*Malays J Pathol 2020; 42(3): 369 – 376*

# ORIGINAL ARTICLE

# FISHing for 1p19q codel in oligodendroglioma

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*Abstract*

Together with isocitrate dehydrogenase (IDH) mutation, co-deletion of 1p19q (1p19q codel) is a pre-requisite for diagnosis of oligodendroglioma, making it imperative that histopathology laboratories introduce testing for 1p19q codel. To date there is still no consensus reference range and cut-offs that confirm deletion of 1p or 19q. We embarked on determining our reference range in 11 formalin-fixed, paraffin-embedded non-neoplastic brain tissue using fluorescence in situ hybridisation (FISH) with the Vysis 1p36/1q25 and 19q13/19p13 FISH Probe Kit (Abbott Molecular Inc., USA). At same time we attempted to validate our methodology in 13 histologically-confirmed IDH-mutant oligodendrogliomas. For 1p, percentage cells with deletion (range=8-23%; mean±SD = 15.73±5.50%) and target: control (1p36:1q25) ratio (range = 0.89-0.96; mean±SD = 0.92±0.03) in non-neoplastic brain, differed significantly ($p<0.000$) from oligodendroglioma (percentage cells with deletion: range = 49-100%; mean±SD = 82.46±15.21%; target:control ratio range:0.50-0.76; mean±SD = 0.59±0.08). For 19q, percentage cells with deletion (range = 7-20%; mean±SD = 12.00±3.49%) and target:control (19q13/19p13) ratio (range:0.90-0.97; mean±SD = 0.94±0.02) in non-neoplastic brain also differed significantly from oligodendroglioma (percentage cells with deletion: range = 45-100%; mean±SD = 82.62±18.13%; target:control ratio range:0.50-0.78; mean±SD = 0.59±0.09). Using recommended calculation method, for diagnosis of 1p deletion, percentage of cells showing deletion should be >32-33% and/or target:control ratio <0.83. For 19q, percentage of cells showing deletion should be >22% and target:control ratio <0.88. Using these cut-offs all 13 oligodendroglioma demonstrated 1p19q codel.

*Keywords:* 1p19q co-deletion, oligodendroglioma, fluorescence in situ hybridisation, reference range, cut-off

## INTRODUCTION

Presently, co-deletion of 1p19q (1p19q codel) is required to diagnose and differentiate oligodendroglioma from other gliomas. This requirement is combined with fulfilling the traditional histological criteria in the presence of isocitrate dehydrogenase (IDH) mutation, and was introduced in the 2016 World Health Organization (WHO) classification of tumours of the central nervous system.[1] This followed on studies which showed that tumours stringently satisfying the criteria were most chemosensitive and had the best prognosis among the gliomas. Thus it is crucial that diagnostic histopathology laboratories which offer neuropathology services introduce this test.[2-4]

1p19q codel in oligodendrogliomas is due to an unbalanced translocation, with deletion of the entire short arm of chromosome 1 and entire long arm of chromosome 19.[5,6] When this occurs, the derivative chromosome der (1;19)(p10;q10) is lost while the derivative chromosome der (1;19)(q10;p10) is retained. To date, how this co-deletion actually effects a more favourable prognosis and enhanced chemosensitivity remains unclear, although 1p19q codel may be part of a "favourable genetic cluster", e.g. oligodendrogliomas are noted to express a proneural genomic signature, as described in The Cancer Genome Atlas Project, which is said to augur for an improved outcome.[7-9] While there are several methods to identify 1p19q

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codel, including multiplex ligation dependent probe amplification, loss of heterozygosity analysis and comparative genomic hybridisation, fluorescence in situ hybridisation (FISH) is the most frequently employed method in most centers.[10-13] Although discussion of the other molecular techniques is beyond the scope of this report, suffice it to say that FISH uses techniques that are fairly similar to immunohistochemistry which are routine tests in the diagnostic histopathology laboratory. Furthermore, it allows morphological identification of cells of interest making it understandable that FISH is preferred in histopathology laboratories for identification of 1p19q codels. However, even though FISH is commonly used to detect 1p19q codels, there is yet a standardised protocol for use or agreed upon consensus cut-offs that signify deletion of 1p or 19q.[12,14] Currently, at least two systems of reporting "deletion" have been used, with one based on the percentage of tumour cells interpreted as having lost 1p and 19q respectively and another based on the ratio of target to control FISH signals in the tumour. Centers vary in the cut-offs adopted for the above. 20-50% tumour cells showing deleted signals have been considered indicative of 1p or 19q deletion using the first system[12,15,16], while target to control signal ratios of ≤0.7 to ≤0.85 and ≤0.8 to ≤0.9 have been used to signify 1p and 19q deletions respectively.[17-19] Laboratories have also resorted to defining their own normal reference range and in-house cut-offs. In most cases, when percentages of cells with deleted 1p or 19q deletion are used as criteria for defining deletion, the mean + 3 standard deviations (SD) of 1p or 19q deletion observed in non-neoplastic brain cells[14,20,21], would be used as the upper threshold for "normal". Using the same reasoning, mean – 3 SD of 1p or 19q (target) versus respective control signal ratios in non-neoplastic brains would define the "normal" cut-off when target to control signals are used for determining deletion.

