ORIGINAL RESEARCH PAPER
Volume - 9 | Issue - 8 | August - 2020 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

STRATIFICATION OF DIFFUSE GLIOMAS BASED ON IDH MUTATION STATUS – FIRST AND THE LARGEST TERTIARY CARE HOSPITAL STUDY OF RAJASTHAN

Pathology

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ABSTRACT

Background - All astrocytic tumors were first grouped together but are now classified on the basis of IDH1/IDH2 mutation irrespective of astrocytic or oligodendroglial lineage as "diffuse gliomas". The most notable change is the IDH status and 1p19q co-deletion for oligodendroglioma. Most of the genetic parameters can be assessed by immunohistochemistry or FISH. Gliomas with mutations of IDH genes are classified as IDH-mutant and those having unmutated IDH genes are classified as IDH wild-type. **Objective –** To study usefulness of IDH1 mutation status in diffuse gliomas by Immunohistochemical study. This is the first and the largest study done in a tertiary care centre in Rajasthan to the best of our knowledge. **Material & methods –**This is a tertiary hospital based study of sixty cases of diffuse gliomas. IHC was done using primary antibody against the these antigens- IDH1 R132H (1:50 dilution, Nova), ATRX (1:100 dilution, Sigma), p53 (dilution 1:1000, Santa Cruz biotechnology), MIB1 (RTU, Dako) **Results & Conclusion -** IDH1 mutations were found in 60% cases with frequency being higher in oligodendroglial tumors as compared to astrocytic tumors. Diffuse and anaplastic astrocytomas had nearly equal frequency of 78.57% & 80% respectively while frequency of IDH1 mutation in grade II oligodendroglioma was 100% and in grade III was 81.81%. However, in our study, 6 cases which had astrocytic tumor morphology on H&E showed heterogeneous positivity for ATRX and were advised IDH2 sequencing and 1p 19q codeletion. Maximum positivity of p53 was observed in glioblastomas. Our study did not show p53 positivity in most of the diffuse astrocytomas. No correlation of IDH1 and p53 mutation was found.

KEYWORDS

diffuse gliomas, IDH1/IDH2 mutation, 1p19q codeletion, Astrocytomas, oligodendroglioma

INTRODUCTION–

The histological subtyping and grading the gliomas provides the clinicians guidance to the course of the disease and its treatment beyond surgery. There is a profound prognostic impact of this grading but it has been found that tumors identical on morphology may have different outcomes and molecular markers aid in providing further prognostic information.

The WHO 2016 classification incorporates molecular parameters in the classification of diffuse gliomas. The important genetic a160lteration in the pathogenesis of gliomas is isocitrate dehydrogenase mutation causes initiation of glioma genesis and has important diagnostic & prognostic value.

Methods–

This is a tertiary hospital based study. A total of sixty cases of diffuse gliomas who got IHC done were included in the study and graded according to WHO 2016 classification.[1]

Institutional ethical committee approval was obtained prior to study. Written and informed consent was obtained from participants.

IHC was done using primary antibody against the these antigens- IDH1 R132H (1:50 dilution, Nova), ATRX (1:100 dilution, Sigma), p53 (dilution 1:1000, Santa Cruz biotechnology), MIB1 (RTU, Dako)

Results -

Out of 60 diffuse gliomas cases which were evaluated for IDH mutation status, 20 were diffuse astrocytomas, 4 anaplastic astrocytomas, 20 glioblastomas, 8 oligodendrogliomas and 8 anaplastic oligodendrogliomas on routine H & E examination.

Fig 1- Incidence of gliomas according to age

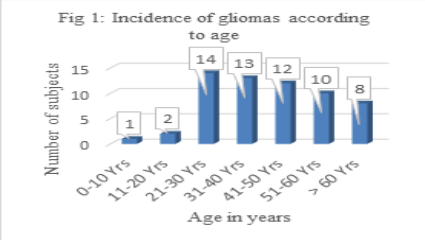
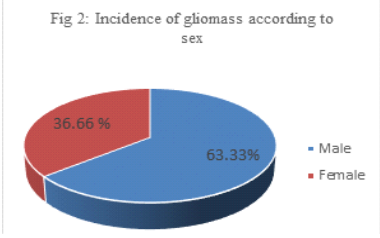
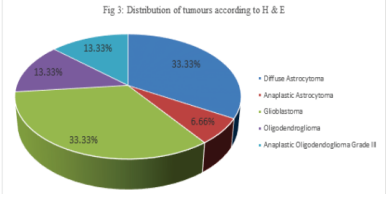


Fig 2- Incidence of gliomas according to sex



Maximum number of diffuse gliomas was found to be in the age group of 21- 30 years (23.33%), mostly being males (63.33%). Females comprised of only 36.66% of the cases.

