Open Access

Original Article

Comparison of IDH and ATRX by immunohistochemistry with 1p/19q co-deletion by FISH in Oligodendrogliomas

Zahoor Hussain, Syed Salman Ali, Sumaira Ilyas*, Qurat Ul Ain Mati, Sidra Shoukat, Sawera Ilyas**

Department of Histopathology, Combined Military Hospital Lahore/National University of Medical Sciences (NUMS) Pakistan,
*Department of Haematology, Combined Military Hospital Lahore/National University of Medical Sciences (NUMS) Pakistan
**Army Medical College/National University of Medical Sciences (NUMS), Rawalpindi Pakistan

ABSTRACT

Objective: To determine 1p/19q co-deletion by Fluorescence in situ hybridization (FISH) based on positive expression of IDH and ATRX by immunohistochemistry (IHC) in oligodendrogliomas.
Study Design: Cross-sectional study.
Place and Duration of Study: Department of Histopathology, Armed Forces Institute of Pathology, Rawalpindi Pakistan, from Oct 2021 to Jun 2022.
Methodology: A total of 58 cases of oligodendrogliomas having positive expression of IDH and ATRX were subjected to FISH for 1p/19q co-deletion. FISH results were correlated with clinicopathological parameters and IHC by using computer software SPSS version 23.
Results: In this study, age range is from 14 to 79 years, with mean of 40 years. 30(52%) cases are <40 years of age and 28(48%) cases are ≥40 years of age. There are 33(57%) males and 25(43%) females with a male to female ratio of 1.3:1. At the time of presentation, 25(43%) patients had headache, 19(33%) had focal neurological deficit, and 14(24%) had seizures. 1p/19q co-deletion by FISH was positive in 51(88%) specimens, whereas it was negative in 7(12%) specimens. 1p/19q co-deletion was not significantly associated with age, gender and clinical symptoms (*p*-value >0.05). Sensitivity & diagnostic accuracy of IHC with FISH as gold standard showed that combined sensitivity of IDH and ATRX was 100% with diagnostic accuracy of 87.93% for oligodendrogliomas.
Conclusion: It's concluded that oligodendrogliomas can be diagnosed with combined positive expression of IDH and ATRX with sensitivity of 100% and diagnostic accuracy of 87.93% where FISH for 1p/19q co-deletion is not available.

Keywords: ATRX, IDH, Oligodendrogliomas, 1p/19q co-deletion.

How to Cite This Article: *Hussain Z, Ali SS, Ilyas S, Mati QUA, Shoukat S, Ilyas S. Comparison of IDH and ATRX by immunohistochemistry with 1p/19q co-deletion by FISH in Oligodendrogliomas. Pak Armed Forces Med J 2026; 76(Suppl-7): S1113-S1117.*
DOI: https://doi.org/10.51253/pafmj.v76iSUPPL-7.12321

INTRODUCTION

In 2016, the World Health Organization (WHO) implemented revisions to the grading and classification framework for brain tumors. Prior to this update, tumor grading and categorization were determined solely through histopathological examination of tissue morphology. The most prevalent types of glial tumors include fibrillary astrocytomas and oligodendrogliomas. Accurate differentiation between these tumor entities is essential for prognostication and therapeutic decision-making, as each subtype presents distinct clinical behaviours and responses to treatment.[1] At the same grade, oligodendrogliomas generally exhibit better outcomes and more favourable responses to treatment compared to other types of brain tumors. The 2016 WHO classification identifies oligodendrogliomas by their rounded cells with minimal cytoplasm, mutations in isocitrate dehydrogenase (IDH-1 or IDH-2), and co-deletion of chromosomal arms 1p and 19q. In contrast, while some astrocytomas also show IDH mutations, this feature is not universally present across all cases.[2] Evidence of deletions on chromosomes 1p and/or 19q is observed in only a limited number of astrocytoma cases, with co-deletions being particularly infrequent. Given the notable disparities in prognosis and therapeutic approaches between astrocytomas and oligodendrogliomas, precise differentiation between these tumor types is essential.[3]

