Original Article
Middle East Journal of Cancer; July 2018; 9(3): 179-185

Prognostic Significance of MGMT Promoter Methylation in Patients with Glioblastoma Undergoing Surgical Intervention: A Retrospective Study in Northeastern Iran

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Abstract

Background: The standard of care for glioblastoma is concurrent chemoradiotherapy and adjuvant chemotherapy with Temozolomide. In this trial, we have investigated the impact of MGMT promoter methylation on prognosis and benefit from Temozolomide based chemotherapy in a group of patients with glioblastoma in our region.

Methods: This retrospective study included glioblastoma patients treated in our institute between 2006 and 2011. We used methylation specific PCR to detect methylation in the promoter region of the MGMT gene. The Kaplan-Meier technique was used to calculate overall survival from the time of diagnosis to the time of death. We utilized the log-rank test and Cox-regression model for univariate and multivariate analyses of potential prognostic factors.

Results: There were 78 patient participants with a median age of 50 (range: 20 to 75) years and a male to female ratio of 56/22. All patients underwent minimal surgical resection which was considered as a biopsy. All patients received adjuvant radiotherapy with a median dose of 60 Gy (54-60 Gy) and 25 patients received concomitant Temozolomide. The MGMT promoter methylation was found in 19 (24.4%) patients and was relatively more frequent in men (28.6%) compared to women (13.6%; P=0.16).This genetic change was associated with a significantly higher 2-year survival in men (57.2%) compared to women (16.8%; P<0.001). Multivariate analysis indicated that male sex and MGMT promoter methylation were independently associated with more favorable prognosis.

Conclusion: In our series, MGMT promoter methylation was a significant independent prognostic factor. The finding of sex as an independent prognostic factor would need further validation.

Keywords: Glioblastoma, MGMT gene, Promoter, Methylation

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Middle East Journal of Cancer

Received: February 04, 2017; Accepted: October 28, 2017

Introduction

Glioblastoma, the most common primary brain tumor in adults, comprises 75% of all glial tumors. Despite multiple treatment modalities that include surgery, radiotherapy, and chemotherapy, the prognosis remains dismal with a long-term survival of 3% to 5% in these patients.[1]

Nitrosourea based chemotherapy only modestly improved survival in patients with glioblastoma.[2,3] However, in recent years, introduction of Temozolomide with its efficient CSF penetration and higher activity[4] has improved the role of chemotherapy in glioblastoma. A final analysis of a randomized trial by Stupp et al. showed that patients with glioblastoma who underwent radiotherapy concomitant with Temozolomide had a 5-year survival of 10% compared to 2% in those who received radiotherapy alone.[5] Currently, the standard of care in treatment of patients with glioblastoma is tumor resection, radiotherapy concomitant with Temozolomide, and adjuvant Temozolomide.[6,7]

Temozolomide is an alkylating agent with the ability to alkylate/methylate DNA which occurs at the N-7 or O-6 position of the guanine residue. The methylated guanine binds with thymidine instead of cytidine during the next mitosis which promotes apoptosis. Some tumor cells are able to repair this kind of DNA damage and become resistant to therapeutic effects of Temozolomide by expressing a DNA repairing enzyme, O6-alkylguanine DNA alkyl transferase (AGT), encoded by the MGMT gene located on chromosome 10q26. This enzyme removes alkyl adducts from the O-6 position of guanine. Meanwhile, epigenetic MGMT gene silencing through methylation in the promoter region enhances tumor cell sensitivity to alkylating agents which can provide more favorable survival.[8,9]

MGMT silencing has been detected in other neoplasms such as colon and lung cancer;[10,11] however, the effect of this epigenetic change on survival has only been documented in patients with glioblastoma. Previous trials reported that 40% to 50% of patients with glioblastoma contained MGMT methylation, which was significantly associated with more favorable survival compared to those with tumors without MGMT methylation.[4,5,12,13]

In this retrospective study, we investigated the incidence of MGMT methylation in patients with glioblastoma in our region. We also assessed the effect of MGMT methylation on the prognosis as well as the response to chemotherapy in these patients.

