Histological spectrum of oligodendroglial tumors: Only a subset shows 1p/19q codeletion

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Abstract

Background: Canonical oligodendroglial tumors (ODGs) are characterized genetically by chromosomes 1p/19q codeletion. Aims: This study was essentially aimed at the detection of frequency of 1p/19q codeletion in the different histological spectrum of ODG tumors in a large cohort of Indian patients. Materials and Methods: All the ODG tumors evaluated for 1p/19q by fluorescence in-situ hybridization (FISH) during 2009–2015 were correlated with histology, immunohistochemical expression for p53 protein and clinical features. Results: A total of 676 cases included both pediatric (n = 18) and adult (n = 658) patients. Histologically, 346 pure ODGs [oligodendroglioma (OD) and anaplastic oligodendroglioma (AOD)] and 330 mixed ODGs [oligoastrocytomas (OA), anaplastic oligoastrocytomas (AOA) and glioblastoma with oligodendroglioma component (GBM-O)] were included. 1p/19q co-deletion was noted in 69% (60/87), 55.9% (145/259), 18.2% (18/99), 10.5% (18/172), and in 5.1% (3/59) cases of OD, AOD, OA, AOA, and GBM-O, respectively. In the pediatric age-group, 1p/19q codeletion was seen in 25% (2/8) of pure ODGs and in 10% (1/10) of mixed ODGs. In adults, it was observed in 60% (203/338) cases of pure ODGs and in 11.9% (38/320) cases of mixed ODGs. In adults, pure ODG histology (P = 0.00), frontal location (P = 0.004), calcification [in pure ODGs] (P = 0.03), and lack of p53 protein overexpression (P = 0.00) showed significant statistical correlation with 1p/19q codeletion. Conclusions: This study is unique in being one of the largest on ODGs for 1p/19q co-deletion including both pediatric and adult age groups of Indian patients. The results showed co-deletion in 60% of adult ODGs and 25% of pediatric pure ODGs. This reemphasizes the occurrence of 1p/19q codeletion, even though rare, in the pediatric age group.

How to cite this article:

How to cite this URL:
Oligodendrogliomas (ODGs), first described by Bailey and Cushing in 1926, are relatively uncommon diffuse gliomas with relative male preponderance and preference for cortical location.[1],[2] Chromosomes 1p/19q codeletion, first reported in ODG tumors in 1994,[3] is now an established molecular signature for canonical ODG tumors with prognostic and predictive utility.[3],[4],[5],[6] This is an important diagnostic marker for identifying the typical ODGs among the histological heterogeneous pure and mixed ODG tumors, which are inherently endowed with histomorphological interpretative variability. An unbalanced t(1;19) (q10;p10) translocation, with the chromosomal breakpoints located close to the centromeres of both chromosomes, is one of the plausible cytogenetic mechanisms of codeletion .[7] The present study is aimed at the evaluation of frequency of 1p/19q codeletion by fluorescence in-situ hybridization (FISH) in the diverse histological spectrum of ODG tumors; and, attempts to establish a correlation of the same with clinical and pathological parameters.

**Materials and Methods**

The present study was undertaken after the approval of the Institutional Ethics Committee. All ODG tumors evaluated for 1p/19q by FISH during the period of 2009–2015 in the division of Molecular Pathology of our Institute formed the study sample. Clinical details were retrieved from the Electronic Medical Records and/or patient's records. The original hematoxylin and eosin (H & E) stained slides of all cases were reviewed and classified according to the 2007 World Health Organization (WHO) classification of primary central nervous system tumors.[2] The cohort was divided into two broad histological groups of pure ODG tumors [oligodendroglioma grade II (OD), anaplastic oligodendroglioma grade III (AOD)] and mixed ODG tumors [oligoastrocytoma grade II (OA), anaplastic oligoastrocytoma grade III (AOA), glioblastoma with oligodendroglioma component grade IV (GBM-O)]. The histological groups were further segregated into broad age groups of pediatric (≤18 years) and adult (>18 years) patients.