Oligodendroglioma is molecularly characterised by the concurrent presence of an IDH mutation and the complete loss of the 1p and 19q chromosomal arms.[4] In line with the WHO 2021 classification for IDH-mutant astrocytomas, IDH-mutant oligodendrogliomas with 1p/19q co-deletion can be classified as either grade 2 or grade 3, which impacts survival outcomes significantly. While homozygous deletions

Correspondence: Dr Zahoor Hussain, Department of Histopathology, Combined Military Hospital, Lahore Pakistan
Received: 06 Jun 2024; revision received: 11 Mar 2025; accepted: 13 Mar 2025

of CDKN2A and/or CDKN2B are infrequent in these tumors, their correlation with poor prognosis—regardless of histology—has established them as a marker for high grade oligodendrogliomas.[5]

1p/19q co-deletion is a key molecular feature that sets oligodendrogliomas apart from astrocytomas. This unique change occurs due to an unbalanced exchange between chromosomes 1 and 19, leading to the loss of entire segments from these chromosomes, specifically arms 1p and 19q.[6] Partial deletion of one or both of the 1p and 19q arms does not meet the criteria for diagnostic 1p/19q co-deletion. Such partial deletions can occur in other central nervous system tumors, including IDH-wildtype glioblastoma.[7] Oligodendrogliomas frequently present with additional molecular abnormalities, including mutations in the TERT promoter and alterations in the capicua transcriptional repressor (CIC) and far upstream element-binding protein 1 (FUBP1).[8]

Interestingly, the genomic localization of CIC on chromosome 19q and FUBP1 on chromosome 1p supports the idea that the 1p/19q co-deletion may disrupt critical tumor suppressor genes located on these chromosomal arms.[9] FISH is the most frequently used method for detecting 1p/19q co-deletion. This technique typically utilizes paired probes: one targeting the deleted arm and the other targeting the retained arm. Commonly used commercial probes are specific to 1p36 and 1q25 on chromosome 1, and 19p13 and 19q13 on chromosome 19. While FISH is both cost-effective and sensitive, relying on probes for localized genomic regions to represent entire chromosomal arms introduces the risk of potential false-positive results.[10]

There is limited information about 1p/19q co-deleted oligodendrogliomas in the Pakistani population. This study aims to explore the clinicopathological features and frequency of these tumors and compare the results with those from other studies. The goal is to improve how we identify, diagnose, assess, and manage these tumors in our local healthcare setting.

METHODOLOGY

The present study was conducted in the Department of Histopathology, Armed Forces Institute of Pathology, Rawalpindi Pakistan over a period of 9 months from 20 July 2022 to 20 April 2023 "FC-HSP20-23/READ-IRB/24/2422". A total of 58 cases of newly diagnosed oligodendroglioma were included by using non-probability consecutive sampling who had space occupying lesion of the brain. Sample size was determined using the WHO sample size calculator, based on prevalence rate of 81.8% cases of 1p/19q co-deleted oligodendrogliomas,[13] with confidence level of 95% and precision of 10%.

Inclusion Criteria: All newly diagnosed cases of oligodendroglioma on brain biopsies, irrespective of the age and gender were included.

Exclusion Criteria: Poorly fixed specimens with inadequate tissue were not included.

All the lab specimens were received after patients' consent. Data collection Performa was filled. Immunohistochemistry and FISH analysis were performed as per the manufacturer's instructions on these cases of oligodendroglioma. Oligodendrogliomas that showed positive expression for IDH and ATRX were tested for 1p/19q co-deletion using FISH. The analysis was performed with a ZEISS Imager.Z2 upright fluorescence microscope, which had filters to detect orange and green signals. The system also included a CoolCube1 metasystems camera and Metafer-4 image analysis software. For each sample, at least 100 non-overlapping tumor cell nuclei were examined for both 1p and 19q arms, with each being assessed separately. The findings were categorized as either 1p/19q co-deleted or 1p/19q non-deleted. Results of the immunohistochemistry and FISH were verified by the consultant histopathologist to minimize the bias.

