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IDH1R132H GENE-A DIAGNOSTIC AND PROGNOSTIC MARKER IN GLIOMAS

Pathology

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ABSTRACT

Aim: To study the frequency of Isocitrate dehydrogenase1 R132H [IDH1R132H] gene mutations in phenotypic lobar gliomas by Immuno histochemistry [IHC] and establish R-132H as a prognostic and diagnostic marker in gliomas. **Material and method:** This study was a Bidirectional correlative study on the clinical, pathological, and molecular characteristics of adult gliomas, carried out in the Dept of Laboratory sciences at Base Hospital, Delhi Cantt-110010.All consecutive (optd) cases of gliomas from 2015 to 2018 were included Immunohistochemistry was used to detect IDH1-R132H instead of sequencing, aiming to provide a mature and simple tool for clinical practice. In the light of the "ISN-Haarlem" consensus, the routine approach to all diffuse astrocytic and oligodendroglial gliomas begins with performing IHC for IDH1-R132H expression. **Results:** A negative control, in which the primary antibody was excluded was incorporated with each batch of staining. Staining intensity was graded as +2 (strong positive staining), +1 (weak positive staining) & 0 (negative/ no staining). In our study only a staining intensity of +2 ie., strong positive staining was considered to label the tumour for the biomarkers studied. The labeling index (LI) for IDH1R132H to be labeled as positive was > 10% and the tumor was considered negative for IDH1R132H if the Labeling index was <10%. **Conclusion:** It was concluded that the IDH1-R132H is necessary to provide the basic molecular information for the "integrated diagnosis" of gliomas.

KEYWORDS

INTRODUCTION

Tumours of the Central Nervous System affect individuals of all age groups and cause severe morbidity and mortality. One of the commonest brain neoplasms, the gliomas originates from the constituent glial cells of the brain, or their precursors which occupy 90% of brain tissue, have an incidence of 12 per 100,000 population and include a variety of different histological tumour types and malignancy grades(1). Among these, malignant gliomas are the most common type of primary brain neoplasms in adults and they diffusely infiltrate surrounding brain tissue, making curative surgical resection almost impossible. The currently accepted classification of these tumours is the World Health Organization (WHO) 2016. These tumours are graded on a scale of II–IV of increasing malignancy(2–5). The high-grade gliomas or anaplastic gliomas (AG; WHO grade III) and glioblastomas (GBM, WHO grade IV), have a dismal prognosis, with median survival of a few years for the former and 12-15 months for the latter(2).

The WHO 2016 classification of CNS tumors defines gliomas by both histological and molecular parameters with the generation of an integrated diagnosis based on phenotypic and genotypic features of each tumor entity. According to this classification, Isocitrate dehydrogenase (IDH) gene mutations (IDHmut) and 1p19q codeletion are tumor defining factors for gliomas. Diffuse astrocytomas (DA), anaplastic astrocytomas (AA) and secondary Glioblastomas (GBM) are defined as tumors that are IDH mutated whereas oligo dendrogliomas are defined as tumors that have both IDH mutations and are 1p19q codeleted. Given the results of molecular testing, phenotypic oligoastrocytomas, astrocytomas, glioblastomas and oligodendrogliomas remain as a 'Not Otherwise Specified (NOS)' entity.

These discoveries have led to a radical change in classification of gliomas. For the first time, the 2016 World Health Organization Classification of Tumors of the Central Nervous System, uses molecular parameters in addition to histology, to define many tumor entities and all diffusely infiltrating gliomas are classified based not only on behaviour and growth pattern, but on IDH mutations. Thus tumours in one group share prognostic markers and it guides treatment of genetically similar entities(4). Biomarkers have now become an established component of the neuropathologic diagnosis of gliomas, since molecular alterations aid in classification, prognostication and prediction of therapeutic response and Isocitrate dehydrogenase (IDH) mutations are a major discriminate of biologic class (1).