Materials and Methods

Patients

In this retrospective cohort study, we evaluated patients with pathological confirmed diagnosis of glioblastoma who referred to the Oncology Department of Omid Hospital affiliated with Mashhad University of Medical Sciences (MUMS) between 2006 and 2011. Patients that had available suitable paraffin blocks and completed their radiation course (at least 55 Gy) were eligible. A control MRI was ordered 6 to 8 weeks following radiation therapy. Patients who completed treatment were followed every 3 months for 2 years, then less frequently. Patients that received less than 55 Gyor unconventional dose fractions, those with very poor performance status (WHO: 4), and uncontrolled diabetes mellitus were excluded.

DNA extraction and bisulfite conversion

Genomic DNA was extracted from patient tissue biopsies by the standard phenol/chloroform method. Bisulfite treatment and purification of genomic DNA was performed with the Epitect Bisulfite kit (Qiagen) according to the manufacturer's instructions. For each conversion reaction, we used 1µg of genomic DNA; after conversion and purification, DNA was eluted in 70 µL of the provided elution buffer.

Quantitative methylation specific PCR (QMSP)

We utilized methylation specific PCR (MSP) to assess methylation in the promoter region of the MGMT gene.[14] Quantitative real-time PCR was performed for MGMT using the following primer/probe set (Metabion):[14] forward 5' –CGAATATACTAAAACAACCCGCG-

3',reverse 5'-GTATTTTTTCGGGAGCGAG GC-3', and probe 5'-FAMAATCCTCGATACGC ACCGTTTACGTAMRA-3' which yielded a122bp amplicon. A primer/probe set specific for the unmethylated promoter region of the ACTB gene was used as the reference gene as follows: forward 5'TGGTGATGGAGGAGGTTTAG-TAAGT-3', reverse 5'-AACCAATAAAACC TACTCCTCCCTTAA-3', and probe 5'FAMAC-CACCACCCAACACACAATAACAAACACAT A-MRA-3' which yielded an amplicon size of 133bp. Leukocyte DNA from a healthy blood donor was methylated *in vitro* with excess SssI methyltransferase (New England Biolabs, Inc.) to generated completely methylated DNA (mDNA). Serial dilutions (pure up to 1:10000) of this DNA were used to construct calibration curves for MGMT and ACTB.[15]

For each 20 µl PCR, 4µl eluted that contained the bisulfite converted DNA,10µl perfect real-time premix EX Taq (PR039; Takara, Kyoto, Japan), 0.5 µl Rox reference dyeII (Takara), 1.5µl each of the forward and reverse primers at a concentration of 10 pmol/µl that resulted in a final concentration of 0.0156 µM were used. PCR conditions were as follows: one step at 95°C for 30 s,50 cycles at 95°C for 4 s, and 60°C for 30s.

We performed the PCR analyses in 48-well plates in a Step One real-time PCR system (Applied Bio-systems).Each plate included calibration curves for the ACTB and MGRT genes, patient DNA samples, positives control [CG genome universal methylated DNA (mDNA) S7821;Millipore], negative control [CG genome universal unmethylated DNA (uDNA) S7822; Millipore], and multiple water blanks. The relative level of mDNA was determined as a ratio of MGMT to ACTB and subsequently multiplied by 1000 for easier tabulation as the average value of triplicates of the gene of interest/average value of triplicates of ACTB*1000. For each sample, quantitative methylation-specific PCR (QMSP) analysis was repeated on three separate plates, and we considered the median values for statistical analyses.

Statistical analysis

We used the chi-square test to compare the frequency of MGMT promoter methylation between groups. Overall survival curves were estimated by the Kaplan-Meier technique from the time of diagnosis to the time of death. We used the log-rank test to compare survival curves between groups. The Cox-regression model was used for

