RESEARCH ARTICLE

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Immunohistochemical Expression of PD-L1 and IDH1 with Detection of MGMT Promoter Methylation in Astrocytoma

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Abstract

Objective: Programmed death ligand 1 (PD-L1) expression was suggested as a poor prognostic predictor for glioblastoma. While isocitrate dehydrogenase (IDH) has been linked to enhanced overall survival in glioma cells. In glioblastoma patients receiving treatment with alkylating drugs, the methylguanine-DNA methyltransferase (MGMT) promoter's methylation status has been discovered as a potent and distinct predictor of good survival. In this study, we aimed to investigate the expression rate of PD-L1, IDH1, and MGMT methylation in patients with different grades of astrocytoma. **Methods:** The present retrospective study retrieved the data and archived paraffin blocks of 60 cases of astrocytoma. Immunohistochemical evaluation was done to assess the expressions of PD-L1 and IDH1, Methylation-specific-PCR was used to investigate the MGMT promoter. **Results:** This study included astrocytoma grade II 18% (11/60), grade III 22% (13/60), grade IV 60% (36 cases). PD-L1 expression was detected in 82% of all studied cases (49/60) while IDH1 mutant astrocytoma were 73% (44/60) & methylation was reported in 58.3% (35 cases). High grade astrocytoma showed highrer expression of PD-L1 & IDH1 but with insignificant correlation (p=0.989). **Conclusion:** There is a relatively high expression of PD-L1 and IDH1 in patients with astrocytoma. More than half of the patients presented with MGMT promoter methylation. Further studies with larger sample size are required to investigate the association between these biomarkers and characteristics of patients with astrocytoma.

Keywords: Astrocytoma- PD-L1- IDH1- MGMT Promoter Methylation

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Introduction

Gliomas are the most common central nervous system (CNS) tumors, which are classified into astrocytic, oligodendroglia, oligoastrocytoma, and other tumors (komori, 2017; Benmelouka et al., 2021). It was found that 35.5% of all CNS tumors in Egypt were gliomas (Zalata et al., 2012). The World Health Organization (WHO) grading categorizes gliomas into four phenotype and genotype classes I, II, III, and IV (Louis et al., 2016). WHO grade IV glioblastoma is the most prevalent subtype and is characterized as a malignant tumor with higher mortality risk (Berghoff et al., 2015). Astrocytoma is considered to be CNS tumor with poor prognosis especially glioblastoma (Martinez-Garcia et al., 2018). Although low grade astrocytoma has better prognosis, it finally progresses to higher grade. The main therapeutic modality is comprehensive treatment including surgical resection, radiotherapy and chemotherapy (Paolillo et al., 2018).

Both Programmed Death- Ligand 1 (PD-L1) and MethylGuanine Methyle Transferease (MGMT) methylation are used as a treatment guidance (July et al., 2020). PDL1 and PD-1 antibodies were approved by the United States Food and Drug Administration (U.S. FDA) for the treatment of multiple cancer types (Cha et al., 2019) and are gradually becoming an effective therapeutic strategy for glioblastoma (Wang et al., 2020). Clinical studies of PD1/PD-L1 checkpoint inhibitors for treating glioma are required since PD-L1 is elevated in glioblastoma and predicts considerably poorer patient survival. Several forms of cancer, including bladder cancer, lung cancer, and melanoma, have responded well to a new family of immune modulatory antineoplastic drugs known as immune checkpoint inhibitors (Topalian et al., 2015). To enhance the antitumor T-cell immune response, these drugs work by inhibiting immunosuppressive receptors.

