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Non-methylated MGMT as Predictive Factor in Newly Diagnosed Glioblastoma Multiforme Treated with Bevacizumab Concurrent with Radiotherapy Followed by Adjuvant Bevacizumab plus Irinotecan versus Temozolomide Concurrent with Radiotherapy Followed by Adjuvant Temozolomide

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Abstract

Background and Purpose: Temozolomide is the standard treatment for patients with newly diagnosed glioblastoma multiforme that had methylated O₆-methylguanine–DNA methyltransferase promoter, but it had a limited efficacy in non-methylated MGMT. Thus the aim of this study is to compare bevacizumab plus irinotecan versus standard temozolomide in newly diagnosed non methylated MGMT glioblastoma multiforme.

Patients and Methods: This study was carried out in oncology department, Tanta university hospital. Patients were randomized into two groups with ratio 2:1, group A received bevacizumab (BEV) (10 mg/kg every 2 weeks) during radiotherapy, followed by maintenance BEV (10 mg/kg every 2 weeks) plus irinotecan (IRI) (125 mg/m² every 2 weeks) until progressive disease. Patients in the group B received 75 mg/m² daily temozolomide (TMZ) during RT followed by adjuvant chemotherapy six cycles of TMZ (200 mg/m² once daily for 5 days every 4 weeks). In recurrence in group B; patients could receive second-line BEV+IRI. The primary end point was the progression-free survival rate.

Results: There was improvement in progression free survival, overall response and overall survival in favour of BEV+IRI versus TMZ. In univariate analyses for progression free survival, age, sex, performance status, extent of resection and line of treatment was statically significance while in multivariate they remained statistically significant. As regard overall survival all prognostic factors were significant in univariate analysis but only line of treatment was statically significant in multivariate analysis.

Conclusion: BEV+IRI could be a good alternative to TMZ in nm MGMT newly diagnosed GBM but required larger studies.

Keywords: Temozolomide; Glioblastoma; Temozolomide; Adjuvant chemotherapy

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Introduction

The most frequent primary tumor reported in adult is glioblastoma multiforme. It is responsible for 15% of all intracranial tumors and 60–75% of astrocytoma's [1]. The duration of survival has

not changed much over the past few decades (4%) [2,3]. Since the publication of EORTC trial temozolomide (TMZ)-based chemo radiation has become the standard treatment but short median survival has been reported in patients with non-methylated O₆-methylguanine–DNA methyltransferase (non-methylated

MGMT) [4,5]. Thus, few trials are applied to test the response GBM with non-methylated MGMT [6]. Glioblastoma multiforme is characterized by high degree of neovascularization caused by secreted vascular endothelial growth factor A (VEGF-A) [7,8]. The blood brain barrier is highly disrupted due to formation of dysfunctional new vasculature leading to focal brain edema [9]. Bevacizumab (BEV) is a monoclonal humanized antibody of the immunoglobulin G1 (IgG1) against VEGF-A was developed, inhibiting neovascularization and reducing vascular permeability then reducing focal brain edema [10]. In recurrent glioblastoma multiforme, a progression free survival benefit but not overall survival benefit was reported with bevacizumab from several non-randomized trials [11,12]. As first line setting the same results were reported as improved progression free survival but not overall survival [13,14]. The most astonishing trial is Glarius trial which randomized 182 patients with glioblastoma multiforme to bevacizumab concurrent with radiotherapy followed by sequential bevacizumab plus irinotecan bimonthly versus daily temozolomide concurrent radiotherapy then six cycles of temozolomide. It resulted in improvement in progression of survival not translated in improvement of overall survival [15]. Irinotecan has marginal activity in recurrent glioblastoma multiforme with progression free survival at 6 months 16% [16]. Irinotecan active metabolite (100-1000 times more active inhibitor of topoisomerase) is 7-ethyl-10-hydroxycamptothecin (SN-38). Gliomas cells can convert irinotecan to SN-38 directly which decrease proliferation with resultant apoptosis [17]. So our trial was to test the role of bevacizumab with irinotecan versus temozolomide in non-methylated MGMT newly diagnosed glioblastoma multiforme.

