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Prognostic significance of O6-methylguanine-DNA-methyltransferase (MGMT) promoter methylation and isocitrate dehydrogenase-1 (IDH-1) mutation in glioblastoma multiforme patients: A single-center experience in the Middle East region

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ABSTRACT

Objectives: To determine the prevalence and prognostic value of MGMT promoter methylation and IDH1 mutation in glioblastoma multiforme (GBM) patients from the Middle East.
Patients and methods: Records of patients diagnosed between 2003 and 2015 were reviewed. MGMT promoter methylation was measured using methylation-specific polymerase chain reaction and IDH-1 mutation was reported. The primary endpoint was overall survival (OS).
Results: A total of 110 patients were included. The median age was 51 years and 71 patients (64.5%) were males. The median diameter of GBM was 4.6 cm and 29 patients (26.4%) had multifocal disease. Gross total resection was achieved in 38 patients (24.9%). All patients received adjuvant radiation therapy, and 96 patients (91.4%) received concomitant temozolomide. At a median follow up of 13.6 months, the median OS was 17.2 months, and the OS at 1 and 2 years were 71.6% and 34.8%, respectively. On multivariate analysis, age at diagnosis (HR 1.019; P = 0.044) and multifocality (HR 2.373; P = 0.001) were the only independent prognostic variables. MGMT promoter methylation was found in 28.2% of patients but did not significantly correlate with survival (HR 1.160; P = 0.635). IDH-1 mutation was found in 10% of patients was associated with a non-significant trend for survival improvement (HR 0.502; P = 0.151).
Conclusion: Patients with GBM from the Middle East have adequate survival outcomes when given the optimal treatment. In our patient population, MGMT promoter methylation did not seem to correlate with outcomes, but patients with IDH1 mutation had numerically higher survival outcomes.

Abbreviations: AUBMC, American University of Beirut Medical Center; EORTC, European Organization for Research and Treatment of Cancer; GBM, glioblastoma multiform; IDH-1, isocitrate dehydrogenase-1; IQ, interquartile range MENA: Middle East and South Africa; MGMT, O6-methylguanine-DNA-methyltransferase; MSP, methylation-specific; PCR NCIC, National Cancer Informative Center; RT, radiation therapy; TMZ, temozolomide; WHO, World Health Organization

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1. Introduction

Glioblastoma Multiforme (GBM) is the most common and aggressive primary tumor of the central nervous system, accounting for 40% of all brain malignancies. The standard treatment consists of maximal safe resection, followed by radiation therapy (RT), with concomitant and adjuvant chemotherapy [1]. Despite this multimodal approach, the prognosis of patients with GBM remains dismal, with a median survival of 14.6 months [2]. Although significant progress in the understanding of the genetic alterations in GBM was made, prognostic molecular markers continue to be rare. O6-Methylguanine-DNA-methyltransferase (MGMT) is a DNA repair enzyme which, in the course of tumor development, may be silenced by methylation of its gene promoter. This methylation was found to correlate with an increase in median survival in patients with GBM [3]. Isocitrate Dehydrogenase-1 (IDH-1) is an enzyme that catalyzes the conversion of isocitrate into alpha-ketoglutarate in the Krebs cycle. Mutations in IDH-1 have been reported in patients with high grade gliomas, ranging from 5% in patients with primary GBM, up to 75% in patients with secondary GBM, and are associated with better survival outcomes [4].

Ethnic disparities in cancer outcome have been reported across many types of cancers, including GBM [5]. There is a gap in the literature on the outcomes of GBM tumors in the Middle East region, and the prevalence and prognostic significance of the MGMT promoter methylation and the IDH1 mutation. Due to the commonly found extended consanguineous family structure in the region, it is unclear whether one can extrapolate the reported prevalence estimates and prognostic significance of the MGMT and IDH1 status to our population. In this study, we aim at reviewing our patients with GBM to determine their survival outcomes, and the prevalence and prognostic value of the MGMT promoter methylation and the IDH1 mutation.

