Clinical Impact of IDH1 Mutations and MGMT Methylation in Adult Glioblastoma

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Abstract

Background. Genetic aberrations and epigenetic alterations have been reported in different types of cancer. Impact of Isocitrate dehydrogenase1 (*IDH1*) and O6-methylguanine-DNAmethyltransferase (*MGMT*) in glioblastoma (GB) 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 GB patients versus non-neurooncological diseases (NND) patients and their impact on survival criteria.

Methods. Formalin-Fixed Paraffin-Embedded (FFPE) tissue sections of 58 GB and 20 non-onconeurological diseases patients were recruited and *IDH1* mutation were detected using Cast-PCR technology and *MGMT* methylation was detected using Methyl II quantitative PCR approach. Their results were assessed with other clinicopathological criteria and correlated with survival patterns (progression free survival [PFS] and overall survival [OS]).

Results. *IDH1* mutation was detected among 15 GB cases (15/58) and it was not reported among NND (P=0.011). Receiver operating characteristic (ROC) curve were plotted to discriminate between *MGMT* methylation among studied groups. Patients with *MGMT* methylation ≥ 66% was reported as high methylation, which was recorded significantly in 51.7% and 100% of GB 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.

Background

Glioblastoma (GB) is the prevalent malignant brain tumor with poor prognosis and aggressive development without identifiable precursor lesions [1]. Advances have been made in therapeutic strategies after addition of temozolomide (TMZ) chemotherapy to maximal safe tumor resection and radiotherapy. However, median survival is still limited to 15 months [2, 3].Thus, there is a great need to unravel oncogenic mechanisms of GB since there are two types of this kind of malignancy either displayed rapidly de novo with unknown precursor 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 GB pathogenicity [5].

On the genetic level several alterations have been reported in glioblastoma as alteration in TP53 [6], truncated of activated form of EGFR (i.e. EGFRvIII) [7], deletions in *PTEN* [8] and mutation in *BRCA* genes [9]. Also, by using genome - wide sequencing it has been reported that isocitrate dehydrogenases genes (*IDH1* and *IDH2*) are mutated among GB patients especially in younger patients and among secondary GB patients [10] and were independent prognostic marker.

Moreover, GB presents with a range of epigenetic changes which are mitotically heritable alterations in gene expression that are not related to alterations in DNA sequence [11]. Among the studied epigenetic changes is CpG island hypermethylation which has been reported in several types of cancer [11–13]. O-6-methylguanine-DNA methyltransferase (*MGMT*), one of the DNA repair enzymes which participate in GB resistance to TMZ by *MGMT* methylation in the promoter region [2]. Silencing of *MGMT* has been correlated with increased survival independently of treatment strategy [14].

Although both genetic and epigenetic variations in GB have been considered independently, there is an indication that these mechanisms interact on signaling pathways, certain genes, and chromosomal domains [4].

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

After obtaining ethical approval from Medical Ethical Committee (National Research Centre ID#20110), to recruit patients who fulfilled the inclusion criteria as 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-neurological 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-neurooncological diseases (NND) were recruited (n = 20). 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 [15]. 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 nono-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 EpiTect Methyl II quantitative polymerase chain reaction (qPCR) System (Qiagen, Germany) which based on assessment of residual input DNA after cleavage with restriction enzyme 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 lab: 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) then they were incubated at 37°C for 6 h then at 65°C for 20 min using 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 mixed directly with qPCR master mix (RT2 qPCRSYBR Green/ROX Master Mix, Cat number 330520) and were dispensed into a PCR plate containing pre-aliquoted *MGMT* primeras follows Left primer ATTTTTGTGATAGGAAAAGGTATGG Right primer CTAAAACAATCTACACATCCTCACT. Real-time PCR is carried out using specified cycling conditions,95°C for 10 min (1 cycle), then 99°C for 30 and 72°C for 1 min (3 cycles), and finally 97°C for 15 sec and 72°C for 1 min (40 cycles). Finally, the raw ΔCTvalues were collected for each PCR reaction tube (UD and D) for each sample as shown in Figure (1A-B). Because in the qPCR reaction the UD was used hence the DNA in which all CpG sites are methylated will be detected by real-time PCR [16] through following equations:

ΔCT = disgestedΔCT – undigested ΔCT

Methylation %= $2^{-\Delta CT}$ fold change X100

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 [17, 18].Qualitative assessment of 6 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; cyclingstage 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* R132H (SNVs) allowing 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. 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 4 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. 3–4). II) For preparation of PCR testing, freshly cut sections of paraffin-embedded tissue, each with a thickness of up to 10 ums. Up to 8 sections, each with a thickness of up to 10 um and a surface area of up to 250 mm2, 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/m2 during the RT course, for 6 weeks followed by the sequential TMZ schedule (150–200mg/m2 for 5 days every 28 days) until disease progression (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 [19]. 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 three 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. 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, 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 [20].

