Egyptian Journal of Medical Human Genetics

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Clinical impact of *IDH1* mutations and *MGMT* methylation in adult glioblastoma

Magda Sayed Mahmoud[1], Mohamed K. Khalifa[2], Amira M. Nageeb[1], Lobna R. Ezz El-Arab[3], Manal El-Mahdy[4], Amal Ramadan[1], Maha Hashim[1], Noha M. Bakr[1] and Menha Swellam[1*]

Abstract

Background Impact of Isocitrate dehydrogenase1 (*IDH1*) and O6-methylguanine-DNA methyltransferase (*MGMT*) in glioblastoma (GBM) have been of great interest due to their implications in prediction of prognosis of several types of cancer. It was aimed to investigate the clinical role of *IDH1* mutation and *MGMT* methylation pattern among GBM patients versus non-neuro-oncological diseases (NND) patients and their impact on survival criteria.

Methods Formalin-fixed paraffin-embedded (FFPE) tissue sections of 58 GBM and 20 non-neuro-oncological diseases patients were recruited and *IDH1* mutation and *MGMT* methylation was detected using Cast-PCR technology and Methyl II quantitative PCR approach, respectively. Results were assessed with other clinicopathological criteria and survival patterns.

Results *IDH1* mutation was detected among 15 GBM cases (15/58) and it was not reported among NND ($P=0.011$). Receiver operating characteristic (ROC) curve was plotted to discriminate between *MGMT* methylation among studied groups. Patients with *MGMT* methylation ≥ 66% were reported as high methylation, which was recorded significantly in 51.7% and 100% of GBM cases and NND, respectively. Both showed significant difference with performance status, while *MGMT* methylation was significantly related with tumor size and tumor location. *IDH1* mutation and *MGMT* methylation reported significant increase with GB patients revealed complete response to treatment. Survival pattern was better for *IDH1* mutation and *MGMT* high methylation as compared to *IDH1* wild type or *MGMT* low–moderate methylation, respectively, and favorable survival was detected when both were combined than using either of them alone.

Conclusion Detection of *IDH1* mutation and *MGMT* methylation among GB patients could aid in prediction of their response to treatment and their survival patterns, and their combination is better than using any of them alone.

Keywords Glioblastoma, *IDH1*, *MGMT*, Temozolomide, Prognosis

*Correspondence:
Menha Swellam
menhamswellam@gmail.com
[1] Biochemistry Department, Biotechnology Research Institute, High Throughput Molecular and Genetic Laboratory, Central Laboratories Network and the Center for Excellences for Advanced Sciences, National Research Centre, Dokki, Giza 12622, Egypt
[2] CSO at Omicsense, Molecular Pathology Lab Children Cancer Hospital 57357, Cairo, Egypt
[3] Clinical Oncology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt
[4] Pathology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt

Background

Glioblastoma (GBM) is the prevalent malignant brain tumor with worse prognosis and aggressive development without identifiable precursor lesions [1]. Advances have been made in therapeutic strategies after adding of the temozolomide (TMZ) chemotherapy, the maximal safe cancer resection and radiotherapy. However, average survival is still restricted to about 15 months [2, 3]. Thus, there is a great need to unravel oncogenic mechanisms of GBM since there are two types of this kind of malignancy either displayed rapidly de novo with unknown precursor

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lesion or from low-grade tumor [4] although they cannot be histopathologically distinguished but both with different molecular alterations due to different genes have been reported to be involved in the process of GBM pathogenicity [5–7].

In addition to other genetic actions, the most interesting recent output in glioma oncogenic events has been the recognition of IDH1 and IDH2 genetic mutations. IDH1/2 are homodimeric isozymes that rely on NADP+and have a highly similar protein structure and significant sequence similarity [8]. *IDH*1 is the cytoplasmic constituent that is produced obviously in the liver and other tissues, while *IDH*2 is completely limited to the mitochondria and displays the considerable expression in heart and lymphocytes, and muscle tissues [9]. Every mutation in *IDH*1 or -2 can cause enhancing oxidative stress through its mutagenic act that damages the DNA [1]. This event is constant by an enhanced quantity of DNA damage in the *IDH*1-mutated cancerous glioma cells and in that way *IDH*1/2 mutations function as driver genetic alterations in glioma carcinogenesis, though their crucial function is still unexplored [1].

Both *IDH*1/2 genetic alterations are further exclusively linked to glial-type phenotype of brain cancer and are determined in about 5% of primary and around 50% of secondary GBM that has been validated to give an better prognosis [10]. Additionally, The Cancer Genome Atlas (TCGA) has allowed categorization of different molecular alternatives of GBM with several findings with proneural reporting a better prognosis, while neural, mesenchymal and classical display a worse prognosis [1]. Lately, the proneural variation is related to a good response to the antiangiogenic agent as bevacizumab [11].

Section of cancerous gliomas defies the chemotherapeutic drug as temozolomide (TMZ), effective sensitive molecule that results in the cell death [4]. TMZ is considered an alkylating factor that cross-links DNA through the DNA-repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT) [12]. *MGMT* fixes alkylating lesions of the DNA frequently produced by the TMZ [2, 13].

Hypermethylaterd *MGMT* revealed cbettwer response to TMZ while cells with unmethylated *MGMT* gene display the resistance to the drug [13, 14].

This procedure is believed to make the *MGMT* methylation as promising prognostic benefit in glioma cases received alkylating drugs [15, 16]. *MGMT* promoter methylation is reported in 35–45% of glioma patients (WHO grades III and IV), though it seems in around 80% of gliomas with low-grade (WHO grade II) [17, 18]. This category of glioma patients may have improved sensitivity to the TMZ as result of the enzyme deficiency, which influences its clinical consequence [1, 12].

In the current study, authors aimed to investigate both *IDH1* genetic mutation and *MGMT* epigenetic methylation of *MGMT* among GB patients and investigate their correlation with each other as well as their relation as predictive prognostic markers among Egyptian patients when tested alone or in combination.