2. Methods

2.1. Study population

Records of GBM patients who were diagnosed between January 2003 and December 2015 and who received radiation therapy as part of their initial management at the radiation oncology department of the American University of Beirut Medical Center (AUBMC) Naef K. Basile Cancer Institute, were reviewed. The year 2003 was chosen to coincide with the start of usage of Temozolomide (TMZ) as concomitant and adjuvant therapy at our institution. The study was approved by the institutional review board of the American University of Beirut.

2.2. Selection criteria

Patients were eligible if they originated from the Middle East region, had a de novo diagnosis of unifocal or multifocal glioblastoma, and presented to AUBMC for management. Pathological confirmation of the diagnosis was required, and patients had to have received radiation therapy at the department of Radiation Oncology at AUBMC. We included patients with a known history of prior lower grade glioma but had not previously received radiation therapy to the brain. We included patients with any WHO performance status at diagnosis for more generalizability and a better representation of the general population. Patients who had previously received radiation or systemic therapy, were excluded. Patients with an active second malignancy were also excluded.

2.3. Evaluations and interventions

Medical charts of eligible patients were reviewed, and data was collected regarding patient demographics, tumor characteristics, interventions, and outcomes. Patients were evaluated with a magnetic resonance imaging (MRI) of the brain prior to any intervention. Pathological confirmation of the GBM diagnosis was achieved through a biopsy, subtotal resection or gross total resection. Radiation therapy targeted the tumor bed, surgical bed and any residual lesions, to a median dose of 60 Gy. All patients' surgical specimens were stored in the department of pathology and molecular medicine.

2.4. Methylation-specific PCR for MGMT promoter and IDH-1 mutation

MGMT promoter methylation was detected using Methylation-specific PCR (MSP) method as per Esteller et al. [6] and Takahashi et al. [7] with minor modifications. The PCR cycling conditions included an initial denaturation step followed by an annealing step. PCR product was then loaded on 3% agarose gel containing ethidium bromide (VWR, Pa, USA) and visualized under UV-light. Samples showing a band with the unmethylated reaction only were considered MGMT promoter unmethylated, while samples showing bands with the methylated or both methylated and unmethylated reactions were considered MGMT promoter methylated [8]. All samples were evaluated at least 3 times, and only those that revealed consistent results were included in the analysis. IDH-1 mutations were obtained using whole exome sequencing of an undergoing separate study (manuscript in preparation).

2.5. Outcome measures

The primary endpoint was overall survival (OS). OS and progression-free survival (PFS) times were calculated from the date of diagnosis, defined as the date of biopsy or resection of the index lesion. Progression was defined as any recurrence within the brain, whether inside or outside of field of radiation treatment. Upon recurrence and/or progression, imaging of the brain was reviewed to determine the type of recurrence. A second primary endpoint was to determine the prevalence of the MGMT promoter methylation and IDH-1 mutation in our cohort and assess for a potential correlation with survival.

2.6. Statistical methods

Statistical analyses were performed using SPSS 23.0 (SPSS inc., Chicago, IL, USA). Survival outcomes were calculated using Kaplan-Meier methods and compared using the log-rank test. A multivariable Cox model was used to examine associations between patients' demographic, tumor, and treatment characteristics and OS. P-value less than 0.05 was considered statistically significant.

3. Results

3.1. Patient and tumor characteristics

A total of 110 patients were eligible and were included in the study. Table 1 summarizes baseline patient and tumor characteristics. The median age at diagnosis was 51 years (Interquartile range (IQ) 41–64 years). Seventy-one patients (64.5%) were males, and 69 (62.7%) had a WHO performance status of 0. A total of 71 patients (64.5%) were Lebanese. Eighteen patients (16.4%) were from Iraq and 15 patients (13.6%) were from Syria. The median largest diameter of GBM was 4.6 cm (IQ range 3.8–5.8) and was associated with a midline shift in 68 patients (61.8%) and with multifocality in 29 patients (26.4%). The most common presenting symptom was motor deficit in 38 patients, (34.5%) followed by seizure in 17 patients (15.5%) and headache in 15 patients (13.6%). Thirteen patients (87.3%) had an altered mental status at presentation. Genomic DNA was available for 83 samples of which 71 revealed consistent MGMT promoter results on MSP. Of those, 20 (28.2%) had an MGMT promoter methylation. The IDH-1 mutation status was available on a total of 60 samples, of which 6 (10%) had a missense mutation leading to a change of Arginin to Histidine (p.Arg132His).

