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Original Article

The efficacy and safety of extended adjuvant temozolomide following concurrent radio-chemotherapy among Egyptian patients with newly diagnosed glioblastoma multiforme

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Abstract: Although concurrent radio-chemotherapy and adjuvant temozolomide (TMZ) treatment for 6 cycles has been established as a standard of care for newly diagnosed glioblastoma multiforme (GBM) patients, the recommended duration of adjuvant TMZ remains a matter of debate. Hereby, we aimed to report for the first time our experience from Upper Egypt through comparing survival and toxicity profile between two treatment modalities of adjuvant TMZ (> six cycles versus six cycles) and delineating factors of prognostic significance in Egyptian patients with newly diagnosed GBM treated by radiation therapy with concomitant and adjuvant TMZ. Between June 2016 and February 2018, the medical records of 121 patients were eligible to be retrospectively reviewed to extract the study relevant data. All patients received concurrent radio-chemotherapy, followed by TMZ for 6 cycles in 29 patients (Group 1) and for >6 cycles in 26 patients (Group 2). Patients in Group 1 had a median PFS of 15 months (95% CI: 10.215-19.785), while those in Group 2 had a median PFS of 18 months (95% CI: 16.611-19.389). After a median follow up duration of 20 months (range: 12-41), the median OS was 18 months (95% CI: 13.420-22.580) in Group 1 and 22 months (95% CI: 18.777-25.223) in Group 2. There was no statistically significant correlation between the number of chemotherapy cycles and PFS (P=0.513) or OS (P=0.867). The extent of surgical resection was the only independent prognostic factor for both PFS (P=0.015) and OS (P=0.028) by multivariate analysis. Three grade ≥3 hematologic toxicity were encountered in 3 patients. One in the six-cycle group (neutropenia), and two in the extended cycles group (one had neutropenia and the other one developed thrombocytopenia). No statistically significant difference in the toxicity profile between both groups. The results of our study suggest that extended TMZ therapy is safe and tolerable, however it did not significantly improve PFS or OS as compared to the standard six-cycle course. Larger randomized studies are required to shed more light on this issue.

Keywords: Glioblastoma multiforme, concurrent radio-chemotherapy, adjuvant temozolomide, survival, magnetic resonance spectroscopy

Introduction

Glioblastoma multiforme (GBM), is the most common primary central nervous system tumor in adults with an incidence of 3-4 cases per 100,000 persons each year [1]. The incidence increases with age, with the peak incidence being in the fifth or sixth decade [2]. The prognosis of GBM is generally poor despite advancements in radiation therapy over the years such as decrease in radiation volumes, inverse planning and dose modulation with intensity-modulated radiation therapy which allowed for more-precise targeting and sparing of critical and normal structures in the brain [3].

Currently the Stupp protocol [4] is the standard treatment of newly diagnosed GBM which consists of maximal safe resection then concurrent temozolomide (TMZ) and radiotherapy followed

by six cycles of adjuvant TMZ (five days per month). At a median follow-up of 28 months, he reported a median overall survival (OS) of 14.6 months with concurrent TMZ and radiotherapy compared to 12.1 months with radiotherapy alone. Aiming at improving the survival in patients with GBM, extending the duration of maintenance TMZ for patients without tumor progression after six cycles of adjuvant TMZ has been evaluated by several randomized trials, but the results are conflicting [5-12]. Hereby, we aimed to report for the first time our experience from Upper Egypt; providing a preliminary overview on the current state of GBM in Egypt at the national level through comparing survival and toxicity profile between two treatment modalities of adjuvant TMZ (> six cycles versus six cycles) and delineating factors of prognostic significance in Egyptian patients with newly diagnosed GBM treated by radiation therapy with concomitant and adjuvant temozolomide (TMZ). Meanwhile, comparing our results with reference to those reported from other oncology centers.

Methods

This retrospective study was conducted at the Radiation Oncology Department and Medical Oncology Department of South Egypt Cancer Institute, Neurosurgery Department and Clinical Oncology Department of Assiut University Hospital, in the period from June, 2016 to February, 2018. Our institutional database was screened for all patients who were newly diagnosed histologically confirmed GBM, 18 years or older, performance status (PS) of ≥2 according to Eastern Cooperation Oncology Group (ECOG) [13], underwent neurosurgical resection of the tumor [Gross total resection (GTR), subtotal resection (STR) or biopsy], and had magnetic resonance imaging (MRI) and magnetic resonance spectroscopy imaging (MRSI) as a part of treatment follow up evaluation that were available for review by radiologists (**Figure 1**). Patients with history of previous malignancy, previous treatment with radiotherapy or chemotherapy, recurrent disease, metastatic disease, discontinuation of concurrent radio-chemotherapy for any cause, and tumor progression during first six cycles of adjuvant TMZ, were excluded from the study. Methylation status of O6-methylguanin-DNA methyltransferase (MGMT) and isocitrate dehydrogenase (IDH1/2) mutation status were not available in patients' files; they were not analyzed as in upper Egypt they are not covered by the public health system.

The medical records of 121 patients were eligible to be retrospectively reviewed to extract the study relevant data. Collected data for the study included the following: patients' age; gender; primary tumor site; extent of surgical resection; treatment modality, the treatment period; radiologically assessed response to combined therapy; systemic and local recurrences.

This study was approved by the Committee of Medical Ethics of the Faculty of Medicine with IRB no: 17300481. However, the consent was waived.

Initial diagnosis

All patients were subjected to initial diagnostic workup included magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) and histopathologic evaluation.

Treatment strategies

Surgical reports were revised by neurosurgeon for evaluation of the extent of surgical resection [Gross total resection (GTR), subtotal resection (STR) or biopsy]. Microsurgical GTR was done for patients with circumscribed GBM, solitary lesion or tumor located in non-eloquent regions. STR was the treatment of choice for multicentric GBM or glioma located in eloquent areas. Stereotactic biopsy was done for lesions located in the thalamic or basal ganglion.

Concurrent radio-chemotherapy

All eligible patients started concurrent radio-chemotherapy within four to six weeks of histologic diagnosis of glioblastoma. Patients who presented with seizures received antiepileptic treatment. Patients presented with neurological deficits, received corticosteroid therapy. Prophylactic antibiotic (400 mg sulfamethoxazole, 80 mg trimethoprim, three times per week) was prescribed to all patients during treatment course.