Materials and methods

Patient selection

The current study has been performed in accordance with the Declarations of Helsinki and approved by Medical Ethical Committee (National Research Centre ID#20110), participants who fulfilled the inclusion criteria were recruited from 2020 till 2022. The inclusion criteria were as follows: adult persons (age > 18 years) newly diagnosed GB with performance less than or equal 2 according to the ECOG (Ester Clinical Oncology Group); which assesses disease progression affecting on patient's daily living abilities and patients with non-neuro-oncological diseases and have not reported other malignancies, while GB patients who have not fulfilled these criteria were excluded. Accordingly, GB patients ($n = 58$) patients were recruited after signing their informed consent. Also, a group of non-neuro-oncological diseases (NND) were recruited ($n = 20$). After obtaining signed informed consent from all participants, surgically resected tumor tissue samples were taken by stereotactic/open biopsy of brain tumors, then fixed in neutral buffered formalin and embedded in paraffin stained with hematoxylin–eosin (HE) reviewed by neuropathologists (MM) to confirm diagnosis according to WHO classification 2016 [17, 18]. Then, 5–10 sections from FFPE were transferred to Eppendorf tubes for further processing of DNA extraction.

DNA extraction

DNA was extracted from FFPE samples using QIAamp FFPE kit (Cat no. 56404) as per manufacturer instructions and both purity and concentration were detected using nano-drop spectrophotometer (Quawell, Q-500, Scribner, USA) by measuring the absorbance at 260 and 280 nm and checked on 1%agarose gel, the extracted DNA samples were stored in − 20 for further processing to detect *MGMT* and *IDH1* mutation.

Detection of MGMT methylation pattern using Methyl II quantitative PCR system

MGMT methylation pattern was detected in DNA extracted samples using the following steps of EpiTect Methyl II quantitative polymerase chain reaction (qPCR) technique (Qiagen, Germany) which relay on assessment of residual DNA input after cleavage by restriction enzyme, and then, the remaining DNA will be quantified

by real-time PCR using specific primers for the desired gene that flanks a promoter region of interest. Thus, reaction was performed in two phases with some modifications in our laboratory: phase I: carried out using EpiTect Methyl II DNA Restriction Kit (cat. no. 335452), briefly input genomic DNA was aliquoted into two equal portions into 2 PCR reaction tubes and they were designated as follows: no-enzyme (UD, i.e., no restriction enzyme was added), methylation-sensitive restriction enzyme (D, i.e., restriction enzyme sensitive to methylation hence digest unmethylated DNA) and then, they were incubated for 6 h at 37 °C, afterwards for 20 min at 65 °C for 20 min by thermal cycler (SureCycler 8800, Agilent, Santa Clara, CA, USA). Then, the remaining genomic DNA sample in each tube (UD and D) is quantified through phase II which was carried out using real-time PCR (Max3005P QPCR system; Stratagene, AgilentTechnologies, CA, USA). Briefly, 5ul from the remaining DNA was directly mixed with Master Mix (RT2 qPCRSYBR Green/ROX Master Mix, Cat number 330520) and was distributed into a PCR plate with pre-aliquoted *MGMT* primer: Left primer 5\-ATTTTTGTGATAGGAAAAGGTATGG-3\ Right primer 5\-CTAAAACAATCTACACATCCTCACT-3\. Real-time PCR reaction is done via specified cycling conditions, for 10 min at 95 °C (1 cycle), then for 30 s at 99 °C, and for 1 min at 72 °C (3 cycles), and finally 40 cycles with following conditions: 15 s at 97 °C and for 1 min at 72 °C for 1 min. Finally, the raw ΔCT values were collected for each PCR reaction tube (UD and D) for each sample as shown in Fig. 1A–B. However, in the qPCR reaction the UD was used, and hence, the DNA in which all CpG sites are methylated will be detected by real-time PCR [19] through following equations:

$$\Delta CT = \text{digested } \Delta CT - \text{undigested } \Delta CT$$

$$\text{Methylation \%} = 2^{-\Delta CT} \text{fold change } \times 100$$

Determination of IDH1 mutation using Cast-PCR technology

Competitive allele-specific TaqMan PCR (Cast-PCR) technology was used to detect *IDH1* mutation as it is sensitive, specific and fast method for detection of mutant allele since it permits not only the discriminating amplification of minor alleles, but it also blocks the amplification of non-mutant allele [20, 21]. Qualitative assessment of six mutations within *IDH1* mutation codon 132 (the 2 major R132H and R132C mutations, and 4 "*IDH1*-other": R132G, R132S, R132L, R132V), one within *IDH1* codon 100 (R100Q), Amplification Refractory Mutation System (ARMS) PCR technology was combined to selectively identify the most frequent *IDH1* R132H/R132C. The Master Mix was prepared as recommended by the supplier. A total of 50 ng of gDNA per reaction and the probes described above were used. The cycling conditions were as follows: pre-PCR read 60°C for 30 s; holding stage 50°C for 2 min, 95°C for 10 min; cycling stage 95°C for 15 s, 60°C for 1 min for 40 cycles; and post-PCR 60°C for 30 s. For each of the analyzed *IDH1/2* mutations, the limit of detection (LOD) of castPCR TM was determined by constructing dilution curves of samples from patients with and without *IDH1/2* gene mutations. Each point was determined using different dilutions (1:1 to 1:50) of the mutated sample and a non-mutated sample (Fig. 2A-B). Sensitivity and specificity of the Cast-PCR for *IDH1*

