ORIGINAL ARTICLE

Bali Medical Journal (*Bali MedJ*) 2021, Volume 10, Number 2: 644-647
P-ISSN.2089-1180, E-ISSN: 2302-2914

Bali Medical Journal
www.BaliMedicalJournal.org
Published by Bali Medical Journal

O^6-Methylguanine-DNA Methyltransferase (MGMT) promoter methylation status of high-grade and low-grade gliomas

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Received: 2021-04-10
Accepted: 2021-07-14
Published: 2021-07-26

ABSTRACT

Background: O^6-methylguanine-DNA methyltransferase (MGMT) is a DNA-repair enzyme that correlates with tumor resistance mechanism to chemotherapy. Methylation of the MGMT promoter inhibits the cells from producing MGMT and is useful to predict chemotherapy's effectiveness with alkylating agents. This study aims to evaluate the MGMT promoter methylation of low-grade and high-grade glioma in the Neurosurgery Department of Cipto Mangunkusumo National General Hospital.

Methods: We evaluated MGMT promoter methylation status using methylation-specific polymerase chain reaction in low and high-grade glioma patients who underwent surgical resection in the Neurosurgery Department of Cipto Mangunkusomo Hospital Jakarta. The result then correlated with age, sex, Karnofsky Performance Scale (KPS), and glioma grading. Data were analyzed using SPSS version 20 for Windows.

Results: MGMT promoter methylation was observed more often in patients diagnosed with age more than 40 years old than in patients less than 40 years old (85.7% vs. 50.0%), also more in men than women (77.7% vs. 50.0%). In patients with KPS more than 70 and KPS 70 or less, methylation of MGMT promoter was observed in 70.0% and 57.1%, respectively. Based on tumor grading, MGMT promoter methylation was observed more often in low-grade gliomas (WHO grade II) than high-grade gliomas (WHO grade II and IV) (85.7% vs. 50.0%). There was no significant relationship between gender, age, KPS, malignancy degree, and Overall Survival (OS) to the MGMT promoter methylation ($p>0.05$).

Conclusion: MGMT promoter methylation was observed less in the higher grade of tumors (grade IV), lower KPS, younger age at the time of diagnosis, and female patients, although the differences were not statistically significant. MGMT promoter methylation was observed more often in gliomas with oligodendroglioma components.

Keywords: Low-grade glioma, High-grade glioma, MGMT, Promoter, Methylation.
Cite This Article: Ichwan, S., Ningsih, H.L., Aman, R.A., Tandian, D., Ashari, S., Gunawan, K., Nugroho, S.W. 2021.O^6-Methylguanine-DNA Methyltransferase (MGMT) promoter methylation status of high-grade and low-grade gliomas. *Bali Medical Journal* 10(2): 644-647. DOI: 10.15562/bmj.v10i2.2316

INTRODUCTION

Glioma is the largest primary brain tumor in adults. Gliomas counted for nearly 80% of all primary brain tumor cases.[1,2] Glioma can be divided into low-grade and high-grade gliomas. Low-grade gliomas are found in about 15-26% of all primary brain tumors. Glioblastoma is the most common primary malignant tumor.[2,3] According to data obtained from Surveillance, Epidemiology, and End Results from WHO, the incidence of glioblastoma accounts for 50% of all new cases of primary brain tumors. Of all cases of glioma, glioblastoma accounts for 50-75%. These tumors are aggressive and have a 5-year life expectancy of less than 3%.[1-4]

Several factors can be used in assessing the prognosis of a patient with glioma.[5] These prognostic factors include age, Karnofsky Performance Scale (KPS), how far the tumor is resected, and the presence of DNA repair enzyme in the tumor (O^6-methylguanine-DNA methyltransferase or MGMT).[1,5,6] MGMT is a DNA-repair enzyme that can inhibit the killing process of tumor cells due to the alkylation process by alkylating agents. Alkylating agents work by alkylating DNA and causing cross-linking of adjacent DNA strands, which will ultimately inhibit cell replication and eventually lead to cell death. This alkylating agent can be found in chemotherapy ingredients. MGMT acts in creating resistance to alkylating agents.[2,7]