Radiotherapy

Target volume delineation: Gross tumor volume 1 (GTV1) included T2/fluid attenuated inversion

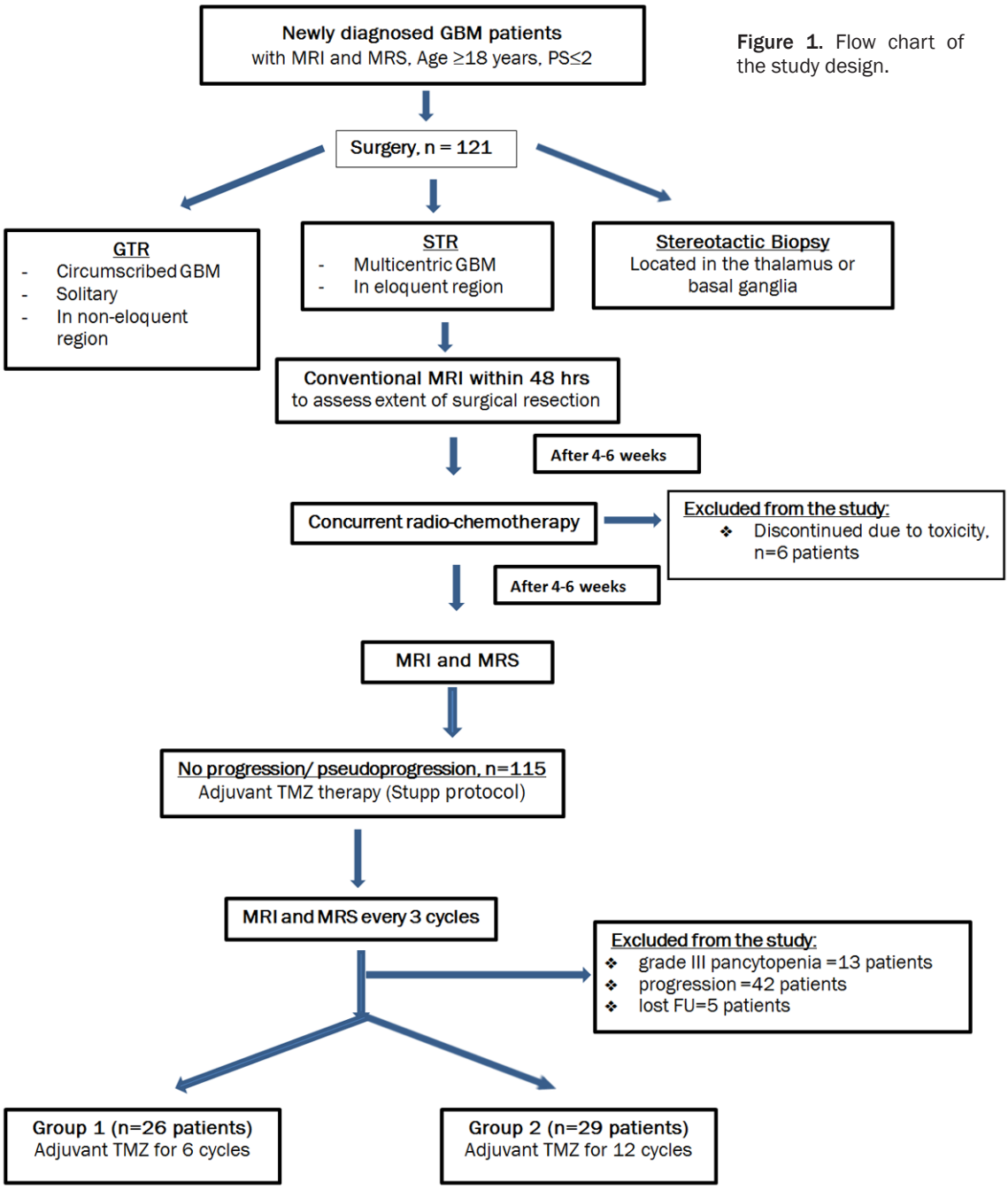

Figure 1. Flow chart of the study design.

recovery (FLAIR) abnormality and surgical cavity if present.

GTV2 included T1 contrast enhanced abnormality and surgical cavity if present.

The clinical target volume 1 (CTV1) and CTV2 were generated by adding 2 cm margin to GTV1 and GTV2 respectively. Margin was reduced around natural barriers.

The planning target volume 1 (PTV1) and (PTV2) were generated by adding 5 mm margin around the CTV1 and CTV2 respectively.

Target dose and energy: Fractionated conformal therapy was delivered to PTV1: for a total dose of 46 Gy in 23 fractions/2 Gy per fraction/once daily/five days per week. PTV2: for boost dose of 14 Gy in 7 fractions/2 Gy per fraction/once daily/five days per week.

All patients were treated using megavoltage linear accelerator and photon energies of 6 MV or more.

Chemotherapy

TMZ (75 mg/m^2/day) was given concurrently with radiation therapy, started from the first day of radiotherapy until the end of radiation.

Adjuvant chemotherapy: Four weeks after the end of radiotherapy, adjuvant TMZ therapy was initiated four weeks after the end of radiotherapy. Initially, patients received six cycles of adjuvant TMZ therapy according to Stupp protocol [4]. However, survival benefit of long term TMZ administration [5, 7-9], relative tolerability of TMZ and absence of effective second line therapies, were the important elements supporting physicians' decision to extend TMZ therapy in patients with good PS (according to ECOG PS) [13] both after surgery and during follow up, manageable toxicity, stable or responsive disease according to Response Assessment in Neuro-Oncology (RANO) criteria [14]. The dose of adjuvant TMZ was 150 mg/m^2/day for five days in the first cycle and increased to 200 mg/m^2/day for five days in the subsequent cycles if no hematologic toxicity had occurred.

Follow up

Clinical and laboratory evaluations: During radiotherapy, patients were followed up weekly in the clinic, and one month after completion of radiotherapy. During adjuvant TMZ therapy, patients were evaluated before each cycle and every three months thereafter.

Patients' follow-up evaluations during treatment included, history, neurological examinations, laboratory investigations (full blood counts and blood chemistry). Assessment of treatment related toxicity was done using common Terminology Criteria for Adverse Events (CTCAE) version 3 [15]. Toxicities were monitored weekly during the concomitant course and once every cycle during the adjuvant course and every three months thereafter. During concurrent radio-chemotherapy, treatment was interrupted if neutrophil count was ≥0.5 - <1.5×10^9/L, platelet count was ≥10 - <100×10^9/L, or grade 2 non-hematologic toxicity (except for alopecia, nausea, vomiting) was observed. Treatment was stopped if neutrophil count was 0.5×10^9/L, platelet count was <10×10^9/L, or ≥ grade 3 non-hematologic toxicity (except for alopecia, nausea, vomiting) was observed. During adjuvant treatment, TMZ dose was reduced from 200 to 150 mg/m^2 or from 150 to 100 mg/m^2 if neutrophil count was <1×10^9/L, platelet count was <50×10^9/L, or grade 3 non-hematologic toxicity (except for alopecia, nausea, vomiting) was observed. Treatment was discontinued if adverse events necessitate reduction of TMZ dose bellow 100 mg/m^2, patient refusal, and disease progression.