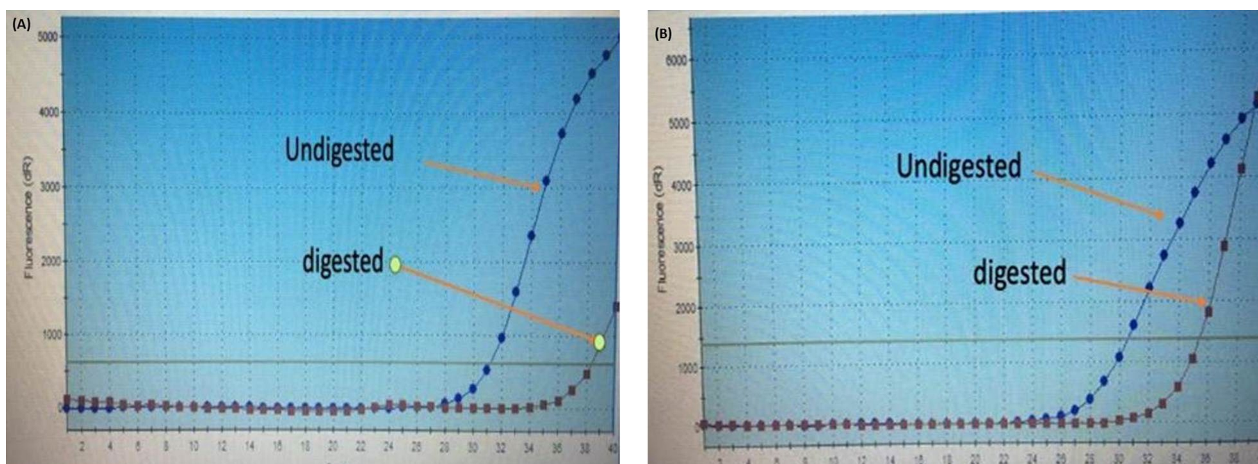

Fig. 1 Amplification plots of the MGMT qPCR reaction, A: showing plots of 100% unmethylated sample, and B: showing partially methylated sample

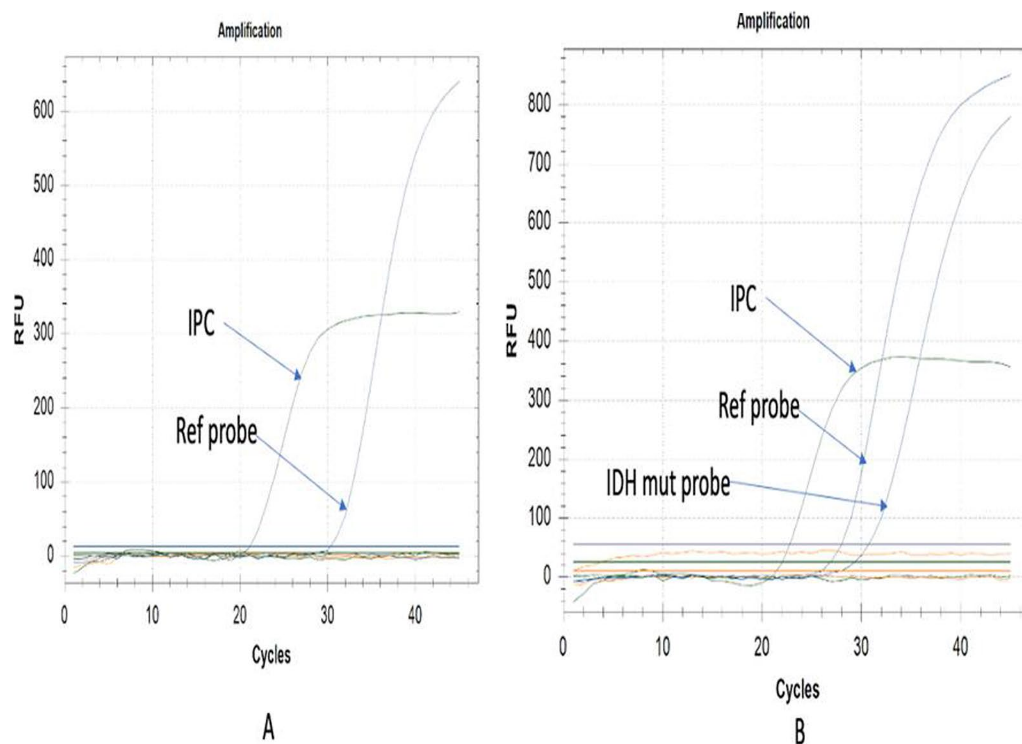

Fig. 2 Amplification plots of the IDH1 mutation by Cast-PCR reaction, A: showing plots of wild-type sample, and B: showing positive mutation sample

R132H (SNVs) allow over 99% confidence of detecting down to 5% mutant DNA in a wild-type background.

Histopathologic preparation

This study included formalin-fixed paraffin-embedded tissue blocks from patients with glioblastoma and the histopathologic criteria of glioblastoma according to WHO classification 2016 were cellular features of neoplastic astrocytic cells as marked pleomorphism, hyperchromatic nuclei and abnormal mitoses and scattered apoptosis, as well as microvascular proliferation and tumor necrosis [17, 18]. The inclusion criteria for selecting the tumor tissue blocks are as follows: (1) histopathological diagnosis of glioblastoma with more than 80% viable tumor tissue, (2) available archival paraffin-embedded tissue blocks and (3) available clinical follow-up data. All studied glioblastoma cases and non-neoplastic control cases were subjected to the following: (I) The paraffin-embedded tissue blocks of the studied glioblastoma cases were cut at full sections with a thickness of four microns and stained for routine hematoxylin and eosin H&E stain. The H&E-stained slides of the tissue specimens were prepared to confirm the diagnosis based on the 2016 CNS Tumors WHO classification and to assess viability of the submitted tumor tissue (Figs. 3 and 4). (II) For preparation of PCR testing, freshly cut sections of paraffin-embedded tissue, each with a thickness of up to 10 ums. Up to eight sections, each with a thickness of up to 10 um and a surface area of up to 250 mm^2 can be combined in one preparation.

