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Original Article

Correlation of histomorphologic prognostic markers and proliferative index with loss of heterozygosity 1p/19q and MGMT status in diffusely infiltrating gliomas

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ABSTRACT

Background: Loss of heterozygosity (LOH)1p/19q, and epigenetic silencing of O⁶-methyl-guanine-DNA methyltransferase (MGMT) gene, displayed promising role as predictive and prognostic markers in brain tumours. The present study correlated both with conventional histomorphologic prognostic markers and proliferative index in diffusely infiltrating gliomas (DIG).

Methods: Tissues from 45 patients were evaluated for LOH1p/19q using polymerase chain reaction based microsatellite analysis; and for MGMT using immunohistochemistry. Results were correlated with age, histologic type, WHO grade, and proliferation index.

Results: Mean MIB-1 LI showed significant correlation with tumour grade. MGMT-staining in grade II and IV tumours were 31.1% and 16.8%, respectively, while in DA and GBM it was 88.2% and 19.0%, respectively, which were statistically significant. Sixteen cases showed LOH 1p and/or 19q of which 10 (5 oligodendrogial, 3 GBM, AA, DA) had combined LOH; while three each showed 1p (all GBM) and 19q (2 DA and GBM) loss. In the MIB-1LI ≤ 5% and >5% groups LOH 1p and/or 19q was encountered in 6 and 10 cases, respectively. A significant inverse association was noted between LOH with MGMT.

Conclusions: LOH1p/19q and MGMT shows good correlation with conventional histomorphologic and proliferation markers, and should constitute part of the optimal diagnostic workup of DIG.

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Introduction

Primary malignant central nervous system (CNS) neoplasms are rare. Of these diffusely infiltrating gliomas (DIG) constitute approximately 42%, among which over three quarters are glioblastoma multiforme (GBM). Despite advancements in management the overall survival (OS) in newly diagnosed GBM is reported to be 42.4% at 6 months, 17.7% at 1 year and 3.3% at 2 years.¹

Rapid progress in molecular biology has unravelled various mechanisms of tumorigenesis and tumour progression of CNS tumours. Of these, loss of heterozygosity (LOH) 1p/19q, and epigenetic silencing of O⁶-methylguanine-DNA methyltransferase (MGMT) gene, has displayed promising role as predictive and prognostic markers.^{1,2} Studies have shown that 70%–85% of oligodendrogliomas and 20%–30% of astrocytomas have allelic losses on chromosome 1p and 19q. Combined LOH 1p/19q has been associated with prognostically favourable outcome and enhanced response to 'PCV' chemotherapy (procarbazine, CCNU, vincristine) in oligodendroglial tumours.² The status of LOH 1p/19q in isolation or in combination has however not been extensively studied in astrocytic tumours.

The MGMT gene located on chromosome 10q26, codes for a critical DNA repair protein, which protects normal cells from exogenous carcinogens, while conferring protection to the tumour cells from alkylating and methylating chemotherapeutic agents. Tumours with promoter methylation causing epigenetic silencing of MGMT gene were found to respond favourably to radiotherapy and alkylating chemotherapy [carmustine or Temozolomide (TMZ)], with longer overall survival (OS). Most studies have analysed MGMT status in GBM unlike other DIG. Considering the potential side effects and high cost factor of TMZ therapy, MGMT evaluation is critical in selecting the therapeutic protocol.³

The aims of this study were to evaluate LOH 1p/19q and MGMT status in DIG and compare this with conventional histologic and proliferation markers of prognosis.

Materials and methods

The study included forty-five DIG cases managed at this tertiary care institute during 2009–2011. This included astrocytic (grades II–IV), oligodendroglial (grades II–III), and mixed gliomas (grades II–III). Relevant clinical features were noted for all cases.