**Immunohistochemistry**

p53 protein overexpression (monoclonal mouse, clone D07; 1:50, Dako, Glostrup, Denmark) was performed on a 4-µm-thick representative formalin fixed paraffin embedded (FFPE) tumor section and were interpreted under 40× magnification. The details of the interpretation of the p53 protein was done as illustrated in our prior study.[8]

**Fluorescence in-situ hybridization**

1p/19q FISH test was performed using commercial fluorochrome-labeled Vysis locus specific identifier (LSI) dual color probes (Abbott Molecular, Illinois, United States) localizing to 1p36 Spectrum Orange/1q25 Spectrum Green and 19q13 Spectrum Orange/19p13 Spectrum Green.

**Interpretation**

Sections were viewed under an Olympus BX53F upright fluorescence microscope equipped with appropriate excitation and emission filters (allowing visualization of the orange and green signals), QCam Olympus camera, and QCapture pro 7.0 image analyzer software. For all cases, at least 100 nonoverlapping tumor cell nuclei were counted for 1p and 19q, with each parameter being assessed separately. Cases with a ratio (1p/1q and 19q/19p) of <0.8 were considered as deleted for 1p and/or 19q.[9] The results were thus expressed as "1p/19q co-deletion," “isolated 1p deletion,” “isolated 19q deletion,” and “1p/19q non-deleted”. The tumor was considered to have polysomy if ≥30% of nuclei showed 3 or more signals for both 1q and 19p.[10] In cases with absent/weak interpretable signals, the tests were repeated before considering them as "uninterpretable."

**Statistical analysis**

1p/19q FISH results were correlated with the clinical and pathological parameters in pediatric and adult...
patients using the Statistical Package for the Social Sciences (SPSS), Version 16.0. Chicago, SPSS Inc; chi-square tests were applied, with a P value of <0.05 as significant. Statistical calculations were performed for 1p/19q codeleted and nondeleted groups by excluding isolated 1p and isolated 19q deletions.

**Results**

**Sample size**

780 gliomas were evaluated for 1p/19q by FISH from 2009–2015 in the Division of Molecular Pathology of our Institute. 104 cases (13.3%) were excluded [(a) 57 cases (7.3%): absent interpretable signals, (b) 43 cases (5.5%): histologically reclassified as non-ODG tumors on review, (c) 3 referral cases (0.4%): lack of relevant clinical details, and (d) additionally 1 case of a 37-year-old female patient with posterior cervical lymph nodal metastases of AOD from a parietooccipital primary operated at 12 years of age (i.e., 25 years back) was also excluded due to the availability of the primary tumor tissue sample; however, it showed an isolated 1p deletion on evaluation of the metastatic tissue]. The final study group consisted of samples from 676 patients.

**Clinical features**

The clinical features are summarized in [Table 1], [Table 2], [Table 3], [Table 4]. The age range was 6–76 years. Eighteen (2.7%) patients were ≤18 years (age range: 6–18 years, median age: 15 years) and 658 (97.3%) were adults [19–25 years: 48/676 (7.1%), 26–40 years: 290/676 (42.8%), 41–60 years: 284/676 (42%), >60 years: 36/676 (5.3%); median age: 40 years]. The male: female ratio in the pediatric age group was 2:1 (males = 12, females = 6), and in adults was 1.8:1 (males = 428, females = 230). Overall, the most common location was frontal (n = 317, 46.8%), followed by temporal (n = 97, 14.3%). Other locations included parietal (n = 64, 9.4%), frontoparietal (n = 63, 9.3%), temporoparietal (n = 37, 5.4%), frontotemporal (n = 39, 5.7%), insular (n = 23, 3.4%), parietooccipital (n = 12, 1.7%), occipital (n = 9, 1.3%), thalamus (n = 8, 1.2%), cerebellum (n = 4, 0.6%), and corpus callosum (n = 3, 0.4%).