Treatment strategies

The clinical target volume (CTV) was contoured on computerized tomography (CT) and postoperative magnetic resonance imaging (MRI) image fusion and integrated residual tumor mass (T1 gadolinium-enhanced lesion) and/or postoperative cavity (i.e., GTV) plus a 15–20mm margin without reflection for peri-tumoral edema. Volume contouring took into account anatomical barriers, as ventricular spaces, cranial bones and the midline excluding for the region of the corpus callosum. An isotropic margin of 5mm was added around to obtain the planning target volume (PTV-1). Radiotherapy treatment (RT) was delivered with a linear accelerator 6–10 MeV beam and 3D-conformal or intensity modulated techniques up to a planned total dose of 60 Gy and with a standard fractionation (2Gy/day for 5 days per week). All patients received also temozolomide (TMZ), concurrently administered *per os* during RT, according to Stupp's protocol (daily TMZ 75mg/m^2 during the RT course, for 6 weeks followed by the sequential TMZ schedule (150–200mg/m^2 for 5 days every 28 days) until disease progression

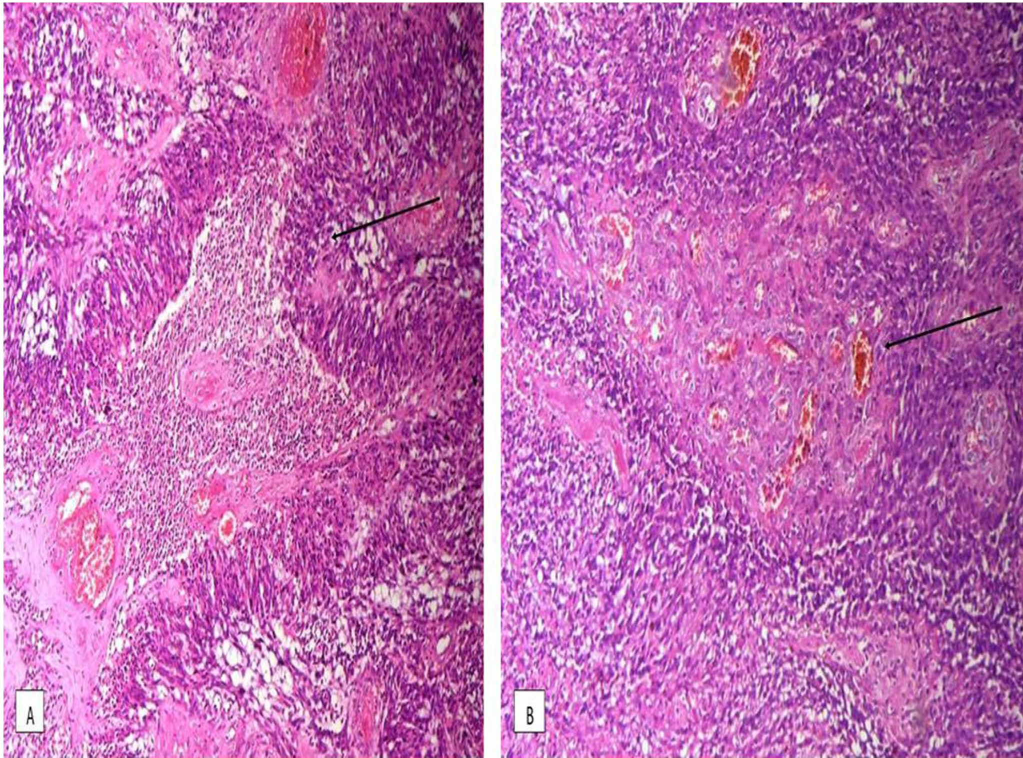

Fig. 3 Histopathology of glioblastoma showing: A—High cellularity and foci of palisading tumor necrosis (Arrow), and B—Vascular endothelial proliferation (Arrow), (Hx&E, X100)

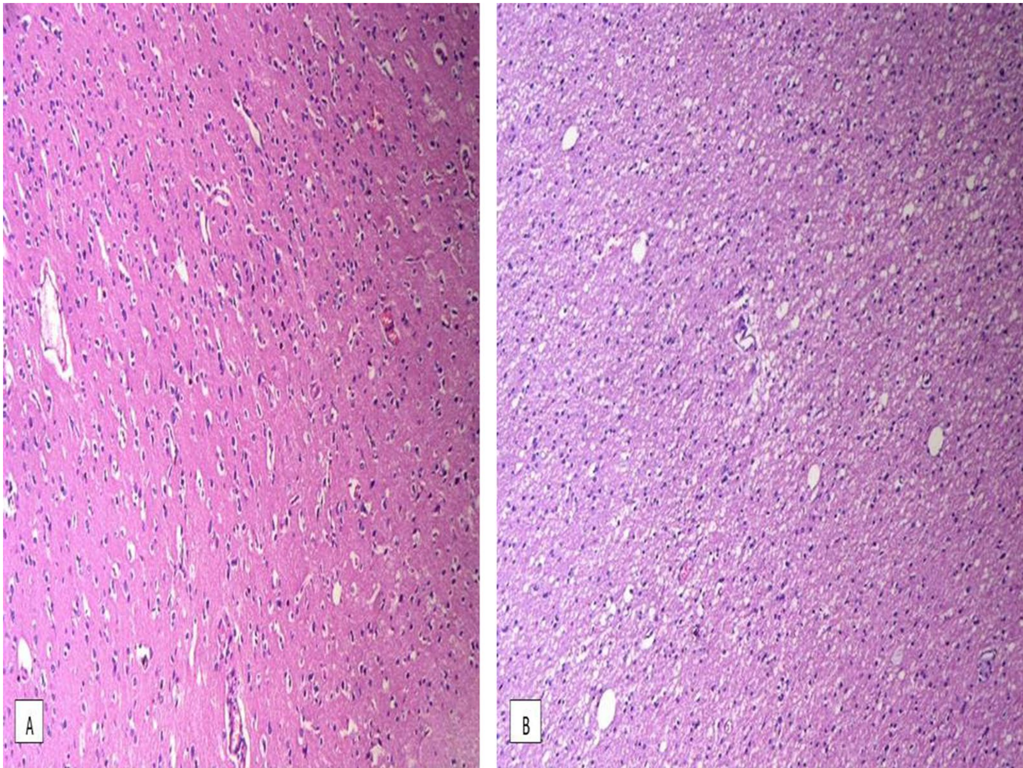

Fig. 4 Non-neuro-oncological diseases, Reactive gliosis (A and B)

