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Subgroup stratification of adult diffuse gliomas and outcomes: an adaptation of the updated WHO classification in a resource-constrained environment

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Original Article

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Abstract

Introduction: The updated World Health Organization 2016 classification of central nervous system tumours recommends the addition of molecular parameters to histological diagnosis. In a resource-constrained setting, molecular testing such as gene sequencing and fluorescence in situ hybridisation is not feasible for all the patients. We assessed the utility of immunohistochemistry (IHC) for isocitrate dehydrogenase (IDH1/R132H) gene and alpha thalassemia/mental retardation syndrome X linked gene (ATRX) to stratify adult diffuse gliomas into subgroups and analysed the outcomes.

Materials/Methods: Fifty-eight patients with grades III/IV astrocytic gliomas were tested by IHC for IDH1/R132H and ATRX mutation as per the standard protocol and were later stratified into three subgroups based on IHC. IDH1/R132H positive/ATRX retained gliomas were stratified as group 1 (G1), IDH1/R132H positive/ATRX lost were grouped as G2 and IDH1/R132H negative (with or without ATRX loss) as G3. All patients underwent adjuvant therapy as per the Stupp regimen. Outcomes and survival were analysed by Kaplan–Meier analysis using SPSS 21.v.

Results: Median age of the cohort of 58 patients (male: 39, female: 19) was 40 years. Histologically, glioblastoma multiforme (GBM), anaplastic astrocytoma (AA) and anaplastic oligodendroglioma (AOD) were seen in 23:17:18 patients. Forty-eight percent were tested positive for IDH1/R132H, 62% had retained ATRX protein stratifying patients into three subgroups (G1:14, G2:14, G3:30). The G3 group contained both AA and GBM cases. At median follow-up of 18 months, overall survival (OS) of the entire cohort was 76%, higher in G1, compared to G2 and G3 (log-rank $p = 0{\cdot}01$). In comparison to various factors such as age, gender, location of the lesion and presenting symptom on survival among various groups, we found that gender of the patient in group I (men vs. women, $p = 0{\cdot}02$), laterality of the tumour in group II (right vs. left, $p = 0{\cdot}07$) and age of the patient in group III (<45 vs. >45, $p = 0{\cdot}01$) demonstrated significant impact on OS.

Conclusion: Subgroup stratification of adult diffuse gliomas based on IHC for IDH/R132H and ATRX demonstrates that group 1 was the most favourable prognostic factor. In a resource-constrained environment, IHC alone may guide appropriate management decision for the majority of adult diffuse gliomas, gene sequencing reserved for IDH1/R132H negative GBM in patients less than 45 years of age.

Introduction

The World Health Organization (WHO 2016) classification of central nervous system (CNS) tumours has now included an algorithm for diffuse glioma classification based on three clinically relevant biomarkers, namely isocitrate dehydrogenase (IDH 1/2) gene mutations, α thalassemia/mental retardation syndrome X-linked gene (ATRX) mutation and 1p/19q co-deletion.[1,2] Based on this, oligodendrogliomas and anaplastic oligodendrogliomas (AODs) are defined by IDH1/2 mutations and 1p/19q co-deletion. Diffuse astrocytomas and anaplastic astrocytomas (AAs) lack the 1p/19q co-deletion and demonstrate ATRX loss often with p53 immunopositivity.

Immunohistochemistry (IHC) can be performed for the most common IDH mutation, that is, for IDH1 (R132H). DNA sequencing is necessary for the rare IDH1/2 mutations whenever IDH1 (R132H) is negative on IHC. The tumours that are negative for IDH1/2 mutations by the above-mentioned techniques are the IDH wild-type (IDH wt) tumours. 1p/19q co-deletion status is mostly assessed by the fluorescence in situ hybridisation (FISH) technique. There is available literature in the Indian context, demonstrating that histo-molecular diagnosis and subgroup stratification of anaplastic gliomas are of prognostic significance and superior compared to WHO histological diagnosis.[3] However, in a resource-constrained setting, gene