Results

The current study was carried out on FFPE samples from 20 NND and 58 GB cases, their full clinical data were 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 were as represented in Table (2). By plotting the ROC curve to report the methylation status between investigated groups the best cutoff point (methylation percentage) that discriminates between them was 66%, hence those < 66 were represented as low - moderate methylation while those ≥ 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 *IDH* wild type at significant level $P = 0.011$, as shown in Table (2).

Distributions of *IDH1* mutation and *MGMT* methylation among GB cases were presented in Table (3). For *IDH1* mutations significant levels were reported between *IDH1* mutation with 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 were 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).

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 Figure (6 A-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 Figure (7A-B).

Discussion

Alteration of many genes have been found to be implicated in pathogenesis of GB, hence they may play an important role in predicting prognosis and response to treatment strategies [21]. 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 GB among elderly which agree with previous reported studies [22, 23] which may be attributed to the fact that aging may gradually suppresses immunosurveillance and hence contributes to GB cell initiation and/or outgrowth [22].

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 [24]. As it is significant to detect the occurrence of *IDH1* mutations in a rapid method thus 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% *versus* 10–25%, respectively) [25] and high specificity as minimal quantities of mutated DNA in a sample that have large quantities of normal wild-type DNA [17] since this technique uses oligonucleotides for the mutated allele so as to repress the normal allele [26]. 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 [27] who reported that detection of *IDH1* mutation points to the presence of glioma and it cannot be attributed to non-neoplastic diseases. For GB cases *IDH1* mutation was detected in 15 out of 58 (25.9%). These results are in concordance with Kalkan and his colleagues [28] 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* reduce the action of NADPH which is important for cellular protection against oxidative stress giving rise to tumor genesis because of oxidative DNA damage [29].

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 [30]. 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 [31], others reported that precise cutoff value might reflect their response to treatment [32]. 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 [33].

Among the GB 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 [28], for *MGMT* methylation; significant levels were reached with factors like tumor size and tumor location which agreed with previous reports [34, 35] as GB patients with tumor size less than 5cm reported high methylation than others with mass more than 5cm, moreover it is generally recognized that tumor location, as significant image feature related to genetic features, is associated with patient prognosis [35]. Also, both *IDH1* mutation and *MGMT* methylation were reported at significant levels in GB patients with ECGO < 2 which may indicate their usefulness as prognostication markers among GB 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 [28] that *IDH1* mutations can be used as a prognostic marker for primary GB patients since it is primary event in tumorigenesis. Regarding GB patients with *MGMT* high methylation reported better PFD and OS as compared to those with low-moderate methylation, these results in concordance with [32].When GB 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 GB patients to treatment than any of them alone this was agreed with previously reported finding [36] 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 GB as predictive and prognostic markers than using either of them alone. In addition, the method

used for *MGMT* methylation and *IDH1* mutation detection reported to be highly sensitive than previously reported techniques.

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-neurooncological diseases; *MGMT*, O6-methylguanine-DNAmethyltransferase, PTV-1, planning Target Volume.

Declarations

Ethics approval and consent to participate: Ethical approval of the study was obtained from the Medical Ethical Committee of National Research Centre (ID#20110), Egypt, and all participants signed their informed consent.

Consent for publication: All authors agreed for sending the paper for publication.

Availability of data and material: no data available to be shared.

Competing interests: Authors declare no competing of interest.

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Authors' contributions: Study conception and design: MSM and MS. Provision of samples and clinical follow-up: LRE and ME, Acquisition of data: AR, NB, MH and AMN. Analysis and interpretation of data: MSM, MS; LRE, MKK, ME and MS. Drafting of manuscript: MS; LRE; MH and AR. Critical revision: all authors.

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Tables

Table (1): Clinical and demographic data for studied cases.

Factors	Non-Neurooncological patients (n=20)	GBM (n=58)
Age (Mean)		
< 60 years	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		
< 5cm		22 (37.9%)
≥ 5cm		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-Neurooncological 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	

Table (3): Distribution of *IDH1* mutation and *MGMT* methylation status among GBM cases.

