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Clinical Profile, Pathology, and Molecular Typing of Gliomas with Oligodendroglial Morphology: A Single Institutional Experience

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Abstract

Background: Diffuse gliomas are represented in the 2007 WHO classification of CNS tumors as astrocytomas, oligoastrocytoma, and oligodendroglioma of grades II/III and glioblastomas WHO grade IV, which was a pure morphologic classification. WHO 2016 classification combines morphology with molecular markers like IDH, ATRX, and 1p/19q codeletion to give an integrated diagnosis. **Methods:** The study was carried out on formalin fixed paraffin embedded tissues from 54 patients including three pediatric patients. Molecular studies were performed to know the 1p/19q codeletion status, IDH1R132H, and ATRX immunoexpression. Also, the IDH1R132H status was correlated with survival data. **Results:** The study included 54 tumors with oligodendroglial morphology. IDH1R132H positivity was seen in 85% of total cases and codeletion was seen in 72%. The integrated diagnosis revised the cases into oligodendroglioma (39), astrocytoma (5), and glioblastoma (6).IDH mutant tumors were found to have better survival than negative ones which was statistically significant. **Conclusion:** This study emphasizes the need for molecular work up of tumors with oligodendroglial morphology with readily available techniques like IHC and Fluorescence *in situ* hybridization.

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Full Text

The diagnosis and grading of gliomas has largely been dominated by their histomorphologic features giving scope for institutional as well as individual variations. However, the updated 2016 classification has brought in an integrated morphologic and molecular basis to the classification of gliomas and other brain tumors.[1] These molecular alterations are robust diagnostic, prognostic, and predictive markers providing clinically relevant information for appropriate management. This change also promises to streamline the diagnostic variations in glioma with layered pattern of reporting.[1],[2],[3],[4],[5],[6],[7],[8] Oligodendrogliomas are

chemoresponsive tumors with better prognosis and have many morphologic mimics.[5] The WHO 2016 classification of CNS tumors classifies oligodendrogliomas with essential molecular work up into grade II and grade III tumors and those with incomplete molecular work up into oligodendroglioma grade II/III NOS. It is now essential to grade and type oligodendrogliomas by a combination of IDH, ATRX, and 1p19q deletion status to overcome the "NOS" category.[1] Many validation studies have already emphasized the need for integrated diagnosis.[2],[3],[4],[5],[6],[7],[8],[9] In the present study, we put forth our experience of 54 diffuse gliomas with oligodendroglial morphology with respect to their molecular genetic features as per the WHO 2016 classification of CNS tumors.

Materials and Methods

The study included 54 cases of gliomas with oligodendroglial morphology that underwent surgical resection between January 2014 and October 2016. The inclusion criteria were availability of tissue blocks for histopathologic and molecular features, and inadequate tissues were excluded from the study. The study was approved by institutional ethics committee. The tumors were initially classified according to the WHO 2007 criteria. The clinical details including demographic features, imaging features, and location of the tumor were obtained from medical records.

Immunohistochemistry

IHC was performed on all the cases on formalin-fixed paraffin-embedded tissue blocks using IDH1R132H (Dianova, dilution 1:200), ATRX (Sigma Aldrich, dilution 1:500), and Ki67 (Biogenex, Ready to use) and p53 (Biogenex, Ready to use). IHC was performed using the poly HRP technique on a fully automated immunostainer (Biogenex, X-Matrix).

Cytoplasmic staining for IDH1R132H and nuclear staining for ATRX, P53, and Ki67 were interpreted as positive. Positivity for endothelial cells, microglia, and lymphocytes served as internal positive control for ATRX. Ki67 labeling index was counted for every tumor. IDH mutation analysis was not available.

Fluorescence in situ hybridization (FISH)

FISH was performed on FFPE sectionsusing dual color locus specific probes for 1p36 and 19q13 paired, respectively, with the reference probes for 1q25 and 19p13 (Vysis).Following pretreatment, probes were added to the sections and the slides were subjected to denaturation at 78°C for 5 min and hybridization at 37°C for16 h in a thermobrite chamber. After counter staining with DAPI, slides were examined under fluorescent microscope.The hybridization signals were scored in at least 200 nonoverlapping nuclei. The ratio of 1p/1q and 19q/19p was calculated by taking the number of test and control signals. Typically, 1:2 ratio of signals in >50% cells was taken as a criteria for codeletion.[10]

Results

Clinical data

The details are enlisted in [Table 1]. The study included 54 cases in patients with an age range of 12–67 years (mean 42.6 years) with male predominance (M:F = 2.3:1). These tumors constituted 7% of primary brain tumors in the study period. The patients commonly presented with seizures and headache. Most of these oligodendrogliomas were located in frontal lobe (n = 25) followed by temporal lobe (n = 11).{Table 1}

Histopathology

Forty-four of the 54 tumors showed "classic" histomorphology.The other morphologies observed were nodules and lobules of cells separated by vascular channels, spindle cells, and absence of perinuclear halo. The

anaplastic oligodendrogliomas showed mitotic count of > 6/10 hpf with 15 cases showing necrosis and microvascular proliferation in addition