Patient and Methods

It was a randomized, controlled, trial in Oncology Department, Tanta University Hospital and Neurosurgery Department, al Azhar University, Cairo approved by Medical Ethic Committee of Tanta University Hospital between August 2013 and August 2015. All patients gave written informed consent. Flow chart of patients was shown in **Figure 1**. The study included newly diagnosed glioblastoma multiforme with age more than 18 years, eastern cooperative oncology 0-2, adequate healing of craniotomy flap, non-methylated MGMT (ratio<0.6); stable or decreasing corticosteroids within 5 days before random assignment; and adequate hematologic, hepatic, renal, and coagulation function. Patients with any of the following criteria presence of overt recent haemorrhage detected by magnetic resonance imaging with contrast; history of thromboembolic disease, bleeding diathesis, coagulopathy, stomach ulcer, gastrointestinal perforation or serious non-healing wound or fracture were excluded from the study. First the specimen was reread by pathologist in pathology department, Tanta University to confirm the glioblastoma multiforme diagnosis. After confirmation it was analysed for MGMT promoter methylation using a real-time methylation-specific polymerase chain reaction (PCR) [18,19].

According to manufacturer's recommendations, assessing DNA methylation of the MGMT promoter region by applying methylation specific PCR is done. Firstly treat Genomic DNA

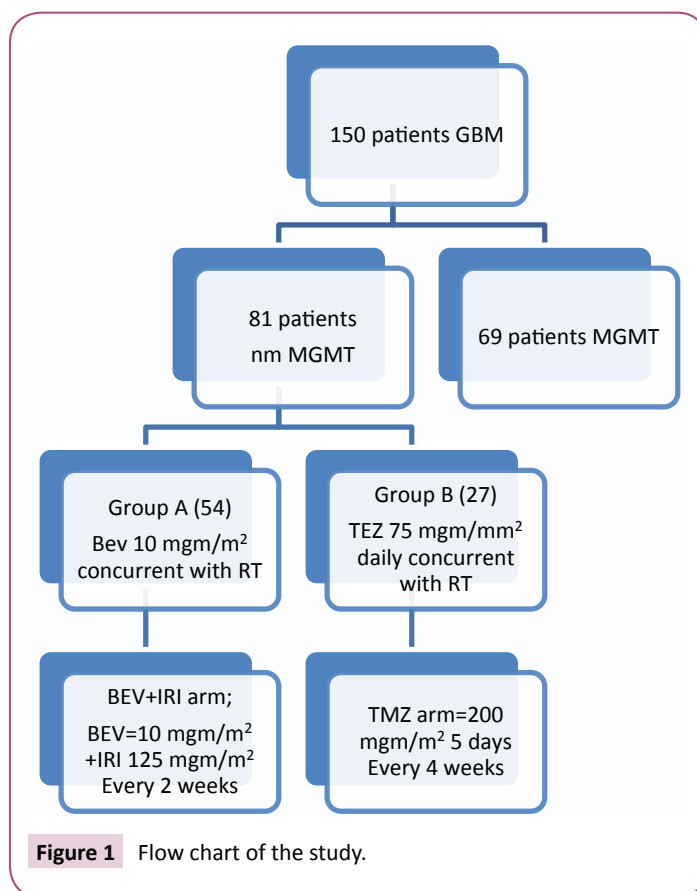


Figure 1 Flow chart of the study.

(1 µg) with sodium bisulphite then the EpiTect Bisulphite Kit (Qiagen) for purification. Then the extracted DNA was amplified with published PCR primers that distinguish methylated and unmethylated DNA [18,19]. Ratio less than 0.6 is considered nm MGMT (non-methylated MGMT). Radiotherapy was given to all patients 4 weeks postoperative in the form of involved field radiotherapy (radiotherapy dose All patients received involved-field radiotherapy) (RT; 33 × 180 cGy). The patient were randomized into two groups, group A received bevacizumab (BEV) (10 mg/kg every 2 weeks) during radiotherapy, followed by maintenance BEV (10 mg/kg every 2 weeks) plus irinotecan (IRI) (125 mg/m² every 2 weeks) until progressive disease. Patients in the group B received 75 mg/m² daily temozolomide (TMZ) during RT followed by adjuvant chemotherapy six cycles of TMZ (200 mg/m² once daily for 5 days every 4 weeks). In recurrence in group B; patients could receive second-line BEV+IRI.