A number of methods have been tried to detect IDH1 mutations including DNA sequencing, immunohistochemistry(IHC), PCR-restriction fragment length polymorphism, Allele specific PCR, PCR-single strand conformation polymorphism, pyrosequencing and high-resolution melting curve analysis (4,5),though IHC is the most common method to detect IDH1R132H mutations. Further studies in Indian population are very few with one study also suggesting racial differences(6). Hence this study aims to study the frequency of Isocitrate dehydrogenase 1R132H [IDH1R132H] gene mutations in phenotypic adult lobar gliomas by Immunohistochemistry [IHC] in an Indian cohort"

MATERIAL & METHODS:

The sample size worked out to be 79 glioma patients, however in this study we propose to study 150 cases.The patients were selected based on the inclusion criteria: adult (> 18 years age) glioma patients operated during years 2015-2018 and have adequate amount of tissue for analysis. All consecutive 150 cases of gliomas (optd) at BHDC fulfilling all the above inclusion criteria were included in the study. Exclusion criteria was paucity of tumor tissue in the paraffin block.

Pathology review:

Hematoxylin and Eosin (H&E) stained slides and formalin fixed paraffin embedded tissue (FFPE) blocks of the cases were retrieved from the department of Laboratory sciences at Base Hospital. Slides were reviewed independently by two pathologists for confirmation of diagnosis and grading according to the current WHO 2016 classification and evaluation/ suitability/ adequacy of available tissue specimen for inclusion in the study.

Fresh specimens were fixed in 10% buffered formalin and then embedded in paraffin. Serial 5-mm sections were stained with haematoxylin and eosin (H&E) for pathological diagnosis and grading. Controls were taken from normal brain at autopsy which were received in lab. IHC was performed using standard Avidin-Biotin-Complex (ABC) method on the selected slides.IHC procedure was done as detailed below.

Preparations of poly-L-lysine coated slides: Frosted glass slides were first cleaned by placing them in acid alcohol solution (1% HCL in 70% ethanol) overnight. Poly-L-lysin solution available as 0.1% w/v solution was diluted 1:10 with deionized water and stored in Coplin's jars (for a maximum of six months). The cleaned air-dried slides were placed in the lysine solution at room temperature for 5 to 10 minutes. The slides were then drained and dried in 60degreeC oven for 1 hour. 4µm sections from the selected blocks were taken on 0.01% poly-L-lysine coated frosted slides. Sections were dewaxed in hot air oven overnight followed by two changes in xylene, each lasting for 10 minutes and dried. They were then treated with decreasing grades of alcohol (100, 90, 70%) each for 5 minutes and then placed in running water for 10 minutes. Endogenous peroxidase blocking was done with 3% hydrogen peroxide solution in methanol for 20 minutes. Antigen Retrieval- antigens in the tumor get masked/ blocked during the process of tissue processing. We performed antigen retrieval by using standard technique as recommended by the manufacturer of the

primary antigen. Antigen retrieval for IDH1R132H– was done by by boiling in a stainless steel pressure cooker for about 15 minutes in EDTA TRIS-buffer prepared at a pH of 7.4. Antigen retrieval was done at pH of 7.6 in TRIS-buffer by boiling in a stainless steel pressure cooker for about 15 minutes.

After the antigen retrieval, the slides were allowed to cool on their own. They were then flooded with the wash buffer, which we used as TRIS buffer solution (TBS) for 10 minutes. Slides were then dried at the edges with filter paper carefully to avoid wiping off the section. Then the respective primary antibody was added and kept for one hour.

Primary antibody

Monoclonal Mouse anti-human DIA-H09 antibody in a dilution of 1:20. Slides were washed with TBS two times for 5 minutes. Secondary antibody i.e biotin labelled goat anti-rabbit and goat anti-mouse Immunoglobulin's (DAKOCYTOMATION) was added for 30 minutes. Slides were washed with TBS two times for 5 minutes. Then slides were dried. Horse-Radish-Peroxidase (DAKOCYTOMATION) linked Streptavidin was added for 30 minutes. Slides were washed with TBS two times for 5 minutes. Then sections were treated with substrate i.e. DAB (3, 3-diaminobenzidine in substrate buffer) for 20 minutes with constant monitoring to prevent over staining. Slides were washed with de-ionized water two times for 5 minutes. Slides were counter stained with Hematoxylin for 5 minutes followed by bluing in running water for 10 minutes. Sections were dried; dehydrated in graded alcohol, cleaning done in xylene and mounted.

RESULTS:

The study cohort comprised 150 patients with lobar Gliomas with a male to female (gender) ratio of (1.67:1) and age ranging from 19 to 78 years with a mean value of 45.96 years and median as 46 years. To test the impact of IDH1-R132H on routine diagnostic neuropathology, we analyzed a series of 150 cases of glioma tissues, including 88 cases of GBM, 23 cases of DO, 19 cases of AA,16 cases of DA,04 cases of AO.