Immunohistochemistry

IDH1R132H was positive in 12/15 (80%) grade II and 28/39 (71.7%) grade III tumors. The median age group of IDH negative tumors was 50 years. All the IDH negative tumors showed retained ATRX. ATRX was retained in 49 tumors whereas five of these showed loss of ATRX expression. These five were also found to be positive for IDH1R132H and reclassified as astrocytomas. None of these five tumors had necrosis or microvascular proliferation. There were two tumors that were IDH mutant with retained ATRX expression and negative for codeletion. These were possibly astrocytomas. Five of the tumors were negative for IDH1R132H with retained ATRX and were non codeleted. These were classified as glioblastomas. Three of these patients were more than 55 years of age. Mutation analysis for IDH in other two tumors was not available. P53 was positive in 15 cases that included the astrocytomas (n = 5) and glioblastomas (n = 6) in the final integrated diagnosis. Four of the oligodendrogliomas also showed immunohistochemical expression of p53. There were three pediatric tumors in the study that showed classic oliogdendroglial morphology. Two of these were grade II and one was Grade III tumor. These three tumors were immunonegative for EMA ruling out a histopathologic overlap of clear cell ependymoma. One of these tumors in a 16 year old showed the molecular phenotype of adult oligodendroglioma, whereas the other two did not show any positive molecular markers. The molecular profile of all the tumors is given in [Table 1].

1p19q codeletion

All 39 oligodendrogliomas showed 1p19q codeletion. The cases of glioblastoma that were negative for IDH1R132H were noncodeleted. The 1p19q codeletion and ATRX mutations were mutually exclusive.

Survival and statistical analysis

The follow-up was available from 6 months to 3.5 years with a median of 2.7 years. Twelve patients died in the study period. Statistical analysis was performed using the "Statistical Package for the Social Sciences (SPSS) software," Windows, version 16. Survival was calculated using COX regression analysis and Kaplan–Meir survival curves were plotted. On cox regression analysis, it was identified that IDH mutant tumors clearly had better survival in comparison to IDH negative tumors, and this was found to be statistically significant (P = 0.005) as shown in [Chart 1]. The hazard ratio was 0.129, indicating an 87% increased risk of death among IDH-negative patients as compared to IDH-positive cases. The 1p19q codeleted tumors also showed better overall survival as compared to non codeleted tumors. Multivariate Cox-regression analysis is given in [Table 2]. It was [Chart 2] observed that statistically significance was seen only for IDH mutations (P value 0.028). Increasing age of the patients and grade III tumor showed higher hazard ratio.[INLINE:1]{Table 2}[INLINE:2]

Kaplan–Meier survival analysis

The mean survival in this study was 3.8 years. The mean survival for IDH mutant tumors was 49 months and for IDH negative ones was 27 months. However, the median survival for both groups was 25 months. Around 70% cases lived upto 2.5 years, and 50% of the cases lived upto 4 years [Chart 1]. The patients who expired included glioblastoma (5), anaplastic oligodendroglioma (4), oligodendroglioma (1), and anaplastic astrocytoma (2). The final integrated diagnosis is depicted in [Chart 3].[INLINE:3]

Discussion

Oligodendrogliomas encompasses about 3–5% of all primary brain tumors.[1] There are few published studies of histologically verified, molecularly proven pure oligodendrogliomas.

The molecular work up, diagnosis, and grading of oligodendrogliomas are critical for optimal patient care, risk assessment, prognostication, and predictive outcomes of tumor.[11],[12],[13],[14],[15],[16],[17],[18]

In the present study, oligodendrogliomas comprised 7% of all the primary brain tumors. Patients in the present study presented at a mean age of 43.7 years, which is comparable to the epidemiological data of WHO.[1] The male to female ratio was 2.3:1 showing a male preponderance similar to world epidemiology.[1] The most common clinical presentation was seizures followed by headache. The most common location of tumor was frontal lobe, which is comparable to the WHO data.[1]

Though the importance of molecular studies in gliomas is unquestionable, the analysis always begins with morphology thatforms the foundation of the "layered "approach of the diagnostic algorithm for gliomas. According to the WHO criteria, grading for oligodendroglioma is dictated by the presence of mitotic activity, necrosis, and microvascular proliferation. The absence of these are considered as WHO grade II, while the presence of one or more of these features designate the tumor as anaplastic oligodendroglioma (WHO grade III).Grading of oligodendroglioma with similar molecular genetics (IDH+/1p19q codeletion) has direct bearing on management protocols as well as median survival.