Response and Toxicity

After two months from the end of radiotherapy evaluation of patients using neurological examination and magnetic resonance imaging with contrast were done. Response evaluation was done using RECIST version 1.1 [20]. After radiotherapy progressive disease was diagnosed only if the tumor progressed outside the radiation field. Then evaluation was done every three months until death. Neurological examination, corticosteroid use and MRI brain with contrast were used to evaluate progressive disease. Determination of progressive disease (PD) was based on MRI, clinical assessment, and corticosteroid use [21]. Adverse

events were assessed according to the Common Terminology Criteria for Adverse Events (CTCAE, version 4.0) [22].

End Points and Statistical Analysis

The primary end point was the progression free survival using Kaplan Meier version 21 [23]. Correlation with different prognostic factor including age, sex, performance status, extent of resection, and type of treatment received. Secondary survival end points were overall response and OS. Survival analyses were done using Kaplan-Meier method, log-rank test for group comparisons.

Results

Patient Characteristics

Between august 2013 and August 2015, one hundred and fifty patients with newly diagnosed GBM were analysed MGMT promotor methylation status. Of them 81 (54%) and 69 (46%) patients had an nm MGMT ratio less than 0.6, and methylated MGMT respectively. The non-methylated group were then randomized in ratio 2:1 to group A (BEV+IRI arm; n=54), group B (TMZ arm, n=27); baseline characteristics listed in **Table 1**.

Treatment

In the group A (BEV+IRI arm), number of biweekly BEV was from 5-80 cycles with median of 22 biweekly course. Three year progression free survival with BEV+IRI versus TMZ was 81.5%, 38.6% respectively which was statistically significant (p-value=0.001) with median survival was 21 and 17 in group A and B respectively (**Figure 2**). According to female sex, three progression free survival was 83.3%, 80% in the BEV+IRI and TMZ group respectively; also according to male sex three progression free was 81.6%, 27.3% in the BEV+IRI and TMZ group respectively which was statistically significant (p-value=0.001). As regard age, three year progression free survival was 92.4% versus 32% for patients aged less than 50 years in group A and B respectively ; also in patients aged more than 50 yrs, it was higher in group A versus group B (70.1% versus 46.3% respectively and this was statistically sigficant (p-value=0.001). In patients with performance status 0-1, three progression free survival was 82.1%, 27.3% in the BEV+IRI and TMZ group respectively; also in performance status 2 it was 79.6% in group A versus 50% 50% which was statistically significant (p-value=0.001). According

to resection, three year PFS biopsy, partial resection, and total resection it was 86.5%, 75.7, 100% versus 80%, 33.3, 0% in group A and B respectively which was statistically significant (p-value=0.029). Multivariate analysis of progression free survival revealed the prognostic factors with statistical significance were performance status (0.043), degree of resection (0.032) and type of treatment (0.003). Three year overall survival was 74.9% and 41.9% in relation to line of treatment BEV+IRI versus TMZ respectively which was statistically significant (p-value=0.011) with median survival for group A was 21.5 months versus 19 for group B (**Figure 3**). According to female sex, three overall survival was 71.4%, 60% in the BEV+IRI and TMZ group respectively ; also according to male sex Three overall survival was 76.2%, 37.1% in the BEV+IRI and TMZ group respectively which was statistically significant (p-value=0.007) As regard age, three year OS was 72.8% versus 45.2% for patients aged less than 50 years in group A and B respectively ; also in patients aged more than 50 yrs it was higher in group A versus group B (78.4% versus 37% respectively and this was statistically significant (p-value=0.015). In patients with performance status 0-1,three overall survival was 72.6%, 38.8% in the BEV+IRI and TMZ group respectively; while in patients with performance status 2 it was 80.8% versus 53.6% in group A and B respectively which was statistically significant (p-value=0.017). According to resection three year OS biopsy, partial resection, and total resection were 86%, 62%, 100% versus 80%, 37.7%, 0% in group A and B respectively which was statistically not significant (p-value=0.213). Multivariate analysis of overall survival revealed the prognostic factors with statistical significance was type of treatment (0.037). Complete response, partial response and overall response were higher in group A (OAR=81.5%) versus group B (OAR=44.4%) which was statistically significant (P-value=0.001) as shown in **Table 2**. Correlation of overall response with line of treatment according to different prognostic factors, the factors with statistical significance were male sex (p-value=0.001), age less than or equal 50 yrs (p-value=0.001), performance status 0-1 (p-value=0.003) and partial resection (p-value=0.001) (**Table 3**). In BEV+IRI group three cerebral haemorrhages occurred of which one was fatal. In TMZ group, high-grade hematotoxicity reported in 5 (18.5%) patients. Grade 3 nausea, vomiting and diarrhoea were higher with BEV+IRI [20 (37.03%) patients] than with TMZ [5 (15%) patients], may be attributed to IRI. Grade 4 vomiting and diarrhoea was reported only in IRI group [3 patients (5.6%)].

