RESEARCH ARTICLE

MGMT promoter gene methylation and neurological scale improvement in glioma: a cohort study [version 1; peer review: awaiting peer review]

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Abstract

Background: Glioma is one of the most common primary brain tumours and conveys a dismal prognosis despite aggressive treatment. Several biomarkers have been studied in the hope of yielding better diagnostic accuracy and improving patient management. Besides survival, functional and neurological disability are concerns that have no lesser importance. In 2017, a disease-specific assessment tool – the Neurologic Assessment in Neuro-Oncology (NANO) scale – was developed to measure neurologic function in neuro-oncology cases. We sought to determine biomarkers that might be associated with neurological scale improvement in glioma patients.

Methods: Glioma grade II-IV patients were recruited from three major hospitals in Jakarta-Tangerang. Isocitrate dehydrogenase (IDH) mutation and O6-methylguanine-DNA methyltransferase (MGMT) promoter gene methylation were tested, as well as patients' neurological function before surgery and three months after. Improvement in neurological scale (NANO scale) was considered positive if there was a decrement of ≥ 1 of the scale.

Results: There were 54 patients included in the study. Mean age was 43.63 (14.723) years old, and 61.1% were male. As much as 16 (29.6%) carried a mutation in codon 132 of the IDH1 gene, and 33 (61.1%) were MGMT methylated. Median NANO scale score before and three months after surgery was 4 (0-12) and 3 (0-12), respectively. Neurological improvement was found in 44 (81.5%) of the patients. Among patients with MGMT promoter gene

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methylation, 90.9% showed neurological improvement (p=0.035; OR=5; 95%CI 1.122-22.272).
Conclusions: Gliomas with MGMT promoter gene methylation are more likely to show neurological improvement three months after surgery.

Keywords
MGMT promoter gene methylation; Glioma; Neurological Scale; NANO Scale

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Introduction

Glioma is one of the most common primary brain tumours, in which 80% of the cases are found to be malignant (Alifieris & Trafalis, 2015; Schwartzbaum *et al.*, 2006; Stupp *et al.*, 2010). Grade II glioma has a median survival ranging from four to more than ten years (Bell *et al.*, 2018; Claus & Black, 2006). However, glioblastoma – a grade IV glioma – accounts for the majority of gliomas and carries a poor prognosis with median survival of six to 14 months despite aggressive treatment with radiotherapy plus chemotherapy, and less than 12 months with radiotherapy alone (Stupp *et al.*, 2005).

Molecular characteristics of glioma have undergone extensive research in the last decade, and some biomarkers have even been integrated into the 2016 glioma classification. Isocitrate dehydrogenase (IDH) mutation and O6-Methylguanine-DNA methyltransferase (MGMT) promoter gene methylation has been associated with better survival. Nonetheless, patients' quality of life and neurological deficit have not been considerably measured and correlated with these prognostic biomarkers.

In 2017, the Neurological Assessment of Neuro-Oncology (NANO) scale was developed to objectively measure neurological deficit in patients with brain tumours (Nayak *et al.*, 2017). Previous studies have shown that a good initial NANO scale score was significantly associated with improvement in neurological deficit two months after surgery (Gunawan *et al.*, 2020) and a more powerful method to predict prognosis during initial diagnosis and disease progression (Lee *et al.*, 2018). In this study, we aimed to elucidate biomarkers that might be associated with neurological scale improvement three months after surgery.

Methods

Patient collections and ethics statement

This is a cohort study, with a purposive sampling method. There were 72 suspected brain tumour patients, aged more than ten years old, who presented at three major hospitals in Jakarta and Tangerang over a period of one year (July 2019–July 2020). After a brain MRI, 5 patients were excluded due to tumour location (brainstem or infratentorial). Written consent was obtained from every patient or their caregiver (for patients who are unable to give consent). Karnofsky Performance Scale (KPS) as well as neurological scale were assessed before surgery. Following tumour resection, 62 patients were histologically confirmed as having glioma grade II-IV. Tumour tissue was tested for IDH mutation as well as MGMT promoter gene methylation. Patients underwent standardised therapy, and neurological function was re-assessed three months after surgery. Six patients were deceased before the second neurological scale assessment, and two patients were lost to follow-up, hence were excluded from the study. The study was approved by Medical Ethical Research Committee, Universitas Hasanuddin, No: 1232/UN4.6.4.5.31/PP36/2019.