MRI and magnetic resonance spectroscopy (MRS): The extent of surgical resection was assessed by conventional MRI within 48 hours after surgical resection. Conventional MRI included axial and sagittal pre-contrast and post-contrast T1-weighted spin echo, axial FLAIR and axial and coronal T2-weighted fast spin-echo images. Diffusion weighted imaging (DWI) helped to determine whether new enhancement developing in the subsequent weeks was secondary to ischemia or caused by tumor recurrence. The mean apparent diffusion coefficient (ADC) values were evaluated in areas with contrast enhancement on T1WI or in suspected non-enhancing areas to detect tumor recurrence.

Patients were evaluated for response using MRI and MRS, which were performed immediately before the first cycle, after every three cycles of adjuvant TMZ and every three months after termination of treatment using 1.5T scanner (Achieva, Philips Healthcare; Amsterdam, Netherlands) equipped with the standard head coil. Two neuroradiologists with 12 and 11 years of experience, reviewed the pre-treatment and follow-up MRI scans (blinded to clinical and MRSI data) for all patients and diagnosis was made in consensus. Radiologic response assessment was defined according to response assessment in neuro-oncology RANO criteria [14]. Progression is verified by the presence of steady growth of the enhancing lesion. If progression has occurred, the date of progression was recorded as the date of original suspicion.

MRS was achieved using Point Resolved Spatial Selection (PRESS) at long echo time (TE) 135 msec. The spectroscopic grid was extended and manually adjusted to include lesion,

perilesional edema if present, and normal brain tissue. The mean Choline (Cho)/N-acetyl aspartate (NAA) and Cho/Creatine (Cr) ratio were calculated in regions of suspected tumor recurrence where the ADC values were also measured. Cho/NAA ratio >2 was considered tumor recurrence while lactate peak with a reduction of all other metabolites was considered treatment-induced necrosis.

Statistical analysis

Patient characteristics between groups were compared using chi square test for categorical variables and Mann-Whitney U test for continuous variables. OS was calculated from the date of surgical resection to the date of death from any cause or last follow up. Progression free survival (PFS) was calculated from the date of surgical resection to the date of progression or date of last follow up or death. Kaplan-Meier methods [16], were used to determine OS and PFS and comparison of survival between treatment groups was determined by log rank test. Univariate analysis was performed to identify the potential prognostic factors for OS and PFS. For multivariable analysis, the Cox proportional hazards model was used and adjusted for age, gender, performance status, tumor location, resection extent, corticosteroid use at initiation of concomitant course, antiepileptic medication uses at initiation of concomitant course and number of adjuvant TMZ cycles.

Receiver Operating Characteristic (ROC) curve analysis was used to correlate the results of the spectroscopic metabolite ratios with the results of follow-up imaging. The area under the curve (AUC) was used to calculate the optimal cutoff values of the metabolite ratios for differentiating tumor recurrence from treatment induced changes. All tests were 2-tailed and differences at *P*-values ≤0.05 were considered statistically significant. Statistical data were performed by Statistical Package for Social Sciences software (version 21, SPSS, Chicago, IL).

Results

Between June 2016 and February 2018, 121 patients were eligible for inclusion in the present study (**Figure 1**). Of 121 patients, 66 patients (54.5%) were excluded from the study due to the following reasons:

-6 patients (5.0%) discontinued concurrent radio-chemotherapy as they developed grade 4 neutropenia.

-13 patients (10.7%) discontinued adjuvant TMZ because of grade 3 pancytopenia during the six-cycle course.

-42 patients (34.7%) had tumor progression during the six-cycle course of maintenance TMZ therapy.

-5 patients (4.1%) lost to follow-up after cycle six of maintenance TMZ.

Overall, a total of 55 patients were eligible for retrospective analysis of treatment outcome and toxicity; maintenance TMZ treatment was administered for six cycles (group 1) in 29 patients (52.7%) while it was continued (group 2) for median cycles of 11.5 (range: 9-18) in 26 patients (47.3%).

Patient characteristics

Patient characteristics were matched between the two treatment groups and are summarized in **Table 1**. There was no statistically significant difference in the median duration of symptoms between the two treatment groups (*P*=0.076). Both treatment groups had a median interval time of one and a half months between diagnosis and surgery.

All 55 patients completed concurrent radio-chemotherapy as planned. The median interval between surgery to the start of concomitant course was 41 days (range: 35-68) in Group 1 and 37 days (28-65) in Group 2. The median total dose of radiotherapy for the entire cohort was 60 Gy (range: 54-66 Gy). Thirty-seven patients (67.3%) received corticosteroid at initiation of concomitant course (22 patients {75.9%} in Group 1, 15 patients {57.7%} in Group 2) at a dose of 4 mg, twice daily to alleviate symptoms of increased intracranial tension. Corticosteroid dose was gradually decreased to be stopped when symptoms subsided. Nineteen patients (34.5%) had significant edema during the entire course of treatment therefore, they remained steroid dependent. Of the 17 patients (30.9%) who received antiepileptic medications, eight patients (47.1%) continued their antiepileptic medications throughout the adjuvant treatment course. After four weeks, all patients

Table 1. Baseline characteristics of 55 patients with GBM

Variables	Group 1 6 cycles 29 (100%)	Group 2 >6 cycles 26 (100%)	Total 55 (100%)	*P*-value*
Age				0.648
Median	58	60.5	59	
Range	25-68	30-66	25-68	
Gender				0.813
Male	22 (75.9)	19 (73.1)	41 (74.5)	
Female	7 (24.1)	7 (26.9)	14 (25.5)	
ECOG PS				0.855
0	6 (20.7)	7 (26.9)	13 (23.6)	
1	15 (51.7)	12 (46.2)	27 (49.1)	
2	8 (27.6)	7 (26.9%)	15 (27.3)	
Symptoms at presentation				
Headache	13 (44.8)	7 (26.9)	20 (36.4)	0.168
Cognitive deficits	12 (41.4)	7 (26.9)	19 (34.5)	0.260
Seizure	4 (13.8)	9 (34.6)	17 (30.9)	0.070
Motor deficits	11 (37.9)	6 (23.1)	13 (23.6)	0.234
Duration of symptoms				0.076
Median	2.5	4.3	3.5	
Range	2-19.5	3-18	2-19.5	
Tumor location				0.243
Frontal	14 (48.3)	8 (30.8)	22 (40.0)	
Temporal	6 (20.7)	10 (38.5)	16 (29.1)	
Parietal	8 (27.6)	5 (19.2)	13 (23.6)	
Occipital	1 (3.4)	3 (11.5)	4 (7.3)	
Extent of surgical resection				0.812
GTR	12 (41.4)	9 (34.6)	21 (38.2)	
STR	12 (41.4)	13 (50.0)	25 (45.5)	
Biopsy	5 (17.2)	4 (15.4)	9 (16.4)	
Corticosteroid use at initiation of concomitant course				0.126
Yes	22 (75.9)	15 (57.7)	37 (67.3)	
No	7 (24.1)	11 (42.3)	18 (32.7)	
Antiepileptic medications use at initiation of concomitant course				0.234
Yes	11 (37.9)	6 (23.1)	17 (30.9)	
No	18 (62.1)	20 (76.9)	38 (69.1)	

GBM, glioblastoma multiforme; TMZ, temozolomide; ECOG PS, Eastern Cooperation Oncology Group performance status; GTR, Gross total resection; STR, Subtotal resection. *Chi-square test was used for all comparisons except age and duration of symptoms (Mann-Whitney U test).

received at least six cycles of adjuvant TMZ therapy with a median interval of 37 days (range 31-47).