Table 1 Patient characteristics.

Patient Characteristics		Total (81)	BEV + IRI arm (54)	TMZ arm (27)	P-value
Age		50 yr	52 yr	50 yr	0.993
Median Range		26-80 + 13.20	26-80 + 12.89	26-78 + 13.99	
Sex	Male	64 (79%)	42 (77.8%)	22 (81.5%)	0.7
	Female	17 (21%)	12 (22.2%)	5 (18.5%)	
Performance status	0-1	57 (70.4%)	38 (70.4%)	19 (70.4%)	1
	2	24 (29.6%)	16 (29.6%)	8 (29.6%)	
Extent of resection	Biopsy	33 (40.7%)	28 (51.9%)	5 (18.5%)	0.015*
	Partial resection	43 (53.1%)	23 (42.6%)	20 (74.1%)	
	Complete resection	5 (6.2 %)	3 (5.6%)	2 (7.4%)	

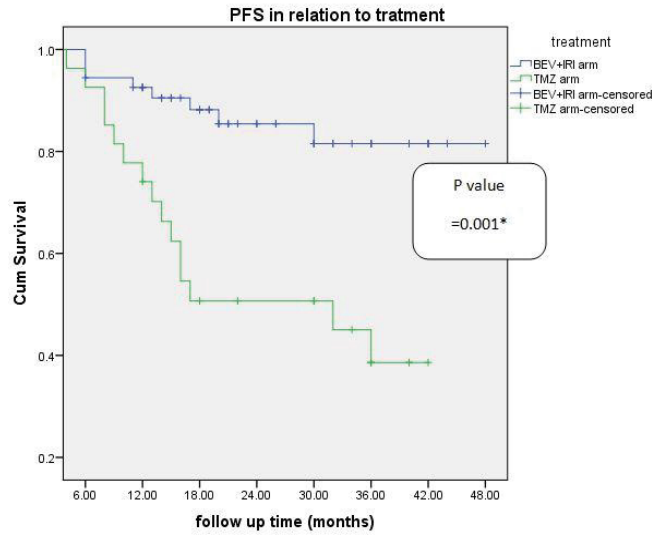


Figure 2 Progression free survivals with treatment.

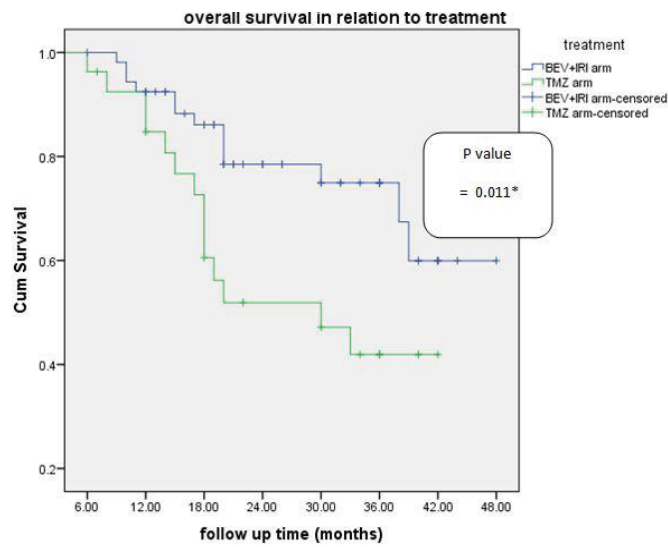


Figure 3 Overall survivals with treatment.

Table 2 Treatment response according to line of treatment.