Isocitrate dehydrogenase (IDH) is crucial for cellular energy metabolism. The most frequently altered gene in grade II–III glioma and secondary glioblastoma is IDH1, which has been linked to enhanced overall survival in glioma cells (Philip et al., 2018). The IDH1 mutation lowers the capacity of cancer to proliferate and invade while increasing its susceptibility to treatment. Additionally, it was shown that IDH1 mutations in various stages of glioma had a better prognosis than their wild-type

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IDH1 counterparts. It was proposed that IDH1 mutations may be optimized as novel targets to enhance the prognosis of glioma patients in light of these results (Cohen et al., 2013). Our understanding of the origins of gliomas, the assessment of their prognosis, and the formulation of effective treatments have all been significantly improved by the discovery of molecular genetic biomarkers (Bleeker et al., 2012). DNA repair protein O6, which is also known as methylguanine-DNA methyltransferase (MGMT), is one of these molecular biomarkers. Reduced MGMT expression and a lower ability to repair DNA are linked to the epigenetic silencing of the gene via the methylation of the promoter, which increases the sensitivity to alkylating agents (Cabrini et al., 2015). In glioblastoma patients receiving treatment with alkylating drugs, the MGMT promoter's methylation status has been discovered as a potent and distinct predictor of good survival (Rivera et al., 2010). In this study, we aimed to investigate the expression rate of PD-L1, IDH1, and MGMT methylation in patients with glioma.

Materials and Methods

Study Design and Patients'data

The present retrospective study retrieved the data and archived paraffin blocks of 60 cases of astrocytoma from the Pathology Department of Kasr Al-ainy University Hospital, Cairo, Egypt. The data were collected through the period from December 2018 to December 2019. Cases were included if they have confirmed diagnosis of grade II-IV glioma, adequate viable tissue for PD-L1 testing (≥ 100 tumor cells), and blocks with high tumor cell content (>80%). We excluded cases with missing demographic data, cases with non-diffuse types of astrocytoma, and cases with significant oligodendroglial components.

The following data were collected from the records of eligible cases before our histopathological reevaluation and immunohistological staining: sex, age at presentation, site of tumor, histologic diagnosis of the tumor, World Health Organization (WHO) grading, and state of recurrence.

Histopathological Examination

The paraffin blocks were sectioned at 3-4 μm thick and stained with routine Hematoxylin and Eosin stain. The grading of the tumor and histopathological classification were done in concordance with the WHO classification of central nervous system tumors 2007 .

Immunohistochemical Evaluation

Two other 3-4 μm thick sections were placed into positively-charged glass slides. After xylene deparaffinization, slices were hydrated in graded alcohols, distilled water, and PBS. Peroxidase was blocked with 3% H2O2. Microwave heating in citrate buffer extracted antigens from slides (pH 6.0). The sections were incubated for one hour at room temperature with PD-L1 (abbexa, cambeirage, UK) and IDH 1 (abbexa, cambeirage, UK) monoclonal mouse antibodies. After washing in PBS, samples were incubated with a biotin-conjugated secondary antibody and the Dako Envision system (Dako Envision labelled polymer, peroxidase) was employed for detection. Haematoxylin was used to stain the counters, which were subsequently rinsed in tap water, ethyl alcohol, and pure alcohol. After drying the slides, cover slips were fixed with DPX [a combination of distyrene, tricresyl phosphate, and xylene]. Positive control sections for PD-L1 and IDH1 were human tonsillitis and liver cancer tissue respectively.

Semiquantitative evaluation of the slides was performed where more than 1% of tumor cells with membranous or fibrillary PD-L1 staining were declared positive, while in IDH1 stained slides, cytoplasmic staining of any density in more 10% of stained tumor cells was considered positive. Positive cases were considered IDH mutant, while negative cases were considered not otherwise specified (NOS) as IDH mutations weren't properly sequenced.

PCR

Using the QIAamp DNA Mini Kit (Qiagen), genomic DNA was extracted from tumor paraffin slices. NanoDrop 2000 measured DNA (Thermo Fisher Scientific). Bisulfite-treated genomic DNA (1 g) was used to convert unmethylated cytosine to uracil, leaving 5-methylcytosine unmodified. Methylation-specific-PCR was used to investigate the MGMT promoter (MS-PCR). MS-PCR was used to amplify methylation and unmethylated MGMT promoter alleles.. The primer sequence for unmethylated reactions was 5′-TTTGTGTTTTGATGTTTGTAGGTTTTTGT-3′(forward), 5′AACTCCACACTCTTCCAAAAACAAAACA-3′ (reverse); for methylated reactions it was 5′-TTTCGACGTTCGTAGGTTTTCGC-3′ (forward), 5′- GCACTCTTCCGAAAACGAAACG-3′ (reverse).