(PD) or complete response (CR) after 12 cycles. After the completion of RT and concurrent TMZ administration, patients entered a scheduled follow-up program. Brain MRI scans were repeated at 4 weeks, 12–16 weeks, and then every 6 months or in any case showing clinical signs suggesting progressive disease (PD). Taking into account the fact that no patient of this series received antiangiogenic treatment, PD after RT-TMZ treatment was assessed using the RANO Criteria [22]. A diagnosis of pseudoprogression was made in cases showing an increase in tumor size and/or T1-contrast enhancement within 3–6 months after the end of concomitant RT-TMZ, without worsening of neurological status and with stabilization or resolution in subsequent further MRIs studies. Imaging findings suggestive of radionecrosis were recorded. All the MRI examinations were revised for the compilation of this paper by a neuroradiologist (LEA). General and neurological examinations and blood counts and chemistry were obtained every 3 months.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS Inc, Chicago IL, USA). Kaplan–Meier survival curves were done and differences in PFS and OS were tested for statistical significance using the log-rank test which is a statistical methodology used to test whether there is a difference between the distribution of survival times until the occurrence of an event of interest in independent groups. Significance level was set at p < 0.05. Cutoff value for *MGMT* methylation status was obtained by plotting receiver operating characteristic (ROC) curve by plotting true positive (sensitivity) versus false positive (100-specificity) for investigation of diagnostic efficacy of *MGMT* by considering GB versus NND. The area under the ROC curve (AUC) assessed the accuracy, and hence: if equals 1 means accurate test; < 1 − 0.8 a good test; < 0.8 – 0.7 a fair test, < 0.7 − 0.6 a poor test, while < 0.5 as worthless test [23].

Results

The current study was carried out on FFPE samples from 20 NND and 58 GB cases, their full clinical data are summarized in Table 1. No significant level was reached when considering gender between the two groups (NND *versus* GB), while significant level was reached when age of the two groups were considered, for both groups *IDH1* mutation and *MGMT* methylation are as represented in Table 2. To determine the methylation level, ROC curve was plotted and the best cutoff point (methylation percentage) that discriminates between them was 66%, and those reported level of methylation blow the < 66% were represented as low–moderate methylation while individuals reported methylation level above ≥ 66% were highly methylated (Fig. 5). Accordingly, all NND patients were highly methylated (100%) while 30 out of 58 (51.7%) GB cases reported high *MGMT* methylation and the remaining were low–moderate methylation at significant level $P < 0.0001$. For *IDH1* mutation, it was detected in 15 GB cases (25.9%) while the remaining (43, 74.1%) reported *IDH* wild type, and all NND patients reported

Table 1 Clinical and demographic data for studied cases

Factors	Non-neuro-oncological patients (*n* = 20)	GBM (*n* = 58)
Age (Mean)		
< 60 ψεαρσ	20 (100%)	42 (72.4%)
≥ 60 years	0 (0%)	16 (27.6%)
	$X^2 = 6.9$, $P = 0.008$	
Gender		
Male	10 (50%)	35 (60.3%)
Female	10 (50%)	22 (39.7%)
Pathology		GBM (Grade IV)
ECGO		
< 2		19 (32.8%)
2		39 (67.2%)
Tumor site		
Lt		29 (50%)
Right		23 (39.7%)
Multiple		6 (10.3%)
Tumor size		
< 5χμ		22 (37.9%)
≥ 5 cm		36 (62.1%)
Surgical intervention		
Biopsy		36 (62.1%)
Resection		
Total		19 (32.8%)
Sub-total		3 (5.2%)

Table 2 Investigated *MGMT* methylation and *IDH1* mutation among studied groups

Investigated items	Non-neuro-oncological patients (*n* = 20)	GBM (*n* = 58)
MGMT methylation		
< 66% (low methylation)	0 (0%)	28 (48.3%)
≥ 66% (highly methylated)	20 (100%)	30 (51.7%)
	$X^2 = 15$, $P < 0.0001$	
IDH1 mutation		
Wild type	20 (100%)	43 (74.1%)
Mutant type	0 (0%)	15 (25.9%)
	$X^2 = 6.4$, $P = 0.011$	

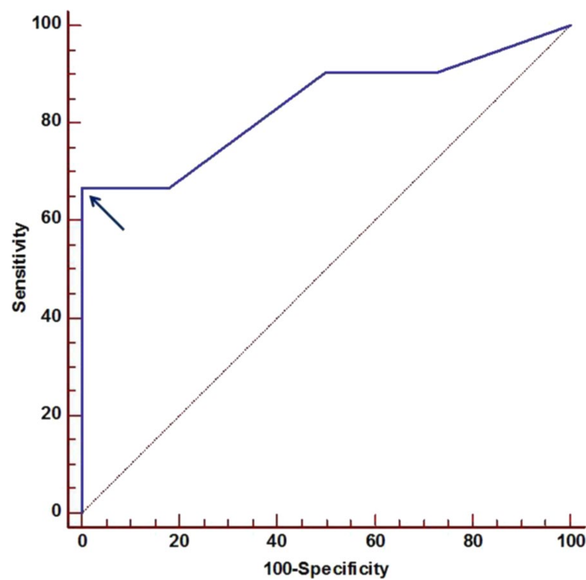

Fig. 5 Receiver operating characteristic (ROC) curve for MGMT methylation among investigated groups. Arrow contributes to the best cutoff point that discriminates between high methylation (≥66%) versus low–moderate methylation at area under the curve (AUC) = 0.837, 95%CI = 0.723–0.917, at P = 0.0001

Table 3 Distribution of *IDH1* mutation and *MGMT* methylation status among GBM cases

Factors	IDH1 mutant	MGMT methylated (≥66%)
Age (Mean)		
<60 ψεαρσ	11 (73.3%)	24 (8%)
≥60 years	4 (26.7%)	6 (20%)
	$X^2=7.7$, $P=0.005$	
Gender		
Male	8 (53.3%)	16 (53.3%)
Female	7 (46.7)	14 (46.7)
ECGO		
<2	11 (73.3%)	19 (63.3%)
2	4 (26.7%)	11 (36.7%)
	$X^2=15$, $P<0.0001$	$X^2=26$, $P<0.0001$
Tumor site		
Lt	8 (53.3%)	17 (56.7%)
Right	7 (46.7)	13 (43.3%)
Multiple	0 (0%)	0 (0%)
		$X^2=7.2$, $P=0.027$
Tumor size		
<5χμ	7 (46.7)	17 (56.7%)
≥5 cm	8 (53.3%)	13 (43.3%)
		$X^2=9.3$, $P=0.002$
Surgical intervention		
Biopsy	8 (53.3%)	13 (43.3%)
Resection		
Total	4 (26.7%)	14 (46.7%)
Sub-total	3 (20%)	3 (10%)
	$X^2=9$, $P=0.011$	$X^2=9.98$, $P=0.007$

IDH wild type at significant level $P=0.011$, as shown in Table 2.