The 1p/19q FISH test results were analysed in conjunction with clinicopathological data and immunohistochemistry using Statistical Package for the Social Sciences version 23. Statistical significance was determined using the chi-square test, with a threshold of $p \leq 0.05$. Qualitative variables such as gender, clinical symptoms (e.g., headache, seizures, and focal neurological deficits), immunohistochemical expression of IDH and ATRX, and FISH results were expressed as percentages. Quantitative variables, like age, were presented as Mean±SD. Age and gender were considered as potential confounding factors in the analysis. The sensitivity and diagnostic accuracy of immunohistochemistry for IDH and ATRX were evaluated using a 2x2 table and compared to the gold standard of FISH for 1p/19q co-deletion.

RESULTS

In our study, patients were aged between 14.00 and 79.00 years, with a mean age of 40.78 ±16.00 years. In this study, 30 cases (52%) were under 40 years of

age, while 28 cases (48%) were 40 years or older. The cohort comprised 33 males (57%) and 25 females (43%), resulting in a male-to-female ratio of 1.3:1. Among clinical symptoms, 25(43%) patients presented with headache, 19(33%) with focal neurological deficit, and only 14(24%) had seizures at the time of presentation. 1p/19q co-deletion by FISH was positive in 51(88%) specimens, whereas it was negative in 7(12%) specimens. The results of a 1p/19q co-deleted oligodendroglioma case are shown in the attached image (Figure). The Figure shows one red signal(1p) and two green signals(19q) indicating codeletion of 1p region while retaining two copies of 19q region. The analysis indicated that 1p/19q co-deletion was not significantly associated with age, gender, or clinical symptoms, as the *p*-value was greater than 0.05 (Table-I).

Figure: FISH results of 1p/19q co-deleted oligodendroglioma

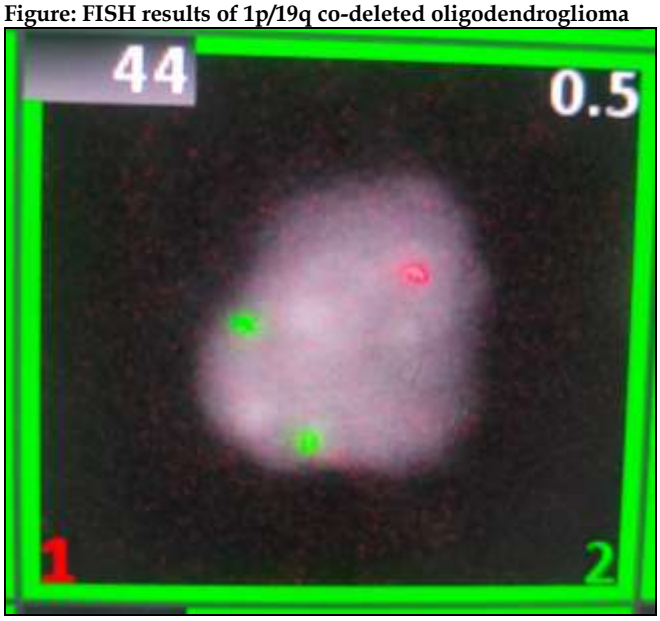

Table-I: Association of 1p/19q co-deletion by FISH in Oligodendroglioma with Age Groups, Gender and Symptoms (n=58)

Clinicopathological Variables	FISH for 1p/19q co-deletion		*p*-value
	Positive 51(88%)	Negative 7(12%)	
Age Groups (years)			
<40 (n=30)	27(90%)	3(10%)	0.617
≥40 (n=28)	24(86%)	4(14%)	
Gender			
Male (n=33)	30(91%)	3(9%)	0.424
Female (n=25)	21(84%)	4(16%)	
Symptoms			
Headache (n=25)	21(84%)	4(16%)	0.695
Seizures (n=14)	13(93%)	1(7%)	
Focal neurological deficit (n=19)	17(89%)	2(11%)	