As a preliminary to the introduction of 1p19q codel analysis into our routine service, we embarked on determining our reference range and cut-off for interpretation of 1p and 19q deletion respectively in formalin-fixed, paraffin-embedded non-neoplastic brain tissue. In addition, we attempted to validate our methodology by using the same method to determine 1p19q codel in histologically-proven, IDH-mutant oligodendrogliomas.

## MATERIALS AND METHODS

The study was approved by the Institutional Review Board (IRB) of the University of Malaya Medical Center (MREC ID NO: 2019717-7656) and carried out in compliance with the Declaration of Helsinki. All patients undergoing surgical procedures have provided informed and written consent. The Medical Research Ethics Committee/IRB of the University of Malaya Medical Center accepts that additional informed consent from patients was not required for studies that utilised archived surgical and biopsy material. Nonetheless, patient identification would be anonymised, information limited to the authors, and data confidentiality protected.

*Cases*

Thirteen histologically re-confirmed, oligodendrogliomas diagnosed between 2014 till 2018 were selected from the archives of the Department of Pathology, University of Malaya Medical Center. 11 brain biopsies carried out between the same period and histologically re-confirmed to be non-neoplastic were also selected and would serve as the "normal" for determination of the reference range and cut-offs for 1p and 19q deletions. Demographic data of the cases were retrieved from the histopathology request forms. All sections of the oligodendrogliomas and "normal" brains were histologically reviewed and one paraffin block of the tumour and non-neoplastic brain was selected for study. For the oligodendrogliomas which had no prior confirmation of its IDH mutant status, IDH mutation was first confirmed by immunohistochemical staining for the mutant protein (Fig.1), before enrolment into the study. Staining for the mutant IDH protein was carried out on the automated Ventana BenchMark ULTRA (Ventana Medical Systems Inc., Tucson, Arizona) using a rabbit monoclonal anti-IDH antibody (1:300; EPR12296, Abcam, Cambridge, UK) which detected mutations of Arg-132 to Cys of the IDH1 gene that encompasses about 90% of IDH mutations in oligodendrogliomas.[22]

For FISH analysis, the best area of the tumour (with at least 60% tumour in the section) and the best area of the non-neoplastic brain would be marked out as regions of interest (ROI) on the selected H+E stained slide. Areas of necrosis or haemorrhage would be avoided. Two 4 $\mu$m sections were cut from the corresponding paraffin block of each case on to Polysine slides® (Thermo Scientific, USA). A diamond pen was used to score underside of the slide with the ROI