Factors	IDH1 Mutant	MGMT methylated (≥66%)
Age (Mean)		
< 60 years	0 (0%)	24 (8%)
≥ 60 years	15 (100%)	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		
< 5cm	7 (46.7)	17 (56.7%)
≥ 5cm	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

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	

Figures

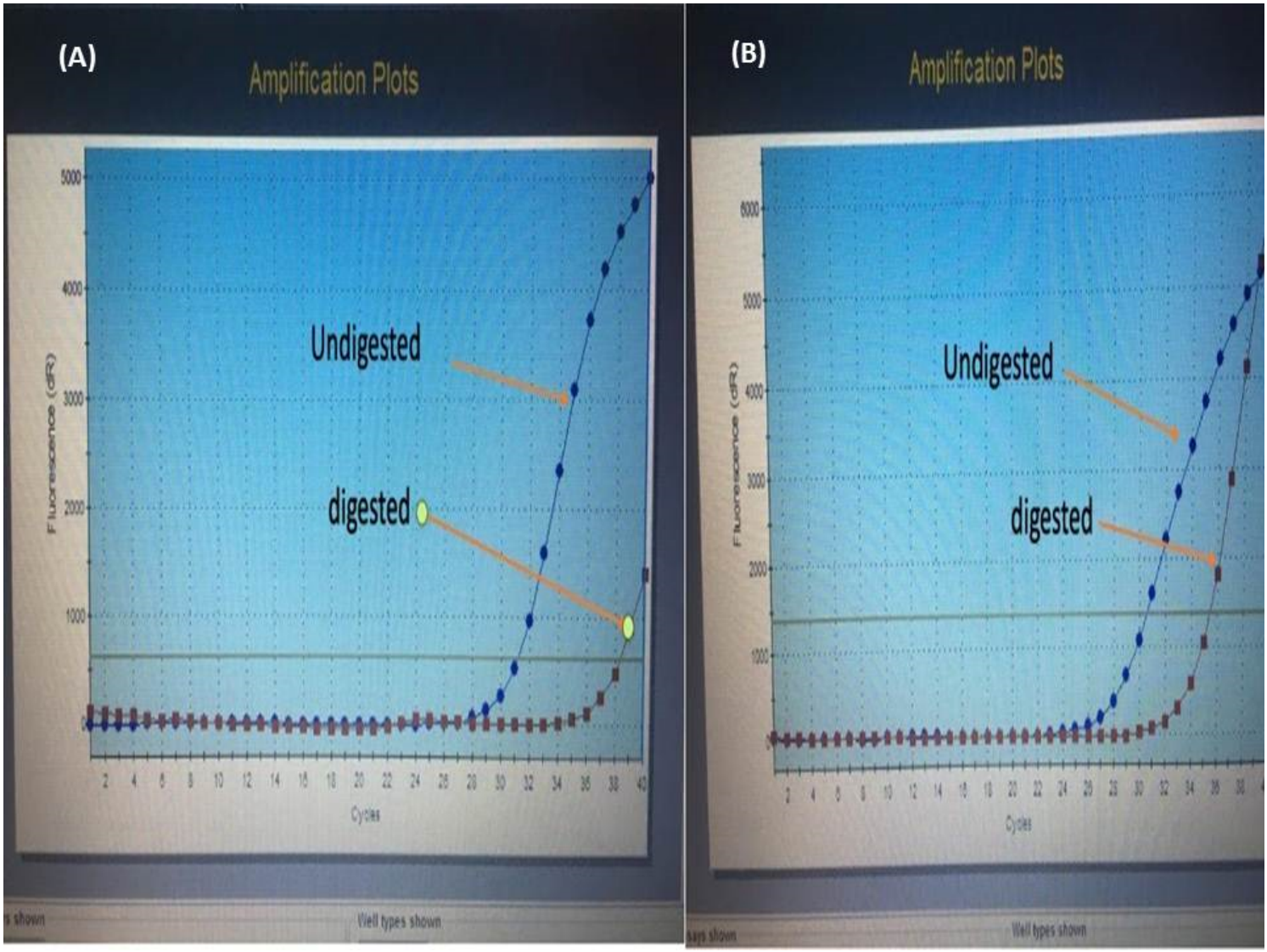

Figure 1

Amplification plots of the *MGMT* qPCR reaction, A: showing plots of 100 % unmethylated sample, and B showing 12.5 % methylated sample.