A combined analysis of IDH, ATRX, and 1p19q codeletion has been emphasized and validated as an essential step in resolving the subtype of glioma to give the four-layered diagnosis. Isocitrate dehydrogenase (IDH1 and IDH2) are seen in diffuse grade II and III gliomas and forms a standard of care in analysis of these tumors. IDH mutations are present in 100% oligodendrogliomas.[19] Majority of these mutations are R132H type, which is most reliably detected by immunohistochemistry.[20]

We identified a high frequency of IDH mutations in our study. Similar has been reported from other studies.[14],[20],[21] A similar study by Geever T et al.hasshown IDH mutations in 46 of the 50 cases in their study. Among the IDHR132H immuno negative tumors included six cases of oligodendroglioma that were codeleted. These tumors are known to harbor IDH mutations, and further sequencing for IDH mutations is not necessary as has been put forth by the ISNO consensus guidelines developed for resource limited settings.[22] ATRX mutations are the hallmark of astrocytic tumors and are not seen in oligodendrogliomas.[1],[2] However, ATRX mutations have been reported in minority of anaplastic oligodendrogliomas (which are 1p19q codeleted) in some published series. Positive ATRX expression in IDH mutant diffuse gliomas showing classic morphologic features of oligodendrogliomas can be classified as oligodendrogliomas even without the results for 1p19q deletion status.[1] We observed that ATRX expression and codeletion were mutually exclusive similar to that given in literature.[1]

Two of the tumors that were IDH mutant and non codeleted showed retained ATRX expression. The presence of such tumors has been explained by Reuss et al.[3] as these tumors may carry an ATRX mutation not resulting in the abrogation of protein expression (e.g., a damaging missense mutation).

Oligoastrocytoma was a well-defined entity in WHO 2007. However, it has been shown that these tumors can be reliably classified as astrocytoma or oligodendroglioma on the basis of molecular data.[22],[23] Five of the tumors showed ATRX loss and were reclassified as astrocytomas as per the WHO and ISNO guidelines.[24] Common histopathologic patterns like fired egg appearance and chicken wire capillaries are known to have significant correlation with 1p/19q codeletion status.[6],[10] In our study, we observed that 84% of the codeleted tumors showed these classic morphologic features. However, also seen 67% of the non codeleted tumors and hence perhaps morphology alone cannot be taken as predictor of the codeletion status.[10]

Five of the tumors that were negative for all the molecular markers were classified as glioblastoma. These perhaps belong to the category of glioblastoma with oligodendroglial component (GBM-O). GBM-O is known to have better prognosis than IDH wild-type glioblastomas. The presence of 1p19q codeletion is crucial to the tumors showing the morphology of oligodendrolglioma with necrosis into anaplastic oligodendroglioma vs GBM-O. However, the terminology of GBM-O no longer is entertained in WHO. Tumors with GBM-O morphology are shown to have diverse molecular markers including IDH mutations, EGFR, and PDGFRA amplifications and 9p deletions thathave different prognostic implications.[24]

Unlike the adult oligodendrogliomas, the molecular definition of the pediatric counterparts still remains a challenge since these tumors do not express either IDH mutations or 1p19q codeletion.[11],[24] In the pediatric age group, the other low-grade gliomas showing oligo-like morphology needs to be excluded. The absence of IDH mutations as well as codeletion creates a diagnostic dilemma as WHO 2016 mandates

molecular genetics for diagnosis of oligodendroglioma. Goel N et al.have published the largest series including 346 patients of pediatric oligodendrogliomas.[25] They have reported 85% of overall survival owing to more number of low-grade tumors as 72% of their patients were grade II oligodendrogliomas. This is a learning lesson where grade still holds its prognostic importance in tumors even in the absence of the conventional good prognostic molecular genetics like IDH mutations and 1p19q codeletion. Rare childhood tumors with IDH mutations and 1p19q codeletions almost always occur in adolescents similar to a 16-year-old patient seen in our study, suggesting that they indeed carry the molecular phenotype of adult oligodendroglioma.

IDH mutation is an independent prognostic parameter for diffuse gliomas.[13],[15],[16],[18] The same was observed in our study. IDH mutation was found to be an independent survival marker irrespective of the tumor grade. The absence of IDH mutations, necrosis, as well as non codeleted tumors showed bad survival, and 12 such patients died on follow up.

The study validates the integrated diagnosis of gliomas with the help of molecular genetics. The cost factors can be resolved to a great extent by using monoclonal antibodies that are robust markers for mutation analysis.

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Conflicts of interest

There are no conflicts of interest.

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Table 1: Molecular profile of all the cases

	Total cases (*n*=54)	IDH + (*n*=40)	IDH- (*n*=14)	ATRX+ (*n*=54)	ATRX- (*n*=4)	P53 + (*n*=15)	P53- (*n*=39)	1p19q Codeleted (*n*=39)	1p19q Non codeleted (*n*=15)
Age<40 years	25	19	6	25	nil	3	22	14/25	9/25
>40 years	29	21	8	29	nil	12	17	23/29	6/29

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Table 2: Multivariate COX-regression analysis

Variables in the Equation

	B	SE	Wald	df	Sig.	Exp(B)	95.0% CI for Exp(B)	
							Lower	Upper
age	.009	.023	.152	1	.697	1.009	.964	1.056
gradeanap1	12.983	221.893	.003	1	.953	434757.132	.000	3.264E+194
IDH	2.020	.919	4.827	1	.028	7.537	1.244	45.680

It was observed that statistical significance was seen only for IDH mutations (*P*=0.028). Increasing age of the patients, grade III tumor showed higher hazard ratio