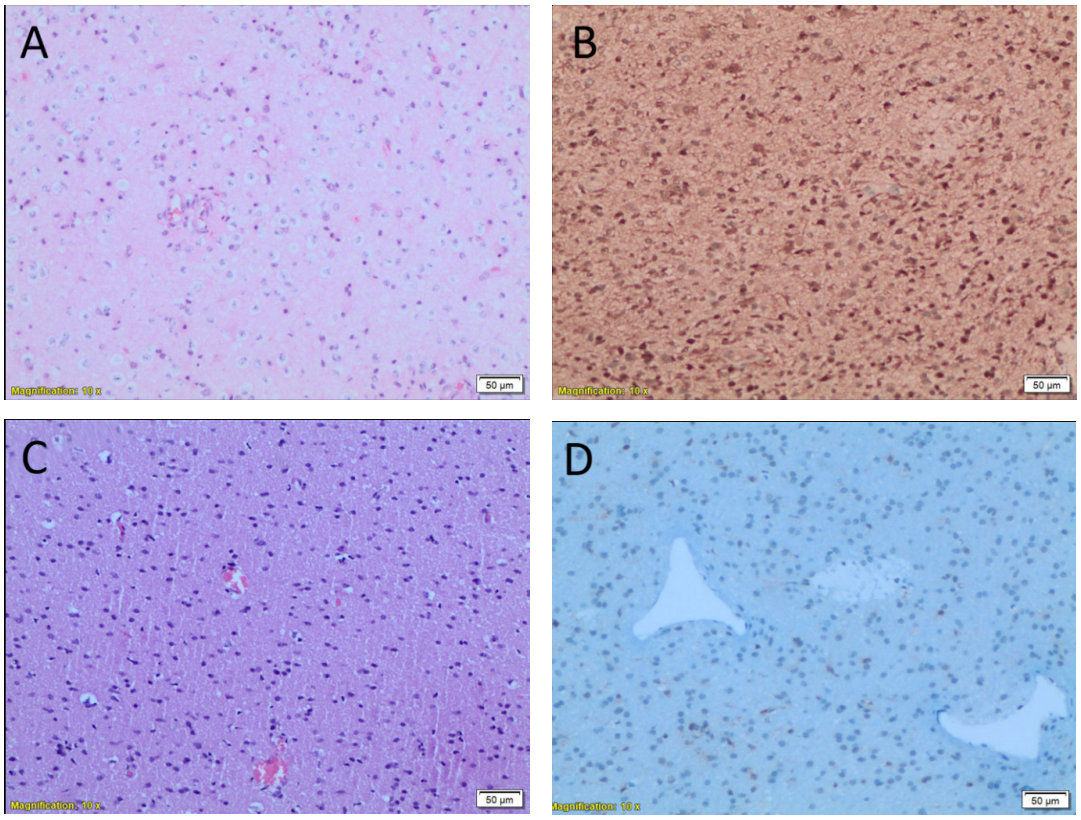


FIG. 1: (A) Oligodendroglioma, WHO grade 2 demonstrating classical "fried egg" tumour cells amidst a fibrially glial background with numerous small calibre blood vessels (H+E) and exhibiting (B) strong cytoplasmic as well as some nuclear IDH immunopositivity. (C) shows non-neoplastic, "normal" brain tissue demonstrating gliosis (H+E) and (D) only faint IDH immunostaining in the microglia / macrophages (x100).

that corresponded with that marked out on the H+E section. The scored slides with the tissue sections were dried at 37°C overnight, and finally at 60°C for 60 min before being ready for FISH analysis.

*Fluorescence in situ hybridisation (FISH)*

FISH was carried out according to the manufacturer's instructions using the Vysis 1p36/1q25 and 19q13/19p13 FISH Probe Kit (Abbott Molecular Inc., USA), together with the Vysis Paraffin Pretreatment IV and Post-Hybridisation Wash Buffer kit (Abbott Molecular Inc., USA). The probes supplied were spectrum orange-labelled 1p36 (435 Kb) and spectrum green-labelled 1q25 (618 Kb) probes in a hybridisation buffer premix, and spectrum orange-labelled 19q13 (380 Kb) and spectrum green-labelled 19p13 (502 Kb) probes as a separate premix. Prior to commencing the analysis, the tissue sections were dewaxed in xylene, dehydrated in two changes of absolute ethanol before pre-treatment at 80°C for 30 min in the Vysis Pretreatment Solution. Digestion followed at 37°C for 20 min with Vysis Protease Solution, and terminated by immersing in deionized water for 3 min at room temperature (RT). This was followed by dehydration through increasing concentrations of ethanol, i.e., 70%, 85%, 100% (1 min each). 5 $\mu$l of the 1p36/1q25 premix was applied on the tissue section corresponding to the ROI of one slide, and 5 $\mu$l of the 19q13/19p13 premix was similarly applied on the second slide. The areas of probe application were cover-slipped and periphery of the cover-slips sealed with rubber cement. This was followed by co-denaturation of the tissue DNA targets with the 1p36/1q25 or 19q13/19p13 probes for 5 min at 73°C. Subsequent hybridisation was carried out at 37°C overnight for the two slides in a StatSpin ThermoBrite system (Iris International Inc., USA). Post-hybridisation washing with Vysis Wash Buffer II at RT for 5 min (at which time the