The polymerase chain reaction (PCR) was run on a 2720 thermal cycler (Applied Biosystems). Initial melting was performed for 10 minutes at 95°C, followed by 35 cycles of 30 seconds at 95°C, 30 seconds at 60°C, and 60 seconds at 72°C, and finally, a seven-minute elongation at 72°C. The amplification products were put onto 16 percent polyacrylamide gels and observed by UV light ethidium bromide staining. At 93 bp, an unmethylated state, the bands appeared on the polyacrylamide gel. Alternatively, bands at 81 bp are regarded as methylated.

Statistical Analysis

Data were analyzed using the SPSS V0.25 software for Windows. We used frequencies to summarize categorical data, while continuous data were presented as mean ± standard deviation (SD). The association between categorical variables was tested using the Chi-square test at a $p < 0.05$ for statistical significance.

Results

Sixty patients were included, with slight female predominance (52%). The mean age of the study group was 41.85 ±14.1 years; the majority of the cases were less than 50 years old (61.7%). The most common site was

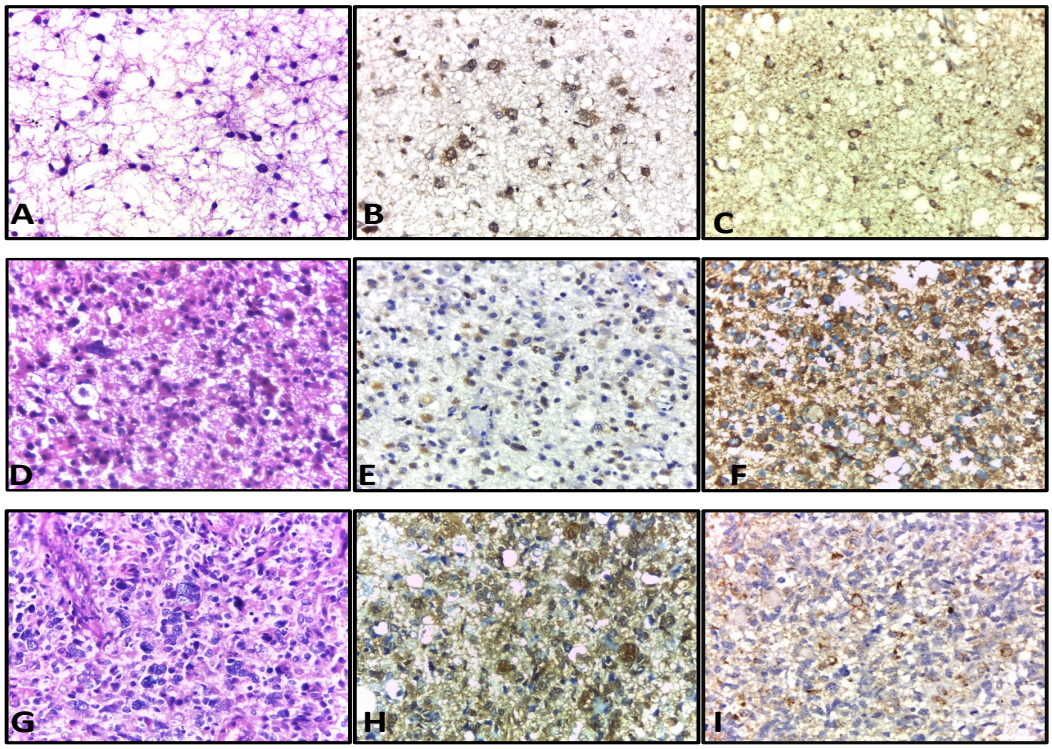

Figure 1. Different Grades of IDH Mutant Astrocytoma. figures (A, D & G); low grade astrocytoma with fibrillary background (WHO G II), anaplastic astrocytoma with neoplastic astrocytes (WHO grade III) and glioblastoma (WHO grade IV) respectively (H&E; (A)X400, (D)X400 &(G)X400 original magnification). Figures (B,E & H) show positive cytoplasmic staining by IDH 1 antibody in > 10% of neoplastic astrocytes (IHC; (B)X400, (E)X400 & (H) X400 original magnification). Figures (C,F& I) show positive membranous staining of > 1% of tumor cells (IHC; (B) X400, (E)X400 & (H)X400 original magnification).

Table 1. Demographic and Clinicopathological Characteristics of the Patients

Variables	Patients (N =60)
Age in years, mean ±SD	41.85 ± 14.1
Age group. No. (%)	
<50 years	37 (61.7%)
>50 years	23 (38.3%)
Gender	
Female, No. (%)	31 (52%)
Male, No. (%)	29 (48%
Tumor anatomic Site, No. (%)	
Frontal	13 (20.3%)
Temporal	14 (23.7%)
Parietal	18 (30%)
Occipital	1 (1.7%)
> One Segment[1]	10 (16.7%)
Others	4 (6.7%)
Tumor grade, No. (%)	
Low grade (Grade II)	11(18%)
High grade Grade III & IV)	49 (82%)
Recurrent glioblastoma cases[2], No. (%)	
Recurrent	7 (19.4%)
Newly diagnosed	29 (81%)