MGMT activity increases in tumor cells, but its activity is influenced by the promoter, where the methylation of the promoter causes genes in tumor cells to stop producing MGMT.[7] High MGMT can result in a lack of tumor response to alkylating substances and lower MGMT is considered to increase the sensitivity of tumor cells to alkylating substances from chemotherapy. The methylation of the MGMT promoter was associated with a better response to alkylating agents.[2,7]

Based on those mentioned above, this study aims to evaluate the methylation status of the MGMT promoter in glioma patients undergoing surgery at the Neurosurgery Department of Cipto Mangunkusumo National General Hospital Jakarta as one of the prognostic factors of glioma patients.

METHODS

During 1 year, patients who underwent surgery at the Neurosurgery Department of FKUI-RSCM with low and high-grade glioma anatomical pathology results were included in the study sample. Tumor tissue examined in the Anatomical Pathology section with low or high-grade glioma results will be followed by a methylation examination of the enzyme promoter O^6-methylguanine-DNA methyltransferase (MGMT) using a methylation-specific polymerase chain reaction (MPS) test. This test is particular for testing MGMT promoter methylation in glioma. The DNA from the tumor was isolated and ran on the gel electrophoresis, it will show if there was methylated or unmethylated cytosine on the gel based on the DNA sequence. If there is methylated DNA, it will show a line in the gel on the specific DNA length.

Patient information obtained through medical records was collected in the form of gender, age, and KPS. Data from the MGMT promoter methylation examination were processed and associated with patient characteristics such as age, sex, KPS and the type of anatomical pathology, and the degree of tumor malignancy. All samples with high-grade glioma and low-grade glioma underwent further treatment in the form of radiotherapy. Patients had a follow-up period of at least 1 year to assess survival rates. The data were analyzed statistically using SPSS version 20 for Windows by Fisher Exact Test.

RESULTS

In one year, there are 17 patients with a pathological finding of low-grade and high-grade gliomas that met the inclusion criteria. Of the 17 glioma patients, 9 (52.9%) were male and 8 (47.1%) were female. A total of 10 patients (58.8%) were less than 40 years old at diagnosis and 7 patients (41.2%) were aged 40 or more. From the Karnofsky Performance Score (KPS) assessment before surgery, there were 7 patients (41.2%) with KPS ≤ 70 and 10 patients (58.8%) with KPS> 70 (Table 1).

Table 1. Patient characteristic and malignancy degree

Characteristics	Patient (N=17)	
	Total	%
Gender		
Men	9	52.9
Women	8	47.1
Age (Years)		
< 40	10	58.8
≥ 40	7	41.2
Karnofsky Performance Score (KPS)		
≤ 70	7	41.2
> 70	10	58.8
Malignancy Degree		
Low-grade	7	41.2
High-grade	10	58.8
Anatomical Pathology		
Diffuse astrocytoma	4	23.5
Oligoastrocytoma	3	17.6
Astrocytoma/anaplastic glioma	3	17.6
Glioblastoma	7	41.2

Table 2. Analysis of methylation and characteristic of subjects

Characteristics	Patients (N=17)		p
	Methylated (%)	Unmethylated (%)	
Gender			
Man	77.7	22.3	0.335
Woman	50.0	50.0	
Age (Years)			
< 40	50.0	50.0	0.304
≥ 40	85.7	14.3	
KPS			
≤ 70	57.1	42.9	0.644
> 70	70.0	30.0	
Malignancy Degree			
Low-grade	85.7	14.3	0.304
High-grade	50.0	50.0	
Overall Survival (OS) (Years)			
< 1	36.4	50.0	0.644
≥ 1	63.6	50.0	

Fisher Exact Test; *Statistically significant if p-value less than 0.05

From a total of 17 glioma patients, 7 (41.2%) were found with low-grade glioma and 10 (58.8%) other with high-grade gliomas. The distribution of the types of anatomical pathology, namely diffuse astrocytoma, was found in 4 patients (23.5%), oligoastrocytoma in 3 patients (17.6%), astrocytoma/anaplastic glioma in 3 patients (17.6%), and glioblastoma in 7 patients (41.2%) (Table 1).