Histopathological evaluation

Two pathologists (PD & NST) independently reviewed all haematoxylin and eosin (H&E) slides along with relevant immunohistochemical (IHC) stains, and reconfirmed the original histopathological diagnoses, viz. tumour type and grade, as per WHO 2007 classification system for CNS tumours.⁴

Immunohistochemistry for MGMT/MIB-1

One representative block of formalin-fixed paraffin-embedded (FFPE) tissue was selected, 5-micron thick sections cut and IHC performed by LSAB technique (M/s Dakopatts, Denmark) using

monoclonal antibodies [(MIB-1 LI, prediluted, M/S Dako, Denmark)/(MGMT Clone MT 3.1, prediluted, M/S Neomarkers, Fremont, CA)]. Sections were incubated overnight at 4 °C. Antigen was retrieved in TRIS-EDTA buffer pH 9.0 in a domestic microwave oven for 20 min at 750 W. Sections were treated with diaminobenzidine (DAB) and counterstained with haematoxylin, dehydrated and mounted, prior to visualisation.

Evaluation

- MIB-1LI: Proliferation index was evaluated by manually counting nuclear immunoreactivity among 1000 cells from at least 5 representative microscopic fields, at 400× magnification.
- MGMT: was semiquantitatively assessed by evaluating at least 100 cells for nuclear staining, as per the following: 0: <5%; 1+: 6–25%; 2+: 26–50%; 3+: >51% positive. Endothelial cells and perivascular lymphocytes showing nuclear reactivity served as internal controls, but were omitted from the analysis. A weak nuclear staining was considered positive, unlike cytoplasmic staining.

Analysis of LOH 1p/19q

DNA extraction

Tumour DNA was obtained either from 10 serial FFPE sections of 40 µm thickness (for retrospective cases); or from snap-frozen fresh tissue (for prospective cases). Corresponding constitutional DNA was obtained either from blood collected in EDTA-vacutainers (for prospective cases) or from normal brain parenchyma in FFPE tissue sections (for retrospective cases). DNA was extracted using commercially available extraction kit (M/s Qiagen, Hilden, Germany), as per the manufacturer's protocol. It was quantified by a spectrophotometer and stored at –30 °C.

PCR amplification of DNA using microsatellite markers for LOH 1p/19q

Extracted DNA was amplified using a set of five microsatellite markers (M/S Sigma) each for 1p and 19q from the most frequently deleted sites⁴ [Table 1]. PCR amplification was performed using 25 ng of DNA, 20 pmol of each primer, 12.5 µL of ready to use master mix (Dream Taq™ 2× master mix M/s Fermentas) and RNase free water to make a volume of 25 µL. Initial denaturation was carried out at 94 °C for 3 min followed by 30 cycles on an automated thermal cycler (Eppendorf). The sequence of amplification included denaturation at 94 °C for 30 s, annealing as per primer-specific temperature [Table 1], followed by extension at 72 °C for 40 s. A final extension step of 72 °C for 1 min was added.

Analysis of LOH using polyacrylamide gel electrophoresis (PAGE)

PCR products were studied using 8% PAGE utilizing a vertical electrophoresis at 65 W for 2 h, and finally visualized by the silver staining protocol. The intensities of the signals in tumour DNA were compared with those of the constitutional DNA. LOH was defined as loss of one of the two bands or abnormal

Table 1 – Nucleotide sequences of the microsatellite markers along with product size and specific annealing temperature.

Marker name	Nucleotide sequence	Product size (bp)	Temp
D1S1608	F: 5'-GAT GGC TTT TGG GGA CTA TT-3' R: 5'-CAC TGA GCC AAG TGA CAC AG-3'	270	58 °C
D1S548	F: 5'-GAA CTC ATT GGC AAA AGG AA-3' R: 5'-GCC TCT TTG TTG CAG TGA TT-3'	167	
D1S1592	F: 5'-GGT GAC AGG TAT TGA CTG CC-3' R: 5'-TTG AGG GCA GAG ATT GTC TC-3'	243	
D1S1161	F: 5'-AGA ATG GTG TGA ACC CAG G-3' R: 5'-AAG GAT TAT ACA GCA GCT GTT-3'	291	
D1S1184	F: 5'-AGCCAAGATCATGCCACTG-3' R: 5'-CCTCCTGGCAAAATATCCAT-3'	251	52 °C
D19S433	F: 5'-CCT GGG CAA CAG AAT AAG AT-3' R: 5'-TAG GTT TTT AAG GAA CAG GTG G-3'	221	56 °C
D19S431	F: 5'-GGCAGAAATGGTTGAGCTTA-3' R: 5'-ACACATCCTCGGTGGATAGA-3'	296	52 °C
D19S718	F: 5'-CTG AGG GAA CAG CAA GGT AA-3' R: 5'-AGA GCA AGA CTC TGA CTC TAT AAA T-3'	340	57 °C
D19S559	F: 5'-ACT CCA GTC TGT GTG ACA G-3' R: 5'-CAT AAT AGG TTT GAG GTA TCT-3'	182	55 °C
D19S601	F: 5'-CAA TGT GAG GCT GGT CTC TT-3' R: 5'-ATC ATA TGG CCT TCA GTG GA-3'	228	