[1], It represent tumors involving more than one lobe as pareito-occipital, frontoparietal or temproparital; [2], All recurrent cases were glioblastoma, but not all glioblastoma cases were recurrent. 29 glioblastoma cases were newly diagnosed.

the parietal region (30%), followed by the temporal and frontal regions (23.7% and 20.3%, respectively). Overall, 36 cases were diagnosed as glioblastoma (60%), 13 were anaplastic astrocytoma (22%), and 11 cases were diffuse astrocytoma (18%) according to WHO classification. Regarding the recurrence state of astrocytoma cases, seven cases (19.4%) were reported as recurrent, and all were diagnosed as glioblastoma (Table 1).

The positive expression of PD-L1 was identified in 49 cases (81.73%) of astrocytoma (Figure 1). On the other hand, IDH1 immunohistochemical expression was detected in 44 cases (73.3%) of astrocytoma (Figure 1). On detecting MGMT promoter methylation by MS-PCR, 35 cases (58.3%) were methylated (Figure 2).

There was no statistically significant association between high-grade astrocytoma and age (P-value = 0.350). In studying the relationship between tumor grade and PD-L1 expression, a higher expression of PD-L1 was detected in high-grade astrocytoma (81.6%) than in low-grade cases (18.4%). Yet, this relation was statistically insignificant (P value= 0.989). Concerning the relation between IDH1 expression and tumor grade, a higher rate of IDH1 expression was noticed in high-grade cases (84.1%) compared to low-grade cases (15.9%). However, this relation was statistically insignificant (P value=0.421). Similarly, a higher rate of methylated cases was detected among high-grade cases (80%). But it was a statistically insignificant relation as well (P value=0.748), Table 2.

It was found that all seven recurrent cases were PD-L1 positive (100%); still, high expression among newly

Table 2. Association between Tumor Grade and Other Variables

		Tumor grade		P value
		Low grade	High grade	
Age	< 50, No. (%)	8 (21.6%)	29 (78.3%)	0.35
	≥50, No. (%)	3 (13%)	20 (87%)	
PD-L1 expression	Positive, No. (%)	9 (18.4%)	40 (81.6%)	0.989
	Negative, No. (%)	2 (18.2%)	9 (81.8%)	
IDH 1 expression	Positive (IDH1 mut), No. (%)	7 (15.9%)	37 (84.1%)	0.421
	Negative (IDH1 NOS),No. (%)	4 (25%)	12 (75%)	
MGMT promoter methylation	Methylated, No. (%)	7 (20%)	28 (80%)	0.748
	Unmethylated, No. (%)	4 (16%)	21 (84%)	

