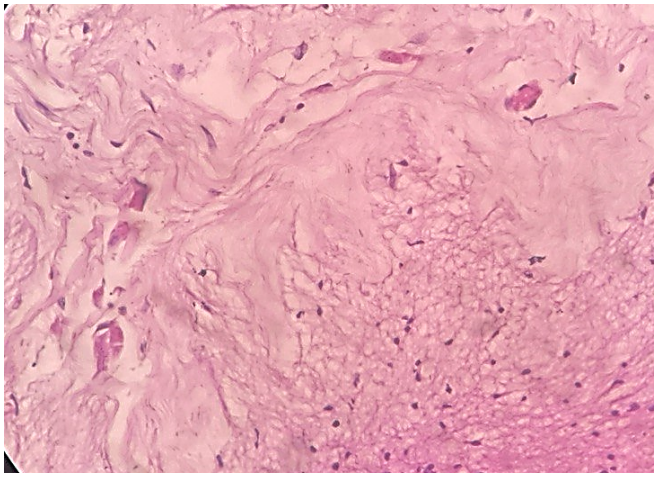

Figure 1: H&E - Pilocytic astrocytoma showing Rosenthal fibres (40X magnification).

Discussion

In our study 42.5% of specimens were from age group 21-40 followed by 29.7% in age group 41-60, 18.5% in age group 1-20 and 9.3% in age group 61-80. The mean age of the study participants was 37.8 (SD-15.3) while the age range of the study participants was between 4-75. Similarly, in a study by Sharma S [17] et al. in Rajasthan among 77 cases of astrocytomas and oligodendrogliomas age range was 4 to 71 years with a mean age of 39.84 (SD-15.84) which was almost identical to our results. In a study by Chatterjee D [15] et al. in Chandigarh among 44 primary astrocytoma cases mean age was 46.4 years with a range of 11-68. From these studies, we can understand that the incidence of astrocytoma is not specific to any age group [18, 19, 20].

In our study among 54 specimens of astrocytomas, 57.4% were from male patients and 42.6% were from female patients. In the study by Chatterjee D [15] among 44 primary cases of gliomas 65% of the subjects were males and 35% were females.

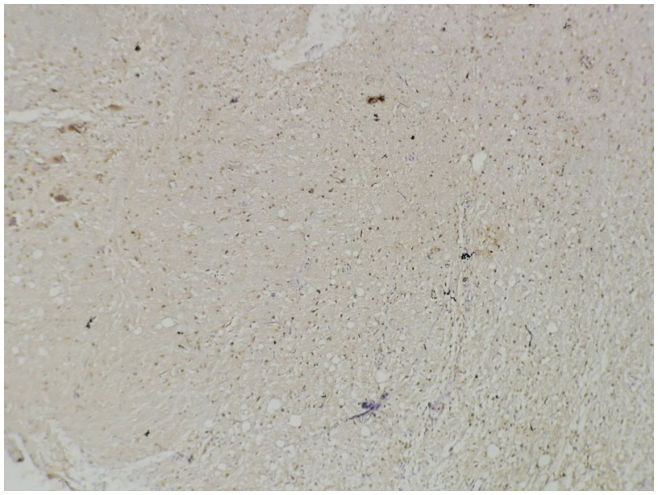

Figure 2: ATRX IHC - Retained expression in tumor cells of pilocytic astrocytoma (10X magnification).

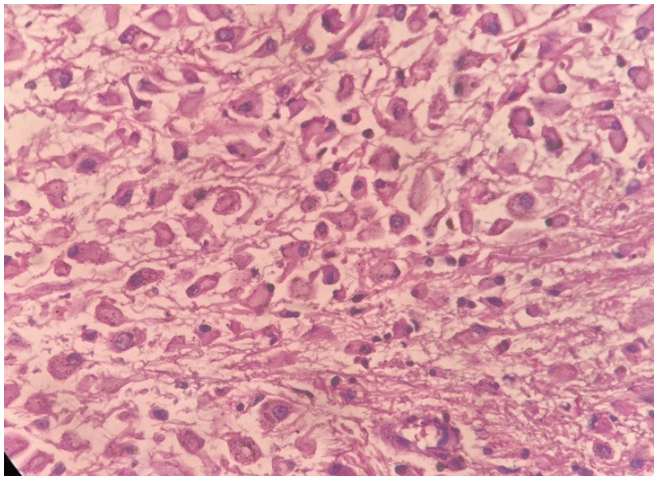

Figure 3: H&E - Gemistocytic astrocytoma (40X magnification).

