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IMMUNOHISTOCHEMICAL STUDY OF GFAP, IDH1, ATRX IN GRADING ASTROCYTIC TUMORS OF CENTRAL NERVOUS SYSTEM.

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ABSTRACT Primary brain tumors are among the top ten causes of cancer-related deaths in the world. The incorporation of molecular subtyping in glioma patients in 2021 WHO classification, there is a need to understand the immunohistochemistry (IHC) marker expression in various Astrocytoma patients and to clinically correlate them into various subgroups. The judicious use of a panel of selected immunostains is helpful in the selection of appropriate therapy for the individual patient. **Material And Methods:** A retrospective study of 30 Astrocytoma cases of our institution . IHC markers (GFAP, Isocitrate dehydrogenase [IDH] 1, ATRX antibody) were done in cases were histopathology diagnosis were inconclusive. **Results:** GFAP mutation is positive in all astrocytic tumors. IDH1 mutation is frequent in Grade 2 and Grade 3 tumors of Astrocytic tumors. ATRX mutation is specific to astrocytic lineage, Grade 2, Grade 3, and GBM patients. **Conclusion:** Molecular nature of DA and AA cases can be accurately confirmed by combined IDH1 and ATRX IHC thereby avoiding costly investigations such as fluorescence in situ hybridization. In astrocytic tumors, p53 can act as a surrogate marker. IDH-mutant glioma patients have better prognoses than IDH wild gliomas.

KEYWORDS : Astrocytomas, IHC, GFAP, IDH1, ATRX

INTRODUCTION

The primary solid tumors of the central nervous system are Astrocytomas that are classified by their genotypic and phenotypic characteristics, according to the World Health Organization[1]. Men are more prone to have malignant CNS tumors. Biomarkers are examined to identify healthy, pathological processes and pharmacological responses to a treatment intervention[2]. GFAP is the most commonly employed marker in diagnostic neuro-oncology. Positive expression to GFAP has been demonstrated in astrocytomas. Isocitrate dehydrogenase 1 (IDH1), an enzyme of lower-grade gliomas linked with prognosis. Any distinct subtype of Astrocytoma is indicated by the expression of IDH1 mutations and ATRX-inactivating mutations combined. IDH1 mutations are common and early genetic alterations in grade II and III Astrocytomas as well as secondary glioblastomas, and they serve as a significant biomarker with diagnostic, prognostic, and predictive significance. As a result, the advent of molecular characterization, which enables the treatment of astrocytomas as distinct entities, will enable the creation of more specialised therapeutics for each subtype.

MATERIALS AND METHODS

A retrospective study is undertaken in department of Pathology, Dr.Pinnamaneni institute of medical sciences and research foundation from January 2022 to December 2022. Thirty cases were included whose histopatholological findings were suggestive of Astrocytomas and who underwent surgery at our institution. Hematoxylin and eosin-stained slides were evaluated and the diagnosis was confirmed as per the WHO classification of CNS tumors 2021. All samples were fixed in 10% neutral buffered formalin and paraffin embedded. IHC staining was done using one representative block in all cases. It was performed using primary antibody against the antigens for GFAP, IDH1, ATRX. Positive control was run with each batch of IHC staining. All the procedures were done as per standard protocols.

GFAP expression was determined using IHC protein GFAP (RP014 concentrated) GFAP staining is Cytoplasmic in glial cells.

IDH-R132H mutation was determined using IHC of mutation-specific anti 1DH antibodies (Mob580R)(PDM580R). Cases showing cytoplasmic positivity for IDH1 in >10% tumor cells were considered positive and labeled as IDH-R132H mutant.

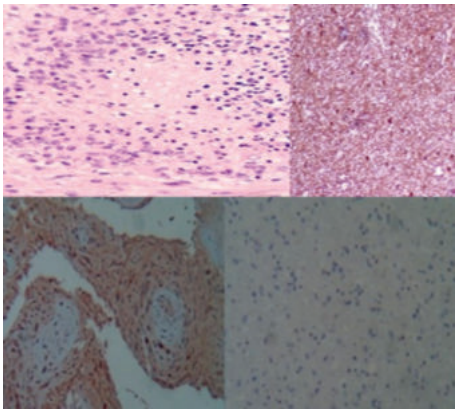
ATRX mutation was determined by IHC using polyclonal antibody (HPA001906-100 UL). Near total loss of nuclear staining for ATRX in tumor cells (>90%) was considered positive for ATRX mutation.