The cases that tested positive for 1p/19q co-deletion by FISH were diagnosed as Oligodendroglioma, IDH mutant, 1p/19q co-deleted, according to WHO guidelines. A 2x2 table assessing the sensitivity and diagnostic accuracy of immunohistochemistry (IHC) with FISH as the gold standard indicated that the combined sensitivity of IDH and ATRX IHC was 100%, with a diagnostic accuracy of 87.93% for oligodendrogliomas (Table-II).

Table-II: Two by two Contingency Table between FISH for 1p/19q co-deletion and Immunohistochemistry results for IDH and ATRX in Oligodendrogliomas (n=58)

Immunohistochemistry (IDH, ATRX)	FISH for 1p/19q co-deletion (Gold Standard)	
	Yes/Positive	No/Negative
Yes/Positive	51(100%)	7(100%)
No/Negative	0(0%)	0(0%)

Sensitivity= True Positive/(True Positive +False Negative)= 100%
Diagnostic Accuracy= (True Positive +True Negative)/All Patients = 87.93%

DISCUSSION

In our study, out of 58 patients with retained ATRX expression and positive staining for IDH, 51(88%) showed 1p/19q co-deletion, and 7(12%) cases were negative. Our results are comparable to study conducted at Mayo clinic USA. Diffuse astrocytomas frequently exhibit mutations in the Tp53 and ATRX genes, whereas oligodendrogliomas are characterized by chromosome 1p/19q eco-deletion and mutations in the TERT promoter. Identifying these molecular characteristics is essential for formulating treatment strategies, which are guided by both the molecular subtypes and the WHO tumor grade.[11] IDH mutations are a critical molecular marker with significant prognostic value and are now used to classify diffuse gliomas. These mutations arise early in glioma tumorigenesis, as evidenced by their presence in both astrocytic and oligodendroglial gliomas.[12] In IDH mutant cases, 81.8% demonstrated 1p/19q co-deletion and were classified as oligodendrogliomas. Importantly, ATRX loss was not detected in any of the oligodendrogliomas with 1p/19q co-deletion.[13] IDH mutant gliomas are generally classified into two main subtypes: one that displays 1p/19q co-deletion and other, more common, subtype that features ATRX loss.[14] ATRX encodes a chromatin remodelling protein. The absence of ATRX protein expression, as detected by immunohistochemistry, can be used as an indirect indicator of ATRX gene inactivation and assists in confirming diagnosis of astrocytoma.[15] However, a study conducted at Aga Khan University Hospital analysed 99 oligodendroglioma patients between 2013 and 2016, finding that 35% exhibited 1p/19q co-deletion, with a male-to-female ratio of 2:1 and a mean age of 39 years.[19] A recent review from Pakistan highlighted that the presence of 1p/19q chromosomal co-deletion in oligodendrogliomas not only serves as a

marker of better prognosis but also indicates a more favourable response to chemotherapy.[20]

