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RESEARCH ARTICLE

Prognostic Significance of MGMT Promoter Methylation in Egyptian GBM Patients: A Single-institution Experience

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Abstract

Background: Glioblastoma Multiforme (GBM) is the highest-mortality tumor of the central nervous system. Epigenetic silencing of the MGMT gene by promoter methylation is associated with loss of MGMT expression and deficiency in MGMT-mediated DNA repair, which is affiliated with improved survival in patients treated with alkylating agents such as TMZ. **Purpose:** This is a retrospective work, studying the MGMT promoter status in a group of GBM patients; correlating this status to time to progression (TTP) and overall survival (OS). **Methods:** Thirty-nine patients with GBM, treated in Kasr El-Ainy Center of Clinical Oncology and Nuclear Medicine (NEMROCK) between January 2014 and January 2015, were included in our study. The QIAamp DNA FFPE Tissue Kit (Qiagen, USA) was used for genomic DNA extraction from Formalin-fixed paraffin-embedded tumor tissues of the 39 patients. Bisulfite modification of DNA was performed for detecting methylation in MGMT promoter using EpiTect Bisulfite Kit (Qiagen, USA). Specific primers were used to match methylated & un-methylated DNA that was visualized by loading its PCR products in gel electrophoresis system. All statistical analyses were carried out using SPSS version 20.0. **Results:** The mean age for our patients was 48 years; with a male to female ratio of 1.3:1. MGMT promoter methylation was found in 27 patients (69.2%) compared to 12 (30.8%) with un-methylation. TMZ was received in 71.8 % (28) of our patients throughout their treatment. The median OS for all patients was 20.03 months; while the median TTP 15.03 was months. Although the OS was statistically significantly higher for patients with the methylated promoter (p-value = 0.004) compared to the un-methylated group; yet the TTP difference did not reach a statistically significant value (p-value = 0.048). **Conclusions:** The epigenetic silencing of the MGMT gene by promoter methylation has been associated with longer OS & TTP in patients with GBM.

Keywords: GBM- epigenetic silencing- un-methylation- overall survival

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Introduction

Glioblastoma Multiforme (GBM) is the highest-mortality tumor of the central nervous system. The role of temozolomide (TMZ), along with maximal safe resection and radiation, was established for the treatment of newly diagnosed GBM patients years ago [1].

The MGMT (O6-methylguanine–DNA methyltransferase) gene is located on chromosome 10q26 and encodes a protein which is responsible for DNA repair via removal of the alkyl groups from the O6 position of guanine, an important site of DNA alkylation. The unrepaired, chemotherapy-induced lesions trigger cytotoxicity and apoptosis [2]. The high activity level of MGMT in the malignant cells creates resistance by blunting the therapeutic effect of alkylating agents and may be an important determinant of treatment failure [3]. Epigenetic silencing of the MGMT gene by promoter methylation is associated with loss of MGMT expression and result in a deficiency in MGMT-mediated DNA repair [4].

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Subset analyses had confirmed the sensitivity of tumors deficient in MGMT (defined by MGMT promoter methylation) to temozolomide, compared to those with adequate MGMT expression (defined by an un-methylation promoter) [5]. Several reports have concluded that epigenetic silencing is affiliated with improved survival in GBM patients who were treated with alkylating agents such as TMZ [6].

This is a retrospective work, studying the MGMT promoter status in a group of GBM patients; correlating this status to time to progression (TTP) and overall survival (OS).

Materials and Methods

Thirty-nine patients with GBM, who had been treated in Kasr El-Ainy Center of Clinical Oncology and Nuclear Medicine (NEMROCK) in the period between January 2014 and January 2015, were included in our study. All the patients (39) underwent surgical intervention followed by postoperative radiation therapy. Temozolomide (TMZ) was used only in 28 patients as concurrent and adjuvant treatment.

MGMT testing

The Formalin-fixed paraffin-embedded tumor tissue blocks were collected from the patients. The QIAamp DNA FFPE Tissue Kit (Qiagen, USA) was used for genomic DNA extraction from Formalin-fixed paraffin-embedded tumor tissues of the 39 patients. Bisulfite modification of DNA was performed for detecting methylation in MGMT promoter using EpiTect Bisulfite Kit (Qiagen, USA). Specific primers were used to match methylated & un-methylated DNA that was visualized by loading its PCR products in gel electrophoresis system.