F: forward primer, R: reverse primer, bp: product size in base pairs.

localization of the bands in the tumour DNA lane as compared to two distinct bands in the constitutional DNA lane.⁵

Clinicopathological correlation

Clinicopathological data were statistically correlated with histopathology, proliferation index, as well as LOH and MGMT status. Unpaired 't' test was used to evaluate the difference in quantitative variables in the two groups, while Pearson Chi-Square test was applied for qualitative variables. ROC curve was utilized to determine the best cut-off values for MGMT percentage score. All calculations were done using the SPSS software version 14.0 for Windows.

Results

Clinical profile

The present study consisted of 45 DIGs diagnosed during 2009–2011. The mean age at surgery was 45.6 years (range: 7–80 years) with male predominance, and predilection for frontal lobe (37.8%). Clinically headache (37.8%) and generalized tonic clonic seizures (33.3%) were most common.

Histopathological features and proliferation index

GBM was the most common tumour (46.7%), followed by diffuse astrocytoma (DA: 37.9%), two cases (4.4%) each of anaplastic astrocytoma (AA) and oligoastrocytoma (OA), and one case (2.2%) each of oligodendroglioma (ODG), anaplastic oligodendroglioma (AO) and anaplastic oligoastrocytoma (AOA).

Mean MIB-1 LI of grades II, III and IV neoplasms were 1.4% (range: 1–3); 6.8% (range: 5–8) and 17.1% (range: 10–26), respectively. A statistically significant difference was noted between MIB-1LI and WHO grades (p value = 0.0001).

Immunohistochemistry: MGMT

Pattern of MGMT-immunoreactivity

Majority demonstrated moderate to strong nuclear immunostaining in at least >5% of cells. Cytoplasmic staining (diffuse, granular or droplet-like) was considered negative. In three cases each of DA and GBM, MGMT-expression was weak and hence excluded. Unlike the uninfiltrated grey matter, glial cells in uninfiltrated white matter demonstrated higher MGMT-immunopositivity. Neuronal cells were generally negative. In GBM, the interface with normal brain parenchyma displayed higher MGMT-staining as compared to tumour centre. Overall, in decreasing order MGMT-expression was highest in the uninfiltrated white matter, followed by the infiltration zone and then the tumour centre [Fig. 1].

Correlation of MGMT with patient age, histologic subtype, WHO grade, & MIB-1LI

MGMT with age: MGMT-immunopositivity (2+/3+) was seen in 11/15 (66.7%), 7/16 (43.8%) and 2/8 (25%) cases in the age groups 21–40 years, 41–60 years and 61–80 years, respectively. Difference between the ≤40 years and >40 years age group was statistically significant [Table 2].

MGMT with histologic subtype

Of the total DA, 2+/3+ MGMT-staining was noted in 88.2% cases (range 26%–58%; mean: 34%) as compared to 19.0% in GBM (range 8%–32%; mean: 16.8%). Of the five cases with oligodendroglial morphology, two cases (anaplastic oligodendroglioma and anaplastic oligoastrocytoma) were discarded due to equivocal staining, while mean of the remaining was 16.3%. The solitary case of oligodendroglioma showed 1+ staining, while the two cases of oligoastrocytoma showed 1+ and 2+ staining. There was a statistically significant association between histologic subtype (for DA and GBM) and MGMT score (p = 0.000). Though oligodendroglial tumours

Fig. 1 – (A–E): Immunohistochemistry patterns of MGMT expression: nuclear in tumour cells (A: $\times 400$), inflammatory cells (B: $\times 200$) and endothelial cells (C: $\times 200$); cytoplasmic droplets in tumour cells (D: $\times 400$); mixed nuclear and cytoplasmic in tumour cells (E: $\times 200$).

showed lesser MGMT-immunoexpression as compared to astrocytomas, but owing to the paucity of oligodendroglial neoplasms, statistical analysis could not be performed [Fig. 2].