In our study, 58.7%cases were alive cases, 34.7% cases were dead and 6.7%cases were lost to follow up. Out of total cases, 62% were IDH1R132H positive and 38% were negative. Out of 93 IDH1R132H positive cases 85 cases were alive and 3 cases were lost to follow up. 57 IDH1R132H negative cases showed poor survival with 47 dead cases,03 alive cases and 07 cases were lost to follow up The majority of positive cases demonstrated a strong perinuclear cytoplasmic staining.

Response to chemotherapy was seen in 52% IDH1R132H positive cases.79% IDH1R132H Positive cases showed no recurrence post surgery whereas 41% IDH1R132H negative cases showed post surgery recurrence.

Total number of cases found IDH1R132H positive-93, negative-57 Total number of deaths in IDH1R132H positive cases-05, negative cases-47(fig-1)

In our study, IDH1R132H positive cases showed better survival. 88 cases out of 90 were alive. This correlation was significant with (p<-0.005). Fig 2 shows correlation between IDH status and survival

Out of 88 cases of GBM, only 37 were IDH1R132Hpositive, Out of 23 cases of DO, 22 cases were IDH1R132H positive, Out of 19 cases of AA, 16 cases were IDH1R132H Positive, Out of 16 cases of DA,15 cases were IDH1R132H Positive, Out of 04 cases of AO,3 cases were IDH1R132H Positive. This correlation is highly significant with p value=0.00 as calculated by Pearson's Chi-square test. Fig-3 & fig -4 shows the correlation.

Mean age group with IDHR132H positivity was between 32-49 years This correlation was significant (p value=0.00) as calculated by Spearman correlation coefficient. Fig -5 shows correlation of IDH1R132H with age

Most common presenting symptoms with IDH1R132H positivity was raised ICT and seizures was found to be most common in IDH1R132H negative cases. This correlation was found insignificant with p value =0.020. Fig-6 shows this correlation

61IDH1R132H positive cases found on right side. 32 IDH1R132H positive cases found on left side. This correlation was found to be insignificant as (p>/0.005). Fig7 depicts this correlation

In our study, 10 cases were lost to follow up, out of which 7 were IDH1R132H Positive and 03 cases were negative. Out of 88 alive cases, 85 were IDH1R132H positive .This correlation came out to be statistically significant with a p value=0.00 as calculated by Pearsons Chi-square test. Fig-8 depicts the correlation.

52% of IDHR132H positive cases were given chemotherapy only, 7%cases were given radiotherapy only and 34% were given radiochemotherapy. 37% of IDH1R132H negative cases were given radiotherapy only, 16% were given chemotherapy and 05% were given radiochemotherapy. This correlation is statistically significant with p value=0.00 as calculated by Chi-Square test. Fig-9 shows this correlation

Cases with IDH1(R132H) positivity showed less recurrence post surgery as compared with IDH1(R132H) negative cases.79% IDH1R132H Positive cases showed no recurrence post surgery 41% IDH1R132H negative cases showed post surgery recurrence.This correlation was found to be significant with p value=0.00. Fig-10 depicts the correlation

DISCUSSION

It is accepted that *IDH1R132H* mutations are used as diagnostic and prognostic biomarkers for molecular classification of gliomas [4].However,very few studies have been done to study the frequency and distribution of adult lobar gliomas in an Indian cohort.

IDH mutation is thought to be an early if not the initial event in the development of low-grade astrocytomas and oligodendrogliomas. In support of this hypothesis, *IDH* mutation is found in secondary glioblastomas but rare in primary glioblastomas (7). *IDH1* mutations have been shown to alter the enzymatic activity of the encoded protein, leading to up-regulation of hypoxia inducible factor-1α (HIF-1α)(3,8). HIF-1α plays an important role in the process of angiogenesis while also supporting tumor cell survival and proliferation(2,3). Although the properties of the *IDH1* mutation would promote tumor growth, *IDH* mutation commonly indicates a favorable prognosis independent of WHO grades(4).