RESULTS

A total of 30 glioma patients were stained for IHC markers GFAP, IDH1-R132H, ATRX antibody. In our study we found a definite male preponderance of 2:1. Most of the cases occurred in 41-50 years age group. Pilocytic astrocytomas were mostly occurred between 4-20 years, whereas glioblastomas were most common in 40-50 years. The most common clinical presentation was seizures, headache in 43.3% of cases.

Most common histological type was Glioblastoma multiforme(46.6%) followed by diffuse astrocytoma(30%). All pilocytic astrocytomas were negative for IDH1 mutation. IDH1 mutation was detected in 100% (9/9) DA and 75% (3/4) AA cases. Glioblastomas showed 64.2% postivity in (9/14) cases. IDH1 R132H was the commonest IDH1 mutation (70%). Loss of nuclear ATRX expression was found in 100% (9/9) and 75% (3/4) DA and AA cases, respectively. Glioblastomas showed 12/14 cases (85.7%) with ATRX loss. IDH1 mutant DA patients had longer overall survival than IDH1 wild type cases.

Tumor Type	GFAP (%Postivity)	IDH (%Postivity)	ATRX LOSS (%Postivity)
Pilocytic Astrocytoma	100%	NIL	100% (3/3)
Diffuse Astrocytoma	100%	100%(9/9)	100% (9/9)
Anaplastic Astrocytoma	100%	75%(3/4)	75%(3/4)
Glioblastoma	92%(13/14)	64.2%(9/14)	64.2%(9/14)



H&E Image Of Glioblastoma At 40x Mag, Image Showing Cytoplasmic Postivity For Gfap At 40x Mag, Image Showing Idh1r132h Mutated Protein Identified In Cytoplasm Of Glioblastoma Cells At 40x Mag.

DISCUSSION

Astrocytomas are the most common tumors of CNS in children, and adults . The astrocyte lineage comprises of Pilocytic astrocytoma (PA), Diffuse astrocytoma (DA), Anaplastic astrocytoma (AA), and Glioblastoma multiforme (GBM).

Tumor markers of Astrocytomas have both diagnostic and prognostic importance.

Based on the results of these biomarkers, various clinical decisions are being made whether for screening, diagnosis, treatment, or prediction monitoring.[6]

For diagnosis of Astrocytomas, there are certain molecular parameters which include isocitrate dehydrogenase (IDH) 1-R132H mutation, ATRX mutation, and 1p/19q co-deletion. The commonly used IHC markers in Astrocytic tumors are IDH, ATRX, P53, and Ki-67 antibody index.[7]

Glial fibrillary acidic protein (GFAP) is an intermediate filament protein of 52kD found in glial cells such as astrocytes and ependymal cells[9]. GFAP expression is seen in all astrocytomas. Glial filament expression had been found in all tumor cells of glial origin and in tumor cells with foci of glial differentiation arising within CNS. No glial immunoreactivity was observed outside the CNS[9]. GFAP immunoreactivity is observed in all grade I and II astrocytomas, the GFAP expression may be negative in grade III.

Anaplastic astrocytoma (AA) and DA have identical IHC profile which is characterized by ATRX and IDH mutation.[10]

IDH is well-established molecular marker in both oligodendroglial and astrocytic tumors. IDH1 mutations are seen in a high frequency in grade II and III astrocytomas and oligodendrogliomas and secondary GBM tumors. IDH-1 mutations are observed in more than 80% of secondary glioblastomas, whereas they are rare in primary glioblastomas. IDH mutation is an early event in glioma formation and occurs prominently in low grade tumors of both astrocytic and oligodendroglial lineage. Although research on glioma with an IDH1 mutation has received more attention, multiple studies have shown that primary glioblastoma overexpresses wild-type IDH1. Importantly, these investigations demonstrate that IDH1 overexpression is associated with poor overall survival in more than 60% of patients with primary glioblastoma.[7] The frequent occurrence of IDH1 mutations in secondary glioblastomas and their complete absence in primary glioblastomas emphasise the concept that inspite of their histological similarities, these subtypes are genetically and clinically distinct entities.[8,10]