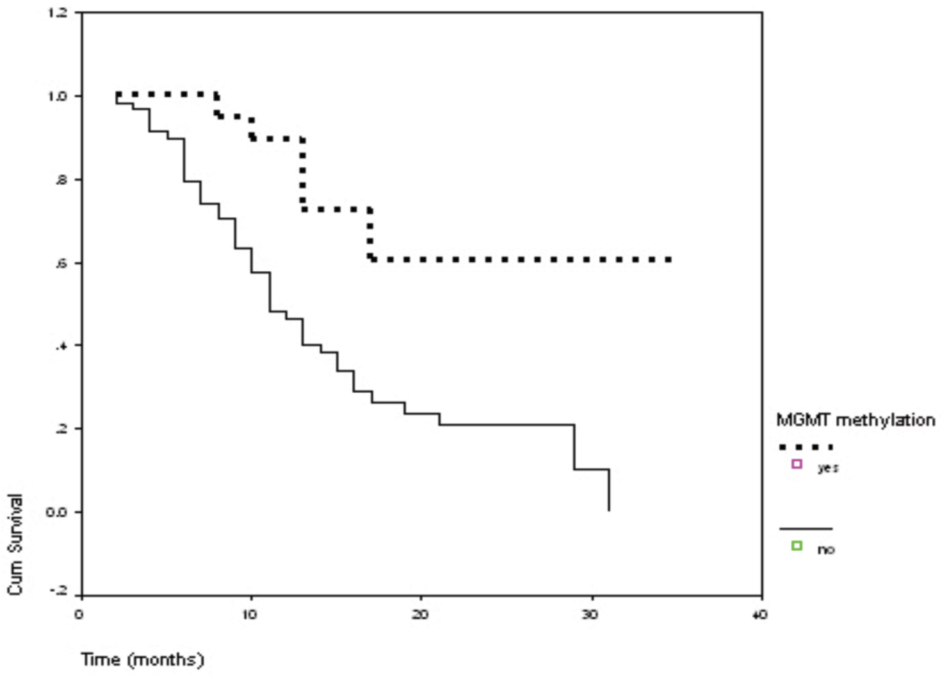

Figure 1. A comparison of overall survival in patients with and without MGMT methylation.

Table 1. Association between some clinical and pathological characteristics and overall survival in patients with glioblastoma.

Characteristics	Number	One-year overall survival % (1 SE*)	2-year overall survival % (1 SE)	Log-rank *P*-value	Cox-regression *P*-value
Age (years)					
<50	37	64.1 (8)	42.5 (8.5)	0.24	-
≥50	41	50.8 (8.1)	21.6 (7.4)		
Sex					
Male	56	63.7 (6.6)	37.9 (7.3)	0.005	0.03
Female	22	40.9 (10.5)	15.9 (8.1)		
MGMT methylation					
Yes	59	89.4 (7.1)	60.5 (11.7)	<0.001	0.001
No	19	46.6 (6.7)	21.1 (6.1)		
Concurrent Radiation therapy-Temozolomide					
Yes	24	69.7 (9.7)	35.6 (10.5)	0.27	-
No	54	51.6 (6.7)	28.6 (7.1)		

*: Standard Error

multivariate analysis to detect independent prognostic factors.

Results

We evaluated 78 patients with a median age of 50 (range: 20-75) years and a male: female ratio of 56/22 (2.54). The tumor location was in the frontoparietal lobe in 39 (50%) patients, temporal lobe in 15 (19.2%), and the remainder were in other regions. All patients underwent either a biopsy or suboptimal surgical resection. The patients received adjuvant radiotherapy with a median dose of 60 Gy (55-60 Gy). A total of 25 (32%) of patients received concomitant chemotherapy with Temozolomide. The remainder received adjuvant chemotherapy with Nitrosourea-based regimens.

In 19 (24.4%) patients, we detected methylation in the promoter region of the MGMT gene. MGMT methylation was relatively more frequent among men (16/56; 84.2%) compared to women (3/22;15.7%; *P*=0.16). There was no significant difference in the rate of MGMT methylation between patients older than 50 (11/40; 26.8%) and less than 50 (8/37; 21.6%; *P*=0.593) years of age.

With a median follow up time of 12.5 months (range: 2-35), 49 patients (62%) succumbed to the disease with a median progression-free survival of 10 months (95% CI: 2-56 months) and median overall survival of 13 months (95% CI: 10-16 months). The one-year overall survival was 57.2%, whereas the 2-year overall survival was 31.5%.

Table 1 shows the association between some clinical and pathological characteristics and overall survival. Patients with tumors that contained MGMT methylation had significantly higher overall survival compared to those with tumors that lacked MGMT methylation (Figure 1). Men had a significantly more favorable outcome compared to women. Both factors remained significant predictors of overall survival in multivariate analysis. Among all investigated patients, both age and chemotherapy protocol had no significant effect on survival.

Discussion

In this study, we assessed methylation in the promoter region of the MGMT gene using the MSP method in 78 patients with glioblastoma.