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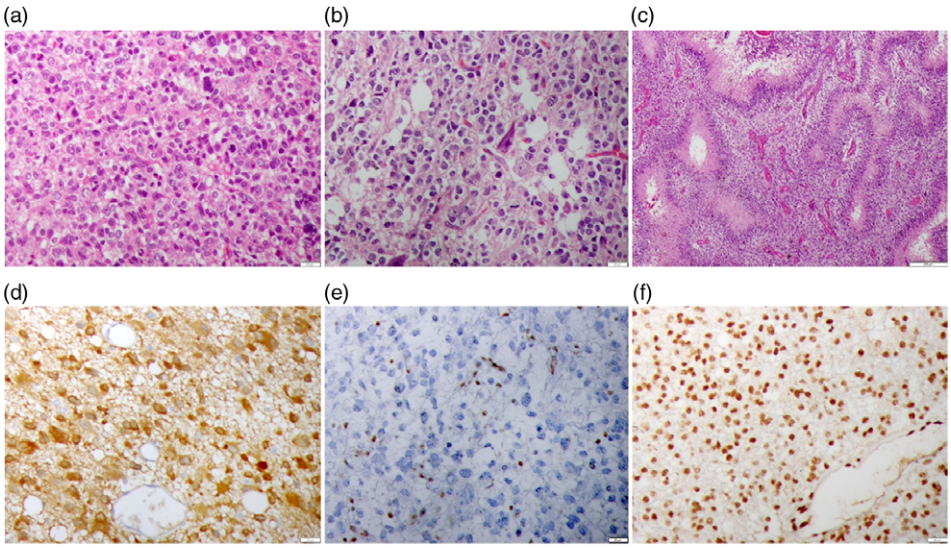

Figure 1. Histology and IHC characteristics of high-grade glioma.

sequencing and FISH testing are not feasible for all the patients and IHC is a methodology which can be carried out in most laboratories.

Our study was conducted to prospectively evaluate the prognostic significance of mutations of IDH and ATRX, tested only by IHC in anaplastic gliomas. Our study emphasises that sub-stratification of anaplastic glioma patients based on histology and IHC has value in prognostication and guiding adjuvant therapy.

Methods and Materials

Between October 2014 and October 2016, 58 patients who were for radical intent of therapy with histological diagnosis of anaplastic glioma (WHO Grade III/IV) after having maximal safe resection underwent adjuvant therapy after a prior informed consent. Histological diagnosis was further supplemented with IHC for IDH R132H and ATRX expression in IHC. Patients underwent adjuvant therapy as per routine protocol. The outcomes of these patients were analysed based on the stratification of the cohort into three groups based on the IHC positivity of IDH and ATRX. Group I comprised patients with IDH1/R132H immunopositivity and ATRX retained anaplastic gliomas. Group II comprised patients with the tumour showing IDH1/R132H immunopositivity and loss of ATRX expression. Group III included patients with IDH1/R132H negative anaplastic gliomas (with or without ATRX loss).

Histopathology and IHC testing

After the routine morphology-based histological classification as AOD, AA and glioblastoma multiforme (GBM), the terminology of oligoastrocytoma is removed in the recent WHO 2016 classification. IHC was carried out using antibodies against IDH1 (R132H) and ATRX. This was performed on formalin fixed paraffin embedded tissue sections using the Ventana Benchmark automated staining system (Ventana Benchmark-XT, Oro Valley, Arizona, USA). The primary antibodies used were as follows: anti-IDH1 (R132H) antibody (1:40, H09; Dianova, Hamburg, Germany), anti-ATRX (1:300, polyclonal; Sigma, St. Louis, Missouri, USA). Relevant positive controls and a negative control were employed with each batch of staining.

Figure 1 depicts histology and IHC characteristics of high-grade glioma. AA showing increased cellularity and mitosis (a), AOD showing increased mitosis (b), GBM showing prominent palisading necrosis (c). Examples of tumours showing IDH1R132H immunopositivity (d), ATRX loss of expression (e) and ATRX retained expression (f) (figure magnification is indicated in the scale bar of each figure).

Radiotherapy and temozolomide planning

All these patients were planned for adjuvant therapy as per the Stupp protocol.[4] Target volume delineation was performed as per European Organization for Research and Treatment of Cancer (EORTC) guidelines[5] and delivered to a dose of 59·4 Gy in 33 fractions or 60 Gy in 30 fractions based on the target volume (1·8 Gy per fraction utilised in patients with larger target volume). Patients underwent 3D-conformal radiotherapy or intensity-modulated radiotherapy based on dosimetrically ideal plan along with concomitant and adjuvant temozolomide. At each follow-up, all patients underwent clinical and neurological evaluation and gadolinium-enhanced MRI initially every 3 months for the first year and later every 6 months until the patient was alive.

Data were tabulated in SPSS version 21.0. Long-term outcomes and survival analysis were performed by Kaplan–Maier analysis and significance tested by log-rank test.

Results

Median age of the cohort of 58 patients (male: 39, female: 19) was 40 years. The most common presenting symptom was raised intracranial pressure (headache/vomiting) followed by seizure or motor deficit (50% vs. 29% vs. 21%). Histologically, GBM, AA and AOD were seen in 23:17:18 patients. IDH1R132H immunopositivity was found in 48% of the cohort and 62% had retained ATRX expression protein, stratifying patients into three subgroups (G1:14, G2:14 and G3:30). The G3 group contained both AA and GBM cases. At median follow-up of 18 months, overall survival (OS) of the entire cohort was 76% as shown in Figure 2, higher in group I compared to group II and group III ($p = 0{\cdot}01$) as shown in Figure 3.