Treatment outcome

Survival: The median follow-up period of the 55 eligible patients was 20 months (range: 12-41). Patients received six-cycle course of adjuvant TMZ treatment had median follow up duration of 16 months (range: 12-41), while patients received > six-cycle course of adjuvant TMZ treatment had median follow up duration of 21 months (12-36). There was no statistically significant difference in the median follow up duration between both treatment groups (*P*= 0.087).

Patients with GTR resection who developed tumor recurrence had a significantly higher Cho/

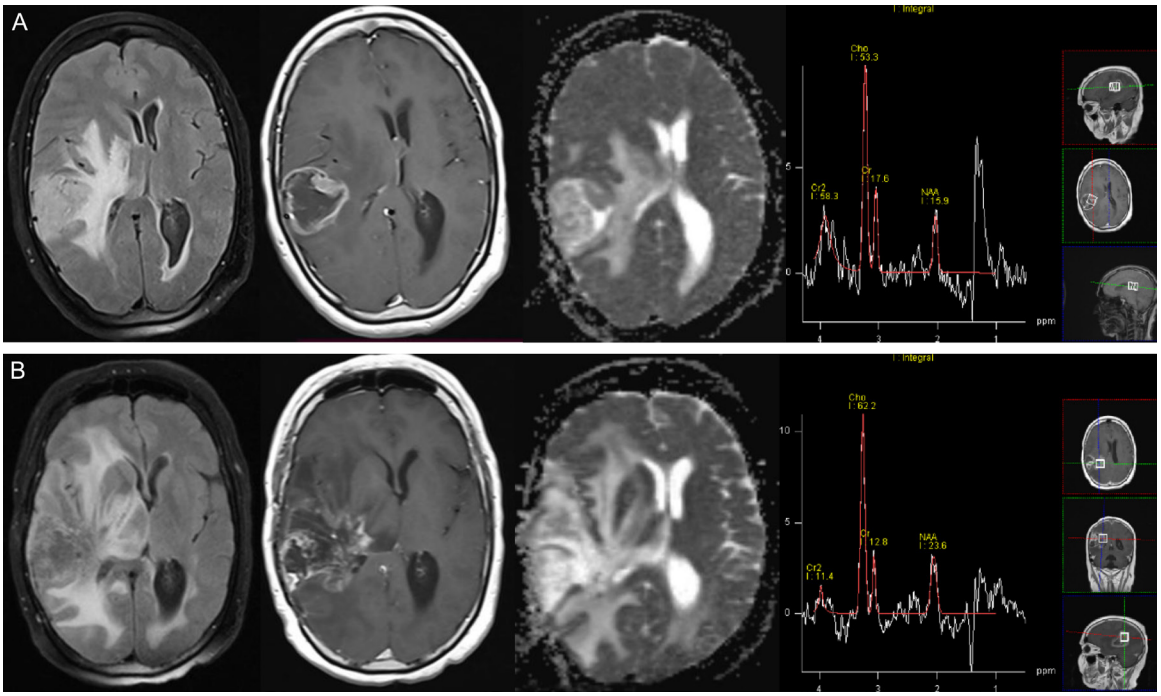

Figure 2. Pre-concurrent radio-chemotherapy and post treatment MRI (FLAIR, T1W+Gd, ADC) and MRSI. Pre-concurrent radio-chemotherapy MRI (FLAIR, T1W+Gd, ADC) and MRSI (A) showing heterogenous enhancement in the wall of the surgical cavity with low ADC nodule and surrounding edema. The VOI was placed at the site of contrast enhancement and low ADC revealing an elevated choline peak and low NAA with CHO/NAA ratio =3.1 and Cho/Cr ratio =2.7 denoting residual disease. The scan done 9 months after six cycles of adjuvant temozolomide (B) shows a heterogenous increase in contrast enhancement surrounding the wall of the surgical cavity with increase in the surrounding FLAIR hyperintensity and hydrocephalus. Metabolite measurement in the surrounding heterogenous enhancement shows Cho/NAA ratio =2.9 and Cho/Cr ratio =3.2 denoting progressive disease.

NAA and Cho/Cr ratios than patients with treatment induced changes (*P*=0.01 and *P*=0.02 respectively). In patients with STR or biopsy who developed tumor progression, the Cho/NAA and Cho/Cr ratios measured at non-enhancing tumor edges were higher on follow up imaging. The cut-off value for Cho/NAA ratio was >2.2 with 87.2% sensitivity and 76% specificity with area AUC=0.92 and *P*=0.01. The cut-off value for Cho/Cr ratio was >1.88 with 82% sensitivity and 71% specificity with AUC=0.86 and *P*=0.02. Regarding DWI, the ADC values were higher in regions of radiation induced changes than in recurrent tumor however, the difference was not significant (**Figure 2**).

A total of 39 patients (70.9%) developed disease progression (20/39 patients were in group 1 and 19/39 patients in group 2). The median time to progression for the entire cohort was 18 months (range: 12-41 months). The median time to progression in Group 1 was 14 months (range 12-41), and in Group 2 was 18 months (range 12-36) with no statistically significant difference between both treatment groups (*P*=0.134). According to Kaplan-Meier analysis [16], the median PFS was 15 months for Group 1 (95% CI: 10.215-19.785) and 18 months for Group 2 (95% CI: 16.611-19.389) with no statistically significant difference between both groups (*P*=0.513) (**Table 2**). PFS rates at 12, 18, 24 and 36 months were 82.8%, 40.0%, 22.5%, 22.5% for Group 1 and 88.5%, 48.4%, 22.6%, 22.6% for Group 2 respectively (**Figure 3A**). Treatment of tumor progression included: best supportive care for most of the patients (n=25, 64.1%), retreatment with TMZ therapy (n=9, 23.1%), and 2nd line chemotherapy (n=5, 12.8%).