diagnosed cases was detected (79.3%; P value= 0.317). As for the relation between IDH1 expression and state of recurrence in glioblastoma cases, it was noticed that most of the recurrent cases (85.7%) were IDH1 mutant. In comparison, 72.4% of newly diagnosed glioblastoma were IDH1 mutant. Again, this relation was statistically insignificant (P value= 0.652). There was no significant correlation between methylation status and state of recurrence in glioblastoma cases (Table 3).

Discussion

In this study, our findings showed that PD-L1 was identified in 81.73% of astrocytoma cases, while IDH1 was detected in 73.3%. Similarly, both Xue et al., (2017) and Bolly et al., (2021) reported expression rate of PD-L1 (78% and 70%, respectively), which was close to our results. As well, Nazari et al., (2020), and Bolly et al., (2021), reported higher PD-L1 expression among the high-grade group (100% and 87.5%, respectively). A lower rate of IDH1 mutation was reported by Nazari et al. (2020) (20%) and July et al., (2020) (17%), which could be attributed to the larger sample size. Another study showed a higher rate of expression where 90.7% of studied cases showed IDH1 mutation (Berghoff et al., 2015). This study did additional genetic sequencing of IDH1 and IDH2 genes to detect less common forms of IDH1 mutations. Regarding MGMT promoter methylation, 58.3% of the cases were methylated, which was close to Bell et al., (2018) and Chen et al., (2022), in which 62% and 55.3% of studied cases were methylated, respectively. On the contrary, Berghoff et al., (2015) and July et al., (2020)

Table 3. Association between Recurrence (Glioblastoma Cases) and Other Variables

		Recurrence		P value
		Recurrent	Newly diagnosed	
PD-L1 expression	Positive, No. (%)	7 (100%)	23 (79.3%)	0.317
	Negative, No. (%)	0 (0%)	6 (20.7%)	
IDH 1 expression	Positive (IDH1 mut) , No. (%)	6 (85.7%)	21 (72.4%)	0.652
	Negative (IDH1 NOS) , No. (%)	1 (14.3%)	8 (27.6%)	
MGMT promoter methylation	Methylated, No. (%)	4 (57.1)	17 (58.6%)	1
	Unmethylated, No. (%)	3 (42.9%)	12 (41.4%)	

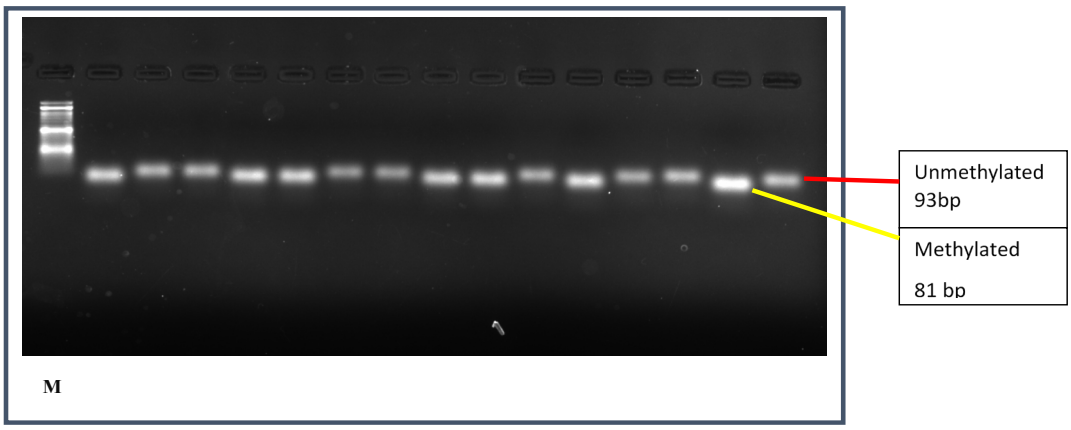

Figure 2. A Polyacrylamide Gel Electrophoresis Showing PCR Product of MGMT Methylation. Lane M represents DNA ladder with 100 bp. Yellow line shows MGMT methylation with 81 bp. Red line represent unmethylation with 93 bp.

showed a higher presentation of unmethylated cases (53% and 70%, respectively). This discrepancy in results could be related to variable sample sizes among studies.