Treatment		Response				Total	P-value
		CR	PR	SD	PD		
Treatment	BEV + IRI arm	Count	19	22	6	4	0.001*
		% within treatment	37.30%	43.10%	11.80%	7.80%	
		% within response	100.00%	64.70%	35.30%	50.00%	
		% of Total	24.40%	28.20%	7.70%	5.10%	
	TMZ arm	Count	0	12	11	4	
		% within treatment	0.00%	44.40%	40.70%	14.80%	
		% within response	0.00%	35.30%	64.70%	50.00%	
	BEV + IRI	OAR	44 (81.5%)				
		Non responsive	10 (18.5%)				
TMZ	OAR	12 (44.4%)					
	Non responsive	15 (55.6%)					

Table 3 Correlation of overall response with line of treatment according to different prognostic factors.

Patient characteristics		Overall response	BEV + IRI	TMZ	P value
			N (%)	N (%)	
Sex	Male	OAR	35 (83.3%)	9 (40.1%)	0.001*
		Non responsive	7 (16.7%)	13 (59.1%)	
	Female	OAR	9 (75%)	3 (60%)	0.536
		Non responsive	3 (25%)	2 (40%)	
Age	< 50 yr or equal	OAR	24 (88.9%)	6 (40%)	0.001*
		Non responsive	3 (11.1%)	9 (60%)	
	50 yr	OAR	20 (74.1%)	6 (50%)	0.536
		Non responsive	7 (25.9%)	6 (50%)	
Performance status	0-1	OAR	31 (81.6%)	8 (42.1%)	0.003*
		Non responsive	7 (18.4%)	11 (57.9%)	
	2	OAR	13 (81.3%)	4 (50%)	0.112
		Non responsive	3 (18.7%)	4 (60%)	
Extent of resection	Biopsy	OAR	22 (78.6%)	3 (60%)	0.372
		Non responsive	6 (21.4%)	2 (40%)	
	Partial resection	OAR	20 (87%)	8 (40%)	0.001*
		Non responsive	3 (13%)	12 (60%)	
	Complete resection	OAR	2 (66.6%)	1 (50%)	0.709
		Non responsive	1 (33.3%)	1 (50%)	

Discussion

Newly diagnosed non methylated MGMT (nm MGMT) GBM treated with BEV+IRI had better overall response, progression free survival and overall survival than standard TMZ. This may provide a good alternative to standard TMZ and this may be good option for patients with nm MGMT. The prognostic factors with statically significance in correlating overall response with line of treatment were male sex (p-value=0.001), age less than or equal 50 yrs (p-value=0.001), performance status 0-1 (p-value= 0.003) and partial resection (p-value=0.001). As regard progression free survival was higher BEV+IRI versus TMZ 81.5%, 38.6% respectively which was statistically significant (p-value=0.00) and this was constant with that reported by Glarius study [15]. The PFS was prolonged by BEV was similar to that reported by the larger phase III AVAglio and RTOG 0825 trials [24] despite different line of treatment as patient received during radiotherapy. In univariate analysis with progression free survival, all prognostic factors including age, sex, performance status and treatment were statistically significant but in multivariate analysis, the prognostic factors with statistical significance were performance status, degree of resection and type of treatment. In our trial the progression free survival was translated into improvement in overall survival in contrast to as reported by Glarius [15], Aglio and RTOG 0825 trials in which improvement in PFS was not translated into improvement in overall survival [15,24]. The progression free survival was not translated into improvement in OS may be attributed to anti edema effect and pseudo progressive effect of BEV and not true anti neoplastic effect. In univariate analysis

of overall survival with different prognostic factors, age, sex performance status and line of treatment were significant but in multivariate analysis, type of treatment was only statistically significant prognostic factor Grade 3 or higher adverse events were higher in the bevacizumab+IRI group than in TMZ group, this may be explained by higher incidence of hypertension in first group. However haematological toxicity was higher in TMZ group but nausea, vomiting and diarrhoea higher in BEV+IRI may be attributable to irinotecan side effect and this was constant with that reported by Glarius trial [15].

Conclusion

It may be concluded that due to improvement in both PFS and OS a new strategic plan for patients with nm MGMT newly diagnosed glioblastome multiforme should search for other alternatives for standard concurrent temozolomide and adjuvant temozolomide in newly diagnosed glioblastome multiforme of which BEV+IRI appeared to be promising.

Conflict of Interest

Authors declared that they had no conflict of interest.

Ethical Approval

All procedures performed in this study involving human participants were in accordance with the ethical standards of the Tanta university hospital and informed consent was taken from patients included in this study.

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