During median follow up duration of 20 months, 34/55 patients (61.8%) died [15/34 patients (44.1%) were in Group 1 and 19/34 patients (55.9%) were in Group 2]. The cause of death was tumor progression during treatment and follow-up period. The median OS estimate by Kaplan-Meier [16], for the entire cohort was 21 months (95% CI: 18.803-23.197). The median OS was 18 months (95% CI: 13.420-22.580) and 22 months (95% CI:

Table 2. Survival analysis according to the number of adjuvant TMZ cycles

Number of CTH cycles	PFS				OS			
	Median			Log Rank (Mantel-Cox) *P*-value	Median			Log Rank (Mantel-Cox) *P*-value
	Estimate	Std. Error	95% CI		Estimate	Std. Error	95% CI	
Six cycles	15.000	2.441	10.215-19.785		18.000	2.337	13.420-22.580	
Twelve cycles	18.000	0.709	16.611-19.389	0.513	22.000	1.644	18.777-25.223	0.867
Overall	18.000	0.577	16.869-19.131		21.000	1.121	18.803-23.197	

TMZ, temozolomide; CTH, chemotherapy; CI, Confidence interval.

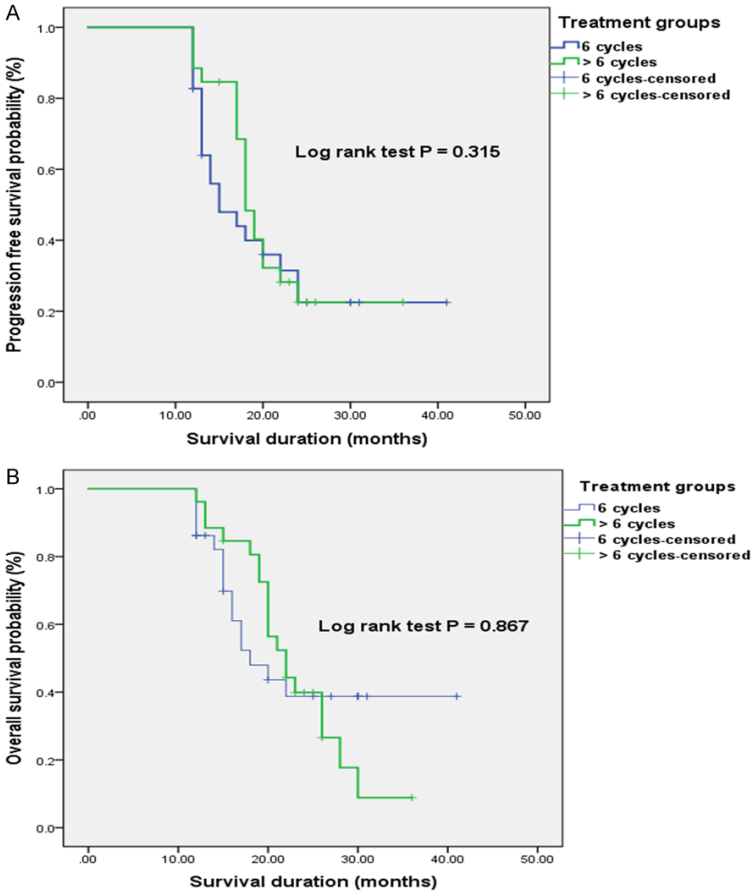

Figure 3. Kaplan-Meier curves showing PFS (A) and OS (B) of GBM patients. PFS and OS curves of the 6C vs. >6C groups demonstrated no difference; *P*=0.513 and *P*=0.867 respectively.

18.777-25.223) for patients enrolled in Group 1 and Group 2 respectively. There was no statistically significant difference in OS between both groups, (*P*=0.867) (**Table 2**). The OS rates at 12, 18, 24 and 36 months in Group 1 were 86.2%, 48.0%, 38.8%, and 38.8% respectively, and it was 96.2%, 80.6%, 39.9% and 8.9% respectively for Group 2 (**Figure 3B**).

There is a statistically significant influence of the EOR on PFS and OS. Patients underwent GTR showed a significantly longer PFS (*P*=0.008) (**Figure 4A**) and longer OS (*P*=0.014) (**Figure 4B**) than those who underwent STR, or biopsy. Cox regression confirmed the prognostic role of the EOR as a significant predictor for PFS and OS. Patients with STR were about three times more likely to progress (HR 2.953, 95% CI 1.381-6.317, *P*= 0.005) (**Table 3**) and four times more likely to die (HR 4.253, 95% CI 1.787-10.123, *P*=0.001) (**Table 4**) as compared to patients with GTR. Age, gender, PS, tumor site, the use of corticosteroid medications, antiepileptic medications, and the number of chemotherapy cycles were not statistically significant predictors of PFS (**Table 3**) or OS (**Table 4**).

According to the CTCAE version 3 [15], a total of 163 toxicities were encountered in 48 patients (87.3%) out of 55 eligible patients during concomitant and adjuvant TMZ therapy. The overall treatment related adverse events were higher during the concomitant course (n=30, 54.5%) than during the adjuvant course (n=21, 38.2%) of TMZ therapy (*P*=0.000012). Thirty-three patients (60.0%) developed hematologic toxicities and non-hematologic toxicities were observed in 46 patients (83.6%). Forty-five patients (81.8%) had grade 1 or 2 treatment related toxicities and 24 patients (43.6%) developed grade ≥3 adverse events. Neutropenia was the most frequently observed treatment related hemato-