**Histology**

The histological subtypes were [Table 1] and [Figure 1], [Figure 2]: OD (n = 87, 12.9%), AOD (n = 259, 38.3%), OA (n = 99, 14.6%), AOA (n = 172, 25.4%), and GBM-O (n = 59, 8.7%). Pure ODG tumors (OD and AOD) were 346 [51.1%; pediatric: 8/18 (44.4%) and adult: 338/658 (51.4%)] and mixed ODG (OA, AOA and GBM-O) were 330 [48.8%; pediatric: 10/18 (55.6%) and adult: 320/658 (48.6%)].

**Calcification**

Calcification was seen in 43.6% (151/346) of pure ODG [Figure 1] and in 14.2% (47/330) of mixed tumors [Table 4]. In the pediatric age group, calcification was seen in 37.5% (3/8) of pure and 30% (3/10) of mixed ODG tumors. In adults, calcification showed a significant statistical association with pure ODG histology (P = 0.00) as compared to mixed ODG tumors [43.8%, (148/338) versus 13.8% (44/320)].

**p53 protein expression**

Data was available in 573 patients and overexpression (positive group) was found in 12.3% (36/291) of pure ODG tumors and in 39.4% (111/282) of mixed ODG tumors. The nonpositive group (focal positive and negative) constituted 87.6% (255/291) of pure ODG [pediatric: 66.7% (2/3); adults: 87.8% (253/280)] as compared to 60.6% (171/282) of mixed tumors [pediatric: 50% (1/2); adults: 60.7% (170/280)].

**1p/19q codeletion**

Codeletion was seen in 59.2% (205/346) of pure ODG tumors and in 11.8% (39/330) of mixed ODG tumors. Co-deletion was noted in 69% (60/87), 55.9% (145/259), 18.2% (18/99), 10.5% (18/172), and in 5.1%
(3/59) cases of OD, AOD, OA, AOA, and GBM-O cases, respectively [Table 1]. Polysomies involving 1p and/or 19q were seen in 5.5% (37/676) {28 pure ODG tumors (OD: 3 and AOD: 25) and 9 mixed ODG tumors (OA: 4 and AOA: 5)}. Relative codeletion and isolated 1p or 19q deletions were seen in 18 and 11 cases, respectively, in the background of either/both polysomies.

Correlation of 1p/19q with clinical and histological parameters

Location

Pediatric: All the codeleted cases were of frontal location whereas 50% (3/6) of frontally located tumors showed the codeletion, and the association was statistically significant (P value: 0.029). No nonfrontal tumors showed codeletion.

Adult: Frontal location showed a significant statistical association (P value: 0.004) with codeletion (45.4%–pure ODG: 69.6% and mixed: 13.3%) as compared to nonfrontal location (33.9%–pure ODG: 61.6% and mixed: 12.4%). Three out of 23 (13.04%) insular pure ODG tumors showed codeletion.

Histology

The histological parameters are represented in [Table 2] and [Figure 1].

Pediatric: Codeletion was noted in 25% (2/8) cases of pure ODG tumors (a 16 year old male and an 18 year female patient, respectively) and in 10% (1/10) cases of mixed tumors (an 8 year old male patient). In addition, 1 case of pure ODG of corpus colossal location showed an isolated 19q deletion (a 10 year old male patient).

Adult: Codeletion was seen in 60% (203/338) of pure ODG tumors [19–25 years: 7/22 (31.8%); 26–40 years: 84/136 (61.8%); 41–60 years: 103/163 (63.2%); >60 years: 9/17 (52.9%)] and in 11.9% (38/320) cases of mixed ODG tumors [19–25 years: 1/26 (3.8%); 26–40 years: 17/154 (11%); 41–60 years: 17/121 (14%); >60 years: 3/19: 15.8%]. The frequency of codeletion in >25 years age group was 67.4% (196/291) in pure ODG and 13.6% (37/272) in mixed tumors (after excluding cases with isolated deletions). Overall, the statistical association between 1p/19q codeletion and pure ODG histomorphology was significant as compared to mixed tumors (64.9% versus 12.7%, P = 0.000). Isolated 1p and 19q deletion was seen in 13 (3.8%) and 16 (4.7%) cases, respectively, in pure ODG; whereas in mixed ODG tumors, isolated 1p and 19q deletion was seen in 4 (1.2%) and 19 (5.9%) cases, respectively.