Statistical analysis

Data analyses were carried out using statistical package for social sciences (SPSS), program version 20. All data entries were checked for accuracy against the original raw data of each patient. The significance level of all statistical analysis was at < 0.005 (P-value).

Results

Patients' and tumor's characteristics were collected and recorded as shown in Table 1. The mean age for our patients was 48 years; with a male to female ratio of 1.3:1. MGMT promoter methylation was found in 27 patients (69.2%) compared to 12 (30.8%) with un-methylation. All patients (39) received postoperative radiation therapy. TMZ was received in 71.8 % (28) of our patients throughout their treatment.

The median OS for all patients was 20.03 months; while the median TTP 15.03 was months. Although the OS was statistically significantly higher for patients with the methylated promoter (p-value = 0.004) compared to the un-methylated group; yet the TTP difference did not reach a statistically significant value (p-value = 0.048).

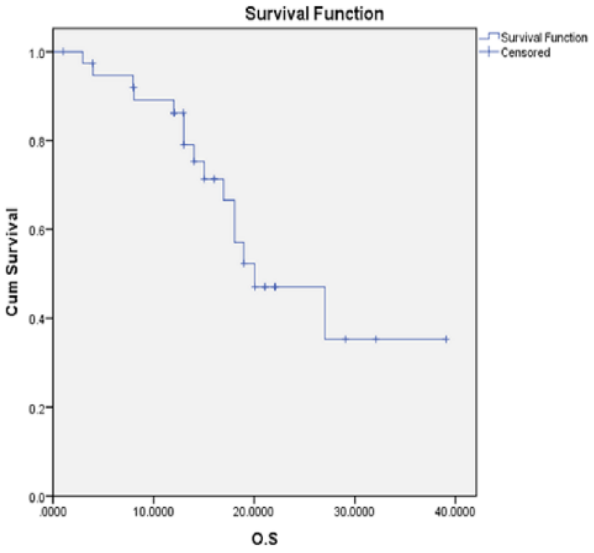

Figure 1. The Overall Survival (OS) Curve

Discussion

Alkylating agents, including temozolomide and the nitrosoureas (carmustine and lomustine), are commonly used cytotoxic chemotherapies for newly diagnosed as well as recurrent GBM patients. They cause apoptosis by methylating guanine at the O6 position, initiating a double-strand break in the DNA, and cell death [7]. The MGMT protein repairs the DNA damage via removing the damaged alkyl groups from the O6 position of guanine. The alkylated protein is then degraded, requiring constant refillment for DNA repair to be fruitful [8].

Table 1. Patients' & Tumor's Characteristics (N=39)

	Number	Percent
Age (years)		
	16	41%
< 48	22	59%
≥ 48	48	
Median (range)		
Sex		
Male	22	56.4%
Female	17	43.6%
Site of lesion		
Frontal	4	10.3%
Fronto-parietal	12	30.8%
Parietal	1	2.6%
Temporal	1	2.6%
Tempo-parietal	15	38.5%
Occipital	6	15.4%
Type of Surgery		
Total resection	6	15.4%
Debulking Surgery	26	64.1%
Biopsy	8	20.5%
MGMT Status		
Methylated	27	69.2%
Un-methylated	12	30.8%