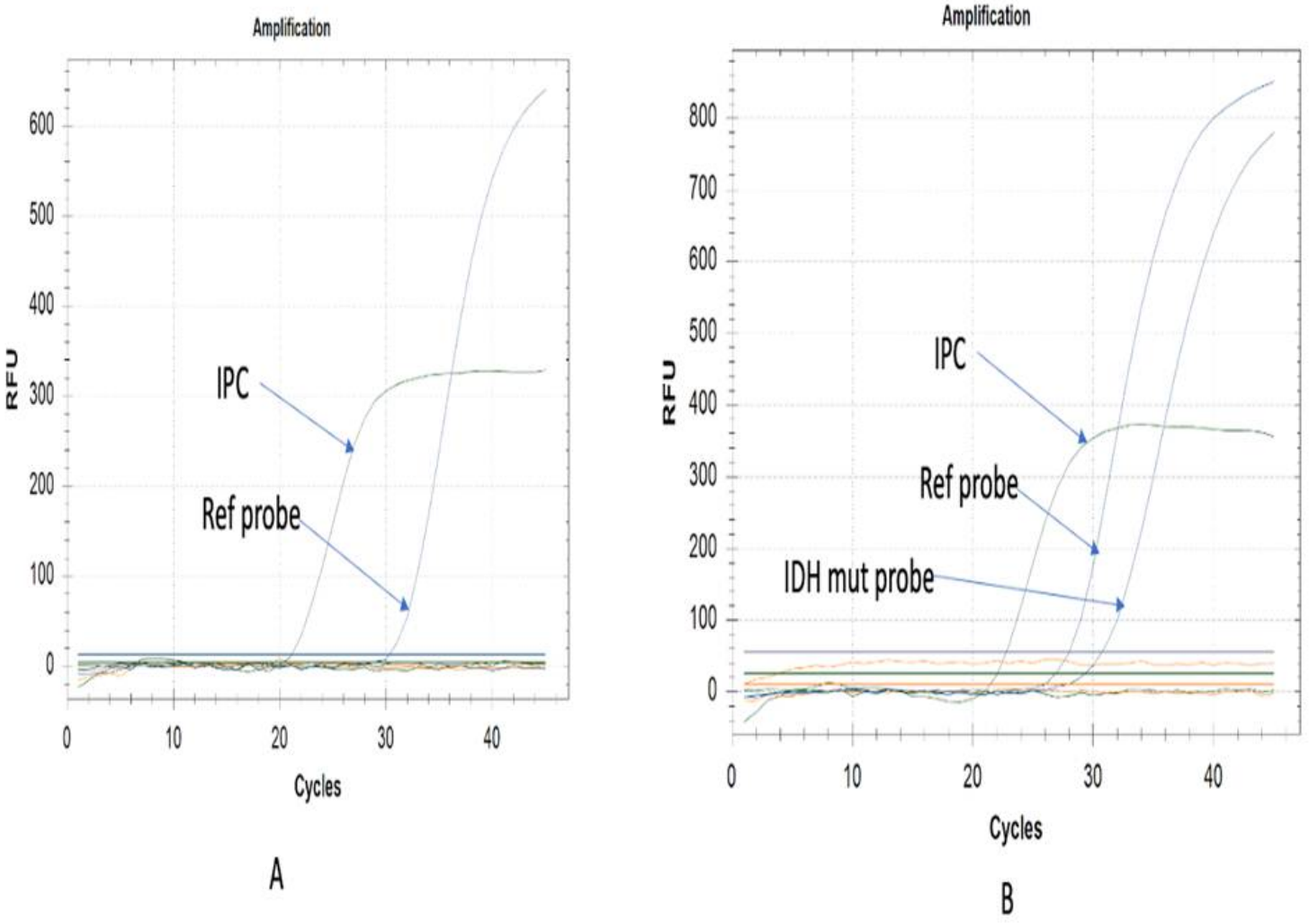

Figure 2

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

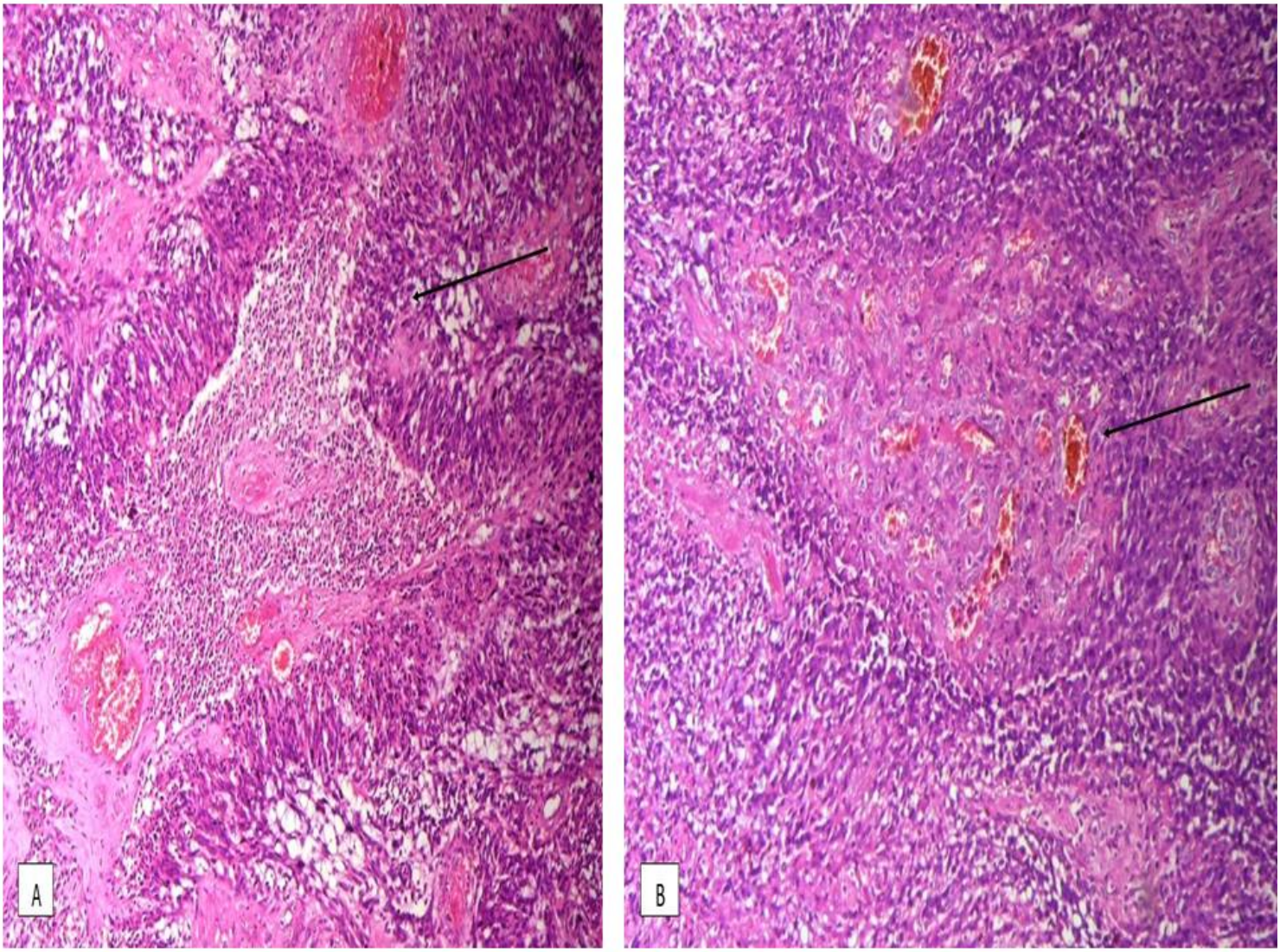

Figure 3