Histopathological and molecular analysis

Histopathological and molecular analysis was carried out in Mochtar Riady Institute Tangerang and Kalgen Innolab Clinical Laboratory, Jakarta. Tissue sample dissected from the tumor was immediately put in formaldehyde 10% buffer. Six to 24 hours later, the desired tumor tissue was stored into cassettes and processed in an automatic tissue processor. After an overnight processing, tissue was put into a mold with paraffin wax. Paraffin block with the embedded tissue was cut using a microtome and placed on glass slides stained with hematoxylin and eosin to determine the diagnosis and grade of malignancy.

DNA isolation

Genomic DNA was extracted from paraffin-embedded tumor tissues using the QIAamp®DNAMicroKit (QIAGEN, catalogue number 56304) following the manufacturers' protocol.

IDH mutation

IDH mutation evaluation was done using high resolution melting (HRM) analysis and Sanger sequencing of polymerase chain reaction (PCR)-amplified fragments, which were generated during the HRM procedure with the PCR primers. Primers used were: IDH1-forward 5'-CGGTCTTCAGAGAAGCCATT-3' and IDH1-reverse 5'-GCAAAATCACATTATTGCCAAC-3' (gBlocks Gene Fragment, Integrated DNA Technologies).

HRM analysis consists of 3 steps: PCR reaction, DNA melting, and data analysis. These analyses were performed using the LightCycler® 480 High-Resolution Melting Master Kit (Roche) according to the manufacturer's instructions. PCR amplification and HRM analysis were carried out in a LightCycler® 480 real-time PCR system (Roche Diagnostics).

PCR amplification started with an initial denaturation of 95°C for 10 minutes (which included activation of FastStart™ Taq DNA polymerase and denaturation of DNA), followed by 45 cycles of amplification (denaturation at 95°C for 10 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 12 seconds). Melting was performed with a denaturation step at 95°C for 1 minute, followed by an annealing step at 40°C for 1 minute and a melt from 70 to 97°C at a ramp rate of 0.03°C/second with 18 acquisitions per degree Celsius. These results were analyzed using LightCycler® 480 software version 1.5.0.

Sanger sequencing was conducted using Applied Biosystems 3500 Genetic Analyzer. For each reaction, 200 ng of genomic DNA was amplified with the following PCR conditions: an initial 10-minute denaturation at 95°C followed by 40 cycles of 30 seconds at 95°C; 30 seconds at 60°C; 30 seconds at 72°C, and a final extension of 7 minutes at 72°C. Sequencing were aligned and edited with the BioEdit Software version 7.2.5.

Samples with conflicting results by HRM and sequencing were re-tested and only HRM-positive samples confirmed by sequencing were considered mutated.

MGMT promoter gene methylation

DNA extracted first underwent bisulphite treatment to convert all unmethylated cytosine to uracil, leaving 5-methylcytosine unaltered. After being eluted in DNase-free water, methylation

analysis was commenced using real-time methylation-specific PCR (MSP). PCR amplification was carried out in a LightCycler® 480 real-time PCR system (Roche Diagnostics). QuantiFast® Multiplex PCR Kit (QIAGEN) was used for PCR and DNA was amplified utilizing HotStarTaq Plus DNA polymerase which was included in the kit. Primers used were: MGMT Methylated-forward 5'-TTTCGACGTTCGTAGGTTTTCGC-3', MGMT Methylated reverse 5'-GCACTCTTCCGAAAACGAAACG-3' and MGMT Unmethylated-forward 5' TTTGTGTTTTGATGTTT-GTAGGTTTTTGT-3', MGMT Unmethylated-reverse 5'- AACTC-CACACTCTTCCAAAAACAAAACA-3'. Cycling conditions were 95°C for 5 min, followed by 42 cycles of amplification (denaturation at 95°C for 30 seconds, annealing at 59°C for 30 seconds, and extension at 72°C for 30 seconds) and 72°C for 5 min. PCR reactions (15 µl) were analyzed on a 2% agarose gel stained with SYBR Safe. Commercial methylated DNA and unmethylated DNA (Zymo Research) served as positive controls. Outcomes were classified as either methylated or unmethylated.