In this study, out of 42 oligodendroglioma patients with retained ATRX expression, 36(85.7%) patients showed 1p/19q codeletion and the same patients showed positive staining for IDH.[16] At the Department of Histopathology, Shaukat Khanum Memorial Hospital and Research Centre in Lahore, 214 diffuse glioma cases were analysed. Over 90% of the IDH1/2 mutant gliomas exhibited the R132 IDH1 mutation. Of the IDH1-mutant cases, 32(44%) retained ATRX expression. FISH analysis for 1p/19q co-deletion was conducted on two of these cases, both of which showed 1p/19q co-deletion, leading to their classification as oligodendrogliomas with IDH mutation and 1p/19q co-deletion.[13] In a study at Zurich University, Germany, which included 405 brain tumor patients, 61 were diagnosed with oligodendrogliomas. The study found that all patients with 1p/19q co-deletion had mutations in IDH1 or IDH2 and retained ATRX expression, except for two cases that, despite having 1p/19q co-deletion, lacked ATRX expression.[17] In comparison to our study, Wick *et al.,* (2020) report that the age, gender, and clinical symptom distribution in glioma patients may not significantly correlate with molecular markers such as 1p/19q co-deletion, further supporting the notion that clinical symptoms are not strongly predictive of 1p/19q co-deletion status in adult gliomas.[18] Our findings align with a recent study that reported 1p/19q co-deletion in 88% of oligodendroglioma cases, with no significant correlation between clinical symptoms and molecular markers.[19] Additionally, a study conducted in Pakistan emphasized the lack of clinical symptoms predicting 1p/19q co-deletion in gliomas, reinforcing the need for molecular testing for accurate diagnosis.[21]

CONCLUSION

In this study, it is concluded that oligodendrogliomas can be diagnosed with combined positive expression of IDH and ATRX with sensitivity of 100% and diagnostic accuracy of 87.93% where FISH for 1p/19q is not available. 1p/19q co-deletion has no statistical association with age, gender and clinical symptoms of the oligodendrogliomas.

Conflict of Interest: None.
Funding Source: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

ZH & SSA: Data acquisition, data analysis, critical review, approval of the final version to be published.

SI & QUAM: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

SS & SI: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Brat DJ, Aldape K, Colman H, Figrarella-Branger D, Fuller GN, Giannini C, et al. cIMPACT-NOW update 5: recommended grading criteria and terminologies for IDH-mutant astrocytomas. Acta Neuropathol 2020; 139(3): 603–608. http://doi.org/10.1007/s00401-020-02127-9
2. Sim J, Nam D-H, Kim Y, Lee I-H, Choi JW, Sa JK, et al. Comparison of 1p and 19q status of glioblastoma by whole exome sequencing, array-comparative genomic hybridization, and fluorescence in situ hybridization. Med Oncol 2018; 35(5): 60. http://doi.org/10.1007/s12032-018-1119-2
3. Huang J, Yu J, Tu L, Huang N, Li H, Luo Y. Isocitrate dehydrogenase mutations in glioma: From basic discovery to therapeutics development. Front Oncol 2019; 9: 506. http://doi.org/10.3389/fonc.2019.00506
4. Geevar T, Pai R, Chacko G, Malepathi K, Patel B, John J. Molecular profile of tumours with oligodendroglial morphology: Clinical relevance. Neurol India 2018; 66(6): 1726–1731. http://doi.org/10.4103/0028-3886.246275
5. Ammendola S, Caldonazzi N, Simbolo M, Piredda ML, Brunelli M, Poliani PL, et al. H3K27me3 immunostaining is diagnostic and prognostic in diffuse gliomas with oligodendroglial or mixed oligoastrocytic morphology. Virchows Arch 2021; 479(5): 987–996. http://doi.org/10.1007/s00428-021-03134-1
6. Kao PY, Ngo T, Zhang A, Chen JW, Manjunath BS. Brain tumor segmentation and tractographic feature extraction from structural MR images for overall survival prediction. International MICCAI Brain lesion Workshop 2018; 16: 128–141. http://doi.org/10.1007/978-3-030-11726-9_12
7. Zachariah MA, Oliveira-Costa JP, Carter BS, Stott SL, Nahed BV. Blood-based biomarkers for the diagnosis and monitoring of gliomas. Neuro Oncol 2018; 20(9): 1155–1161. http://dx.doi.org/10.1093/neuonc/noy074
8. Liu J, Zhang X, Yan X, Sun M, Fan Y, Huang Y. Significance of TERT and ATRX mutations in glioma. Oncol Lett 2018. http://dx.doi.org/10.3892/ol.2018.9634
9. Fan H-C, Chen C-M, Chi C-S, Tsai J-D, Chiang K-L, Chang Y-K, et al. Targeting telomerase and ATRX/DAXX inducing tumor senescence and apoptosis in the malignant glioma. Int J Mol Sci 2019; 20(1): 200. http://doi.org/10.3390/ijms20010200
10. Tesileanu CMS, Vallentgoed WR, Sanson M, Taal W, Clement PM, Wick W, et al. Non-IDH1-R132H IDH1/2 mutations are associated with increased DNA methylation and improved survival in astrocytomas, compared to IDH1-R132H mutations. Acta Neuropathol 2021; 141(6): 945–957. http://doi.org/10.1007/s00401-021-02291-6
11. Chatterjee D, Radotra BD, Kumar N, Vasishta RK, Gupta SK. IDH1, ATRX, and BRAFV600E mutation in astrocytic tumours and their significance in patient outcome in north Indian population. Surg Neurol Int 2018; 9. http://doi.org/10.4103/sni.sni_284_17