To look for the impact of various factors on OS, we compared OS in each subgroup with various factors such as age of the patient (stratified as <45 years or >45 years), gender of the patient (men vs. women), laterality of the lesion (right sided vs. left sided, which

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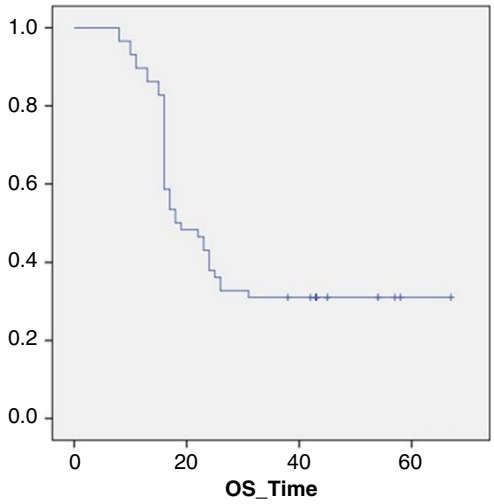

Figure 2. Overall survival (OS) of the entire cohort.

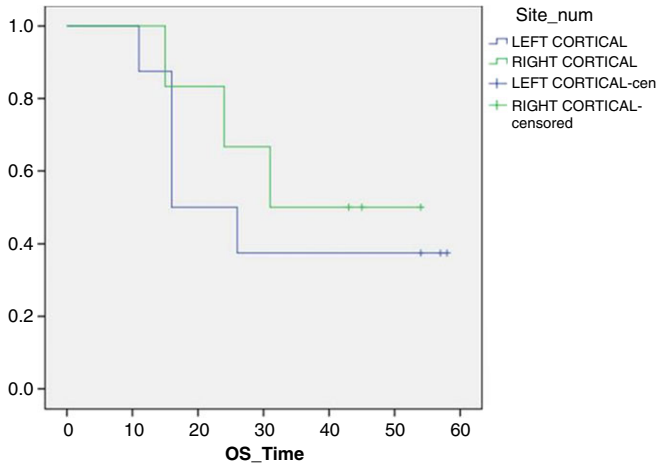

Figure 5. OS difference in group 2.

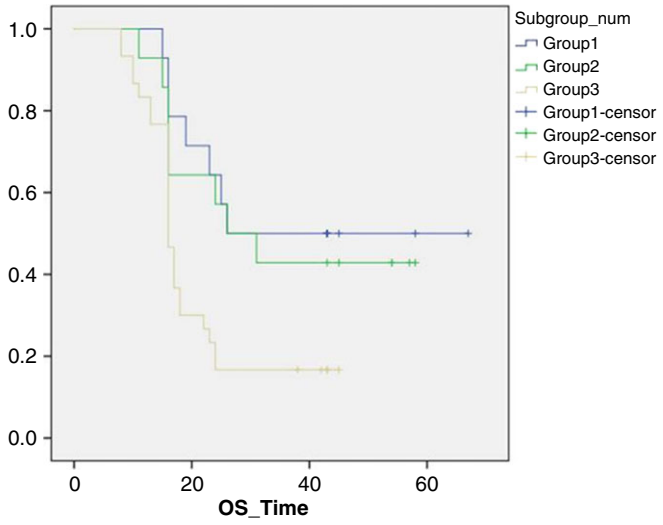

Figure 3. OS between groups.

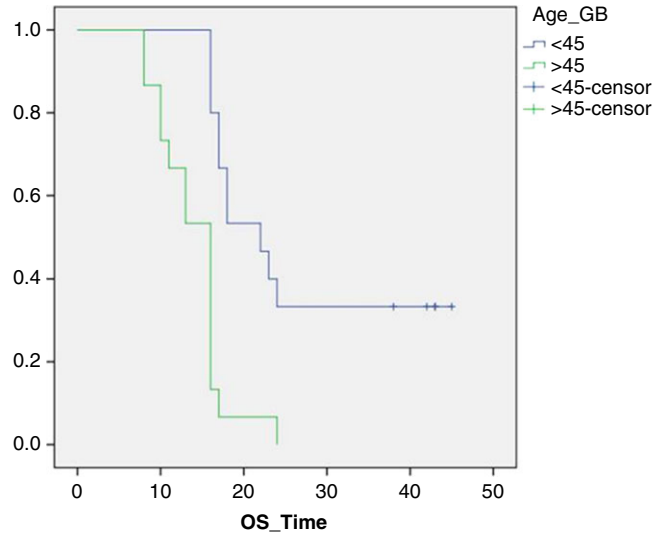

Figure 6. OS difference in group 3.