Of all the glioma patients in the 1 year, high-grade and low-grade gliomas, the results obtained show that 11 patients (64.7%) were methylated and 6 patients (35.3%) were not methylated. Based on age, the methylated MGMT promoter was more common in patients aged ≥ 40 years compared to patients aged <40 years (85.7% vs. 50.0%). Based on gender,

the methylated MGMT promoter was found more in male than female patients (77.7% vs. 50%). Whereas based on KPS, the methylated MGMT promoter was found to be more in patients with KPS> 70 compared to KPS ≤ 70 (70.0% vs. 57.1%) (Table 2).

Based on the degree of malignancy, the methylated MGMT promoter was more common in low-grade gliomas than in high-grade gliomas (85.7% vs. 50%). In high-grade gliomas, the methylated MGMT promoter was found to be more common in anaplastic gliomas (WHO grade III astrocytoma/oligoastrocytoma) than glioblastoma (WHO grade IV) (66.6% vs. 42.8%). In low-grade gliomas, based on the type of anatomical pathology, methylated MGMT promoter was more frequent in oligoastrocytoma than diffuse astrocytoma (100% vs. 75%). Based on the type of anatomic pathology, at both low-grade and high-grade, methylated MGMT promoter was found to be more in oligoastrocytoma than astrocytoma (100% vs. 66 %). There is no significant association between all characteristic variables and MGMT status (p>0.05) (Table 2).

In a minimum follow-up period of 1 year, Overall Survival (OS) was assessed where it was found that in patients with unmethylated MGMT promoter, OS <1 year was 50.0% (3 patients) and ≥1 year was 50.0% (3 patients). In patients with the methylated MGMT promoter, OS <1 year was 36.4% (4 patients) and OS ≥1 year was 63.6% (7 patients) (Table 2). Overall survival ≥ 1 year was more common in glioma patients with methylated MGMT promoter than in glioma patients with unmethylated MGMT promoter (63.6% vs. 50.0%) (Table 2).

DISCUSSION

Several prognostic factors can be used in assessing the prognosis of glioma, including age, Karnofsky Performance Scale (KPS), how far the tumor is resected, and levels of O^6-methylguanine-DNA methyltransferase (MGMT).[5-8] Methylated MGMT was found more frequently in patients aged ≥ 40 years than patients aged <40 years (85.7% vs. 54.5%). These findings are consistent with the prospective study of Brandes AA et al., in which 81% of patients with the methylated MGMT promoter were found to be ≥40 years of age. The methylated MGMT promoter was more common in patients aged ≥ 40 years than patients aged <40 years (55.0% vs. 35.0%).[9]

Based on gender, the methylated MGMT promoter was found more in male than female patients (77.7% vs. 55.5%). From Kamiryo T et al., on high-grade glioma patients, it was found that the MGMT promoter methylation was higher in men than in women (50% vs. 40.3%), but this difference was not statistically significant.[10]

Karnofsky Performance Scale (KPS) is an instrument used to assess functional scores generally associated with the overall quality of life.[11] The methylated MGMT promoter was more in patients with KPS> 70 than those with KPS ≤ 70 (72.7% vs. 57.1%). Several studies have shown that low KPS is associated with poor outcomes.[10,12]

Generally, in most tumors, the grade does not correlate with the activity of MGMT. However, in gliomas, it is found to have higher activity in high-grade tumors, whereas normal brain tissue around the tumor does not usually express MGMT activity. From this study, the methylated MGMT promoter was found in 42.8% of glioblastoma. This consistent with the literature where the methylated MGMT promoter is reported to be 30-60% in glioblastoma.[13] A systematic review of 5 studies found that the methylated MGMT promoter was found in 35-67.9% of glioblastoma patients.[14]