Garrett M et al. found that in IDH1 wild-type glioblastomas were vulnerable to de novo nucleotide synthesis inhibitors, and IDH1 mutant glioblastomas were able to repair their DNA after radiation. Small retrospective series have suggested that the response rate to chemotherapy is also higher in IDH mutated grade 2 tumors than in wild-type tumors and that progression free survival after radiation or alkylating chemotherapy is higher for people with IDH-mutated tumors than for people with wild type tumors.[7,9]

ATRX gene mutation can be identified by immunohistochemistry as loss of nuclear ATRX expression. ATRX mutation is frequent in astrocytic lineage. It occurs predominantly in Grade 2 and Grade 3 astrocytic lineage glioma, i.e., DA and AA IDH and ATRX mutations are rarely in glioblastomas (GB) and pilocytic astrocytomas (PA)[10].

In our study, all astrocytic tumors were reactive for GFAP showing 100% positivity. Goyal R et al[11] observed wide spread GFAP positivity in all primary astrocytic tumors.

In our study, Pilocytic astrocytoma showed no IDH1 postivity, Out of 9 cases of DA, all of the cases were positive for IDH1. In our study, we had 4 cases of AA. Out of which, 75% (3/4) cases were positive for IDH1. In our study, we had 14 cases of glioblastoma. Out of which, 9 cases (64%) were positive for IDH1,. Sarma S et al.[12] studied on 53 glioma cases and observed that IDH1mutation (IDH1 positivity) in 8/9 DA (88.8%), 4/5 AA (80%), and 12/22 GBM (55%). Chatterjee D et al[13] studied on 100 astrocytoma cases and observed that IDH1 postivity in 80%(20/25) cases of DA, 86.7% (13/15) cases of AA, 100% (1/1) case of secondary GBM was positive for IDH1, whereas PA and primary GBM was negative for IDH1.

In our study, Pilocytic astrocytoma showed no ATRX postivity, Out of 9 cases of DA, all of the cases showed ATRX mutation . In our study, we had 4 cases of AA. Out of which, 75% (3/4) cases were positive for ATRX . In our study, we had 14 cases of glioblastoma. Out of which, 9 cases (64%) were positive for ATRX. Sarma S et al.[12] studied on 53 glioma cases and observed that ATRX mutation (loss of staining) was observed in 9/9 DA (100%), 4/5 AA (80%), and 12/22 primary GBM (55%). Chatterjee D et al[13] studied on 100 astrocytoma cases and observed that ATRX loss was observed in 87%(20/23) cases, 100% (14/14) cases and 100%(1/1) loss in secondary GBM, whereas PA and primary GBM was negative for ATRX.

In our study, it was found that mutant IDH1 patients had better prognosis in term of overall survival in both DA and AA case. Among Grade 2 and Grade 3 tumors, Grade 2 tumors had better prognosis in our study, consolidating the importance of grading on the basis of histomorphological features.

CONCLUSION

The molecular IHC characterization with immunomarkers GFAP, IDH1-R132H, ATRX, predicted that the low Grades 1 and 2 gliomas with DAs, pilocytic astrocytoma had best prognosis, followed by molecular group of AAs and glioblastoma who had poor results. Molecular nature of DA and AA cases can be determined with great accuracy by combining IHC analysis of IDH1 and ATRX hence, reducing the need of costly investigations like FISH. All investigations, however, show that IDH1 mutant and IDH1 wild-type gliomas have different metabolic characteristics, indicating that they may have unique vulnerabilities indicating individualized treatment. Regardless, this classification scheme may be able to accurately predict the molecular subgrouping of all astrocytoma cases.

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Conflicts of interest
There are no conflicts of interest.

ABBREVATIONS

PA- Pilocytic Astrocytoma
DA- Diffuse Astrocytoma
AA- Anaplastic Astrocytoma
GBM- Glioblastoma Multiforme

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