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

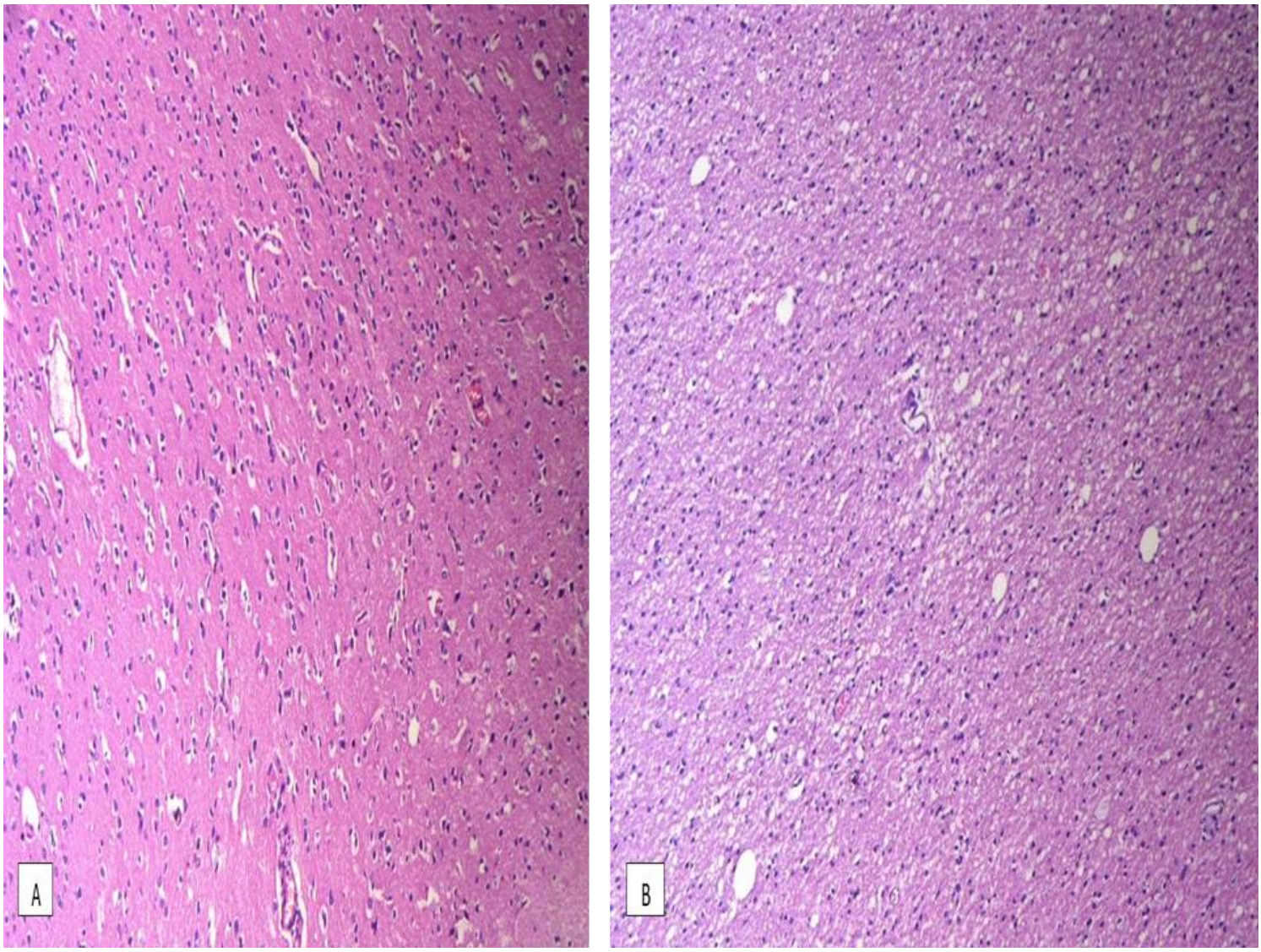

Figure 4

Non-neurooncological diseases, Reactive gliosis (A and B).