Table 1
Patient and tumor baseline characteristics.

Characteristics	No (%)	Characteristics	No (%)
Age (years)	51 (41–64)	Multifocality (n = 1 missing)	
Median (IQ range)		Yes	29 (26.4%)
		No	80 (72.7%)
Gender		Midline shift (n = 4 missing)	
Males	71 (64.5%)	Yes	68 (61.8%)
Females	39(35.5%)	No	38 (34.5%)
Patient country/region of origin (n = 3 missing)		First presenting symptom Motor disturbance	
Lebanon		Seizure	38 (34.5%)
Beirut	18 (16.4%)	Headache	17 (15.5%)
Mount Lebanon	15 (13.6%)	Speech difficulties	15 (13.6%)
North	10 (9.1%)	Sensory disturbances	11 (10.1%)
Beqaa	7 (6.4%)	Cranial nerve deficits	8 (7.3%)
Nabatieh	4 (3.6%)	Cerebellar symptoms	7 (6.4%)
South	17 (15.5%)	Other	6 (5.5%)
Iraq	18 (16.4%)		8 (7.3%)
Syria	15 (13.6%)		
Other	3 (2.7%)		
WHO performance status (n = 2 missing)		Altered mental status (n = 1 missing)	
0	69 (62.7%)	Yes	13 (87.3%)
1	18 (16.4%)	No	96 (11.8%)
2	5 (4.5%)		
3	6 (5.5%)		
4	10 (9.1%)		
Size (cm)	4.6 (3.8-5.8)	Neurological status prior to RT (n = 5 missing)	
Median (IQ range)		Normal physical exam	51 (46.4%)
		Motor disturbances	29 (26.4%)
		Sensory disturbances	2 (1.8%)
		Cranial nerve disturbances	16 (14.5%)
			5 (4.5%)
		Cerebellar signs	2 (1.8%)
		Speech problems	

3.2. Treatment and interventions

Details about patient management are summarized in Table 2. Gross total resection was achieved in 38 patients (24.9%), subtotal resection in 61 patients (56.0%) and a biopsy only in 10 patients (9.2%). All patients received adjuvant radiation therapy to the tumor and surgical bed, as well as any gross residual lesion, to a median total dose of 60 Gy in 2 Gy fractions. One hundred and five patients (95.5%) completed the full course of radiation therapy. A total of 96 (91.4%) patients received concomitant and 74 patients (88.1%) received adjuvant systemic therapy with TMZ.

3.3. Survival outcomes

At a median follow-up time of 13.6 months, the median overall survival was 17.2 months, and the OS at 1, 2 and 3 years was 71.6%, 34.8% and 8.7%, respectively. The median PFS was 8.8 months, and the PFS at 1, 2 and 3 years was 31.6%, 18.8% and 4.1%, respectively. A total of 92 patients (83.6%) progressed, 7 patients (6.4%) did not develop any recurrence or progression of disease, and 11 patients (10.0%) were lost to follow up. Among those who developed disease progression, 58 (63.0%) had an in-field local recurrence, 14 (15.2%) had an out-of-field recurrence, and the type of recurrence could not be determined from available records in 20 patients (21.7%). A total of 78 patients eventually (70.9%) died from their disease, while 11 patients (10.0%) were still alive and 21 patients (19.1%) were lost to follow up.