Neurological scale assessment and follow-up

Neurological function was assessed using the NANO scale. NANO scale is a simple neurological assessment evaluating patients in nine domains: gait, strength, upper extremity ataxia, sensation, visual fields, facial strength, language, level of consciousness, and behaviour. Each domain was scored from 0 to 2 or 3, with higher score indicating worse neurologic function. Neurological scale was assessed by two individual physicians, and subjective complaints not included in the scale were also noted. Any discrepancies between investigators were discussed and optimal scale were determined. NANO scale was first assessed before surgery and then re-assessed three months later during their routine clinical follow up. Patients who were deceased before the second assessment were excluded from the study. Neurological improvement was defined as decrement in NANO scale score by 1 or more, which was calculated from difference of initial NANO and NANO three months after surgery.

Statistical analysis

The association between baseline characteristics and biomarkers were analysed. Categorical data are presented as proportions and interpreted using chi-square or Pearson Fisher's exact test. All calculations were performed using IBM SPSS Statistics version 24. The reported p values are two-sided, and a probability value of <0.05 was considered statistically significant.

Results

In total, 54 patients completed the follow up and were analysed. Half of the cases (50%) were glioma grade IV, followed by grade II (37%) and grade III (13%). Overall mean age was 43.63 (14.723) years old for all gliomas, and mean age for glioblastoma was 50 (12.7) years old. Male to female ratio was 1.57. Median Karnofsky Performance Scale (KPS) score was 55 (30–80). Most of the patients underwent surgery followed by radiation and chemotherapy (79.6%) (Table 1).

The most common presenting symptom was headache (63%). Patients with tumours located in the frontal lobe most commonly presented with headache (58.8%), one-sided weakness (35.3%), seizure (32.4%), cognitive disturbance (20.6%), and aphasia (5.9%).

In 16 patients (29.6%) an IDH1 mutation was found and no IDH2 mutation was found. Younger age and male gender were significantly associated with having an IDH mutation (Table 2).

There were 33 patients (61.1%) that were MGMT methylated (Table 3). Older patients had a higher tendency to have methylated MGMT than younger patients. Patients with IDH mutations were more likely to harbour MGMT methylation (p=0.049; OR=1.54; 95%CI 1.05-2.26).

The number of patients with MGMT methylation who underwent surgery plus chemoradiation, surgery plus radiation only, and surgery only were 26 patients (78.8%), one patient (3%) and six patients (18.2%), respectively.

Median NANO scale score before surgery was 4 (0–12) and three months after surgery was 3 (0–12). Improvement in neurological function, measured using the NANO scale, was found in 44 (81.5%) of the patients. Age, gender, initial KPS, tumour location, grade of glioma and IDH mutation were not associated with improvement in neurological function. Among patients with MGMT promoter gene methylation, 90.9% showed improvement in neurological function (p=0.035; OR=5; 95%CI 1.122-22.272) (Table 4).

Further analysis shows that coexistence of IDH mutation and MGMT methylation were mostly found in grade II patients (53.8%) and 100% of patients with coexistence of both biomarkers showed improvement in neurological scale (p=0.032) (Table 5).

Discussion

Glioma remains to be a challenging tumour, with diverse clinical presentation, phenotype and molecular parameters (Louis *et al.*, 2016). It is not unusual for clinicians to encounter cases of higher-grade glioma with longer survival than lower-grade glioma during conventional treatment. Despite survival, clinical and functional status is another concern in treatment initiation and disease progression. KPS has been generally used to evaluate brain tumours' performance status. However, like tumour grade, there have also been cases in which those with a low KPS score survived longer than those with a high KPS score (Lee *et al.*, 2018).

The NANO scale is a relatively new scale that serve as an objective and quantifiable metric of neurologic function in brain tumour patients (Nayak *et al.*, 2017). A previous study found that performance status estimated by the NANO scale was significantly associated with overall survival, and was a more powerful method to predict the prognosis of GBM than the KPS during both initial diagnosis and disease progression

Table 1. Baseline characteristics.