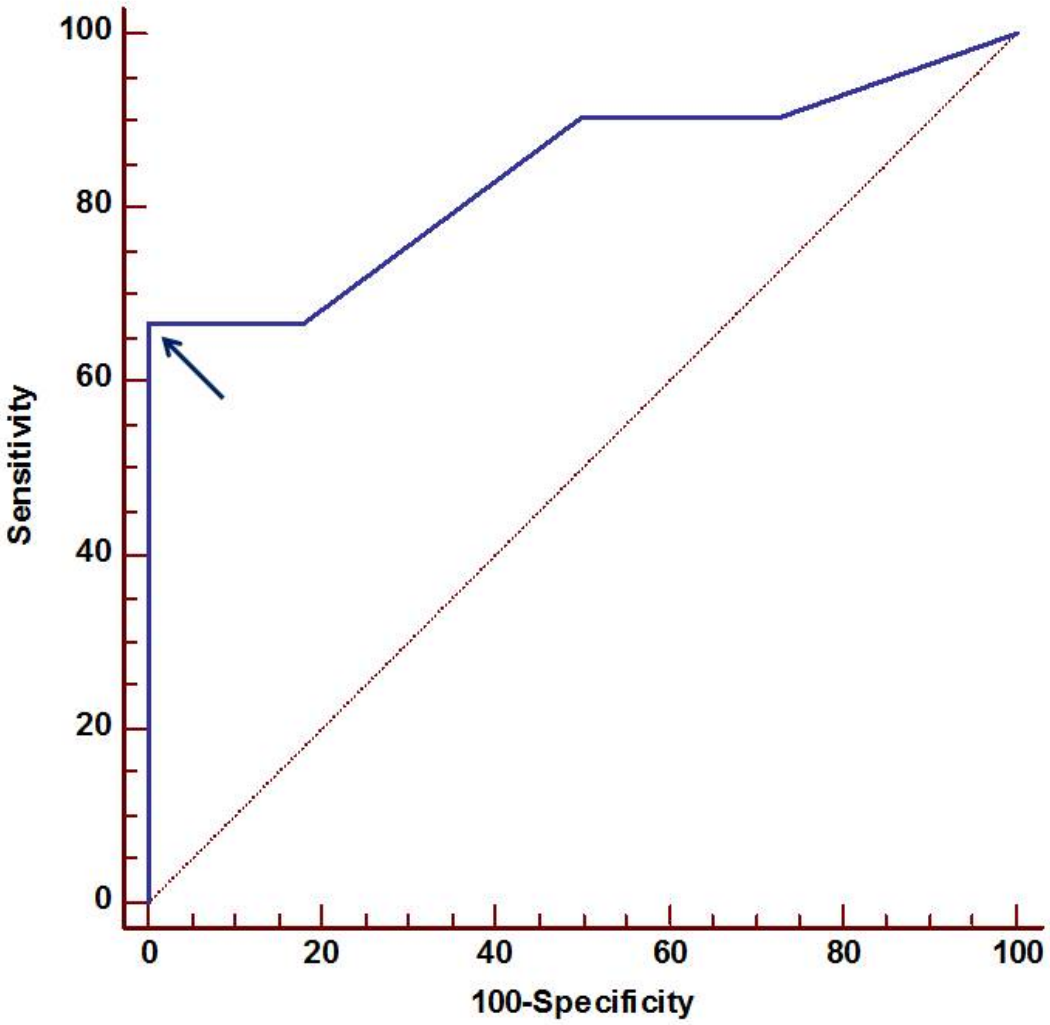

Figure 5

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.