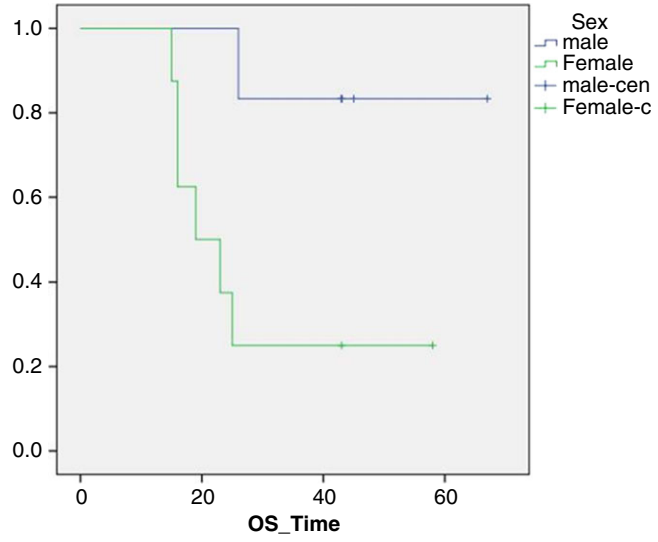

Figure 4. OS difference in group 1.

may be a factor for extent of resection) and presenting symptom (seizure at presentation vs. motor weakness). We found that gender of the patient in group I (men vs. women, $p = 0{\cdot}02$) as shown in Figure 4, laterality of the tumour in group II (right vs. left, $p = 0{\cdot}07$) as shown in Figure 5 and age of the patient in group III (<45 vs. >45, $p = 0{\cdot}01$) as shown in Figure 6 demonstrated significant impact on OS. Histomorphology of the tumours within groups did not demonstrate any survival difference.

Discussion

Mutations in IDH1 were found to be present in a large proportion of Indian patients with diffuse astrocytic and oligodendroglial neoplasms as shown by Sarkar et al., similar to the reported literature. They also demonstrated that IHC was an easy and quick method of detection of IDHR132H mutations. They demonstrated a concordance rate of 88% between DNA sequencing and IHC methods of testing IDH mutation.[6] The outcomes of ATRX mutation in patients with anaplastic gliomas recruited in NOA-04 study were

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analysed by Wick et al. and found that anaplastic oligoastrocytoma (AOA) harbouring ATRX loss shared a similar clinical course with AA, whereas AOA carrying 1p/19q co-deletion shared a similar course with AOD), thereby showing that ATRX status helps better define the clinically and morphologically mixed group of AOA.[7]

The International Society of Neuropathology–Haarlem (ISN-Haarlem) guidelines propose that the diagnosis of CNS tumours be layered with histological classification, WHO grade and molecular information in the form of an integrated diagnosis in order to optimise inter-observer reproducibility, clinic-pathological predictions and therapeutic planning.[8] In the Indian context, Rajmohan et al. and Rajeshwari et al. evaluated and demonstrated that histo-molecular diagnosis and subgroup stratification of anaplastic gliomas are of prognostic significance and superior compared to WHO histological diagnosis.[9,10]

This prospective study was conducted to emphasise and clinically prove the prognostic significance of IDH and ATRX for stratifying anaplastic gliomas into three groups: group 1 (IDH1 mutant and ATRX retained), group 2 (IDH1 mutant and ATRX lost) and group 3 (IDH1/R132H negative). Group 1 correlated with AOD phenotype, group 2 was AA and group 3 was anaplastic glioma, including AA and GBM cases. We did not perform IDH sequencing to look for other rare mutations.

Our study is unique in that all the patients were diagnosed on basic histomorphology and group stratification made upon the protein expressions, rather than DNA sequencing. All the patients were treated uniformly as per the Stupp protocol. The OS of the study cohort was on par with the available world literature. This study provides evidence that in a resource sparing setting, simple IHC itself will enable us to stratify anaplastic gliomas into groups—only for prognosis, as the adjuvant therapy will not differ despite availability of molecular testing. This generates scope for uniformity in reporting, recording and matching diagnostic and treatment standards across various centres in our country.

A possible limitation to the study is that none of the patients in the age group of <45 years underwent further molecular testing to check for possible rare mutations in the IDH gene.

Conclusion

In a resource-constrained environment, IHC alone may guide appropriate management decision for the majority of adult diffuse anaplastic gliomas, gene sequencing reserved for IDH negative anaplastic gliomas and patients younger than 45 years.

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