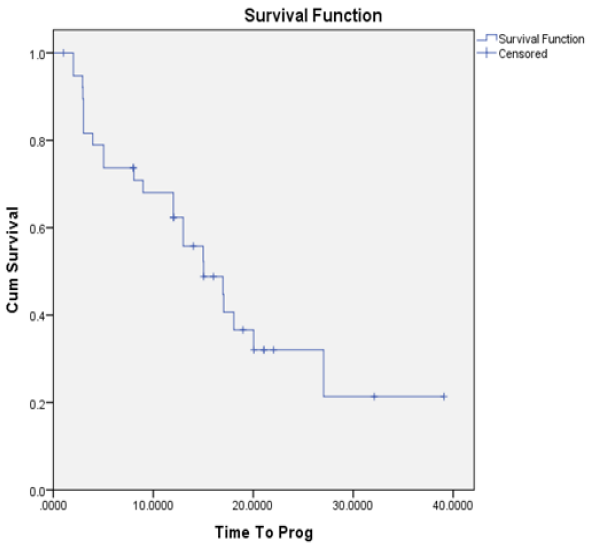

Figure 2. The Time to Progression (TTP) Survival Curve

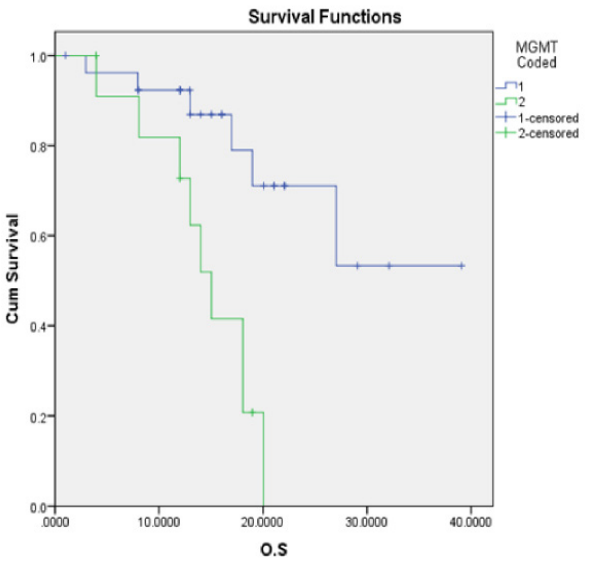

Figure 3. The Correlation Between the OS and the MGMT Promoter Methylation

High expression of MGMT in cancer cells accounts for the primary resistance against alkylating agents [9-10].

Setting the cutoff between MGMT methylation and unmethylation, as defined by methylation-specific PCR, is a little bit complicated by methylation pattern, heterogeneity, and tissue contamination with non-neoplastic-methylated cells. This leads to wide variability among laboratories and lack of standardization, which makes the interpretation of the prognostic and predictive impact of MGMT methylation status difficult. However, the constancy of results showing improved outcomes for methylated patients, irrespective to the treatment given, and an adjoined benefit with temozolomide, suggest that MGMT methylation has both prognostic and predictive impact [11].

The NOA-08 trial was a large, phase 3 study planned to experiment temozolomide monotherapy in patients with newly diagnosed high-grade gliomas >65 years of age. The design was to compare it as a non-inferior treatment to standard radiation (60 Gy). They tested 51 % of samples for MGMT promoter methylation using two distinct methylation-specific PCRs. The authors found that MGMT promoter was methylated in 35% of tested samples; methylation status as a predictive marker of response to temozolomide. They suggested temozolomide monotherapy in elderly patients who are MGMT methylation versus radiation therapy alone in those unmethylated [12].

In the Nordic trial, temozolomide's efficacy was tested as a monotherapy to standard radiation (60 Gy) and hypofractionated radiation (34 Gy in 3– 4 Gy over 2 weeks) in newly diagnosed GBM patients >60 years of age. MGMT methylation status was evaluated in 59 % of patients, and 45 % of the samples were found to be methylated [13]. Results supported previous evidence demonstrating the value of hypofractionated radiation (40 Gy) in elderly patients [14]. They reached similar conclusions to those from the NOA-08 study, with inferior mOS in patients with unmethylated MGMT, regardless of treatment, and no gained survival benefit with chemotherapy [13].

Another study was performed to interpret the prognostic and predictive value of MGMT promoter methylation in Chinese patients with GBM. It is considered a powerful study because of the large sample recruited, its prospective design, the standardization use, and pyrosequencing analysis to assess the MGMT promoter methylation status, and the chance of dissecting the prognostic and predictive aspects of MGMT promoter methylation as a biomarker. This cohort represents patients treated in a single center over a 68-month period [15]. The group with methylation data had a similar median age and range, performance status, and percent of patients with biopsy vs debulking surgery compared with other clinical studies [16-19]. Progression-free survival was 8.2 months compared with 6.9 months reported by SteelFisher et al; as for the overall survival, it was 13.1 months in contrast to 14.6 months. MGMT promoter methylation was prognostic for OS but not for PFS for the whole group of 128 participants [17].

Table 2. The Overall Survival (OS) for the 39 Patients

Variable	OS (months)
Median	20.03
95%CI	(11.61-28.45)
N of Event	15
6ms Survival rate	89.75%

Table 3. The Time to Progression (TTP) for the 39 Patients

Variable	T.T.P (months)
Median	15.03
95%CI	(08.91-21.15)
N of Event	23
6ms Survival rate	71.79%

In conclusion, the epigenetic silencing of the MGMT gene by promoter methylation has been associated with longer OS & TTP in patients with GBM.

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