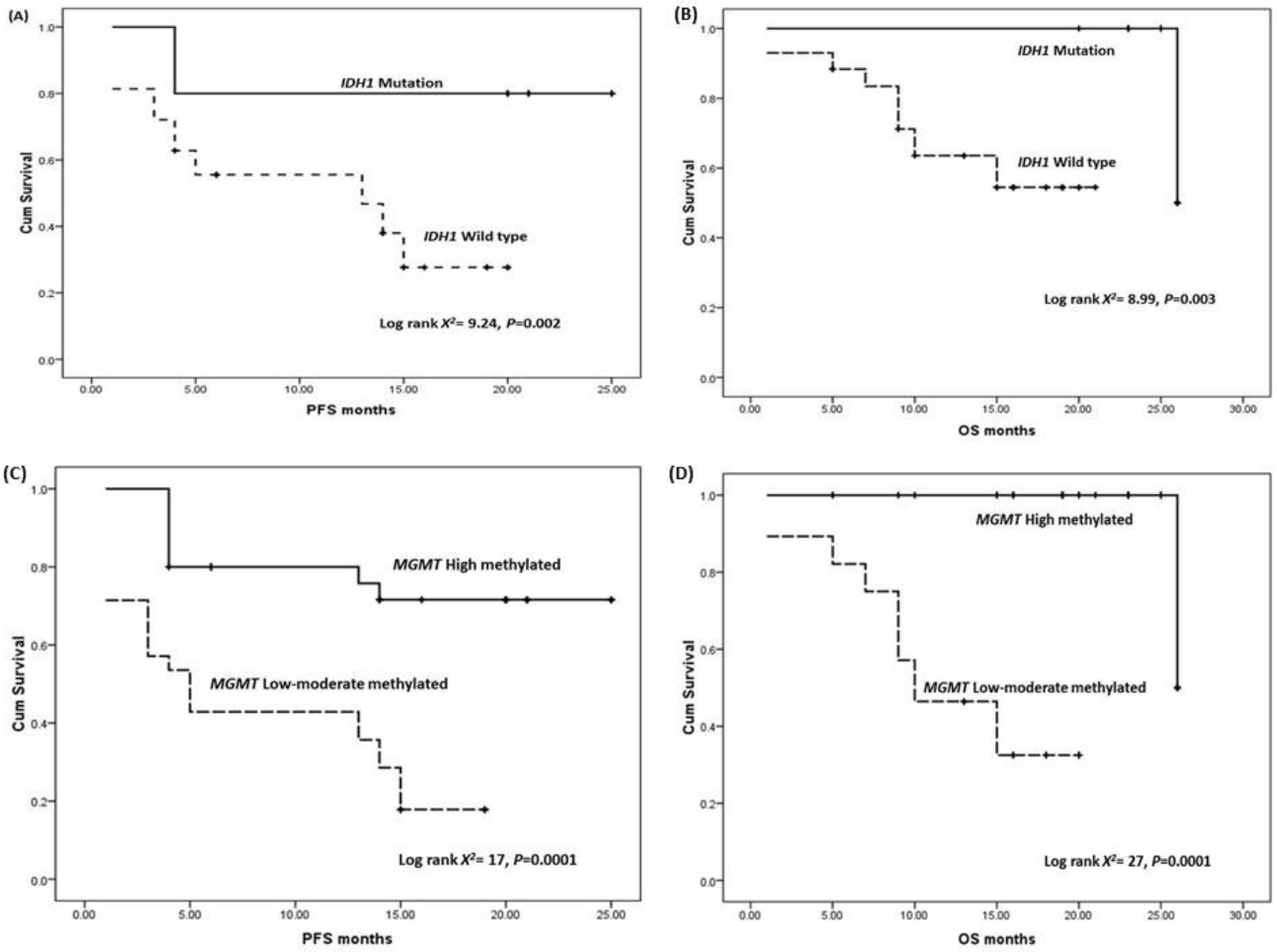

Figure 6

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