We could not identify any association between tumor grade and PD-L1 expression (P = 0.989), IDH1 expression (P=0.421), or MGMT promoter methylation (P= 0.748). Moreover, there was no significant association between the recurrence rate and PD-L1 expression (P = 0.317), IDH1 expression (P=0.652), or MGMT promoter methylation (P= 1.00). Bolly et al.,(2021) reported that most of the high-grade studied astrocytoma cases were IDH1 wild. This could be explained by most of our cases may arise from lower-grade tumors, which unfortunately were unavailable in our records. This study showed a high prevalence of MGMT promoter methylation among high-grade cases (80%). This is consistent with July et al., (2020), who reported that MGMT promoter methylation was more frequent in high-grade astrocytoma (70%). Another study by Kim et al., (2018) reported a lower methylation rate among high-grade astrocytoma (53.3%). Upon studying the relationship between recurrent glioblastoma cases and PDL1 expression, 100% of recurrent glioblastoma cases were PD-L1 positive. This was similar to what was stated by Nazari et al., (2020). Moreover, 72.4% of newly diagnosed glioblastoma were IDH1 mutant in our study; Nazari et al., (2020) reported a slightly higher percentage of IDH1mutant newly diagnosed glioblastoma (83%); this study has a larger sample size (152 glioblastoma cases) compared to our study (36 glioblastoma cases). Regarding the relation between recurrent glioblastoma cases and MGMT promoter methylation in this study, 57.1% of recurrent cases were methylated, while 42.9% were unmethylated. Newly diagnosed glioblastoma showed close results (58.6%) methylated and 41.4% unmethylated. Other studies in the literature were concerned with methylation status changes between initial and recurrent tumors; this could not be applied to our study due to the limitation of proper institutional reporting system. In this study, IDH1 mutant astrocytoma showed a higher rate of PD-L1 expression (77.5%) than IDH1 NOS cases (22.5%). This relation was statistically insignificant. On the contrary, other studies in the literature showed more frequent PD-L1 expression in IDH1 wild-type tumors than in IDH1 mutant ones [5, 20]. This difference might be due to a higher IDH mutation percentage in our studied cases (73.3%). Higher PD-L1 expression was also detected in IDH1 mutant low-grade cases (66.7%) and IDH1 mutant high-grade cases (80%) in relation to IDH1 NOS cases. On the contrary, Wang et al., (2016), Berghoff et al., (2015), and Bolly et al., (2021) reported that IDH wild-type astrocytoma is associated with significantly higher PDL1 expression in all grades of astrocytoma. This discrepancy could be attributed to the that these studies used different both anti IDH1 clones and anti-PD-L1 clones, in addition to the high prevalence of IDH mutation in our cases. Kaminska et al. (2019) found that IDH wild-type astrocytoma is associated with significantly higher PD-L1 expression in all grades of gliomas, so it was likely to be more immunologically active and more suitable for PD-L1 immunomodulator therapy compared to mutant IDH, which could explain the importance of evaluating the molecular status of IDH1/2 in immunotherapy. We also reported that MGMT promoter methylation was detected in (61.4%) IDH1 mutant astrocytoma cases. Similar findings were reported by Pandith et al., (2020), who found that 60.5% of IDH1 mutant astrocytoma cases were methylated. In studying MGMT promoter methylation and IDH1 mutant status in low-grade and high-grade astrocytoma, methylated low-grade astrocytoma (GII) was reported in 85.7% of IDH1 mutant cases. This agreed with Tanaka et al., (2015), who reported a higher rate of methylation among IDH1 mutant low-grade cases (72.7%). In high-grade astrocytoma cases included in this study, 56.8% of IDH1 mutant cases were methylated. Another study reported a contradictory higher methylation rate in IDH1 wild high-grade cases (Molenaar et al., 2014). Those contradictory results could be explained by the usage of the different clones of IDH1 antibody, and most of our IDH1 mutant high grade arose from the previous low-grade initial; however, no records were available to prove that. Based on our data, the rate of PD-L1 positive astrocytoma was high in methylated cases (63.3%) compared to unmethylated cases (36.7%). Also, a higher rate of PD-L1 expression was reported among methylated high-grade cases (62.5%) and methylated low-grade cases (66.7%). However, these correlations were statistically insignificant and were not evaluated by other comparative studies. The limitations of this study included the lack of patient follow-up and correlation with the patient's prognosis and survival and the lack of previous grades of recurrent cases. Additionally, detection of IDH1 and IDH2 mutation by gene sequencing (as recommended by WHO) could not be performed.