Calcification

[Table 4] summarizes the frequency of calcification encountered in different subgroups of OG.

Pediatric: 1p/19q codeletion was seen in 1/3 of pure ODG tumors with calcification as compared to 1/4 tumors without calcification. However, none of the mixed ODG tumors with calcification (0/3) were codeleted.

Adult: 1p/19q co-deletion showed a statistically significant association (P = 0.03) with the presence of calcification in pure ODG tumors as compared to the absence of calcification (72.3% versus 60.5%). In mixed tumors, calcification was more common in the codeleted subset (23.1% versus 11.2%); however, the incidence was not statistically significant (P value = 0.07).

p53 protein expression

p53 protein expression in different tumor subtypes is summarized in [Figure 1].

Pediatric: Of the 2 pure ODG tumors with nonpositivity for p53 protein, 1 was codeleted (50%). p53 protein expression was not available for the other 2 cases of codeleted tumors.

Adult: p53 nonpositivity showed a significant statistical association with codeletion as compared to p53...
positivity (overexpression), both in pure ODG (70.3% versus 30.3%; P value: 0.00) and mixed tumors (21.7% versus 0; P value = 0.000) [Table 4]. However, it is to be noted that 30.3% (10/33) of pure ODGs showed p53 protein overexpression with 1p/19q codeletion.

Discussion

Cairncross et al.,[11] in 1998, first reported the independent prognostic and predictive role of 1p/19q codeletion in high grade ODG tumors. This was followed by many other studies.[4] The RTOG-9402 trial, that included 291 AOD and AOA patients [with the frequency of 1p/19q codeletion being 48% (126/263) and a median follow-up of 11.3 years], showed a significant longer survival (14.7 years) in codeleted cases as compared to 2.6 years in nondeleted cases, and showed an advantage of combined chemotherapy with radiotherapy over radiotherapy alone in codeleted cases.[12] In 2014, a meta-analysis of 28 studies involving 3408 cases concluded that 1p/19q codeletion was associated with a better progression free survival (PFS) and overall survival (OS) and was independent of the detection methods, histological grades, and subtypes of gliomas. Isolated 1p deletion conferred a favorable outcome to a lesser extent, but no significantly favorable outcome was associated with isolated 19q deletion.[5] Recent TCGA (The Cancer Genome Atlas) data of 293 low-grade adult gliomas, stratified into 3 principal molecular subgroups, found that isocitrate dehydrogenase (IDH) mutated and 1p/19q codeleted tumors were enriched with mutations in telomerase reverse transcriptase (TERT) promoter, CIC, and FUBP1 genes and were predominately of pure oligodendroglial histology with a favorable clinical outcome.[13] Dubbink et al.,[14] evaluated 139 diffuse gliomas of the EORTC (European Organisation for Research and Treatment of Cancer) phase III trial 26951 by targeted next-generation sequencing (NGS) and found that most of the 1p/19q codeleted tumors had both IDH (48/49) and promoter TERT mutations (48/49). Eckel-Passow et al.,[15] in their large cohort of 1087 diffuse gliomas (grade II–IV), found that the frequency of triple positive markers (IDH mutation, TERT promoter mutation, and 1p/19q co-deletion) was seen in 29% (181/615) of grade II and grade III gliomas with a strong association with ODG histomorphology and a better overall survival (OS). Thus, it is important to identify a canonical ODG tumor within the broad histological heterogeneous subset. However, this study was limited by the lack of comparison of 1p/19q codeletion with IDH mutations. Evaluation of 1p/19q co-deletion in all glial tumors with an ODG histological component is the current standard practice to identify the canonical type of ODG tumors. It can be done by FISH, polymerase chain reaction (PCR)-based loss of heterozygosity (LOH) analysis, real-time quantitative comparative PCR (RQ-PCR), multiplex ligation-dependent probe amplification (MLPA), array comparative genomic hybridization (aCGH) and next-generation sequencing (NGS). FISH and PCR-based LOH are the most frequently used and time-tested standard methods.[4],[6],[16] FISH is a robust and relatively easy implementable method on formalin-fixed, paraffin-embedded (FFPE) tissue;[4] however, it cannot distinguish between the full chromosomal arm and partial losses that span the probe sites.[17] It is also inherent with false positive results, especially in cases with genomic imbalance (relative deletions of target 1p and 19q in presence of reduplication of control arm).[6] Although PCR-LOH analysis has the advantage of evaluation for multiple loci within each sample, it requires more tissue, a nonneoplastic control sample, and is also not very specific for whole chromosome losses, is more labor intensive, and is a relatively blind procedure.[18] Radiation Therapy Oncology Group (RTOG) and EORTC study trials used the FISH technique for the detection of 1p/19q status. Recently, NGS has been shown to be more sensitive than FISH in the detection of 1p/19q status; however, accessibility of the technique is a major limitation.[14] Despite variability between different centers and trials, the interpretation of FISH data is reproducible in most cases.[18]