3.4. Predictors of outcome

Patient and tumor characteristics that have historically been shown to predict survival were analyzed to look for correlations with outcomes in our patient population. On univariate analysis, factors that were shown to significantly correlate with survival were age at diagnosis when tested as a continuous variable (HR 1.021; 95% CI 1.006–1.036; P = 0.005) and multifocality (HR 2.086; 95% CI 1.250–3.480; P = 0.005), (Fig. 1). Age tested as a dichotomous variable, with a cut-off of 50 years [9], showed borderline significance (Fig. 1). The extent of surgical resection was borderline significant (HR 1.618; 95% CI 0.996–2.628; P = 0.052). Factors such as gender, performance status, size of the lesion, midline shift and mental status did not significantly correlate with outcomes. The use of concurrent and adjuvant TMZ did not significantly impact outcomes, however only a small proportion of patients did not receive TMZ as part of their initial management strategy. MGMT promoter methylation status did not significantly correlate with survival (HR 1.160; 95% CI 0.629–2.138; P = 0.635). Of all patients with a methylated MGMT promoter methylation, only 1 patient did not receive concomitant TMZ; therefore, the correlation between MGMT promoter methylation status and the use of TMZ could not be assessed. IDH-1 mutation was associated with numerically higher survival outcomes, although non-statistically significant (HR 0.502; 95% CI0.179-1.410; P = 0.151), (Fig. 1). On multivariate analysis, age at diagnosis (HR 1.019; 95% CI 1.000–1.038; P = 0.044) and multifocality (HR 2.373; 95% CI 1.401–4.018; P = 0.001) maintained significance and were the only independent prognostic variables (Table 3). Patients 50 years or younger and with unifocal GBM had a 2-year OS of 52.5%, compared to 31.8% in patients above the age of 50 or with multifocal disease (P = 0.018), (Fig. 2).

4. Discussion

To the best of our knowledge, this represents the first study that reports patients with GBM from the Middle East region, examining survival and predictors of outcome. Our population represents a group

Table 2
Treatment strategy.

Intervention	No (%)	Intervention	No (%)
Extent of surgical resection (n = 1 missing)		Use of Anti-epileptic drugs during Radiation Therapy (n = 2 missing)	
Gross total resection	38 (34.9%)		
Subtotal resection	61 (56.0%)	Yes	99 (91.7%)
Biopsy	10 (9.2%)	No	9 (8.3%)
Completion of Radiation Therapy		Use of concomitant Temozolomide (n = 5 missing)	
Yes	105 (95.5%)	Yes	96 (91.4%)
No	5 (4.5%)	No	9 (8.6%)
Median dose of Radiation therapy (Gy)	60.0	Use of adjuvant Temozolomide (n = 26 missing)	
		Yes	74 (88.1%)
		No	10 (11.9%