coverslips would be floated off), was followed by Vysis Wash Buffer I at 73°C for 2 min and finally by Vysis Wash Buffer II at RT for 1 min. The sections were air-dried before counterstaining with 5$\mu$l DAPI II Counterstain (Abbott Molecular Inc., USA) on the ROI for 30 min at -20°C. The slides were viewed on a Zeiss Axio Imager Z2 epi-fluorescence microscope (Carl Zeiss, Germany). Images were analysed via the MetaCyte Lite software ver. 3.11.3 (Metasystems, Germany). The spectrum green-labelled 1q25 and 19p13 probes served as internal controls for the spectrum orange-labelled 1p36 and 19q13 target probes respectively. For purposes of this study 100 consecutive, non-overlapping glial cells of each case of non-neoplastic brain or tumour cells of the oligodendroglioma were used for signal enumeration. To minimise the uncertainty regarding the effects of 1p19q codel in polysomy[23], only diploid cells were counted. To limit the problem of truncation, only cells with 2 green internal control signals were included. Loss of the derivative chromosome der (1;19) (p10;q10) would be represented by loss of one orange signal/cell when using either the 1p36 or 19q13 probe (Fig. 2 & 3). In other words, cells with 1 orange signal and 2 green signals when the 1p36/1q25 premix was applied would mean deletion of 1p36 and similarly 1 orange and 2 green signals when the 19q13/19p13 premix was used would mean deletion of 19q13. Percentage of the selected 100 cells interpreted as demonstrating "deletion" (1 orange and 2 green signals) was tabulated for 1p36 and 19q13 in each case of non-neoplastic brain and oligodendroglioma. In addition, the ratio of total orange (target) to total green (control) signals in the 100 cells was also tabulated for both 1p36 and 19q13 in every case.

*Statistical analysis*

Statistical analysis was performed using SPSS version 24.0 (IBM, Chicago, Illinois, USA). The continuous variables would be tested with Independent Samples T-test for parametric data and Mann-Whitney U test for non-parametric data respectively. Statistical significance was set as $p < 0.05$.

## RESULTS

Table 1 shows the demographic profile of the cases with non-neoplastic brains and oligodendrogliomas entered into the study. Eight of the non-neoplastic brains were biopsied for epileptic seizures and one each for clinical query of toxoplasmosis, cerebral lymphoma and ependymoma. All the 3 cases of clinically suspected toxoplasmosis, cerebral lymphoma and ependymoma were proven negative on biopsy. 10 of the oligodendrogliomas were grade 2, and 3 were grade 3. All the cases of non-neoplastic brains and oligodendrogliomas in this study were conducive for FISH analysis and demonstrated appropriate signals. Table 2 illustrates 1p36 and 19q13 deletions in non-neoplastic brain and oligodendrogliomas by percentage of cells with deletions and target to control signal ratios (1p36/1q25 and 19q13/19p13). Based on the signal counts per 100 cells in each case, 1p36 deletion was detected in 8-23% (mean±SD = 15.73±5.50%) of the 11 non-neoplastic brain cases which was significantly lower ($p<0.000$) than that noted in the 13 oligodendrogliomas (range 49-100%; mean±SD = 82.46±15.21%). Expectedly, non-neoplastic brains also achieved significantly higher ($p<0.000$) target to control signal ratio (1p36:1q25 range: 0.89-0.96; mean±SD = 0.92±0.03) compared with oligodendroglioma (1p36:1q25 range:0.50-0.76; mean±SD = 0.59±0.08). 19q13 deletion was observed in 7-20% (mean±SD = 12.00±3.49%) non-neoplastic brains, which was also significantly lower ($p<0.000$) than oligodendrogliomas (range: 45-100%; mean±SD = 82.62±18.13%). As with 1p36 deletion, 19q13 target to signal control ratio was also significantly higher ($p<0.000$) in non-neoplastic brains (19q13:19p13 range:0.90-0.97; mean±SD = 0.94±0.02) compared with oligodendroglioma (19q13:19p13 range:0.50-0.78; mean±SD = 0.59±0.09). Based on the recommendation of using mean + 3SD of non-neoplastic brains, >32.23 % of tumour cells must show deletion of 1p36 and >22.47% must show deletion of 19q13, for a case to be considered 1p and 19q co-deleted. For target:control signal ratios, the mean - 3SD of 1p36/1q25 and 19q13/19p13 deletions in non-neoplastic brains was 0.83 for 1p36/1q25 and 0.88 for 19q13/19p13 respectively. Hence target:control signal ratios should be <0.83 for 1p36/1q25 and <0.88 for 19q13/19p13 if tumours are to be considered co-deleted for 1p and 19q.