Variables	N = 54 (%)
Age	
≥45 years old	28 (51.9)
<45 years old	26 (48.1)
Gender	
Male	33 (61.1)
Female	21(38.9)
Tumour location	
Frontal lobe	34 (63)
Temporal lobe	14 (25.9)
Parietal lobe	4 (7.4)
Occipital lobe	2 (3.7)
Symptoms	
Headache	34 (63)
Hemiparesis/hemiplegic	31 (57.4)
Seizure	16 (29.6)
Cognitive disturbance	9 (16.7)
Language disturbance	3 (5.6)
Initial KPS	
0–50	27 (50)
60–100	27 (50)
Therapy	
Surgery	8 (14.8)
Surgery and radiation	3 (5.6)
Surgery, radiation and chemotherapy	43 (79.6)
Histopathological diagnosis	
WHO grade II	
Diffuse astrocytoma, IDH-mutant	5 (9.3)
Diffuse astrocytoma, IDH-wild type	3 (5.6)
Oligoastrocytoma, IDH-mutant	2 (3.7)
Oligodendroglioma, IDH-mutant	1 (1.9)
Oligodendroglioma, IDH-wildtype	3 (5.6)
Gemistocytic astrocytoma, IDH-mutant	2 (3.7)
Gemistocytic astrocytoma, IDH-wild-type	1 (1.9)
Pleomorphic xanthoastrocytoma	2 (3.7)
WHO grade III	
Anaplastic oligodendroglioma, IDH-mutant	2 (3.7)
Anaplastic xanthoastrocytoma	7 (12.9)
WHO grade IV	
Glioblastoma, IDH-mutant	3 (5.6)
Glioblastoma, IDH-wildtype	24 (44.4)

KPS: Karnofsky Performance Scale; IDH: isocitrate dehydrogenase; WHO: World Health Organisation.

Table 2. Characteristics of patients and IDH mutation.

Variables	IDH mutation		P value
	IDH mutant	Wild type	
Age			
≥45 years old	5	23	0.049
<45 years old	11	15	
Gender			
Male	13	20	0.049
Female	3	18	
Initial KPS			
0–50	5	22	0.074
60–100	11	16	

KPS: Karnofsky Performance Scale; IDH: isocitrate dehydrogenase.

(Lee *et al.*, 2018). It was also found that initial NANO scale possesses a stronger correlation neurological scale improvement than initial KPS towards functional scale improvement (Gunawan *et al.*, 2020). In this study, we used NANO scale to objectively measure neurological scale improvement.

From 54 patients included in the study, most of the cases were glioma grade IV, glioblastoma, IDH-wildtype (44.4%), followed by grade II and III. This distribution is similar to previous studies analysing different grades of gliomas (Malueka *et al.*, 2020; Ostrom *et al.*, 2018; Theresia *et al.*, 2020). Mean age in this study was 43.63 years old, and patients with glioblastoma have a mean age of 50 (12.7) years old, which is comparable to previous studies conducted in Asian (Lee *et al.*, 2018; Malueka *et al.*, 2020; Theresia *et al.*, 2020), African

Table 3. Characteristics of patients and MGMT methylation.

Variables	MGMT methylation		P value
	Methylated	Unmethylated	
Age			
≥45 years old	20	13	0.291
<45 years old	13	8	
Gender			
Male	13	20	0.924
Female	3	18	
Initial KPS			
0–50	17	10	0.780
60–100	16	11	
IDH Mutation			
IDH mutant	13	3	0.049
Wild type	20	18	

KPS: Karnofsky Performance Scale; IDH: isocitrate dehydrogenase; MGMT: O6-Methylguanine-DNA methyltransferase.

(Senhaji *et al.*, 2017), and Amsterdam (Molenaar *et al.*, 2014) populations and lower than mean age of studies in the United Kingdom (Philips *et al.*, 2018; Sehmer *et al.*, 2014), Greece (Alifieris & Trafalis, 2015) and United States (Ostrom *et al.*, 2018; Schwartzbaum *et al.*, 2006). The male to female ratio was 1.57, which is comparable to previous studies (Malueka *et al.*, 2020; Ostrom *et al.*, 2018; Sehmer *et al.*, 2014). The proportion of low and high initial functional status was equivalent. Tumours were mostly located in the frontal lobe, followed by temporal, parietal, and occipital. The most common presenting symptom was headache, and 58.8% of tumours located in the frontal lobe presented with this symptom. Surgery, radiation, and chemotherapy were underwent by 79.6% of patients.