MGMT with WHO grade. The average levels of MGMT-expression were significantly higher in grade II (31.1%) as compared to grade IV tumours (16.8%). Sixteen grade II tumours (80.0%) showed 2+/3+ positivity, unlike 4 in grade IV (19%) ($p = 0.000$). Of the four grade III neoplasms, two were discarded due to equivocal staining, and mean of the remaining was 15%.

MGMT with MIB –1 LI. On segregating MIB-1LI into $\leq 5\%$ and $> 5\%$ groups, 16/21 (76.2%) in the former showed 2+/3+ MGMT-immunostaining, as compared to 4/24 (16.7%) in the latter group, which revealed statistically significant association ($p = 0.000$).

Results of molecular genetic analysis

Sixteen (35.6%) cases showed LOH 1p and/or 19q of which 10 (5 oligodendroglial, 3 GBM, 1 AA, 1 DA) had combined loss (22.2%). Three GBMs showed LOH 1p, while isolated 19q loss was seen in two DA and one GBM [Fig. 3].

Results of primers D1S1184 and D19S431 were largely uninformative, and hence excluded. This was possibly due to the poor sensitivity of the thermocycler in attaining/maintaining lower annealing temperature of 52°C , as compared to the other primers. However, all samples were informative for at least one of the remaining four microsatellite markers for

each 1p and 19q. The most and least frequently deleted markers for 1p were D1S1592 (13.3%) and D1S548 (4.4%), respectively, while for 19q they were D19S601 (13.3%), and DS19S433 and D19S559 (6.7%).

Correlation of LOH with histologic subtype, WHO grade, MIB-1 LI & MGMT status

LOH with histologic subtype: All five oligodendroglial neoplasms displayed combined LOH 1p/19q, unlike 1 of 17 (5.9%) DA and 3 of 21 (14.3%) GBM. Of the 3 DA with LOH, one had combined, two had isolated 19q and none had 1p loss. The corresponding figures for 7 GBM with LOH were 3, 1 and 3, respectively. A solitary AA revealed LOH 1p and 19q [Table 3].

LOH with WHO grade: LOH 1p and/or 19q was present in 6/20 (30%) grade II; 3/4 (75%) grade III; and 7/21 (33.3%) grade IV tumours

LOH with MIB –1LI: In the $\leq 5\%$ group, LOH 1p and/or 19q was encountered in 6 cases (28.6%), as against 10 (41.7%) in the $> 5\%$.

LOH with MGMT: All LOH 1p cases showed 1+ MGMT-staining, while those with LOH 19q demonstrated 2+ (with one case being discarded). Of these, all the former were GBM, while all the latter were diffuse astrocytoma. Cases with combined LOH 1p/19q showed 1+ in 6 cases and 2+ in one (three being discarded). Of the five oligodendroglial tumours two (anaplastic oligoastrocytoma and anaplastic oligoastrocytoma) were discarded, and the remaining three cases displayed LOH 1p/19q where the solitary case of oligodendroglioma showed 1+ staining, and the two cases of

Table 2 – Correlation of MGMT with patient age, histologic subtype, grade, MIB – 1 LI & patient age [N = 45].