In conclusion, our study showed a relatively high expression of PD-L1 and IDH1 in patients with astrocytoma. All recurrent glioblastoma were PD-L1 positive, most of them were IDH mutant and more than half were MGMT methylated. Further studies with larger sample size are required to investigate the association between these biomarkers and prognosis, overall survival and response to immunotherapy.

Author Contribution Statement

All authors contributed efficiently to the research and approved the manuscript.

Acknowledgment

Approval

This research was approved by by the research committee of pathology department, faculty of medicine, Cairo university and Kasr alainy research ethics committee (REC).

Ethical Declaration

This study obtained the approval of REC that conduct according to appropriate local and institutional regulation.

Data Availability

All data is available upon request according to insititutional regulation and with official permission.

Study registrartion

This study isn't registered in any database (clinical trial, guidelines or metaanalysis).

Conflict of interest

The authors declare that they have no conflict of interest.

References

Bell EH, Zhang P, Fisher BJ, et al (2018). Association of MGMT promoter methylation status with survival outcomes in patients with high-risk glioma treated with radiotherapy and temozolomide: an analysis from the NRG oncology/RTOG 0424 trial. *JAMA Oncol*, **4**, 1405-9.

Benmelouka AY, Munir M, Sayed A, et al (2021). Neural stem cell-based therapies and glioblastoma management: Current evidence and clinical challenges. *Int J Mol Sci*, **22**, 2258.

Berghoff AS, Hainfellner JA, Marosi C, Preusser M (2015). Assessing MGMT methylation status and its current impact on treatment in glioblastoma. *CNS Oncol*, **4**, 47-52.

Berghoff AS, Kiesel B, Widhalm G, et al (2015). Programmed death ligand 1 expression and tumor-infiltrating lymphocytes in glioblastoma. *Neurooncol*, **17**, 1064-75.

Bleeker FE, Molenaar RJ, Leenstra S (2012). Recent advances in the molecular understanding of glioblastoma. *J Neuro Oncol*, **108**, 11-27.

Bolly HM, Faried A, Hermanto Y, et al (2021). Analysis of mutant isocitrate dehydrogenase 1 immunoexpression, Ki-67 and programmed death ligand 1 in diffuse astrocytic tumours: study of single center in Bandung, Indonesia. *J Korean Nerurosurg S*, **64**, 100.

Cabrini G, Fabbri E, Lo Nigro C, Dechecchi MC, Gambari R (2015). Regulation of expression of O6-methylguanine-DNA methyltransferase and the treatment of glioblastoma. *Int J Oncol*, **47**, 417-28.

Cha JH, Chan LC, Li CW, Hsu JL, Hung MC (2019). Mechanisms controlling PD-L1 expression in cancer. *Mol Cell*, **76**, 359-70.

Chen S, Xu Y, Ye M, et al (2022). Predicting MGMT Promoter Methylation in Diffuse Gliomas Using Deep Learning with Radiomics. *J Clin Med*, **11**, 3445.