This method has a much higher accuracy rate than the immunohistochemical (IHC) method to detect this genetic change.[16] We found MGMT methylation in 24.4% of all cases. In previous trials, MGMT promoter methylation has been reported in 35%-45% of malignant gliomas.[17,18]

The effect of combined modality treatment that contained chemotherapy on improving survival in patients with glioblastoma has been proven in a large study conducted by Corle et al.[19] They analyzed 8527 patients treated between 1999 and 2008 using the California Cancer Registry. Patients that received surgery, radiation, and chemotherapy had a significantly better one-year survival rate (62.5%) compared to those that received any other combination (24.3%, $P<0.001$).[20] In recent years, the introduction of Temozolomide as an active agent for concurrent chemoradiotherapy and adjuvant chemotherapy for patients with glioblastoma improved survival rates. Multiple studies documented the positive effect of MGMT promoter methylation in glioblastoma tumors on response to treatment irrespective of the treatment protocol. Stupp et al. reported the final results of a phase III randomized trial with a median follow up of more than 5 years when comparing the results from radiotherapy alone with radiotherapy plus concomitant and adjuvant Temozolomide. They concluded that MGMT promoter methylation was the most important prognostic factor and benefit from concomitant and adjuvant Temozolomide.[5] Our study failed to prove the superiority of concurrent chemo-radiotherapy over radiotherapy alone without respect to MGMT methylation. However, patients harboring tumors with MGMT methylation had significantly more favorable prognosis.

In a trial that used the clinical centers of the German Glioma Network, Krex et al. performed a thorough clinical and molecular assessment on 55 glioblastoma patients with longer than 3-year survival. They found that 74% of these long-term survivors had MGMT methylation which was significantly more frequent than the 141 consecutive patients in the same group.[13] Gorila et al. investigated the prognostic factors in an EORTC and NCIC trial which included 573 patients randomized to receive radiotherapy alone or radiotherapy plus Temozolomide. In all randomized patients, extensive surgical resection, administration of Temozolomide, younger age, Mini-Mental State Examination (MMSE) score of 27 or higher, and no corticosteroid treatment at baseline were associated with higher survival according to multivariate analysis. In cases with available MGMT status, the methylated tumors, better performance status, and higher MMSE scores had an independent association with a more favorable outcome.[20]

The effect of MGMT methylation on patient survival has been documented in older cases. A retrospective study by Grasbon-Frodl et al. on 64 elderly patients (>70 years old) with glioblastoma showed an association of MGMT methylation with reduced hazard for death (HR: 0.475; 95% CI: 0.254, 0.890; $P=0.0283$).[14] Malmströmet al. conducted a multi-center randomized trial to compare Temozolomide versus hypofractionated radiotherapy versus conventional radiotherapy in patients older than 70 with glioblastoma. They found more favorable outcomes in cases that received Temozolomide and better results with hypofractionated radiotherapy compared to the conventional protocol. MGMT methylation was associated with significantly better survival (9.7 months) compared to those without MGMT methylation (6.8 months; HR: 0.56; 95% CI: 0.34-0.93; $P=0.02$). The effect of MGMT promoter methylation was only significant in cases that received Temozolomide.[21] In a retrospective study by Lorimer et al. on 339 patients with glioblastoma over 70 years old, radical chemoradiotherapy that contained Temozolomide decreased the risk of death by 80%. Total resection was also associated with more favorable outcome ($P=0.019$).[22]

MGMG methylation was also a significant favorable prognostic factor in recurrent glioblastoma treated with gamma knife radiosurgery in a study conducted by Kim et al.[23] They retrospectively examined 61 patients

between 2004 and 2015. The median overall survival was considerably higher in 25 (41%) with MGMG methylation (14 months) compared to the unmethylated group (9 months; *P*=0.026). MGMT promoter methylation occurred more frequently in men than women. This finding might partly explain the more favorable outcome in men in our patients. None of our patients have undergone optimal resection which is an adverse prognostic factor. Thon et al. investigated the sub-population with unresectable glioblastoma that underwent external beam radiotherapy plus concurrent and adjuvant chemotherapy with Temozolomide. They found significantly more favorable treatment response and overall survival in cases with MGMT promoter methylation.[24]

Our study, along with previous trials, showed a significantly more favorable outcome in patients with glioblastoma that contained MGMT promoter methylation. The role of MGMT methylation testing in treatment stratification of patients with glioblastoma should be observed, particularly for elderly patients in whom concomitant chemoradiotherapy might have higher toxicity.

Conflict of Interest

No conflict of interest is declared.

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