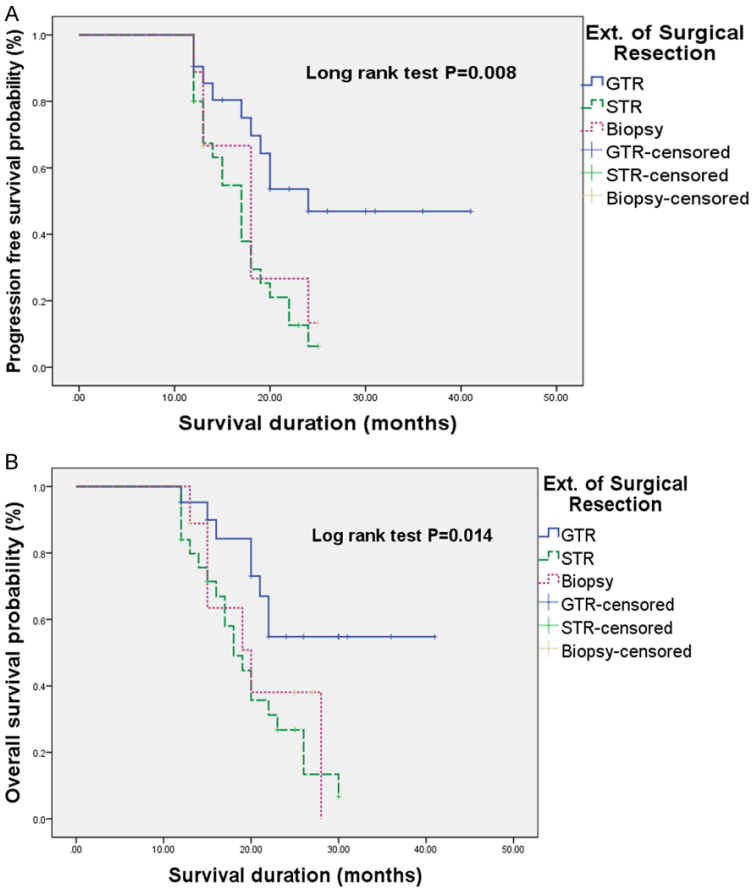

Figure 4. Kaplan-Meier curves of 55 GBM patients showing PFS (A) and OS (B) according to EOR. Patients underwent GTR had longer PFS (*P*=0.008) and OS (*P*=0.014) than those underwent STR or biopsy.

logic adverse events (n=14, 25.5%). The most frequent non-hematologic adverse events were nausea (n=27, 49.1%) followed by vomiting (n=21, 38.2%) and fatigue (n=21, 38.2%) (**Table 5**). In most of the patients, nausea and vomiting occurred as a paired adverse event. There was no statistically significant difference in the toxicity profile between six-cycle group and > six-cycle group (*P*=0.289) (**Table 5**).

There was no statistically significant difference in the rate of grade 3 & 4 treatment related toxicities between both groups (*P*=0.851). During concurrent radio-chemotherapy, 3 grade ≥3 hematologic adverse events were encountered in three patients. One of the three patients was in the six-cycle group (developed neutropenia), while the other two patients were in the extended treatment group (one patient developed neutropenia and the other one developed thrombocytopenia) (**Table 6**). The concomitant course was interrupted in these patients, and it was resumed within 12 days of conservative measures.

During adjuvant TMZ therapy, treatment was delayed in two out of 29 patients (6.9%) in six-cycle group, because of grade 3 or more hematologic adverse events (neutropenia, one; thrombocytopenia, one) (**Table 6**). Maintenance TMZ was restarted after improvement of blood cell count with a reduced dose. Treatment was discontinued in 3/26 patients (11.5%) received extended cycles group (one patient at cycle 10 of chemotherapy, and two patients at cycle 11 of chemotherapy) because of grade 4 neutropenia in one patient, grade 3 leukopenia in one patient and grade 3 thrombocytopenia in one patient (**Table 6**).

Twelve patients (21.8%) developed CTCAE grade 3 nausea (**Table 6**). None of our patients discontinued treatment; only treatment interruption due to grade 3 toxicities and all were resolved by conservative measures. Adjuvant TMZ treatment was resumed in these patients with a reduced dose of 150 mg/m^2 in subsequent cycles. We did not report any morality related to TMZ administration.

Discussion

Despite the great advances in multimodality treatment approach for high grade glioma, the prognosis remains poor. Several modifications are being tried to further improve survival and reduce toxicity based on the proven efficacy of TMZ in the adjuvant treatment of GBM. Extending the duration of adjuvant TMZ therapy is one of the main modifications that has gained special interest in the past few years [5-12]. The main focus being its safety, tolerability and efficacy.

In this study, the percentage of patients (45.5%) who completed maintenance TMZ treatment was comparable to that reported by Skardely et

Table 3. Predictors of progression free survival in patients with GBM by univariate and multivariate analyses using COX regression

Variables	Number	Univariate analysis			Multivariate analysis		
		HR	*P*-value	95% CI	HR	*P*-value	95% CI
Age (years)					Not included in the model		
<50	9	Ref					
≥50	46	1.239	0.630	0.519-2.958			
Gender					Not included in the model		
Male	41	Ref					
Female	14	1.451	0.298	0.720-2.924			
ECOG PS							
0	13	Ref			Not included in the model		
1	27	0.686	0.328	0.323-1.460			
2	15	0.668	0.361	0.282-1.586			
Tumor location					Not included in the model		
Frontal	22	Ref					
Temporal	16	1.131	0.755	0.522-2.450			
Parietal	13	1.475	0.335	0.669-3.255			
Occipital	4	1.295	0.733	0.292-5.743			
EOR							
GTR	21	Ref			Ref		
STR	25	2.953	0.005*	1.381-6.317	2.953	0.005*	1.381-6.317
Biopsy	9	2.231	0.105	0.845-5.894	2.231	0.105	0.845-5.894
Corticosteroid use**					Not included in the model		
No	18	Ref					
Yes	37	0.841	0.605	0.437-1.620			
Antiepileptic medications use**					Not included in the model		
No	38	Ref					
Yes	17	1.532	0.215	0.780-3.009			
Number of CTH cycles					Not included in the model		
Six cycles	29	Ref					
Twelve cycles	26	0.820	0.540	0.435-1.546			

ECOG PS, Eastern Cooperation Oncology Group performance status; EOR; Extent of surgical resection; GTR, Gross total resection; STR, Subtotal resection; CI, Confidence interval; HR, Hazard ratio. *P-values ≤0.05 were considered statistically significant. **At initiation of concomitant course. CTH, chemotherapy.

al. [12] (44.4%) but higher than the percentage reported in the prospective phase III study by Gilbert et al. [17], (35.6% received at least 6 cycles of maintenance treatment, while 19.1% received 12 cycles). Two main reasons were responsible for the early termination of TMZ therapy during the first 6 cycles in this study. Tumor progression was the commoner cause which was encountered in 34.7% of patients, however other prospective studies reported even higher percentages (39-49.3%) [4, 17]. The second reason was toxicity which occurred in 15.7% of our patients and was similarly responsible for early discontinuation of TMZ therapy in 16.4% of patients in the study by Gilbert et al. [17]. In the current study, the median number of cycles in patients received up to 12 cycles of adjuvant TMZ was 11.5 (range: 9-12). This is in accordance to the study conducted by Refae et al. [9] (11 cycles, range: 8-23) and by Delion et al. [6] (11 cycles, range: 7-13).