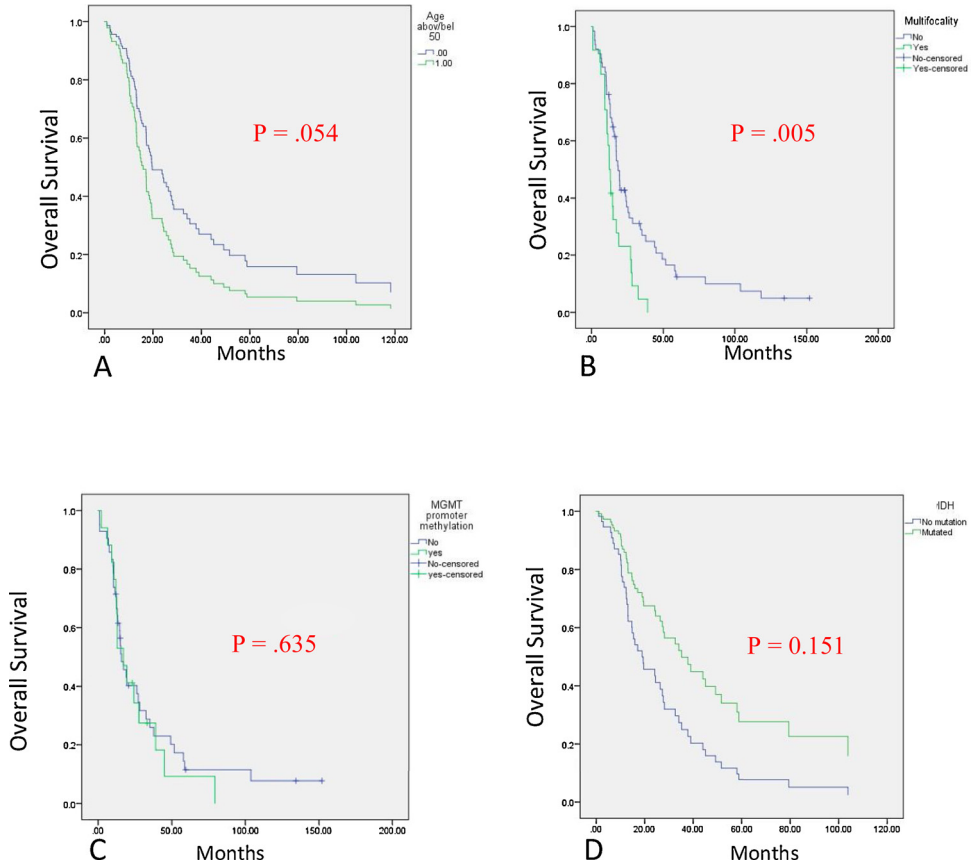

Fig. 1. Overall survival according to age (A), multifocality (B), MGMT promoter methylation status (C) and IDH mutation status (D).

of patients who were offered the standard treatment of care for GBM, and indicates a median overall survival of 17.2 months, which compares favorably to the published literature [2]. Although only 35% of patients achieved a gross total resection, this did not translate into a lower median survival than expected. While 10% of the patients were long term survivors and were alive at the time of assessment, the majority eventually succumbed to their disease, with a 3-year progression-free survival of 4.1%. As predicted, the most common type of recurrence was a radiotherapy in-field local recurrence, outlining the aggressiveness of the disease and the need for optimizing local control.

Several factors have been shown to correlate with survival in patients with GBM, and an exploratory subgroup analysis of the European Organization for Research and Treatment of Cancer (EORTC) and the National Cancer Information Center (NCIC) showed that MGMT promoter methylation status, age at diagnosis, baseline performance status, maximum safe resection, the mini-mental status examination, and successful completion of chemotherapy and radiation therapy were all significant prognostic factors, and are being used in nomograms to predict individual patient outcomes [10]. In our patient population from the Middle East, age and multifocality were both significant prognostic factors, while the extent of tumor resection showed borderline significance. Age has consistently been shown to be a strong predictor of outcome, and a recursive partitioning analysis of prognostic factors in three Radiation Therapy Oncology Group malignant glioma trials suggested that an age cutoff of 50 years was the most significant determinant of survival [9]. Interestingly, while old age is

Table 3
Predictors of outcomes on univariate and multivariate analysis.

Factors	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Gender	0.940 (0.594–1.488)	0.791	—	—
Age at diagnosis	1.021 (1.006–1.036)	0.005	1.019 (1.000-1.038)	0.044
Age (Above vs Below 50)	1.586 (0.992–2.535)	0.054	1.273 (0.670-2.421)	0.461
Midline Shift	0.986 (0.609–1.594)	0.953	—	—
Multifocality	2.086 (1.250–3.480)	0.005	2.373 (1.401-4.018)	0.001
Size of the lesion	1.001 (0.856–1.170)	0.992	—	—
Extent of Resection	1.618 (0.996–2.628)	0.052	1.609 (0.946-2.734)	0.079
WHO Performance Status	0.870 (0.478–1.583)	0.648	—	—
Altered Mental Status	1.010 (0.482–2.118)	0.979	—	—
Use of Concurrent TMZ	0.891 (0.405–1.960)	0.774	—	—
Use of Adjuvant TMZ	0.619 (0.293–1.310)	0.210	—	—
Use of AED	1.300 (0.615–2.748)	0.492	—	—
MGMT promoter methylation	1.160 (0.629–2.138)	0.635	—	—
IDH1 mutation	0.502 (0.179–1.140)	0.191	—	—