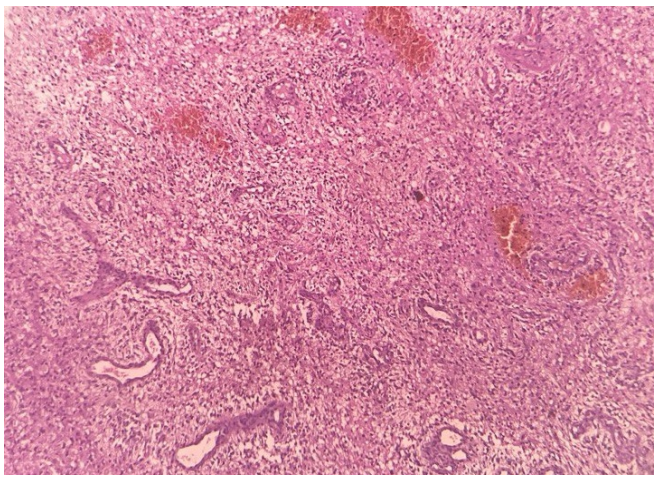

Figure 4: H&E - Astrocytoma grade 4 showing microvascular proliferation (10X magnification).

In the study by Sharma S [17] among 77 cases of astrocytomas and oligodendrogliomas, 47 (61.03%) were males and 30 (38.96%) were females. From the above studies, we can find that astrocytomas were slightly more common among males compared to females. But we cannot conclude this because the aim of the studies was different and not focused on gender comparison.

In our current study, half of the astrocytoma specimens were from the left side of the brain and 38.8% of them were from the right side of the brain. Specimens from intramedullary and intraventricular areas were 3.8% each while specimens from insular area and CP angle were 1.8% each. Inskip PD [22] et al. compared the laterality among 489 gliomas and found that

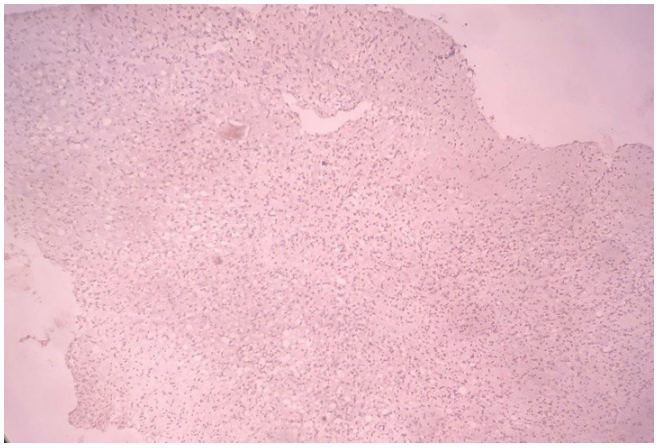

Figure 5: ATRX IHC-Loss of expression in tumor cells of astrocytoma grade 4(10 x magnification).

low-grade gliomas occurred more often on the left side and high-grade gliomas on the right side. Ilgren EB [23] et al. found among 112 cases of cerebellar astrocytomas there was no laterality difference in the occurrence of these tumours. Literature review showed that laterality of astrocytomas is not considered as a significant factor as almost all studies had not mentioned anything about the laterality of the tumours in their results.

We found that among our study specimens, the frontal lobe was the most common involved lobe (25.9%) followed by the temporal lobe (18.5%). Around 14.8% specimens were from the frontotemporal region and another 12.9% were from the parietal lobe. Similarly, Sharma S [17] et al. from Rajasthan in their study among 77 cases of gliomas found that, the most common site for glial tumors was the frontal lobe (23.4%), followed by temporal, parietal, and frontoparietal (10.4%) each. In a study by Chatterjee D [15] et al. in Chandigarh among 44 primary astrocytoma cases they found that 67% of them were located in the frontal lobe. These various studies show that the frontal lobe is the most common site for the occurrence of astrocytomas.

Histopathological grading of specimens from the current study showed that 44.5% of them were grade 2 followed by 25.9% in grade 4, 18.5% in grade 3 and 11.1% in grade 1. Chatterjee D [15] et al. in their study among 80 cases of astrocytoma in Chandigarh found that 34% were Grade 1, 43.7% were Grade 2 and 22.3% were Grade 3. Another study by Sharma S [17] among 55 astrocytoma cases found that 12.7% of their specimens were in Grade 1, 27.3% in Grade 2, 7.3% in Grade 3 and 52.7% in Grade 4. As WHO had changed the grading in 2016 and again 2021 comparing the results of our study with studies done before the changes is difficult.