Cohen AL, Holmen SL, Colman H (2013). IDH1 and IDH2 mutations in gliomas. *Curr Neurol Neurosci*, **13**, 1-7.

July J, Patricia D, Gunawan PY, et al (2020). Clinicopathological associations and prognostic values of IDH1 gene mutation, MGMT gene promoter methylation, and PD-L1 expressions in high-grade glioma treated with standard treatment. *Pan Afr Med J*, **36**.

Kaminska B, Czapski B, Guzik R, Król SK, Gielniewski B (2019). Consequences of IDH1/2 mutations in gliomas and an assessment of inhibitors targeting mutated IDH proteins. *Molecules*, **24**, 968.

Kim HS, Kwon MJ, Song JH, et al (2018). Clinical implications of TERT promoter mutation on IDH mutation and MGMT promoter methylation in diffuse gliomas. *Pathol Res Pract*, **214**, 881-8.

Komori T (2017). The 2016 WHO classification of tumours of the central nervous system: the major points of revision. *Neurol Med-Chir*, **57**, 301-11.

Louis DN, Perry A, Reifenberger G, et al (2016). The 2016 World Health Organization classification of tumors of the central nervous system: a summary. *Acta Neuropathol*, **131**, 803-20.

Martínez-Garcia M, Álvarez-Linera J, Carrato C, et al (2018). SEOM clinical guidelines for diagnosis and treatment of glioblastoma. *Clin Transl Oncol*, **20**, 22-8.

Molenaar RJ, Verbaan D, Lamba S, et al (2014). The combination of IDH1 mutations and MGMT methylation status predicts survival in glioblastoma better than either IDH1 or MGMT alone. *Neurooncol*, **16**, 1263-73.

Nazari PMS, Berghoff AS, Preusser M, et al (2020). Association of programmed cell death ligand 1 and circulating lymphocytes with risk of venous thromboembolism in patients with glioma. *ESMO Open*, **5**, e000647.

Pandith AA, Qasim I, Baba SM, et al., (2020). Favorable role of IDH 1/2 mutations aided with MGMT promoter gene methylation in the outcome of patients with malignant glioma. *Future Sci OA*, **7**, FSO663.

Paolillo M, Boselli C, Schinelli S(2018). Glioblastoma under siege: an overview of current therapeutic strategies. *Brain Sci*, **8**, 15.

Philip B, Diana XY, Silvis MR, et al (2018). Mutant IDH1 promotes glioma formation in vivo. *Cell Rep*, **23**, 1553-64.

Rivera AL, Pelloski CE, Gilbert MR, et al (2010). MGMT promoter methylation is predictive of response to radiotherapy and prognostic in the absence of adjuvant alkylating chemotherapy for glioblastoma. *Neurooncol*, **12**, 116-21.

Tanaka, K, Sasayama T, Mizukawa K, et al (2015). Combined IDH1 mutation and MGMT methylation status on long-term survival of patients with cerebral low-grade glioma. *Clin Neurol Neurosurg*, **138**, 37-44.

Topalian SL, Hodi FS, Brahmer JR, et al (2012). Safety, activity, and immune correlates of anti–PD-1 antibody in cancer. N*ew Engl J Med*, **366**, 2443-54.

Wang J, Shen F, Yao Y, et al (2020). Adoptive Cell Therapy: A Novel and Potential Immunotherapy for Glioblastoma. *Front Oncol*, **10**, 59.

Wang Z, Zhang C, Liu X, et al (2016). Molecular and clinical characterization of PD-L1 expression at transcriptional level via 976 samples of brain glioma. *Oncoimmunology*, **5**, e1196310.

Xue S, Hu M, Li P, et al (2017). Relationship between expression of PD-L1 and tumor angiogenesis, proliferation, and invasion in glioma. *Oncotarget*, **8**, 49702.

Zalata KR, El-Tantawy DA, Abdel-Aziz A, et al (2011). Frequency of central nervous system tumors in delta region, Egypt. *Indian J Pathol Micr*, **54**, 299.