## DISCUSSION

In this study, we set out to first determine the baseline deletions of chromosomes 1p and 19q in non-neoplastic brains by FISH. Based on

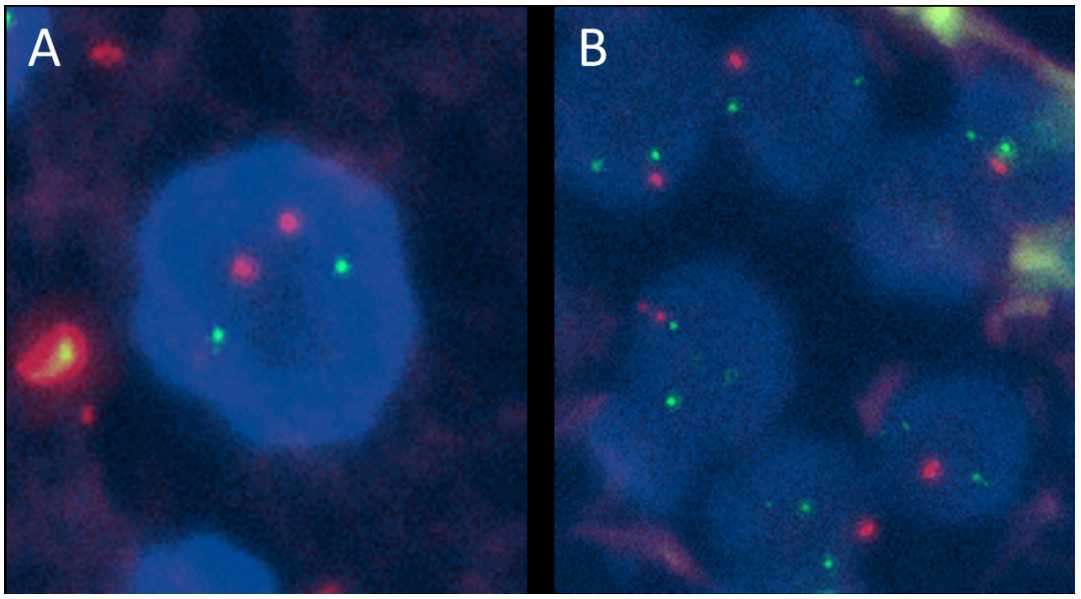


FIG. 2: (A) shows 2 spectrum orange-labelled 1p36 and 2 spectrum green-labelled 1q25 in a "normal" brain (x400); while most cells in (B) show 1 spectrum orange-labelled 1p36 against 2 spectrum green-labelled 1q25 in a case of oligodendroglioma (x400)

Horbinski *et al.'s* recommendation that at least 100 cells/case of 10 non-neoplastic brains be used to derive this[16], and Woehrer *et al.'s*[14], recommendation for using 5-10 cases, we used 11 non-neoplastic brains and enumerated 100 cells/case for 1p36 (orange) versus 1q25 (green) signals, or 19q13 (orange) versus 19p13 (green) signals to determine deletion of 1p or 19q respectively. Based on the two systems of reporting "co-deletion", viz the percentage of cells interpreted as having lost 1p or 19q, or ratio of target to control FISH signals for 1p or 19q, we worked out both the percentage of deleted signals of 1p or 19q as well as the target: control signal ratio for each case of non-neoplastic brain to finally derive the acceptable