The present study is an audit of 676 ODG tumors retrospectively evaluated for 1p/19q by FISH. The average age distribution for various grades of tumors and the male preponderance [Table 1] noted in the study is concordant with the existing literature.[19],[20] The frequency of 1p/19q codeletion in various studies varies from 50–90%, suggesting the histological heterogeneity of ODGs.[21],[22],[23] In the present study, the frequency of codeletion in pure ODG tumors was 59.2% (205/346) and 11.8% (39/330) in mixed ODG, which is lower in comparison to that of 89 and 65% seen in the study by in Miller et al.,[24] and Reddy et al.,[25] in their series of pure ODG tumors, respectively. Singh et al.,[20] reported codeletion in 81.8% of pure ODG tumors and in 35.3% of mixed ODG tumors in their total series of 100 cases of glial tumors evaluated by FISH; Shukla et al.,[26] reported codeletion in 60% of pure ODG tumors (12/20) and in 44.4% (4/9) of mixed...
ODG tumors. The possible reason of a relatively lesser percentage of codeletion in the present study may be due to the inclusion of cases across different age groups and locations; moreover, the cohort under discussion is disproportionately larger. However, the frequency of codeletion in patients >25 years, of pure ODG tumors and mixed tumors, in the present study is 62 and 12.6%, respectively. The low percentage of 1p/19q codeletion in mixed tumors can partly be explained by the inclusion of GBM-O. Fifty-eight cases were adult GBM-O, of which 3 were codeleted (5.2%); this finding is similar to that of a previously reported study. [4],[22] Nineteen nondeleted cases of 58 adult GBM-O were a part of our clinical study of GBM-O.[27] Furthermore, the relative codeletion was noted in 2.7% (18/676) cases with polysomies. Studies have shown that irrespective of tumor grades, polysomies associated with codeleted ODG tumors are associated with an unfavorable outcome with shorter progression free survival (PFS) and OS.[10],[22],[23]

This study also emphasizes the occurrence of 1p/19q codeletion in pediatric and young adults. In the pediatric age group, 25% of the pure ODG cases and 10% of the mixed tumor cases showed codeletion. In the age group of 19–25 years, 31.8% (7/22) of pure ODG cases and 3.8% (1/26) of mixed tumor cases showed codeletion, possibly suggesting the occurrence of a distinctive adult type of ODG tumor in the older pediatric and young adult population. Rodriguez et al.,[28] also found 1p/19q codeletion in 25% (10/40) patients with a pediatric ODG, with 8 of them in the 16–20 years group. Raghavan et al.,[29] reported codeletion in 3 out of 26 patients with a pediatric ODG in the age group of 10–18 years. However, Suri et al.,[30] reported codeletion in 4/7 cases in the age group of 19–25 years and none in the pediatric age group (0/7; age range: 3–18). Similarly, several studies showed no 1p/19q codeletion in patients with pediatric ODGs.[26],[31],[32],[33] However, these findings re-emphasize the occurrence of the adult type (1p/19q mediated) type of ODG in the pediatric age group, especially in the older children.