In our study, we found that among 54 specimens of clinical and radiological evidence of astrocytoma, 39 had lost Atrx expression (72.2%) while the remaining 15 (27.8%) retained ATRX expression. Inclusion criteria of our study is 54 cases of clinically and radiologically suspected astrocytomas, after histopathologic examination and doing ATRX immunohistochemistry which also comprises of tumors other than astrocytomas such as oligodendroglioma (20.4%) and glioneuronal tumor (1.8%). In the study by Chatterjee D [15] et al. in Chandigarh among glioblastoma patients found that among astrocytoma specimens 61.8% had lost ATRX expression. Another study by Sharma S [17] among 55 astrocytoma cases in Rajasthan found that 36 (65.4%) had lost ATRX expression. But Shao LW [27] et al. in their study among 135 astrocytoma patients in China performed immunohistochemistry for 130 specimens and found that only 18.6% had lost ATRX expression. Various studies had showed different proportion of ATRX loss among astrocytoma cases which may be due to demographic change or the grades of astrocytomas included in the studies [25].

Among the 54 study specimens in the current study, it was found that 90.7% were positive for P53 expression while the remaining 9.3% were negative. Another study by Abdel-Maqsoud R [21] et al. found among 45 samples of astrocytoma that P53 mutation was present in the nucleus of 38 samples (84.4%). Noor H [28] et al. in a study done in Australia among 102 cases of gliomas found that among 51 cases of astrocytomas 31 (60%) were positive for P53 mutation. Thus the proportion of P53 mutation in astrocytomas varies in different studies but studies [17] had suggested that they may play a role in malignant progression of astrocytomas [26].

In our study we compared the histopathological grading and ATRX expression among 54 astrocytoma specimens and observed that as the grading increases ATRX expression was lost. Grade 3 and grade 4 specimens had 100% loss of Atrx expressions while in Grade 2 specimens 54.2% had lost Atrx expression and in Grade 1 specimens only 33.3% had lost. This difference in Atrx expression among different histopathological grades was also statistically significant ($p < 0.05$). Sharma S [17] et al. in a study among 55 specimens of astrocytomas found that ATRX loss was significantly associated with the grade of astrocytomas. They found that ATRX loss was found in 0% of Grade 1, 86.7% of Grade 2, 75% of Grade 3 and 34.4% of Grade 4 astrocytomas ($p < 0.001$). Chatterjee D [15] et al. (2018) in a retrospective study among specimens from

80 astrocytoma patients from a tertiary care institute in Chandigarh, analyzed for IDH1 mutant protein, ATRX, p53, and BRAF using immunohistochemistry. ATRX loss was observed in 0% of Grade 1, 87% of Grade 2 and 100% of Grade 3 specimens. Liu J [16] et al. in a study to find association between TERT and ATRX mutations in glioma patients from a tertiary care hospital in China found that ATRX loss was observed in 55.3% of Grade II, 55.9% of Grade III and 72.5% of Grade IV gliomas.

In our study when comparing P53 mutation and histopathological grading no statistically significant difference was observed. Similarly, Chatterjee D [15] et al. (2018) in a retrospective study among specimens from 80 astrocytoma patients from a tertiary care institute in Chandigarh found no significant difference between P53 mutation and histopathological grading. They observed that P53 mutation was absent in all Grade 1, 44% of Grade 2 and 80% of Grade 3 astrocytomas (p-value-0.64). Pardo Fs et al. [24] in study among 74 astrocytomas from Massachusetts General Hospital found that among Grade 1 and 2 cases P53 mutation was observed in 13.7% of specimens and 71.1% of Grade 3 and 4 specimens. It was also statistically significant ($p < 0.01$).

Conclusion

Based on our study we can conclude that ATRX expression is an important IHC marker in astrocytomas and complete loss of which is associated with higher grades. So this can be used as a prognostic marker in astrocytoma cases. In our study, p53 mutation was present in 90.7% of cases. As per WHO, P53 mutations in IDH mutant astrocytomas may enable tumor cell survival in the setting of ATRX loss. Hence diffuse P53 immunopositivity can be used as a surrogate marker for TP53 mutation and support in diagnosis of IDH mutant astrocytoma.

Limitations:

- IDH immunohistochemistry was done only for IDH1 R132H isoform and the other isomers were not included.
- Our study is based on immunohistochemistry but molecular diagnostics are required for confirmation which was not done in our study.
- Further studies incorporating both molecular and immuno-histochemical methods can help us to understand more about the association of these markers with disease prognosis.

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Competing Interests: The authors declare no conflicts of interest.

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