Distributions of *IDH1* mutation and *MGMT* methylation among GB cases are presented in Table 3. For *IDH1* mutations, significant levels were reported between *IDH1* mutation with both age, ECGO and surgical intervention, while for *MGMT* methylation was revealed significant with other factors apart from age and gender. Patients were treated with standard of care treatment protocol and patients were categorized according to their response to treatment as follows; complete response (CR), partial response (PR), stable disease (SD) and progressed disease (PD). Both *IDH1* mutation and *MGMT* methylation reported higher frequency among those patients with CR as reported in Table 4. When response of GB patients was divided into either responders (CR, PR, SD) ($n=30$) *versus* non-responders (PD) ($n=28$) and GB patients with both *IDH1* mutations and *MGMT* methylation were combined in one group ($n=15$) *versus* those GB patients with either mutated, methylated or non in another group ($n=43$), significant level was reached as all of GB patients (15/15, 100%) with both *IDH1* mutated with *MGMT* methylated showed response to treatment as reported in Table 5.

In GBM, PFS and OS are intensely correlated, representing that PFS may be a suitable surrogate for OS. Compared with OS, PFS proposes earlier assessment and advanced statistical power at the time of analysis. Survival patterns (PFS and OS) were calculated using Kaplan–Meier curves as it is the easiest way of analyzing the survival over time in spite of all other difficulties related with subjects or situations. For every time interval, survival probability is computed as the number of

Table 4 Relation between response to treatment and investigated markers

Response	IDH1 mutant ($n=15$)	MGMT methylated (≥66%) ($n=30$)
Complete response (CR) ($n=15$)	12 (80%)	12 (40%)
Partial response (PR) ($n=7$)	3 (20%)	8 (26.7%)
Stable disease (SD) ($n=8$)	0 (0%)	5(16.7%)
Progressed disease (PD) ($n=28$)	0 (0%)	5 (16.7%)
	$X^2=35$, $P<0.0001$	$X^2=26$, $P<0.0001$

Table 5 Distribution of the response of GBM patients when IDH1 mutation and MGMT methylation were combined

Response	Either *IDH1* mutated or MGMT methylated or both are not detected (*n* = 43)	*IDH1* mutation with *MGMT* methylation (*n* = 15)
Responders (*n* = 30)	15 (34.9%)	15 (100%)
Non-responders (*n* = 28)	28 (65.1%)	0 (0%)
Statistics	$X^2 = 18.89$, $P < 0.0001$	

subjects surviving divided by the number of patients at risk.

GB patients were followed up for a median of 10 months and the estimated progression-free survival (PFS) was 13 months, while median overall survival (OS) was 16 months. Relation between survival pattern and estimated markers reported significant difference for *IDH1* mutation with PFS (log rank $X2 = 9.2$, $P = 0.002$) and OS (log rank $X2 = 8.99$, $P = 0.003$), as GB patients reported to have *IDH1* mutations revealed better PS and OS; similarly, *MGMT* methylation reported significant with PFS (log rank $X2 = 17$, $P = 0.0001$) and OS (log rank $X2 = 27$, $P = 0.0001$) as GB patients with methylated *MGMT* showed better PFS and OS as reported in Fig. 6A–D. Moreover, survival pattern for patients with *IDH1* mutation with *MGMT* methylation was better (mean PFS = 20 months, mean OS 26 months) than patients with either *IDH1* mutation or *MGMT* methylation alone (mean PFS = 10 months, mean OS = 15 months) as plotted in Fig. 7A–B.