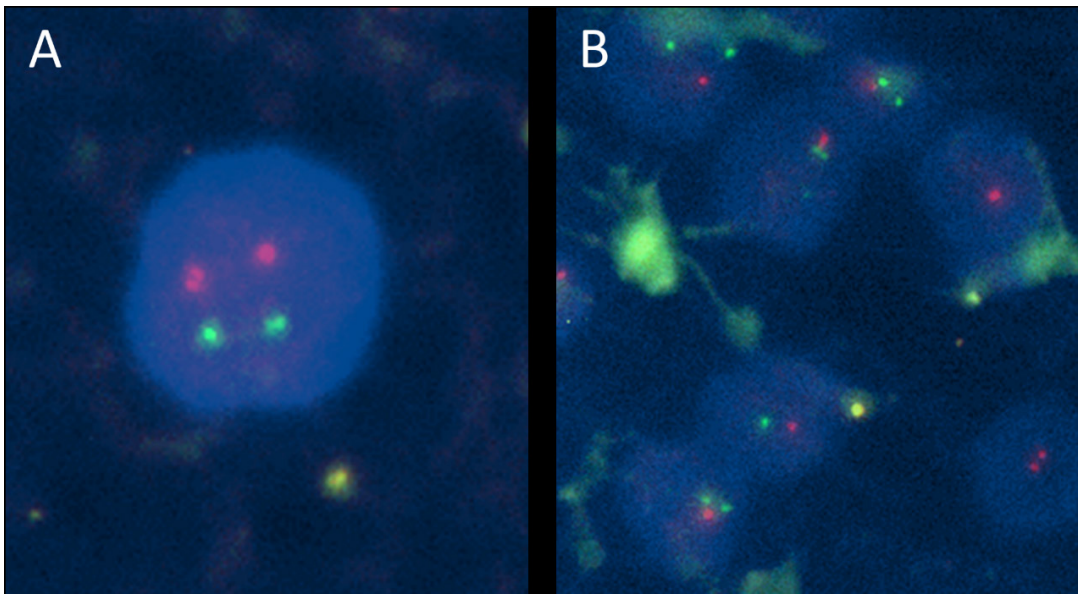


FIG. 3: (A) shows 2 spectrum orange-labelled 19q13 and 2 spectrum green-labelled 19p13 in a "normal" brain (x400); while most cells in (B) show 1 spectrum orange-labelled 19q13 against 2 spectrum green-labelled 19p13 in a case of oligodendroglioma (x400)

**TABLE 1: Demographic data of the cases of non-neoplastic brain and oligodendroglioma**

| | | Non-neoplastic brain (n=11) | Oligodendroglioma (n=13) |
|---|---|---|---|
| Age | Range (years) | 28-62 | 18-69 |
| | Mean±SD (years) | 42.2±10.1 | 49.5±15.7 |
| Gender | Male | 9 | 8 |
| | Female | 2 | 5 |
| | M:F | 4.5:1 | 1.6:1 |
| Ethnicity | Malay | 3 | 1 |
| | Chinese | 5 | 6 |
| | Indian | 3 | 4 |
| | Others | 0 | 2 |

threshold of deletions in "normal" brains. In this study, a total of 1100 non-neoplastic cell nuclei were analysed. Most suggest that 3SD added to the mean[14,20,21] percentage of non-neoplastic brain cells designated as deleted for 1p or 19q be used for cut-off of the normal range. Using the above suggestions, our study showed that tumours with >32.23% tumour cells demonstrating 1p36 deletions can be considered 1p deleted and tumours with >22.47% tumour cells demonstrating 19q13 deletion considered 19q deleted. For the target:control signal ratio cut-offs, tumours with 1p36/1q25 ratio <0.83 and 19q13/19p13 <0.88 would be considered deleted for 1p and 19 q respectively. The normal range cut-offs observed in this study were within the range recommended for use by other authors (20-50% for both 1p and 19 deletion[12,15,16] and target to control ratios of ≤0.7 to ≤0.85 for 1p; and ≤0.8 to ≤0.9 for 19q.[17-19] At this point it may be relevant to reiterate that while the principles of the FISH technique used in various studies to determine the normal range cut-offs were generally similar, although not standardised, the cut-off values reported varied between studies. This was generally due to differing criteria employed to calculate these values. Notwithstanding the above, the possibility of population differences cannot also be completely discounted, underscoring the importance of determining our local values, as carried out in this study, before embarking on using FISH for detection of 1p19q codel in the routine diagnosis of oligodendrogliomas. It should be mentioned that for this study, we restricted the cells that were enumerated for both the non-neoplastic brains and oligodendrogliomas to diploids. By doing so, we tried to minimise the complications of issues of aneuploidy in deriving our normal range cut-off as there is currently still no consensus on how to interpret these results.[12] With conflicting reports from various studies[18,24,25], it is still unclear if polysomy affects survival. However, it is noteworthy that recently, Chen *et al*. have shown that polysomy resulted in shortened progression-free survival in a study of 412 oligodendroglial tumours with 1p19q codel.[23]

Having established a cut-off for the normal

**TABLE 2: 1p36 and 19q13 deletion in non-neoplastic brain (n=11) and oligodendroglioma (n=13)**

| | | Non-neoplastic brain | | | Oligodendroglioma | | | p value |
|---|---|---|---|---|---|---|---|---|
| | | Range | Mean | SD | Range | Mean | SD | |
| 1p36 | % of deleted cells | 8-23 | 15.73 | 5.50 | 49-100 | 82.46 | 15.21 | <0.000 |
| | Target:control | 0.89-0.96 | 0.92 | 0.03 | 0.50-0.76 | 0.59 | 0.08 | <0.000 |
| | | Range | Mean | SD | Range | Mean | SD | |
| 19q13 | % of deleted cells | 7-20 | 12.00 | 3.49 | 45-100 | 82.62 | 18.13 | <0.000 |
| | Target:control | 0.90-0.97 | 0.94 | 0.02 | 0.50-0.78 | 0.59 | 0.09 | <0.000 |

range of deletions of 1p and 19q, we also showed that our cases of oligodendrogliomas exhibited 1p19q co-deletion, at a significantly higher level than those of non-neoplastic brains. Using cut-offs established in this study (>32.23% deleted cells, with target:control signal ratio <0.83 for 1p deletion; >22.47% deleted cells, with target: control signal ratio <0.88 for 19q), all 13 cases of histologically-proven oligodendroglioma demonstrated 1p19q codel, as corroborative validation of the FISH methodology employed in the study.