On the other hand, it is crucial to differentiate tumor progression from pseudoprogression on follow-up imaging to avoid unnecessary termination of extended TMZ therapy. In this study, MRS was used and the Cho/NAA and Cho/Cr ratios were measured at the non-enhancing tumor edges on follow up imaging. The calcu-

Table 4. Predictors of overall survival in patients with GBM by univariate and multivariate analyses using COX regression

Variables	Number	Univariate analysis			Multivariate analysis		
		HR	*P*-value	95% CI	HR	*P*-value	95% CI
Age (years)					Not included in the model		
<50	9	ref					
≥50	46	1.165	0.755	0.448-3.028			
Gender					Not included in the model		
Male	41	ref					
Female	14	1.438	0.336	0.686-3.011			
ECOG PS							
0	13	ref			ref		
1	27	0.505	0.093	0.228-1.120	0.311	0.106	0.134-0.720
2	15	0.572	0.217	0.235-1.389	0.396	0.146	0.160-0.983
Tumor location					Not included in the model		
Frontal	22	ref					
Temporal	16	0.974	0.951	0.426-2.227			
Parietal	13	1.437	0.390	0.628-3.285			
Occipital	4	0.789	0.820	0.102-6.076			
EOR							
GTR	21	ref			ref		
STR	25	3.120	0.007*	1.362-7.147	4.253	0.001*	1.787-10.123
Biopsy	9	2.453	0.100	0.843-7.143	3.441	0.028	1.139-10.395
Corticosteroid use**					Not included in the model		
No	18	ref					
Yes	37	0.672	0.267	0.334-1.355			
Antiepileptic medications use**					Not included in the model		
No	38	ref					
Yes	17	1.436	0.326	0.698-2.956			
Number of CTH cycles					Not included in the model		
Six cycles	29	ref					
Twelve cycles	26	1.059	0.871	0.532-2.106			

ECOG PS, Eastern Cooperation Oncology Group performance status; EOR, Extent of surgical resection; GTR, Gross total resection; STR, Subtotal resection; CI, Confidence interval; HR, Hazard ratio. *P-values ≤0.05 were considered statistically significant. **At initiation of concomitant course. CTH, chemotherapy.

lated cut-off values showed similar results to those reported by Cordova et al. [18] in terms of precise delineation of tumor margins and accurate identification of disease progression from pseudoprogression which also aided in the accurate calculation of PFS.

Regarding median PFS, our results were comparable to those reported by Refae et al. [9], (The median PFS for more than six cycles arm was 18.8 months vs. 12.1 months for six cycles arm). However, our result is far less than the result showed by Darlix et al. [8], it showed PFS of 28.4 months (range 12.8-34.2 months), for patients who received 9 cycles or more. This can be explained by the higher rate of GTR (60.3%) in their study compared to 38.2% in our study [8]. Hau et al. [5], on the other hand reported a lower median PFS, 14 months, in patients with GBM receiving adjuvant TMZ for a median 13 cycles.

Hau et al. [5] reported a median OS of 22.4 months after administration of a median of 13 cycles (range 9-40) which concurred with the results of this study. However, several other studies focusing on the use of extended TMZ therapy have shown a higher median OS ranging between 23.8-30.6 months [9-12]. The lower rate of GTR in our study may explain the

Table 5. Comparison of the toxicities between six-cycle treated patients and > six-cycle treated patients

Adverse events	6 cycles (Group 1) N=29	>6 cycles (group 2) N=26	*P*-value
Hematologic			
Anemia	1 (3.4)	3 (11.5)	0.249
Neutropenia	7 (24.1)	7 (26.9)	0.813
Leukopenia	4 (13.8)	6 (23.1)	0.373
Thrombocytopenia	3 (10.3)	7 (26.9)	0.112
Non-hematologic			
Alopecia	7 (24.1)	3 (11.5)	0.226
Anorexia	6 (20.7)	7 (26.9)	0.587
Nausea	11 (37.9)	16 (61.5)	0.080
Vomiting	9 (31.0)	12 (46.2)	0.249
Constipation	2 (6.9)	1 (3.8)	0.619
Diarrhea	0	2 (7.7)	0.128
Fatigue	11 (37.9)	10 (38.5)	0.968
Insomnia	2 (6.9)	5 (19.2)	0.171
Headache	4 (13.8)	6 (23.1)	0.373
Dizziness	2 (6.9)	4 (15.4)	0.313
Pneumonia	2 (6.9)	3 (11.5)	0.550

lower OS outcome compared to these studies. Meanwhile, we showed a better median OS than Siez et al. [7], who reported a median OS of 15 months and a 2-year OS of 27% in a cohort of patients who received an extended TMZ protocol.

In the present study, there was no statistically significant difference in survival upon extending the duration of maintenance TMZ therapy beyond six cycles. Our data were further confirmed by the recently published manuscript of the GENO-14-01 trial (NCT02209948) [19], in which 159 patients were randomized according to MGMT status and the presence or absence of residual disease to receive extended cycles (80 patients) vs. six cycles (79 patients) of adjuvant TMZ therapy. The author concluded that there was no significant correlation between OS or PFS and extending the duration of adjuvant TMZ beyond 6 cycles. On the other hand, Refae et al. [9], reported a statistically significant correlation between median PFS (12.1 months in patients receiving six cycles versus 18.8 months in patients receiving >6 cycles; *P*=0.015) and median OS (18.1 months for patients receiving six cycles and 24.1 months for patients with more than six cycles; *P*=0.048) and the number of adjuvant TMZ cycles. Darlix et al. [8], also found statistically significant improvement in both OS (*P*=0.01) and PFS (*P*=0.03) in patients received extended adjuvant TMZ treatment compared to those who received standard adjuvant TMZ. A pooled analysis from 4 randomized trials was conducted by Blumental et al. [11], in 2017 for newly diagnosed GBM patients. Patients were randomized to either stop TMZ after 6 cycles (n=333), or to continue TMZ for 12 cycles or until progression (n=291). They concluded that extended TMZ therapy was associated with an improved PFS (*P*=0.03) but without statistically significant improvement in OS (*P*=0.52).

Several studies [20, 21], have shown that the EOR had a significant impact on survival following adjuvant therapy. Similarly, our present study showed that the EOR significantly correlated with survival; patients in whom GTR was achieved had significantly longer PFS (*P*= 0.015) and OS (*P*=0.028) than patients underwent STR or biopsy. Contrary to our findings, Michaelsen et al. [22], did not find significant correlation between the EOR and survival, although he demonstrated a significant impact of patient age, ECOG PS and use of corticosteroid therapy on survival.