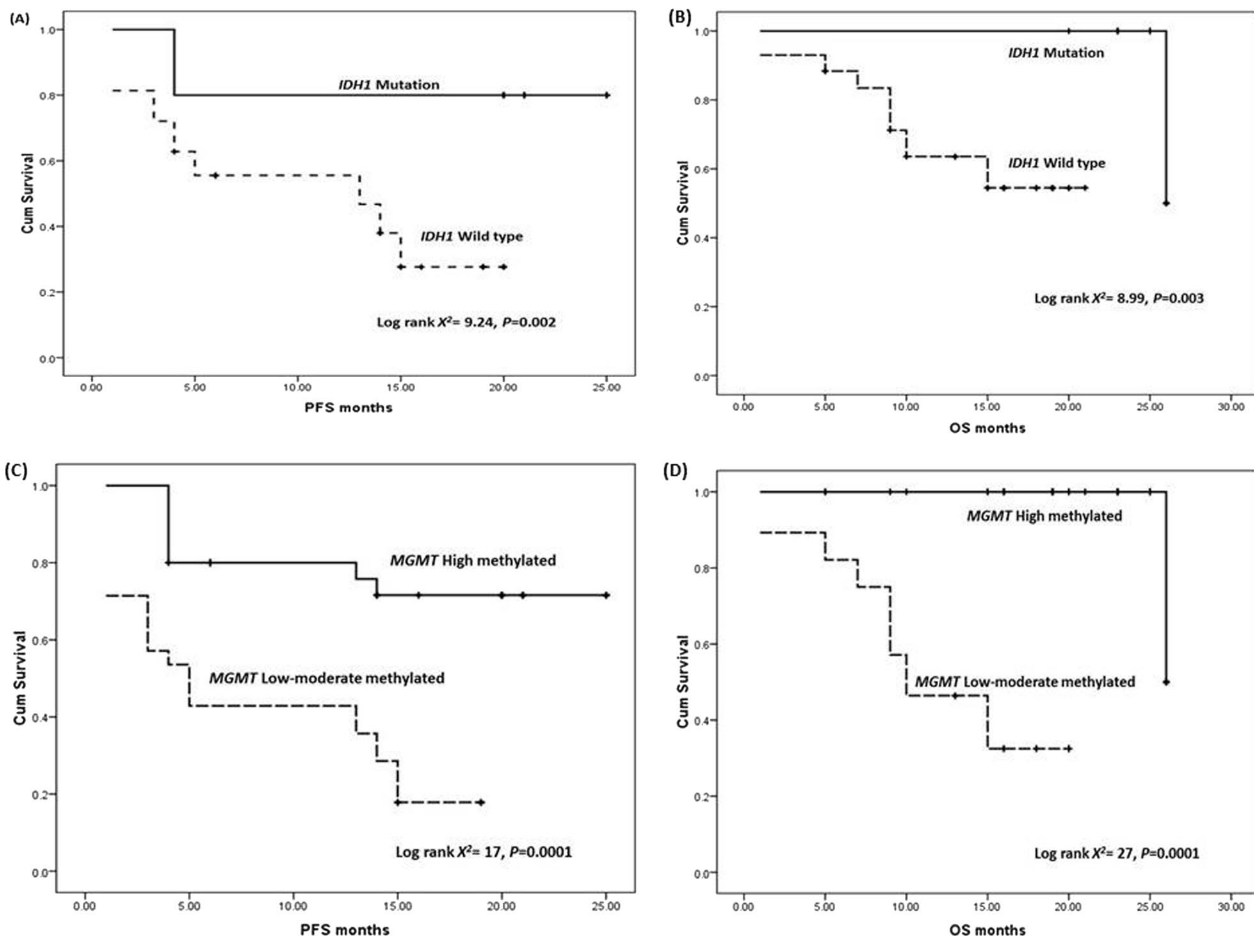

Fig. 6 **A** PFS for IDH1 mutation, **B** OS for IDH1 mutation, **C** PFS for MGMT methylation, **D** OS for MGMT methylation

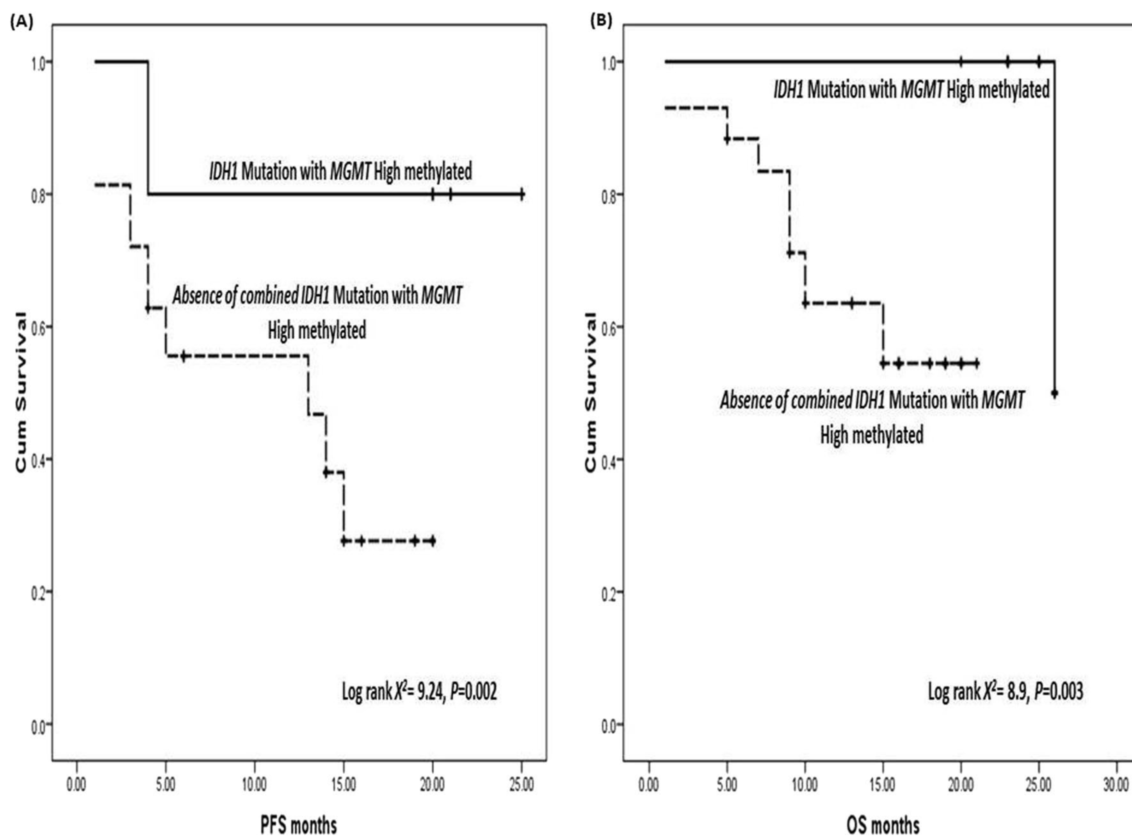

Fig. 7 **A** PFS or IDH1 mutation with MGMT methylation, **B** OS for IDH1 mutation with MGMT methylation

Discussion

Alteration of many genes has been found to be implicated in pathogenesis of GBM; hence, they may play an important role in predicting prognosis and response to treatment strategies [24]. In the current study, the role of *IDH1* gene mutation and *MGMT promoter* methylation status were investigated among Egyptian GB patients as compared to a group of NND. Among the investigated groups, no significant difference was reported between their genders; however, significant difference was reported among their ages as all NND were below 60 years. This result emphasizes the relation between the increase of GBM among elderly which agree with previous reported studies [25–27] which may be attributed to the fact that aging may gradually suppress immunosurveillance and hence contributes to GB cell initiation and/or outgrowth [25].