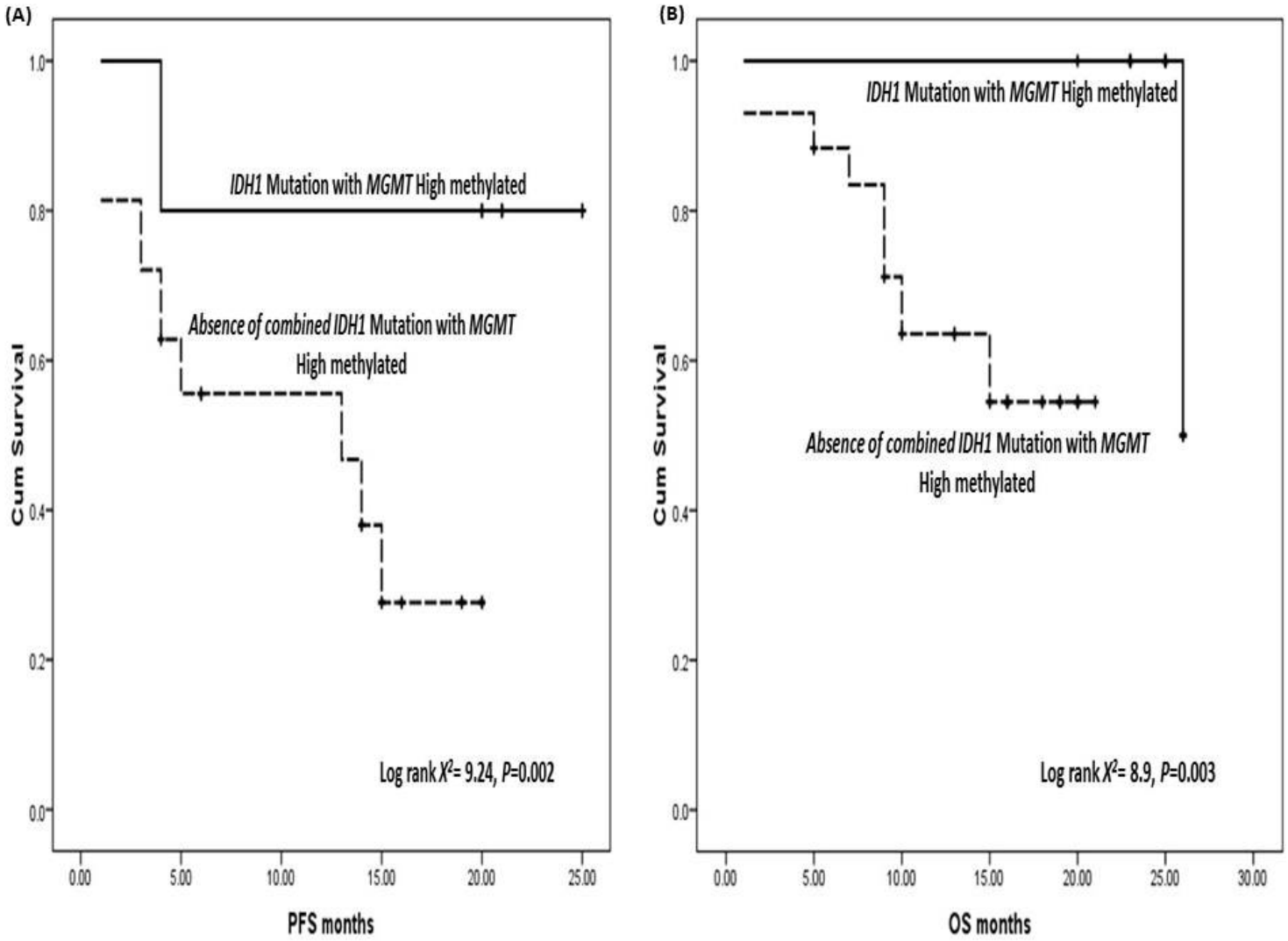

Figure 7

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