While FISH seems a viable method for use in a routine histopathology laboratory for diagnosis and confirmation of 1p19q co-deletions in formalin-fixed, paraffin-embedded brain tumours, it is important to understand some inherent characteristics of the assay so that results can be interpreted in the appropriate context. Even in experienced hands, most have observed that FISH fails in 2.5% of cases[16], while others have only been successful in about 90%.[14] Most failures are likely to be due to the pre-analytical DNA quality of the formalin-fixed, paraffin-embedded tissues which defies hybridisation, despite modifications of analytical protocols.[14,26] Under such circumstances, use of the same material for analysis of 1p19q codel by other known detection methods e.g. multiplex ligation dependent probe amplification, loss of heterozygosity analysis (PCR-based), comparative genomic hybridisation which are also dependent on good quality DNA, may also not yield successful results. For such cases diagnosis of oligodendrogliomas may be inferential and based on other markers e.g. IDH, α thalassemia/mental retardation syndrome X-linked (ATRX) and TP53 mutations.[22] Another relevant point in the use of FISH for detection of 1p19q codel should take into account that in general probes currently used do not cover the whole arm of 1p or 19q. In most cases the probes are designed against "minimally deleted regions" of the chromosomes that range from 100 Kb to 1 Mb in length.[12,16] In our case, 1p36 of 435 Kb and 19q13 of 380 Kb certainly do not cover the full chromosome arms. As small interstitial deletions of 1p or 19q do not provide survival advantage[13], use of such probes can result in false "positive" deletions being reported. Finally and as mentioned earlier, to date there is no consensus on reporting of polysomies. Although in this study, cells with more than 2 control signals i.e. 1q25 and 19p13 were not included in the enumeration, we are of the opinion that it may be judicious to include these in future in view of recent findings that polysomies may affect progression-free survival.

*Acknowledgement:* The authors are grateful to Genomic Medical Science @ UM for supporting this study.

*Authors' contribution:* PLK performed testing, analysed results and wrote manuscript. PLC designed study, validated and analysed results and wrote manuscript. KSM validated results and wrote manuscript. SFC, KHT, NAR validated results. TPL, CCK performed testing. LML designed study and analysed results.

*Conflict of interest:* The authors declare no conflict of interest.

## REFERENCES

1. Louis DN, Perry A, Reifenberger G, *et al*. The 2016 World Health Organization Classification of tumors of the central nervous system: a summary. Acta Neuropathol. 2016; 131(6): 803-20.
2. Chai RC, Zhang KN, Chang YZ, *et al*. Systematically characterize the clinical and biological significances of 1p19q genes in 1p/19q non-codeletion glioma. Carcinogenesis. 2019; 40(10): 1229-39.
3. Jooma R, Waqas M, Khan I. Diffuse low-grade glioma - changing concepts in diagnosis and management: a review. Asian J Neurosurg. 2019; 14(2): 356-63.
4. Tom MC, Varra V, Leyrer CM, *et al*. Risk factors for progression among low-grade gliomas after gross total resection and initial observation in the molecular era. Int J Radiat Oncol Biol Phys. 2019; 104(5): 1099-105.
5. Griffin CA, Burger P, Morsberger L, *et al*. Identification of der(1;19)(q10;p10) in five oligodendrogliomas suggests mechanism of concurrent 1p and 19q loss. J Neuropathol Exp Neurol. 2006; 65(10): 988-94.
6. Jenkins RB, Blair H, Ballman KV, *et al*. A t(1;19)(q10;p10) mediates the combined deletions of 1p and 19q and predicts a better prognosis of patients with oligodendroglioma. Cancer Res. 2006; 66(20): 9852-61.
7. Cooper LA, Gutman DA, Long Q, *et al*. The proneural molecular signature is enriched in oligodendrogliomas and predicts improved survival among diffuse gliomas. PLoS One. 2010; 5(9): e12548.
8. Ducray F, Idbaih A, de Reynies A, *et al*. Anaplastic oligodendrogliomas with 1p19q codeletion have a proneural gene expression profile. Mol Cancer. 2008; 7: 41.
9. Sahebjam S, McNamara MG, Mason WP. Emerging biomarkers in anaplastic oligodendroglioma: implications for clinical investigation and patient management. CNS Oncol. 2013; 2(4): 351-8.