Fig 3- Distribution of tumours according to H & E



On H&E, out of 60 cases of diffuse gliomas most common were glioblastomas (33.33%) and diffuse astrocytomas (33.33%)

Table-1: Evaluation of all diffuse gliomas on MIB1 for final grading

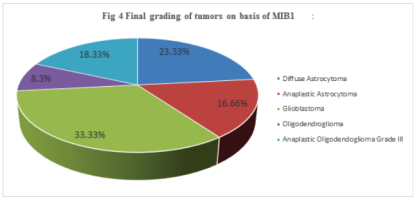
HPE Subtype	Diagnosis by MIB1					Total
	Diffuse Astrocytoma	Anaplastic Astrocytoma	Oligodendro-glioma	Anaplastic Oligodendro-glioma	Glioblastoma	
Diffuse Astrocytoma Grade II	14 (23.33)	6 (10.00)	0 (0.00)	0(0.00)	0(0.00)	20 (33.33)
Anaplastic Astrocytoma Grade III	0 (0.00)	4 (6.66)	0 (0.00)	0(0.00)	0(0.00)	

Oligodendroglioma Grade II	0 (0.00)	0 (0.00)	5 (8.33)	3 (5.00)	0 (0.00)	8 (13.33)
Anaplastic Oligodendroglioma Grade III	0 (0.00)	0 (0.00)	0 (0.00)	8 (13.33)	0 (0.00)	8 (13.33)
Glioblastoma Grade IV	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	20 (33.33)	20 (33.33)
Total	14 (23.33)	10 (16.66)	5 (8.33)	11(18.33)	20 (33.33)	60 (100.00)

6 diffuse astrocytomas had increased MIB1 and were included in anaplastic astrocytomas.

3 oligodendrogliomas had increased MIB1 and were included in anaplastic oligodendrogliomas.

Fig 4- Final grading of tumour according to MIB 1



On the basis of MIB1 labelling index the number of tumors were reclassified as – 14 diffuse astrocytoma , 10 anaplastic astrocytoma, 20 glioblastoma,5 Oligodendroglioma & 11 anaplastic oligodendroglioma.

Table 2: Evaluation of IDH1 mutation status

HPE Subtypes	IDH1 Expression		
	Positive n (%)	Negative n (%)	Total cases n
Diffuse Astrocytoma	11(78.57)	3 (21.42)	14
Anaplastic Astrocytoma	8 (80.00)	2 (20)	10
Glioblastoma	3 (15.00)	17 (85.00)	20
Oligodendroglioma	5 (100)	0 (0.00)	5
Anaplastic Oligodendroglioma	9 (81.81)	2 (18.18`)	11
Total	36	24	60

Table 3: Evaluation of ATRX expression

HPE Subtypes	ATRX Expression			
	Heterogeneous Positive n (%)	Lost n (%)	Retained n (%)	Total n
Diffuse Astrocytoma	2 (14.28)	10(71.42)	2 (14.28)	14
Anaplastic Astrocytoma	3 (30.00)	4 (40.00)	3 (30.00)	10
Glioblastoma	1 (5.00)	1 (5.00)	18 (90.00)	20
Oligodendroglioma	0 (0.00)	0 (0.00)	5(100.00)	5
Anaplastic Oligodendroglioma	0 (0.00)	0 (0.00)	11 (100.00)	11
Total	6	15	39	60

6 cases showed heterogeneous positivity for ATRX and were advised IDH2 sequencing and 1p 19q codeletion.

Table 4: Evaluation of p53 Expression

HPE Subtypes	p53 Expression		
	Positive n (%)	Negative n (%)	Total n
Diffuse Astrocytoma	2 (14.28)	12 (85.71)	14
Anaplastic Astrocytoma	7 (70.00)	3 (30.00)	10
Glioblastoma	8 (40.00)	12 (60.00)	20
Oligodendroglioma	0 (0.00)	5 (100.00)	5
Anaplastic Oligodendroglioma	1 (9.09)	10 (90.90)	11
Total	18	42	60

Maximum positivity of p53 is observed in glioblastomas (13.33%) and was negative in all grade II Oligodendrogliomas One anaplastic Oligodendroglioma showed scattered positivity for p53.All oligodendroglioma cases were advised 1p19q codeletion Most of diffuse astrocytoma cases were negative for p53.