In our study, the overall toxicity profile of adjuvant TMZ therapy was tolerable as most of the patients (n=45, 81.8%) developed only grade 1 or 2 adverse events and only 3/55 patients (5.5%) discontinued adjuvant TMZ therapy due to toxic effects. This low figure is in accordance with that reported by Stupp et al. [4] in 2005 (only 8% of the patients discontinued adjuvant TMZ treatment due to toxic effects). We reported higher incidence of grade 3 and 4 hematologic toxicity (n=8, 14.5%) during concomitant and adjuvant course than what was reported in CATNON trial (8-12%) [23]. Although the reason for this is unclear, treatment was discontinued in only 3/26 patients (11.5%) whose adjuvant TMZ extended to beyond six cycles. Bahandari et al. [10], reported that the incidence of grade 3 and 4 hematologic toxicities was 0% in the six-cycle TMZ group and 5% in the 12-cycle TMZ group during concomitant course. He also reported a higher incidence of grade 3 and 4 hematologic toxicity in the 12-cycle TMZ group compared to six-cycle TMZ group; 15% and 5%, respectively during adjuvant course. Similarly,

Table 6. Grade 3 & 4 CTC that occurred during CRCT and adjuvant course of TMZ

Adverse events	6 cycles (group 1) (n=29) No (%)	>6 cycles (group 2) (n=26) No (%)	Total (n=55) No (%)	*P*-value
Hematologic				
Neutropenia				
CRCT	1 (3.4)	1 (3.8)	2 (3.6)	
Adjuvant TMZ	1 (3.4)	1 (3.8)	2 (3.6)	
Total	2 (6.9)	2 (7.7)	4 (7.3)	0.910
Leukopenia				
CRCT	0	0	0	
Adjuvant TMZ	0	1 (3.8)	1 (1.8)	
Total	0	1 (3.8)	1 (1.8)	0.286
Thrombocytopenia				
CRCT	0	1 (3.8)	1 (1.8)	
Adjuvant TMZ	1 (3.4)	1 (3.8)	2 (3.6)	
Total	1 (3.4)	2 (7.7)	3 (5.5)	0.489
Non-hematologic				
Anorexia				
CRCT	0	2 (7.7)	2 (3.6)	
Adjuvant TMZ	1 (3.4)	1 (3.8)	2 (3.6)	
Total	1 (3.4)	3 (11.5)	4 (7.3)	0.249
Nausea				
CRCT	5 (17.2)	0	5 (9.1)	
Adjuvant TMZ	4 (13.8)	3 (11.5)	7 (12.7)	
Total	9 (31.0)	3 (11.5)	12 (21.8)	0.081
Vomiting				
CRCT	0	1(3.8)	1 (1.8)	
Adjuvant TMZ	0	2(7.7)	2 (3.6)	
Total	0	3(11.5)	3 (5.5)	0.060
Fatigue				
CRCT	0	1 (3.8)	1 (1.8)	
Adjuvant TMZ	0	1 (3.8)	1 (1.8)	
Total	0	2 (7.7)	2 (3.6)	0.128
Insomnia				
CRCT	1 (3.4)	0	1 (1.8)	
Adjuvant TMZ	0	1 (3.8)	1 (1.8)	
Total	1 (3.4)	1 (3.8)	2 (3.6)	0.937

CTC, Common Toxicity Criteria; TMZ, Temozolomide; CRCT, Concurrent radio-chemotherapy.

we observed that 3.4% of the patients in the six-cycle TMZ group and 7.7% of the patients in the >6 cycles TMZ group had ≥ grade 3 hematologic toxicity during concomitant course while during adjuvant course of TMZ, we reported an overall incidence of ≥ grade 3 hematologic toxicity of 6.9% in six-cycle TMZ group and 11.5% in >6 cycles TMZ group. The most frequent hematologic adverse event was neutropenia (n=14, 25.5%). However, Stupp et al. [4] reported that thrombocytopenia (12.0%) was the most common hematologic adverse event of temozolomide.

Nausea (n=27, 49.1%), vomiting (n=21, 38.2%) and fatigue (n=21, 38.2%), were the most frequently reported treatment related non-hematologic adverse events. Our results are in accordance with that reported by Bae SH, et al. [24], who analyzed the data of 300 patients with histologically confirmed WHO grade 3 or 4 glioma who received TMZ therapy as a concomitant, adjuvant, or palliative therapy. They reported that, the most common toxicities were nausea (44.3%) and vomiting (37%).

There are several disadvantages which may limit the potential benefit from extending maintenance TMZ treatment that need to be considered. Prolonged administration of TMZ results in mutational changes in the tumor which in turn leads to resistance to ongoing alkylating therapy [25]. Treatment with alkylating agent resulted in mutation in the mismatch repair (MMR) gene mutS homolog 6 (MSH6) which was found in association with a hypermutator phenotype [26]. Consequently, this hypermutator phenotype may lead to progression to a more malignant tumor phenotype at the time of recurrence [27]. Another disadvantage is toxicity that occurs with increasing cumulative doses of TMZ such as increased risk of myelodysplasia

and leukemia as reported by Momota et al. [28]. Additionally, prolonged administration of TMZ, may suppress the immune system [29, 30] and prevent the possibility for subsequent salvage therapy at the time of recurrence resulting in reduced survival.

The limitations of our study include the small sample size of patients, the lack of assessment of methylation status of MGMT gene and IDH1/2 mutation status as it is not covered by public health centers in Upper Egypt, and finally, salvage therapy was not uniform in all patients with tumor progression. Salvage therapy included best supportive care for most of the patients (n=25, 64.1%), retreatment with TMZ therapy (n=9, 23.1%), and different 2nd line chemotherapy (n=5, 12.8%) inform of Etoposide/Cisplatin, Bevacizumab as single agent or in combination with irinotecan or carboplatin.

Conclusion

The results of our study suggest that extended TMZ therapy is safe and tolerable, however it did not significantly improve PFS or OS as compared to the standard six-cycle course. Larger randomized studies are required to shed more light on this issue.

Acknowledgements

This historical cohort study was approved by the Committee of Medical Ethics of the Faculty of Medicine, Assiut University, Assiut, Egypt with IRB no: 17300481. However, given the retrospective nature of the study the need for informed consent from human was waived by the Committee of Medical Ethics of the Faculty of Medicine, Assiut University, Assiut, Egypt with IRB no: 17300481. All methods were carried out in accordance with guideline and regulations. Confidentiality of patient's records was maintained throughout the study.

Disclosure of conflict of interest

None.

Abbreviations

GBM, Glioblastoma multiforme; TMZ, Temozolomide; OS, overall survival; PS, Performance status; ECOG, Eastern Cooperation Oncology Group; GTR, Gross total resection; STR, Subtotal resection; MRI, Magnetic resonance imaging; MGMT, O6-methylguanin-DNA methyltransferase; IDH, isocitrate dehydrogenase; GTV, Gross tumor volume; FLAIR, fluid attenuated inversion recovery; CTV, clinical target volume; PTV, planning target volume; RANO, Response Assessment in Neuro-Oncology; CTCAE, Common Terminology Criteria for Adverse Events; MRS, Magnetic resonance spectroscopy; DWI, Diffusion weighted imaging; ADC, Apparent diffusion coefficient; PRESS, Point Resolved Spatial Selection; TE, echo time; Cho, Choline; NAA, N-acetyl aspartate; Cr, Creatine; PFS, Progression free survival; ROC, Receiver Operating Characteristic; AUC, Area under the curve; EOR, Extent of surgical resection.

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