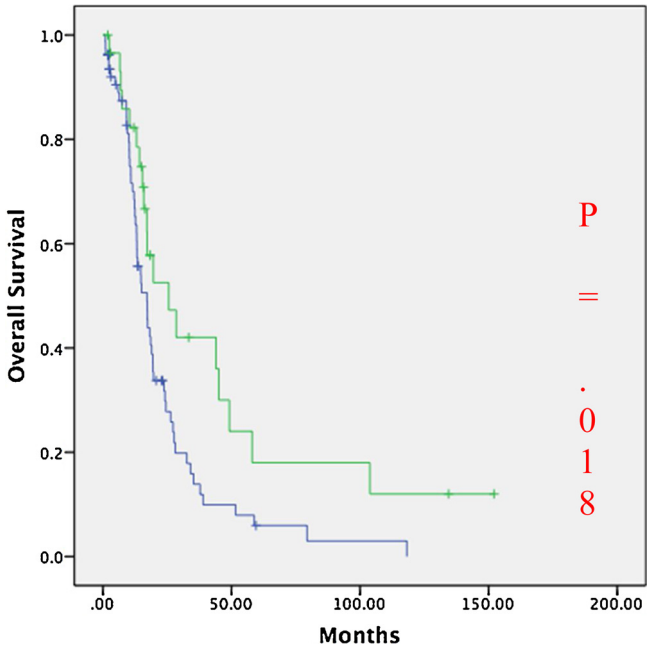

Fig. 2. Overall survival according to age and multifocality.

associated with poor outcomes, it does not imply withholding treatment in the elderly. In fact, Iwamoto et al reported that radiotherapy, combined with TMZ, significantly reduced mortality by 55% in GBM elderly patients [11]. Similarly, multifocal GBM lesions have consistently been shown to be associated with worse outcomes [12,13]. Unsurprisingly, the extent of tumor resection showed a borderline significant association with outcomes in our study. We would have expected a more significant association had the study population been larger. A study by Awad et al. showed that the interaction between the extent of tumor resection and preoperative tumor volume, which represents the reduced disease burden, was the most significant predictor of survival [14].

The role of MGMT promoter methylation in GBM has gained significant importance in recent years. In a recently published study by Gessler et al, almost half (45.7%) of 175 GBM patients displayed MGMT methylation [15], while others have reported significantly lower MGMT promoter methylation rates [16]. Studies have shown that the prevalence of this promoter methylation increases with age and older patients have about 50% chance of MGMT methylation [2,15,16]. Our results showed that among the cohort of patients with available tissue for analysis, 28.2% tested positive for the MGMT promoter methylation. In our patient cohort, the median age was only 51 years which is much lower that typical series from western countries where it is usually about 65 years [16]. Since MGMT promoter methylation is detected in almost half of elderly patients with GBM, our lower-than-expected population median age becomes important in explaining our lower prevalence of MGMT promoter methylation [17–19]. Furthermore, there was no significant correlation between the MGMT promoter methylation and survival. It is difficult to tell if this lack of association reflects a true variability in gene expression and tumor behavior in the Middle East compared to the rest of the world, or can be justified by the relatively small sample size, and lower prevalence of the MGMT promoter methylation in our study cohort, compared to the literature. In a subset analysis of the updated Stupp et al. trial, MGMT promoter methylation status was found to be the strongest prognostic factor for survival (HR 0.49, $p = 0.001$)[3]. The favorable prognostic value of the MGMT promoter methylation was shown to persist irrespective of the use of TMZ, but patients with a methylated MGMT promoter seemed to derive the most benefit from the addition of TMZ [8]. Several other studies have also shown that MGMT promoter methylation significantly correlates with survival outcomes [20–25]. Furthermore, not only does MGMT methylation play an important role in treatment response, but it was also found to predict the incidence and outcome of pseudoprogression after treatment of glioblastoma patients [16]. On the other hand, it is also important to note that similar to our findings, many have reported no significant correlation between MGMT promoter methylation and survival outcomes [26–29]. Nonetheless, a meta-analysis showed a significant improvement in OS and PFS in patients with a methylated MGMT promoter [30].