DISCUSSION

Molecular information helps in grading the glial tumors into different prognostic groups which assist the individualization of the treatment and optimization of the dose. [1][2][3]

IDH mutation is now believed to be responsible for initiation of glioma genesis. Somatic mutation in IDH1 gene was discovered for the first time in glioblastomas in 2008 by Parson et al. [4] The IDH1 gene located at codon 132 is most commonly mutated. [5]

FE Bleeker et al in 2009 studied that IDH1 mutations are gliomas specific. They are not found in any other CNS or non – CNS tumors apart from AML (acute myeloid leukaemia). [6]

IDH 1 gene is located at codon132 and IDH2 at codon 172.

IDH1 is a well-recognised molecular marker in glial tumors of both astrocytic and oligodendroglial differentiation. [2]

Studies show increased frequency of these mutations in low grade gliomas, both of astrocytic and oligodendroglial origin and in secondary glioblastomas. [2][5][7][8]

ATRX mutation is considered to be a feature of astrocytic differentiation and can be determined by loss of nuclear ATRX expression on immunohistochemistry. [9]

MIB 1 labelling index is used for the final grading of the tumor according to the WHO 2016 classification. [1][10][11]In our study we included 60 cases of CNS tumors which were diagnosed as diffuse gliomas on H& E and then were analysed on Immunohistochemistry for assessment of IDH mutation. To the best of our knowledge, ours is the first and largest study in a tertiary care hospital of our state . We could not find any research of the same even after extensive search.

It was found that IDH 1 mutations were most frequent in age group of 21-30 yrs. and then in 31-40 yrs. [3]

Parsons et al., Yan et al and Felsberg et al also found that IDH1 mutations are associated with young age. [9]

Ichimuria et al 2009 have shown that IDH1 mutations are commonly associated with young age and have a longer survival.[12]

Maximum IDH1 mutant cases were found in diffuse astrocytoma ,out of 14 cases of diffuse astrocytoma,11 (78.57 %) were IDH1 mutant and 3 (21.4%) were IDH negative. IDH1 negative cases were advised for IDH2 sequencing for final categorization as mutant or wild type.

Jha et al (2011) also observed IDH1 mutations are more frequent in diffuse astrocytomas.[13]

Out of 10 anaplastic astrocytomas, 8 (80 %) cases were IDH mutant. 2 (20%) were IDH negative, which were advised IDH2 sequencing. [14] Yan et al et al in 2009[4] observed IDH1 mutation in anaplastic astrocytoma is 69.2%.

In this study maximum glioblastomas were in 60-70 age group and most of them were IDH1 negative.17 (85 %) out of 20 glioblastomas were IDH1 negative and 3 (15%) were IDH1 mutant. Though, IDH1 negative cases were advised sequencing, as they are elderly IDH2 sequencing was not mandatory as per WHO 2016, so were considered IDH wild type. MGMT was advised in all cases. [15]

IDH wild type cases are found to be more common in glioblastomas and have poor prognosis as compared to the mutant glioblastomas.[16][17]

Gravendeel et al in 2010[16] found 19.4% IDH1 mutation in glioblastomas. Horbinski et al in 2010 also found 16.7 % IDH1 mutation in glioblastomas. [18]

All our results of IDH1 mutation are in concordance with other studies.

Out of 5 oligodendrogliomas WHO grade II, all were IDH1 mutant and ATRX was retained, p53 was negative.

Out of 11 cases of anaplastic oligodendrogliomas, 9 (81.81 %) were IDH1 mutant and 2 (18.18 %) was negative for IDH1.ATRX was retained and p53 was negative in all.

All cases were advised for 1p19q co-deletion. Follow up of 1p19q result could not be done as our institution did not have the facility of this test.

In other studies also p53 is negative in oligodendrogliomas Horbinski et al in 2010[18] observed IDH1 mutation, 80% in oligodendrogliomas and 88.9% in anaplastic oligodendrogliomas.

ATRX mutation is considered to be a feature of astrocytic differentiation and can be determined by loss of nuclear ATRX expression on immunohistochemistry[9].

In this study we found ATRX loss in 10/14 (71.42 %) of diffuse astrocytomas, 4/10 (40 %) of anaplastic astrocytomas and 1/20 (5%) of glioblastomas.

Similarly Jiao et al in 2012[19] and Wiesler B et al in 2013[20] observed ATRX loss more commonly in diffuse astrocytomas and anaplastic astrocytomas and very rarely in primary glioblastomas.

That's why ATRX mutation as detected by loss of nuclear ATRX expression is considered to be sensitive for determining astrocytic lineage.

Wiesler B et al in 2013[20] observed diffuse gliomas with IDH mutation, 1p/19q co deletion, and retained ATRX expression were diagnosed as oligodendrogliomas and gliomas with IDH mutation, intact 1p/19q and loss of nuclear ATRX expression were diagnosed as astrocytomas .