Sanger sequencing is considered the "gold standard" for detection of *IDH1* mutations because of its high specificity and low false positive results but with some drawbacks as low sensitivity, consumes time and high-quality tissue samples to perform the reaction in addition needs manual interpretation [28]. As it is significant to detect the occurrence of *IDH1* mutations in a rapid method, patients can gain the advantage from targeted therapies. Therefore, authors detected *IDH1* mutation using TaqMan™ competitive allele-specific probes (castPCR™) which has high sensitivity over Sanger sequencing (0.1% vs. 10–25%, respectively) [29] and high specificity as minimal quantities of mutated DNA in a sample that have large quantities of normal wild-type DNA [20] since this technique uses oligonucleotides for the mutated allele so as to repress the normal allele [30]. Accordingly, in the current study *IDH1* mutation was not detect among patients with NND 0 out of 20 individuals (0%), these results were agreed with previously reported data [31] who reported that detection of *IDH1* mutation points to the presence of glioma and it cannot be attributed to non-neoplastic diseases. For GBM cases, *IDH1* mutation was detected in 15 out of 58 (25.9%). These results are in concordance with Kalkan and his colleagues [32] who reported the presence of *IDH1* mutations in 12.5% primary GB cases which reveal that it is an early consequence in tumor genesis and this due to the fact that mutated *IDH1* reduced the action of NADPH which is important for cellular protection against oxidative stress giving rise to tumor genesis because of oxidative DNA damage [33].

Methylation status of *MGMT* is among the most studied molecular biomarkers in neuro-oncology because of its influence in therapeutic management of glioblastoma; thus, its detection has been reported using different techniques [34]. However, debate remains about the most appropriate technique to be used, in the current study authors assessed methylation status using restriction enzyme that cut the unmethylated regions and hence the detected will be the methylated (REF). Although it was previously reported in several neuro-oncological centers as 10% as the biological cutoff [35], others reported that precise cutoff value might reflect their response to treatment [36]. In the current study as for the first time NND were included, the ROC was plotted between both groups as considering NND as reference (control) group; hence, the best cutoff point was 66% methylation (< 66% as low–moderate methylation, ≥ 66% as highly methylated). By using this methylation cutoff, currently studied groups reported all NND patients with high *MGMT* methylation as compared to GB cases as 51.9% were high *MGMT* methylation. Methylation of NND patients could be attributed to the previously reported findings of Teuber-Hanselmann and his colleagues that *MGMT* hypermethylation arises in chronic neurological diseases that are not strictly associated to distinctive pathogens, oncogenic viruses or neoplasms but that lead to destruction of the myelin sheath in several ways [37].

Among the GBM cases; those reported *IDH1* mutation were of younger age (less than 60 years) than those with older ages; these results agreed with previously reported study by Kalkan and his colleagues [32], for *MGMT* methylation; significant levels were reached with factors like tumor size and tumor location which agreed with previous reports [38, 39] as GBM patients with tumor size less than 5 cm reported high methylation than others with mass more than 5 cm; moreover, it is generally recognized that tumor location, as significant image feature related to genetic features, is associated with patient prognosis [39]. Also, both *IDH1* mutation and *MGMT* methylation were reported at significant levels in GBM patients with ECGO < 2 which may indicate their usefulness as prognostication markers among GBM patients.

After patients were treated with standard of care treatment strategy, they were followed up for median 10 months, GBM patients with *IDH1* mutations reported better PFS and OS than those with *IDH1* wild type. A finding that agreed with previously reported study [32] that *IDH1* mutations can be used as a prognostic marker for primary GBM patients since it is primary event in tumorigenesis. Regarding GBM patients with *MGMT*, high methylation reported better PFD and OS as compared to those with low–moderate methylation, these result in concordance with Radke and his colleagues [36]. When GBM patients with both *IDH1* mutations and *MGMT* high methylation were considered, our results emphasized best PFS (20 months) and OS (25 months), indicating that detection of *IDH1* mutation combined with *MGMT* methylation is a better prognostic marker and estimates response of GBM patients to treatment than any of them alone this was agreed with previously reported finding [40] thus using both combined markers for predicting response to treatment and predicting survival pattern is obviously advised than using any of them alone.

Conclusion

Current study reported the superiority of combined detection of *MGMT* methylation and *IDH1* mutation among GBM as predictive and prognostic markers than using either of them alone as both reported to discriminate between non-neuro-oncological disease from GBM cases with a significant impact on prediction of GBM response to treatment. Moreover, the use of these two markers highlights significant impact as prognostic markers. In addition, the method used for *MGMT* methylation and *IDH1* mutation detection reported to be highly sensitive than previously reported techniques which may imply its applicable to be used in clinical routine for superlative as follow-up markers.

Abbreviations

ARMS	Amplification refractory mutation system
BRCA	Breast cancer gene
CTV	Clinical target volume
CR	Complete response
CT	Computerized tomography
DNA	Deoxyribonucleic acid
ECOG	Ester clinical oncology group
EGFR	Epidermal growth factor receptor
GB	Glioblastoma
HE	Hematoxylin–eosin
IDH	Isocitrate dehydrogenase
LOD	Limit of detection
MRI	Magnetic resonance imaging
NND	Non-neuro-oncological diseases
MGMT	O6-methylguanine-DNA methyltransferase
PTV-1	Planning target volume

Acknowledgements

The instruments listed in the current study were purchased through a grant from Science Technology Development Fund (STDF) through Capacity Building Grant Fund (CBG) [No. 4940], Egypt.

Author contributions

MSM and MS were involved in study conception and design. LRE and ME helped in provision of samples and clinical follow-up, AR, NB, MH and AMN contributed to acquisition of data. MSM, MS, LRE, MKK, ME and MS assisted in analysis and interpretation of data. MS, LRE, MH and AR performed drafting of manuscript. All authors helped in critical revision.

Funding

This work was supported through a grant from Science Technology Development Fund (STDF) through Basic and Applied Research Support Grant Project (BARG) [No. 25562], Egypt.

Availability of data and materials
Authors declare that no data will be available.

Declarations

Ethics approval and consent to participate
Ethical approval was obtained from Medical Ethical Committee (National Research Centre ID#20110), and patients included in the study signed their informed consent. The images included in the study are freely available on the internet and may be seen by the general public.

Consent for publication
Consent to publish has been obtained from individuals participated in the current study.

Competing interests
Authors declare no competing of interest.

Received: 18 May 2023 Accepted: 18 March 2024
Published online: 28 March 2024

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