Mutations in IDH-1, on the other hand, have recently been reported in patients with gliomas, and a study by Argawal et al. reported a prevalence of 12% in patients with GBM, ranging from 5% in patients with primary GBM, up to 75% in patients with secondary GBM [31]. Another prospective study by Beiko et al reported IDH-1 mutation in 13% of the 207 GBM patients recruited [32]. Among the 60 patients in our study whose mutations were available for IDH-1 analysis, 10% had mutant IDH-1. This mirrors the reported prevalence of 12% in literature [31,32]. This mutation has been shown to correlate with better prognosis, leading to a significantly higher median survival in patients with IDH1 mutation in all gliomas [4]. In the study by Beiko et al, IDH-1 mutation was significantly associated with complete tumor resection and a median survival of 19.6 months, versus 10.7 months for incomplete resection. This indicated that IDH-1 mutant tumors were more amenable to resection and had a survival benefit associated with maximal resection [32]. In our population, patients who tested positive for the IDH-1 mutation had a higher one and two-year median survival compared to those without the mutation, but it did not reach statistical significance. This could be the result of the small sample size with IDH-1 information. Most studies, including ours, have studied the association between progression-free survival and MGMT promoter methylation and/or IDH-1 mutation [20–25,33]. This approach does not take into consideration the complex pathways that can lead to resistance to chemotherapy and/or radiation therapy [33,34]. To avoid focusing on only two genes, Fatai et al analyzed 558 GBM gene expression profiles generated by the Cancer Genome Atlas and showed that a 35-gene signature can predict overall and progression-free survival[35]. While our study focuses only on MGMT and IDH-1 genes, this 35-gene signature can serve an attractive candidate for future studies to assess its utility as a prognostic GBM biomarker and as a potential therapeutic target.

This is a retrospective analysis and as such, is subject to the inherent biases of such study designs. Errors due to confounding and bias are more common in retrospective studies, especially with a small sample size. Another important limitation is the fact that not all patients had analyzable tissues for the MGMT and IDH-1 mutations, which leads to loss of power and may limit our interpretation of the association between the mutation and survival. The relatively small sample size also likely affected the correlation between several other known prognostic factors and survival outcomes.

5. Conclusions

Despite those limitations, this remains the only study examining patients with GBM from the Middle East region, and shows that when given the optimal treatment, consisting of maximal safe resection, followed by radiation therapy with concomitant and adjuvant TMZ, GBM patients have adequate survival outcomes that are comparable to the reported literature. Age, performance status, multifocality, extent of tumor resection and mental status should all be assessed in patients presenting with GBM. Of importance, age and multifocality showed the most significant correlation with outcomes in our patient cohort. Testing for biologic markers such as the MGMT promoter methylation and the IDH1 mutation can be helpful to better understand the tumor biology and behavior and to help predict outcome, although no significant correlation with outcomes was observed in our patient population. Future prospective studies with larger sample sizes can help assess the prognostic value of these two biologic markers in GBM

patients from the Middle East and determine if the lack of significant correlation with survival we obtained reflects a true variability in tumor behavior in patients from the Middle East.

Ethics approval and consent to participate

Ethics approval for the study was obtained from the American University of Beirut Medical Center's institutional review board. Patients were enrolled in the study after they provided oral consent.

Consent for publication

This manuscript contains no individual person's data in any form, and therefore consent for publication was not required.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors have no conflicts of interest to declare.

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The funding source had no other contribution to the concept, design, data analysis, or manuscript preparation.

Authors' contributions

ZA, HIA and FG have made substantial contributions to conception and design of the study.

ZA, HIA, FG, MN, YC, NK, PK, RM, FIB, TA, FGK, MA and GK: acquisition of data, analysis and interpretation of data. ZA and HIA have been involved in drafting the manuscript. ZA, HIA, FG, PK, FGK, NK, FS and FK: revising manuscript critically for important intellectual content. All authors contributed substantially to its revision. HIA and ZA take responsibility for the paper as a whole. All authors read and approved the final manuscript.

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