10. Franco-Hernandez C, Martinez-Glez V, de Campos JM, *et al*. Allelic status of 1p and 19q in oligodendrogliomas and glioblastomas: multiplex ligation-dependent probe amplification versus loss of heterozygosity. Cancer genetics and cytogenetics. 2009; 190(2): 93-6.
11. Jeuken J, Cornelissen S, Boots-Sprenger S, Gijsen S, Wesseling P. Multiplex ligation-dependent probe amplification: a diagnostic tool for simultaneous identification of different genetic markers in glial tumors. J Mol Diagn. 2006; 8(4): 433-43.
12. Pinkham MB, Telford N, Whitfield GA, Colaco RJ, O'Neill F, McBain CA. FISHing tips: what every clinician should know about 1p19q analysis in gliomas using fluorescence in situ hybridisation. Clin Oncol (R Coll Radiol). 2015; 27(8): 445-53.
13. Rodriguez FJ, Vizcaino MA, Lin MT. Recent advances on the molecular pathology of glial neoplasms in children and adults. J Mol Diagn. 2016; 18(5): 620-34.
14. Woehrer A, Sander P, Haberler C, *et al*. FISH-based detection of 1p 19q codeletion in oligodendroglial tumors: procedures and protocols for neuropathological practice - a publication under the auspices of the Research Committee of the European Confederation of Neuropathological Societies (Euro-CNS). Clin Neuropathol. 2011; 30(2): 47-55.
15. Ambros PF, Ambros IM, Siop Europe Neuroblastoma Pathology B, Bone Marrow G. Pathology and biology guidelines for resectable and unresectable neuroblastic tumors and bone marrow examination guidelines. Med Pediatr Oncol. 2001; 37(6): 492-04.
16. Horbinski C, Miller CR, Perry A. Gone FISHing: clinical lessons learned in brain tumor molecular diagnostics over the last decade. Brain Pathol. 2011; 21(1): 57-73.
17. Kouwenhoven MC, Gorlia T, Kros JM, *et al*. Molecular analysis of anaplastic oligodendroglial tumors in a prospective randomized study: A report from EORTC study 26951. Neuro Oncol. 2009; 11(6): 737-46.
18. Senetta R, Verdun di Cantogno L, Chiusa L, *et al*. A "weighted" fluorescence in situ hybridization strengthens the favorable prognostic value of 1p/19q codeletion in pure and mixed oligodendroglial tumors. J Neuropathol Exp Neurol. 2013; 72(5): 432-41.
19. Smith JS, Perry A, Borell TJ, *et al*. Alterations of chromosome arms 1p and 19q as predictors of survival in oligodendrogliomas, astrocytomas, and mixed oligoastrocytomas. J Clin Oncol. 2000; 18(3): 636-45.
20. Korshunov A, Sycheva R, Golanov A. Molecular stratification of diagnostically challenging high-grade gliomas composed of small cells: the utility of fluorescence in situ hybridization. Clin Cancer Res. 2004; 10(23): 7820-6.
21. Scheie D, Andresen PA, Cvancarova M, *et al*. Fluorescence in situ hybridization (FISH) on touch preparations: a reliable method for detecting loss of heterozygosity at 1p and 19q in oligodendroglial tumors. Am J Surg Pathol. 2006; 30(7): 828-37.
22. Lee SC. Diffuse gliomas for nonneuropathologists: the new integrated molecular diagnostics. Arch Pathol Lab Med. 2018; 142(7): 804-14.
23. Chen H, Thomas C, Munoz FA, *et al*. Polysomy is associated with poor outcome in 1p19q co-deleted oligodendroglial tumors. Neuro Oncol. 2019; 21(9): 1164-74.
24. Jiang H, Ren X, Zhang Z, Zeng W, Wang J, Lin S. Polysomy of chromosomes 1 and 19: an underestimated prognostic factor in oligodendroglial tumors.J Neurooncol. 2014; 120(1): 131-8.
25. Ren X, Jiang H, Cui X, *et al*. Co-polysomy of chromosome 1q and 19p predicts worse prognosis in 1p/19q codeleted oligodendroglial tumors: FISH analysis of 148 consecutive cases. Neuro Oncol. 2013; 15(9): 1244-50.
26. Looi LM, Cheah PL. In situ hybridisation: principles and applications. Malays J Pathol. 1992; 14(2): 69-76.