It was found that 29.6% of patients were positive for IDH1 mutation. Being of a younger age and male were factors associated with having an IDH mutant. This is similar to previous studies that found younger age to be associated with IDH mutation in glioblastoma (Molenaar *et al.*, 2014; Songtao *et al.*, 2012). However, a study in Indonesia showed no differences in age and gender towards IDH mutation (Malueka *et al.*, 2020). Dissimilarity may be caused by differences in characteristics of patients included in their study (which also included grade I glioma). Grade I glioma is frequently found in children, with different characteristics, therapeutic interventions, and prognosis. Therefore, in this study, we did not include grade I glioma and excluded patients less than 10 years old.

MGMT methylation was found in 61.1% of patients. Patients with IDH mutation were more likely to have an MGMT methylation as well (p=0.049; OR=1.54; 95%CI 1.05-2.26). These findings are in accordance with previous studies (Molenaar *et al.*, 2014; Songtao *et al.*, 2012).

Improvement in NANO scale within three months after surgery was found in 81.5% of the patients. From the variables studied, only MGMT promoter gene methylations revealed a significant link to NANO scale improvement three months after surgery (p=0.035; OR=5; 95%CI 1.122-22.272).

Although IDH mutation alone was not significantly related to scale improvement, the combination of IDH mutation and MGMT methylation was associated with improvement in neurological function three months later. This might be due to the significant association of MGMT methylation towards NANO scale improvement, which influenced the relation between its combination with IDH mutation towards scale improvement.

MGMT promoter gene methylation has been commonly accepted to confer survival advantage regardless of therapy (Reifenberger *et al.*, 2014). It carries both prognostic and predictive value. In this study, it was found that gliomas with MGMT gene promoter methylation are five times likely to show neurological improvement three months after surgery.

IDH mutation is one of the molecular tests well known for its prognostic and predictive implications in high-grade as

Table 4. Characteristics of patients and neurological improvement.

Variables	NANO scale improvement		P value
	+	-	
Age			
≥45 years old	23	5	0.897
<45 years old	21	5	
Gender			
Male	27	6	0.936
Female	17	4	
Initial KPS			
0–50	22	5	1
60–100	22	5	
Tumour Location			
Frontal lobe	28	6	0.830
Other than frontal lobe	16	4	
Grade of glioma			
II	17	3	0.729
III	5	2	
IV	22	5	
IDH mutation			
IDH mutant	15	1	0.132
Wild type	29	9	
MGMT methylation			
Methylated	30	3	0.035
Unmethylated	14	7	

KPS: Karnofsky Performance Scale; IDH: isocitrate dehydrogenase; MGMT: O6-Methylguanine-DNA methyltransferase; NANO: Neurologic Assessment in Neuro-Oncology.

Table 5. Combination of IDH mutation and MGMT methylation towards neurological improvement.

Biomarker	NANO scale improvement		P value
	+	(-)	
IDH mutant			
MGMT methylated	13	0	0.032
MGMT unmethylated	2	1	
Wild type IDH			
MGMT methylated	17	3	0.184
MGMT unmethylated	12	6	

IDH: isocitrate dehydrogenase; MGMT: O6-Methylguanine-DNA methyltransferase; NANO: Neurologic Assessment in Neuro-Oncology.

well as some low-grade gliomas. Patients with IDH mutation have been shown to convey a longer overall survival and progression-free survival compared to those in the wild-type group (Malueka *et al.*, 2020; Molenaar *et al.*, 2014; Reifenberger *et al.*, 2014; Songtao *et al.*, 2012). However, it is still unclear if IDH mutational status is a prognostic and predictive measure of response to treatment. Some studies have concluded that IDH mutation is an independent factor towards response to treatment (Hartmann *et al.*, 2011; Houillier *et al.*, 2010), while others linked the relation to other markers such as 1p19q deletion (Iwamoto *et al.*, 2008; Jenkins *et al.*, 2006; Mariani *et al.*, 2006) and G-CIMP phenotype (Reifenberger *et al.*, 2014). Our study found no association between IDH mutation and neurological improvement. This might be due to the difference in study outcome, which is improvement in neurological function rather than survival or progression-free survival. Secondly, since we used the difference between initial NANO scale score and score three months later as a measure for neurological improvement, it is possible that patients with low initial scale scores had a persistent score three months later, which is then assessed as not having improvement after three months. Third, the NANO scale as a measure for neurological improvement does not integrate headaches into the scale, which is the most common presenting symptom in this study.