Parameter	W	1+	2+	3+
No. of cases				
Histologic subtype				
DA	2	Nil	14	1
AA	Nil	2	Nil	Nil
GBM	2	15	4	Nil
ODG	Nil	1	Nil	Nil
AO	1	Nil	Nil	Nil
OA	Nil	1	1	Nil
AOA	1	Nil	Nil	Nil
WHO grade				
II	2	2	15	1
III	2	2	Nil	Nil
IV	2	15	4	Nil
MIB – 1 LI				
≤5%	2	3	15	1
>5%	4	16	4	Nil
Patient age				
≤20	2	Nil	Nil	Nil
21–40	1	4	11	Nil
41–60	2	9	6	1
61–80	1	6	2	Nil

AA: Anaplastic Astrocytoma WHO grade III; AO: Anaplastic Oligodendroglioma WHO grade III; DA: Diffuse Astrocytoma WHO grade II; GBM: Glioblastoma Multiforme WHO grade IV; OA: Anaplastic Oligoastrocytoma WHO grade III; ODG: Oligodendroglioma WHO grade II.

oligoastrocytoma demonstrated 1+ and 2+ staining. Interestingly, on evaluating the association between LOH status (1p and/or 19q) with MGMT expression, a statistically significant ($p = 0.036$) inverse association was noted.

Lack of statistically significant association between LOH 1p and/or 19q with histologic type, WHO grade or MIB-1LI, could possibly be attributed to the high sensitivity of the procedure along with low number of cases recruited in the study.

Discussion

Tumour biology is the resultant of a complex interaction of many histologic, proliferation and genetic factors. The limitations of the prognostic role of histological markers have been aptly highlighted by GBMs with long-term survival exceeding 5 years.⁶ In this regards MIB-1LI, MGMT status and LOH 1p/19q have shown promising results. Schmidt et al⁵ noted LOH 1p/19q in 5 cases of GBM with long-term survival.

Proliferation in DIG, as evaluated by MIB-1LI, shows positive correlation with tumour grade and prognosis,² akin to the present study. Mean MIB-1LI reported in various series were 3.0% for grade II, 11.8% for grade III and 15.7% for grade IV tumours, respectively,² while the corresponding values in the present study were 1.4%; 6.8% and 17.1%, respectively. In order to account for variations due to tissue processing, staining and evaluation, it is recommended that each laboratory should compute their own cut-offs for various types and grades of tumour.²

The present study attempted to correlate MIB-1LI with survival using cutoffs described in the literature.² Cases with MIB-1LI ≤ 5% had a statistically significant ($p = 0.004$) better mean OS than those with >5%. It is therefore recommended that MIB-1LI must be determined in all gliomas, not only for prognostication, but also to correctly determine the WHO grade especially in small and stereotactic biopsies.

Of the various molecular markers evaluated in gliomas, LOH 1p/19q has established itself as a molecular signature of tumours with oligodendroglial differentiation, as also noted in the index study.^{2,7,8} It is also an indicator of better OS and enhanced chemosensitivity.^{1,8,9} However, its utility in astrocytic tumours remains largely unexplored.

The PCR-based microsatellite analysis followed by PAGE adopted in the present study is the most widely practiced method for LOH analysis. Though the assay is tedious and time consuming, it is high reliable and cost effective. The major limitations however are: (a) additional requirement of constitutional DNA; (b) poor DNA quality due to formalin fixation, making PCR amplification difficult (circumvented by using snap frozen tissue); (c) likelihood of missing a chromosomal deletion due to significant contamination with normal tissue or effect of clonal heterogeneity within the tumour.

Amongst the other methods of LOH analysis, fluorescent in situ hybridization (FISH) has gained considerable popularity with comparable results. Though the process is time consuming, very expensive, and requires large number of cells, it eliminates the need for constitutional DNA, and is able to determine gene copy number, which could be of additional benefit in assessing prognosis.¹⁰

In astrocytomas (grade II/III), LOH 1p and 19q is reported to range from 0 to 17%.¹¹ The current study revealed isolated 19q and combined 1p/19q LOH in 17.6% of DA, similar to the Western literature. LOH 1p/19q was encountered in a solitary case of AA, which could perhaps be attributed to the small sample size.

von Deimling et al¹² observed LOH 1p and 19q in 10% and 30% GBMs, respectively. In the current study, LOH 1p, 19q, 1p/19q LOH in GBM was seen in 14.3%, 4.8%, and 14.3%, respectively. Overall, the incidence of combined 1p/19q LOH in GBM varied between 6 and 20% cases,^{5,13–15} while the only study from India reported 12%.¹⁶ The frequency of LOH 1p and/or 19q was higher in GBM with oligodendroglial differentiation (GBM-O) than conventional GBMs, as also noted in 2 of 7 GBM with oligodendroglial differentiation, in the current study.