However, in our study, 6 cases which had astrocytic tumor morphology on H&E showed hetrogenous positivity for ATRX and were advised IDH2 sequencing and 1p 19q codeletion. In these, 2/14 (14.28%) were diffuse astrocytomas, 3 /10 (30%) were anaplastic astrocytoma and 1/20 (5%) was Glioblastoma.

It indicates a subgroup of IDH mutant astrocytic gliomas based on ATRX status with those having ATRX loss having a better prognosis while those having ATRX positive have a worse prognosis. This is however subject to larger studies for confirmation. Also, these cases were advised IDH2 sequencing and 1p19q codeletion to rule out oligodendroglioma. [20]

B Wiestler et al in 2013 observed ATRX loss which refines the classification of anaplastic gliomas identifying a subgroup of IDH mutant astrocytomas with better outcome. [21]

Maximum positivity of p53 is observed in glioblastomas 8/20 (40 %) and 6/10 (60 %) anaplastic astrocytomas and p53 was negative in all Oligodendrogliomas – WHO grade II.

One case of Oligodendroglioma-WHO grade III showed scattered positivity for p53.

All oligodendroglioma cases were advised 1p19q codeletion.

Our study did not show p53 positivity in most of the diffuse astrocytomas as shown in many studies. Only 2/14 (14.28%) cases showed positivity for p^{53}.

Nearly 6o% of WHO grade II diffuse astrocytomas showed TP53 mutation in study done by Sandra Camelo- Piragua et al in 2012[21]

Study done by Subhlakshmi Sengupta et al of brain tumors in paediatric age group in 2012 observed 71.4% of diffuse infiltrating astrocytoma had low p53 score. [22]

However, Similar result as ours was observed by Pal et al in 2000 [23] noted p53 protein overexpression in 58.3% of high grade gliomas in contrast to only 8.3% of low grade glial tumors.

No oligodendroglial tumor was positive for p53 mutation. Studies suggest this is usually seen in IDH mutant 1p19q codeleted oligodendrogliomas suggesting the mutual exclusivity. [14]

The results of p53 mutation in glioblastoma were in concordance with other studies.

MIB-1 Labelling index was done to assess the proliferative activity in diffuse gliomas for confirmation of the WHO grade.

In our study, out of 20 diffuse astrocytomas, 6 cases had increased MIB1 so were included as anaplastic astrocytomas & out of 8 oligodendrogliomas (WHO grade II) 3 cases had increased MIB1 and were included as anaplastic oligodendrogliomas (WHO grade III).

Johannessen et al in 2006[10] analysed and reviewed 16 studies which constituted a total of 915 patients demonstrating a substantial increase in MIB-1 labelling index which was directly proportional to the increasing WHO grade of the tumor .

MIB1 is therefore important to confirm the final grading of the tumor as it affects the treatment & prognosis. [10][11]

Studies show that gliomas having IDH mutation carry a better prognosis than IDH unmutated wild type. [24] We could not do the 5-year survival studies due to less span of time since these cases were diagnosed.

CONCLUSION

Diffuse gliomas in most cases can be successfully characterized by immunohistochemistry of IDH1 and ATRX mutations into molecularly defined groups as IDH mutant & wild types, with mutant ones having a better prognosis. MIB1 labelling index is important for accurate grading.

Diffuse and anaplastic astrocytomas and oligodendrogliomas show more frequency of IDH1 mutation as compared to glioblastomas.

IDH 1 negative cases were further advised IDH2 sequencing and oligodendrogliomas were advised 1p19q codeletion as we did not have this facility.

All our results were in concordance with other studies except that p53 mutation was not common in diffuse astrocytomas and that 6 cases with astrocytic morphology had heterogeneously retained ATRX.

Of the 6 cases, 2 were diffuse astrocytomas, 3 were anaplastic astrocytomas and 1 was glioblastoma. It indicates a subgroup of IDH mutant astrocytic gliomas based on ATRX status with those having ATRX loss having a better prognosis while those having ATRX positive have a worse prognosis. However, this needs to be studied in more number of cases.

On the prognostic front, IDH1 mutations have an independent prognostic significance affecting the treatment of the patient.

Despite IDH1 mutation, WHO grading based on routine H & E carries its own importance in determining the prognosis. It remains important to determine the lineage and grading the tumors on the basis of histomorphology. Final integrated diagnosis should be given after evaluation of all factors.

On-going studies are likely to yield novel biomarkers in future suggesting that molecular neuropathology will have increasing impact in neurooncology.

Key message –

Diffuse gliomas can now nearly be successfully categorized into IDH mutant and wild types on the basis of their IDH mutation status which also has a prognostic impact.

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