There are several limitations of this study. First, we did not analyse other predictive biomarkers such as 1p19q deletion and G-CIMP phenotype. Second, the follow up period of three months after surgery might not reveal changes in neurological improvement, since some patients might still be in the chemotherapy and radiation therapy. Hopefully, future studies could integrate other biomarkers as well and conduct a longer period of follow up.

Conclusions

MGMT promoter gene methylation as well as coexistence of IDH mutation and MGMT methylation shows a significant link to improvement in NANO scale score three months after surgery. Glioma patients with MGMT gene promoter methylation are five times more likely to show neurological improvement three months after surgery.

Data availability

Underlying data

Zenodo: MGMT promoter gene methylation and Neurological Scale Improvement in Glioma. https://doi.org/10.5281/zenodo.4482094 (Gunawan *et al.*, 2021)

References

Alifieris C, Trafalis DT: **Glioblastoma multiforme: Pathogenesis and treatment.** *Pharmacol Ther.* 2015; **152**: 63–82.
PubMed Abstract | Publisher Full Text

Bell EH, Zhang P, Fisher BJ, *et al.*: **Association of *MGMT* Promoter Methylation Status With Survival Outcomes in Patients With High-Risk Glioma Treated With Radiotherapy and Temozolomide: An Analysis From the NRG Oncology/RTOG 0424 Trial.** *JAMA Oncol.* 2018; **4**(10): 1405–1409.
PubMed Abstract | Publisher Full Text | Free Full Text

Claus EB, Black PM: **Survival rates and patterns of care for patients diagnosed with supratentorial low-grade gliomas: data from the SEER program, 1973-2001.** *Cancer.* 2006; **106**(6): 1358–1363.
PubMed Abstract | Publisher Full Text

Gunawan PY, Islam AA, July J, *et al.*: **Karnofsky Performance Scale and Neurological Assessment of Neuro-Oncology Scale as Early Predictor in Glioma.** *Asian Pac J Cancer Prev.* 2020; **21**(11): 3387–3392.
PubMed Abstract | Publisher Full Text

Gunawan PY, Islam AA, July J: **MGMT promoter gene methylation and Neurological Scale Improvement in Glioma [Data set].** *Zenodo.* 2021.

Hartmann C, Hentschel B, Tatagiba M, *et al.*: **Molecular markers in low-grade gliomas: predictive or prognostic?** *Clin Cancer Res.* 2011; **17**(13): 4588–99.
PubMed Abstract | Publisher Full Text

Houillier C, Wang X, Kaloshi G, *et al.*: ***IDH1* or *IDH2* mutations predict longer survival and response to temozolomide in low-grade gliomas.** *Neurology.* 2010; **75**(17): 1560–1566.
PubMed Abstract | Publisher Full Text

Iwamoto FM, Nicolardi L, Demopoulos A, *et al.*: **Clinical relevance of 1p and 19q deletion for patients with WHO grade 2 and 3 gliomas.** *J Neurooncol.* 2008; **88**(3): 293–298.
PubMed Abstract | Publisher Full Text

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): 9852–9862.
PubMed Abstract | Publisher Full Text

Lee J, Park SH, Kim YZ: **Prognostic Evaluation of Neurological Assessment of the Neuro-Oncology Scale in Glioblastoma Patients.** *Brain Tumor Res Treat.* 2018; **6**(1): 22–30.
PubMed Abstract | Publisher Full Text | Free Full Text

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): 803–820.
PubMed Abstract | Publisher Full Text