Several workers have demonstrated an association between MGMT status with survival in gliomas, while others have failed to do so.^{3,17,18} Though GBM with MGMT-expression on combined Temozolomide and radiotherapy displayed an enhanced median survival (MS) of nearly 2 years,^{19,20} it is yet to be adopted in the current therapeutic protocol. Future glioma therapy will possibly consider MGMT status and stratify patients as per their MGMT-expression.

Though methylation-specific PCR (MSP-PCR) is the gold standard, but the heterogenous nature of astrocytic neoplasms combined with false negative methylation results due to formalin-fixation, has precipitated conflicting outcome in many studies.^{21,22} In a recent study, Maxwell²³ noted that MSP-negative (where MGMT is not silenced) tumours have a large fraction of TMZ-sensitive MGMT-negative tumour cells

Fig. 2 – (A–I): MGMT expression in different types of diffusely infiltrating gliomas: nuclear staining of tumour cells in diffuse astrocytoma (A: $\times 200$; B: $\times 400$), oligoastrocytoma (C: $\times 200$); weak nuclear and cytoplasmic staining in oligoastrocytoma (D: $\times 400$); cytoplasmic droplet and lymphocyte nuclear staining of anaplastic astrocytoma (E: $\times 400$); nuclear staining of endothelial, inflammatory and tumour cells in GBM (F: $\times 200$); GBM with predominant staining of inflammatory cells (G: $\times 200$) and predominantly tumour cells (H: 200; I: $\times 400$).

while in MSP-positive neoplasms (where MGMT is silenced) large fractions of MGMT-positive cells can still be found. This contradicts the strict division between MSP-positive and MSP-negative gliomas, since it tends to overlook the heterogeneous nature of the tumour. MSP requires a specialized setup with dedicated manpower trained in molecular techniques, along with high quality DNA (snap frozen tissue as against FFPE).^{21,22} The current study ventured to adopt MSP, but the DNA yield of FFPE tissue was far from satisfactory. This has precipitated inconsistent results in various series, especially in cases of repetitive runs.²¹ Preusser et al²⁴ obtained conclusive results only in 13/24 (54.2%) MGMT MSP analyses, which they attributed to FFPE tissue.

The present study used IHC to evaluate MGMT status, owing to distinct advantages like less cumbersome procedure; needs routine laboratory setup; is cost effective; commercially available antibodies compatible with FFPE; and contains internal positive controls (endothelia and inflammatory cells).^{21,23} However, one of the plausible hurdles in the path of accepting IHC for MGMT into routine clinical practice is the

lack of consistently defined cut-off values for the prognostic groups. Most studies proposing a cut-off level for MGMT have analyzed GBM or AA.^{25,26} Despite limitations of sufficient follow-up data, the current study attempted to determine the best cut-off point for MGMT, in relation to two conventional prognostic markers, viz. MIB-1LI ($\leq 5\%$ / $>5\%$) and tumour grade (grades II/IV). The cut-off value so determined was 10.50%, which was similar to Nakasu et al.²⁷ However it is suggested that a larger population size with proper follow-up data be studied, in order to ensure a better correlation of this value.

MGMT expression was significantly less in grade IV tumours with high MIB-1LI, as compared to grade II tumours with low MIB-1LI, similar to that noted in the present study.^{25,26} Studies by Ohgaki and Kleihues have noted that secondary GBM show higher frequency of MGMT promoter methylation as compared to primary GBM, owing to which they are less aggressive and are associated with longer survival.²⁸ Since MGMT-immunopositivity is an expression of the unmethylated MGMT gene, primary GBM should display higher MGMT-immunoreactivity. Though the present study did not evaluate EGFR and p53 status

Fig. 3 – Images of the polyacrylamide gel electrophoresis after silver staining showing results of LOH 1p/19q according to microsatellite markers in representative cases.

for segregating the primary from secondary GBM, but considering that primary GBM is encountered in elderly population, the age group of the four GBM showing 2+ MGMT-immunostaining were reviewed for their age. It was noted that all the four cases were in their sixth decade of life and more (55, 57, 62, 70 years), thus suggesting that indeed primary GBM display higher MGMT-immunoreactivity.