From this study, it was discovered that the methylated MGMT promoter was found to be more common in low-grade gliomas than in high-grade gliomas (85.7% vs. 50%). Several studies on oligodendroglioma and oligoastrocytoma have given a wide variety of methylated promoter MGMT rates, namely 25-85%.[15,16] Molleman M et al. found that the methylated MGMT promoter was present in 88% of patients with oligodendroglioma tumors.[15] Dong SM et al. found that the methylated MGMT promoter was present in 60% of patients with oligodendroglioma tumors.[16] As much as 67.7% were anaplastic tumors.[16] In Brandes AA et al.'s study, the methylated MGMT promoter was found in 70% of patients with oligodendroglioma tumors and 29.7% in anaplastic oligoastrocytoma.[9] From Kamiryo T et al study, there was 116 high-grade glioma patients which found that 45.2% of the methylated MGMT promoter occurred in patients with anaplastic astrocytoma.[10] In addition, there was 44.6% of Glioblastoma Multiforme (GBM) patients with a statistically significant relationship between methylated MGMT promoter with overall survival (OS) and progression-free time (PFS) in anaplastic astrocytoma patients, but not in GBM.[10] Several other studies have shown that the methylated MGMT promoter is associated with prolonged survival rates for glioblastoma and high-grade glioma patients who are treated with chemotherapy with temozolomide along with radiation.[9,10] They have also shown a role for MGMT methylation status in predicting response to therapy with alkylating agents in patients with diffuse astrocytoma and oligodendroglioma on overall survival.[9,10]

In this study of patients with the unmethylated MGMT promoter, the overall survival <1 year was 50% (3 patients) and ≥1 year was 50% (3 patients). In patients with the methylated MGMT promoter, OS <1 year was 36.3% (4 patients) and OS ≥1 year was 63.6% (7 patients). Overall survival of ≥ 1 year was more common in glioma patients with the methylated MGMT promoter than in glioma patients with the unmethylated MGMT promoter (63.6% vs. 50%).

In a previous study, it was found that the survival rate in patients with the methylated MGMT promoter was higher than the unmethylated MGMT promoter, namely 62% -73.9% vs. 41.2% -65% for 1-year survival and 15.8% - 46% vs. 13.8% -21% for 2-year survival.[14] A meta-analysis of 24 studies assessing the association between methylated MGMT promoter with progression-free time (PFS) and overall survival (OS) showed GBM patients with methylated MGMT promoter had significantly better PFS and OS than non-methylated people.[17] Another meta-analysis of a total of 2,986 patients from 30 studies that looked at the prognostic value of the methylation of the MGMT promoter on GBM showed

that the methylated MGMT promoter was associated with better PFS and OS in GBM patients regardless of the interventions undertaken and associated OS prolongation in GBM patients who undergo therapy with alkylating agents.[18] The prognostic value of MGMT is related to the efficacy of adjuvant therapy in the form of chemotherapy and radiotherapy, which may influence patient selection for more aggressive treatment regimens.[17,18]

CONCLUSION

Fewer methylated MGMT promoter status was found at higher tumor grade (WHO grade IV), low KPS, younger age at diagnosis, and female patients, although the difference is not statistically significant. The methylated MGMT promoter is found more in tumors with an oligodendroglioma component. Further studies with larger sample sizes are needed to determine whether the methylation of the MGMT promoter has a significant association with these factors.

CONFLICT OF INTEREST

There is no conflict of interest in this paper.

FUNDING STATEMENT

This study was self-funded by authors.

ETHICS CONSIDERATION

Ethical approval has been obtained from the Ethics Committee of Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia, prior to the study being conducted.

AUTHOR CONTRIBUTIONS

All authors contributed to drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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