Malueka RG, Dwianingsih EK, Bayuangga HF, *et al.*: **Clinicopathological Features and Prognosis of Indonesian Patients with Gliomas with IDH Mutation: Insights into Its Significance in a Southeast Asian Population.** *Asian Pac J Cancer Prev.* 2020; **21**(8): 2287–2295.
PubMed Abstract | Publisher Full Text | Free Full Text

Mariani L, Deiana G, Vassella E, *et al.*: **Loss of Heterozygosity 1p36 and 19q13 Is a Prognostic Factor for Overall Survival in Patients With Diffuse WHO Grade 2 Gliomas Treated Without Chemotherapy.** *J Clin Oncol.* 2006; **24**(29): 4758–4763.
PubMed Abstract | Publisher Full Text

Molenaar RJ, Verbaan D, Lamba S, *et al.*: **The combination of *IDH1* mutations and *MGMT* methylation status predicts survival in glioblastoma better than either *IDH1* or *MGMT* alone.** *Neuro Oncol.* 2014; **16**(9): 1263–1273.
PubMed Abstract | Publisher Full Text | Free Full Text

Nayak L, DeAngelis LM, Brandes AA, *et al.*: **The Neurologic Assessment in Neuro-Oncology (NANO) scale: a tool to assess neurologic function for integration into the Response Assessment in Neuro-Oncology (RANO) criteria.** *Neuro Oncol.* 2017; **19**(5): 625–635.
PubMed Abstract | Publisher Full Text | Free Full Text

Ostrom QT, Gittleman H, Truitt G, *et al.*: **CBTRUS statistical report: Primary brain and other central nervous system tumors diagnosed in the United States in 2011-2015.** *Neuro Oncol.* 2018; **20**(suppl_4): iv1–iv86.
PubMed Abstract | Publisher Full Text | Free Full Text

Philips A, Henshaw DL, Lamburn G, *et al.*: **Brain Tumours: Rise in Glioblastoma Multiforme Incidence in England 1995-2015 Suggests an Adverse Environmental or Lifestyle Factor.** *J Environ Public Health.* 2018; **2018**: 7910754.
PubMed Abstract | Publisher Full Text | Free Full Text

Reifenberger G, Weber RG, Riehmer V, *et al.*: **Molecular characterization of long-term survivors of glioblastoma using genome- and transcriptome-wide profiling.** *Int J Cancer.* 2014; **135**(8): 1822–1831.
PubMed Abstract | Publisher Full Text

Schwartzbaum JA, Fisher JL, Aldape KD, *et al.*: **Epidemiology and molecular pathology of glioma.** *Nat Clin Pract Neurol.* 2006; **2**(9): 494–503.
PubMed Abstract | Publisher Full Text

Sehmer EAJ, Hall GJ, Greenberg DC, *et al.*: **Incidence of glioma in a northwestern region of England, 2006 – 2010.** *Neuro Oncol.* 2014; **16**(7): 971–974.
PubMed Abstract | Publisher Full Text | Free Full Text

Senhaji N, Louati S, Chbani L, *et al.*: ***EGFR* Amplification and *IDH* Mutations in Glioblastoma Patients of the Northeast of Morocco.** *Biomed Res Int.* 2017; **2017**: 8045859.
PubMed Abstract | Publisher Full Text | Free Full Text

Songtao Q, Lei Y, Si G, *et al.*: ***IDH* mutations predict longer survival and response to temozolomide in secondary glioblastoma.** *Cancer Sci.* 2012; **103**(2): 269–273.
PubMed Abstract | Publisher Full Text

Stupp R, Tonn JC, Brada M, *et al.*: **High-grade malignant glioma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up.** *Ann Oncol.* 2010; **21 Suppl 5**: v190–3.
PubMed Abstract | Publisher Full Text

Stupp R, Mason WP, Van Den Bent MJ, *et al.*: **Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma.** *N Engl J Med.* 2005; **352**(10): 987–96.
PubMed Abstract | Publisher Full Text

Theresia E, Malueka RG, Pranacipta S, *et al.*: **Association between Ki-67 labeling index and histopathological grading of Glioma in Indonesian population.** *Asian Pac J Cancer Prev.* 2020; **21**(4): 1063–1068.
PubMed Abstract | Publisher Full Text | Free Full Text