The most common location of adult ODG tumors in our cohort was frontal followed by the temporal lobe [Table 1] and [Table 3] and 1p/19q codeletion was significantly associated with the frontal location (P = 0.004), as is reported in the literature.[34],[35] Interestingly, insular (7 pure and 16 mixed) ODG tumors showed codeletion in 13.04% patients and all were associated with a pure ODG histology [Table 3], which is in concordance with that reported by Wu et al.[36] However, the literature on insular ODG tumor for 1p/19q is conflicting. Gozé et al.,[37] in their series of insular low grade ODG tumors (n = 12), showed no codeletion and hypothesized the insular gliomas to be of distinct molecular profile with a relatively worsen prognosis and reduced chemosensitivity, as compared to those seen at other sites. Subsequently in 2013, Gozé et al.,[38] reported codeletion in 1/11 (10%) patients with pure insular grade II gliomas and 6/36 (17%) patients in the paralimbic group. Wu et al.,[36] reported codeletion in 57% (4/7) cases of pure insular tumors. This variability is possibly because of the inclusion of parainsular based tumors, rather than those situated in a true insular location. It is also interesting to note that only one nonhemispheric (cerebellar) case showed a 1p/19q codeletion [Table 3], suggesting that deep seated tumors are distinctive from the hemispheric ones.

Interestingly, the presence of calcification within pure ODG tumors in adults (P = 0.03) had shown a statistically significant correlation with 1p/19q codeletion, which is concordant with the findings reported in literature.[21]

The current study reemphasizes the occurrence of lesser frequency of p53 overexpression in pure ODG tumors and of higher frequency of p53 overexpression in high grade tumors, which is concordant with the existing literature.[39] In this study, an inverse correlation was observed between 1p/19q codeletion and p53 protein overexpression [Table 4], which is possibly indicative of mutually exclusive events. This finding is also corroborative with that of McDonald et al.,[40] who reported p53 protein overexpression in 20% (11/55) of 1p19q codeleted and in 48.6% (35/72) of nondeleted cases, respectively.

The present study is unique because it is the largest single-institution based series (n = 676) from India and has representation of the different histological spectrum of ODG tumors across different age groups and locations. In the present study, 60% of pure adult ODG tumors showed 1p/19q codeletion (with the highest frequency (63.2%) in the age group of 41–60 years). However, it is uncommon in the pediatric age group, with 25% cases being seen in the pure ODG and 10% cases in the mixed ODG tumors. The study also demonstrated the occurrence of 1p/19q codeletion (in approximately 13.04% patients) in the insular ODG tumors, which is contrary to the traditional belief. Adult patients with a pure ODG histology, frontal location, presence of calcification, and p53 protein nonpositivity had statistically significant correlation with the 1p/19q
codeletion. Overall, this study reiterates the existence of heterogeneity among the similar histological groups for 1p/19q codeletion; thus, evaluation for 1p/19q codeletion in histologically confirmed ODG tumor is an absolute requisite for identifying the canonical (that has a better prognosis and a more predictive course) type of ODGs.

Acknowledgement

The authors wish to acknowledge Mr Sandeep Dhanavade, Mr Vinayak Kadam, Ms Sonali Tambe, Mrs Rajani Mohite, Mrs Jyoti Bodake, Dr Ranjan Basak and Dr Omshree Shetty for the technical assistance. The authors are indebted to Brain Tumor Foundation (BTF) of India for partially supporting the work.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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