IDH1-R132H status is essential to the diagnosis for gliomas. *IDH1R132H* mutations dominate in WHO grade II/III gliomas (also called as lower grade gliomas) and secondary GBM. According to several studies, IDH1–R132H is the most common mutation (90%). The frequency of *IDH* mutations is rare in primary GBMs that have *TERT* promoter mutations, *EGFR* alteration and *PTEN* loss. Yao et al. analyzed *IDH1* and *IDH2* status at codon 132 of *IDH1* and codon 172 of *IDH2* by direct sequencing and anti-IDH1-R132H immuno histochemistry in 53 paired samples and their recurrences(9). The "Haarlem Consensus Guidelines for Nervous System Tumor Classification"(10) suggest that some entities will require molecular information to provide an "integrated" diagnosis, which is based on several layers comprising (i) the integrated diagnosis as top layer, followed by (ii) histological classification, (iii) WHO grade, and (iv) molecular information. In the present study, we illustrated an evaluation formula for the evolution of gliomas by IDH1-R132H immunohistochemistry and identified a longer progression time interval of gliomas.

CONCLUSION

IDH1-R132H status is essential to the diagnosis for gliomas. IDH1-R132H mutations dominate in WHO grade Ⅱ/Ⅲ gliomas (lower grade gliomas) and secondary GBM. The frequency of IDH1-R132H mutation is rare in primary GBM. Our results showed that IDH1-R132H is essential to provide the basic molecular information necessary for diagnosis and prognosis of gliomas.

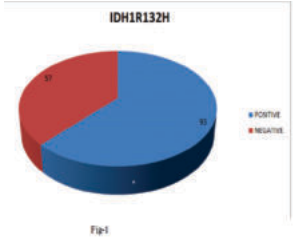


Fig-1

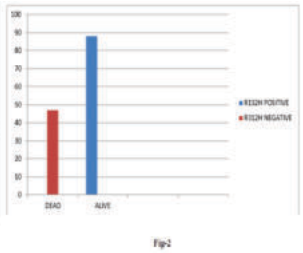


Fig-2

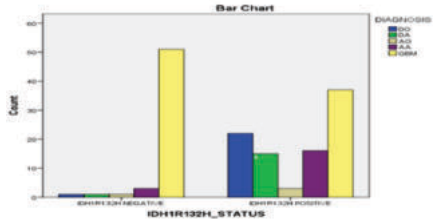
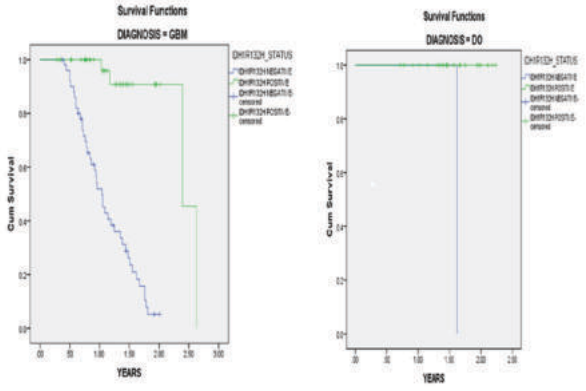


Fig-3



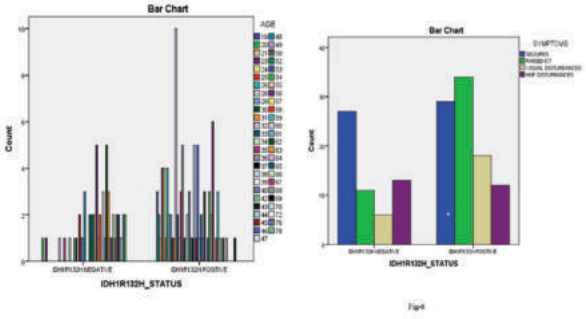


Fig-5 Fig-6

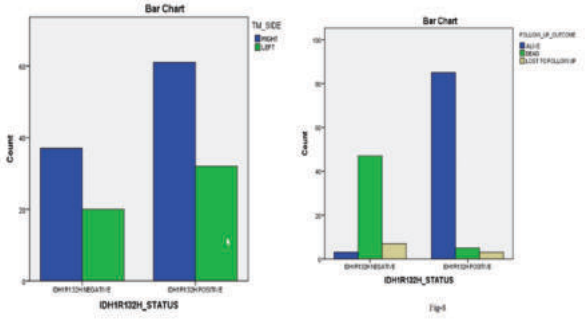


Fig-7 Fig-8

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