The current study also displayed that in decreasing order MGMT expression was highest in the uninfiltated white matter, followed by the tumour-normal tissue interface and then the tumour centre, similar to that noted by Margison et al.²⁹ High expression of MGMT in the uninfiltated white matter indicates that the DNA repair mechanism at this site is functioning at a higher level, as compared to the tumour-normal tissue interface, and is least at the tumour centre. As per tumour biology this is a desirable mechanism, which helps in counteracting spontaneous mutations by endogenous DNA alkylation that may progressively contribute to tumour development and progression. However, this has its inherent drawback in therapeutic planning of low-grade gliomas since several therapeutic substances depend on a reduced DNA repair mechanism of tumour cells.

Capper³⁰ reported a significant positive association of age and MGMT-expression in grade II tumours, while Silber et al observed an inverse correlation of age with MGMT enzyme activity in GBM.³¹ In contrast, the current study noted an inverse relation between age (in the ≤ 40 years and > 40 years age group) and MGMT-expression, a fact that needs to be validated with larger number of cases.

Studies have suggested that favourable prognostic outcome of low-grade gliomas is possibly a function not only of MGMT but also of the aberrant methylation of other genes, possibly including yet to be identified candidate genes whose silencing may contribute to enhanced radiosensitivity of glioma cells.²² In contrast to primary GBMs, MGMT promoter methylation in diffuse gliomas and secondary gliomas is often associated with other prognostically favourable genetic alterations.²² In oligodendroglial tumours, a strong association of MGMT hypermethylation has been observed with LOH 1p/19q, similar to the present study, and IDH1 mutations.¹⁸ Sanson et al also noted that diffusely infiltrating astrocytomas, except GBM, showed strong association between MGMT promoter hypermethylation and IDH1 mutations.³²

Table 3 – Correlation of 1p and/or 19q deletion along with histologic subtype, grade & MIB – 1 LI [N = 45].

Parameter	LOH 1p	LOH 19q	LOH 1p19q	No LOH
No. of cases				
Histologic subtype				
DA	Nil	2	1	14
AA	Nil	Nil	1	1
GBM	3	1	3	14
ODG	Nil	Nil	1	Nil
AO	Nil	Nil	1	Nil
OA	Nil	Nil	2	Nil
AOA	Nil	Nil	1	Nil
WHO grade				
II	Nil	2	4	14
III	Nil	Nil	3	1
IV	3	1	3	14
MIB – 1 LI				
≤5%	Nil	2	4	15
>5%	3	1	6	14
MGMT status				
W	Nil	1	3	2
1+	3	Nil	6	10
2+	Nil	2	1	16
3+	Nil	Nil	Nil	2

AA: Anaplastic Astrocytoma WHO grade III; AO: Anaplastic Oligodendroglioma WHO grade III; DA: Diffuse Astrocytoma WHO grade II; GBM: Glioblastoma Multiforme WHO grade IV; OA: Anaplastic Oligoastrocytoma WHO grade III; ODG: Oligodendroglioma WHO grade II.

However, in order to accurately determine the actual survivals, a longer and closer follow up, with availability of detailed post-operative therapeutic protocol, involving larger number of cases pooled from multiple tertiary care Neurosurgery centres is warranted. Further, in addition to MGMT and LOH 1p/19q the cases should also be evaluated for p53 and IDH1 mutations for a better prognostic and predictive value.

Intellectual contribution of authors

Study concept: Col Prabal Deb, Brig NS Mani, Brig SM Sudumbrekar

Drafting & manuscript revision: Col Prabal Deb, Maj Nitin Taneja

Statistical analysis: Maj Nitin Taneja, Seema Patrikar

Study supervision: Col Prabal Deb, Brig NS Mani, Brig SM Sudumbrekar

Conflicts of interest

All authors have none to declare.

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