12. Haase S, Garcia-Fabiani MB, Carney S, Altshuler D, Núñez FJ, Méndez FM, et al. Mutant ATRX: uncovering a new therapeutic target for glioma. Expert Opin Ther Targets 2018; 22(7): 599–613. http://doi.org/10.1080/14728222.2018.1487953
13. Javed R, Akhtar N, Hassan U, Azam M, Loya A, Hussain M. Idh-1, Atrx and 1p19q co-deletion in diffuse gliomas. Pak Armed Force Med J 2020; 70(2): 571–577. http://doi.org/10.51253/pafmj.v70i2.4235
14. Hu W-M, Wang F, Xi S-Y, Zhang X, Lai J-P, Wu H-Y, et al. Practice of the new integrated molecular diagnostics in gliomas: Experiences and new findings in a single Chinese center. J Cancer 2020; 11(6): 1371–1382. http://doi.org/10.7150/jca.38603
15. Mukherjee J, Johannessen TC, Ohba S, Chow TT, Jones L, Pandita A. Mutant IDH1 Cooperates with ATRX Loss to Drive the Alternative Lengthening of Telomere Phenotype in Glioma Mutant IDH1 Drives ALT and Gliomagenesis. Cancer res 2018; 78: 2966–2977. http://doi.org/10.1158/0008-5472.CAN-17-2269
16. Leeper HE, Caron AA, Decker PA, Jenkins RB, Lachance DH, Giannini C. IDH mutation, 1p19q codeletion and ATRX loss in WHO grade II gliomas. Oncotarget 2015; 6(30): 30295–305. http://doi.org/10.18632/oncotarget.4497
17. Reuss DE, Sahm F, Schrimpf D, Wiestler B, Capper D, Koelsche C, et al. ATRX and IDH1-R132H immunohistochemistry with subsequent copy number analysis and IDH sequencing as a basis for an "integrated" diagnostic approach for adult astrocytoma, oligodendroglioma and glioblastoma. Acta Neuropathol 2015; 129(1): 133–146. http://doi.org/10.1007/s00401-014-1370-3
18. Walach H. Omega. Karger Kompass Onkol 2020; 7(3): 176–176. http://dx.doi.org/10.1159/000510435
19. Tariq MU, Ahmed Z, Moatter T. Role of chromosomal 1p/19q co-deletion on the prognosis of oligodendrogliomas in Pakistan. Ann Oncol 2017; 28(suppl_10): x38. http://doi.org/10.1093/annonc/mdx657.012
20. Laghari AA, Khalid MU, Qadeer N, Shamim MS. Prognostic value of 1p/19q chromosomal codeletion in patients with oligodendroglioma. J Pak Med Assoc 2019; 69(1): 132–134.
21. Rathore S, Iftikhar MA, Mourelatos Z. Prediction of overall survival and molecular markers in gliomas via analysis of digital pathology images using deep Learningarxiv 2019. http://doi.org/10.48550/arXiv.1909.09124

.....