# Immunohistochemical Assessment of MGMT Expression in Human Gliomas. A Clinico-Pathological Study

**Mustafa Hafudh Hamoodi[*], Ban J. Qasim[**]**


## ABSTRACT:

**BACKGROUND:**

Gliomas are the most common primary malignant brain tumors in adults. They can occur anywhere in the central nervous system but primarily occur in the brain and arise in the glial tissue. $O^6$-alkylguanine DNA alkyl transferase is a protein (DNA repair molecule) encoded by the $O^6$-methylguanine DNA methyltransferase (MGMT) gene in human, and are able to remove alkyl adducts from the O6 position of guanine, and the O4 position of thymine, restoring these DNA bases and preventing temozolomide induced cell death.

**OBJECTIVE:**

To assess the immunohistochemical expression of MGMT in human glioma.

**METHOD:**

This retro and prospective study included 56 tissue paraffin blocks of intracranial gliomas assigned as cases group, and 28 tissue paraffin blocks of normal brain tissue as control group. From each block, two sections were taken; one was stained with the routine hematoxylin and eosin stain, and the other was stained immunohistochemically for marker MGMT.

**RESULTS:**

MGMT showed a highly significant difference in its expression between the control group and the disease group (p value<0.001). MGMT showed a highly significant relation between its expression and the age of patients with glioma (p value< 0.001). MGMT expression showed no significant correlation with other clinicopathological parameters like the histological types and gender (p value<0.065 and 0.255) respectively.

**CONCLUSION:**

MGMT revealed a highly significant difference in its expression in brain tumors (gliomas) compared to control group which may reflects the need in proliferating cells for greater capacity to repair O6-alkylguanine adducts before replication. Besides there was a significant correlation between age and MGMT expression which may reflect processes associated with the physical and functional maturation of the CNS during life.


**KEYWORDS:** Brain tumors, Gliomas, O6-methylguanine, O-6-methylguanine-DNA methyltransferase, immunohistochemistry

## INTRODUCTION:

Tumors of neuroepithelial origin comprise a large and diverse group of neoplasms, with a mixture of benign and malignant tumor types. Gliomas which are tumors that arises from the supportive (glia) tissue of the brain (e.g., astrocytoma, oligodendroglioma, and ependymoma) are the largest subgroup within the neuroepithelial class of neoplasms, and are also the most common type of primary brain tumors (PBT).[1,2] The incidence of brain tumors varies with age (higher in ages < 14 years and in >70 years), race (at least twice as great in the white population than in the black population) and gender (higher in men compared with women).[3] In Iraq, according to the latest Iraqi cancer registry in the year 2015, the incidence of brain and other CNS tumors are 6.1 %,( 3.48 per 100,000 population).[4] The causes of glioma are not well known and they appear to occur randomly.[5] Risk factors that have been investigated in epidemiological studies of primary brain tumors included hereditary syndromes ( as tuberous sclerosis, neurofibromatosis types I and II, Turcot's syndrome, and Li- Fraumeni syndrome), any family history of brain tumors, or prior cancers, and ionizing radiation exposure.[6,7]

$O^6$-alkylguanine DNA alkyl transferase is a protein (DNA repair molecule) encoded by the $O^6$-methylguanine DNA methyl transferase (MGMT) gene in human; and is located on

[*] Dept. of Pathology, Laboratories of Al-Imamain Al-Kadhumain Medical city, Baghdad, Iraq.

[**] Dept. of Pathology and Forensic Medicine, College of Medicine, Al-Nahrain University, Baghdad, Iraq.

chromosome 10q26. MGMT mediates control of genome stability.[8] In DNA replication and transcription there is a chance of mismatch and errors, so leads to formation of unwanted derivatives like mutagenic DNA lesion $O^6$ methylguanine (DNA adduct) which formed in DNA by alkylation. If not repaired by MGMT, $O^6$ methylguanine adducts have dramatic biological consequences that include cytotoxicity, mutagenicity, and carcinogenicity. So on the basis of these protective effects of MGMT expression; it is not surprising that loss of MGMT expression can adversely affect survival after exposure to alkylating agents.[9–12]

MGMT is ubiquitously expressed in human cells of different origin. MGMT activity is highly variable between different normal tissues; the highest activity is in the liver while the lowest is in the brain. Also there is highly variability between individuals and between different stages of development; whereas the MGMT activity in small intestine, fetal colon, spleen, kidney, stomach, lung and brain was in the same level as the corresponding adult tissue, the fetal liver showed a 5-times lower MGMT activity than adult liver.(13) Notably, many types of tumors, including brain, breast, colon and lung tumors have increased MGMT activity when compared with the corresponding normal tissue.[14] In particular, numerous studies have found that pediatrics brain tumors exhibit much higher MGMT activity than adult tumors, leading to a poor response of pediatric tumors to alkylating agents such as temozolomide.[12] Several studies have determined the MGMT activity in tumors and compared it with the corresponding normal tissue. In paired samples of brain, breast, colon , bladder, gastric mucosa, colon and lung, MGMT activity was higher in the tumor than the corresponding normal tissue.[15]Normal brain tissue express low to undetectable levels of the protein, whereas significant levels of expression have been observed in malignant brain tumor specimens.[16]

In this study we will assess the immunohistochemical expression of $O^6$-methylguanine DNA methyltransferase (MGMT) in human glioma, and its correlation to clinic-pathological parameter (patient's age and gender, tumor site, and tumor histopathological type, grade).

## METHODS:

A retrospective and prospective study was intended, which included a total of 84 brain tissue paraffin blocks. Fifty-six tissue paraffin blocks, assigned as the case group, included gliomas obtained from patient who underwent craniotomy for malignant brain tumors. These blocks were collected from Specialized Neurosciences Hospital Laboratories from August 2017 to October 2018. Twenty-eight tissue paraffin blocks, assigned as the control group, included normal brain tissue with age and sex matched, obtained from Forensic Medical Institute laboratory during May2018.

The clinicopathological parameters including (age, gender, site, grade & type) were obtained from patients' admission case sheets and pathology reports.

From each block, two sections of 5 µm thickness were taken. One section was stained with the routine H&E stain, examined and the histopathological diagnosis, tumor histological type, and grade according to WHO classification of the tumors of the CNS were evaluated and revised by a specialist pathologist. The other section was deparaffinized and rehydrated at room temperature, antigen retrieval was carried out by autoclave 3 min. then allowed to cool for 20 minutes at room temperature. Hydrogen peroxide block were added till cover the and then were incubated at room temperature for 10 min in a humid chamber, followed by protein block were added and then the slides were incubated for 10 min at room temperature. Mouse monoclonal MGMT antibody [MT3.1] (orb334008) (Biorbyt, UK) (dilution 1.0 mg/ml) applied to sections and were incubated for an overnight. The immunohistochemical specific detection kit [Super Sensitive IHC Detection System Kit (orb219874)] (Biorbyt, UK) reagents were used. Counterstaining of the sections by Mayer's Hematoxylin stain for 20-30 seconds then followed by mounting of the sections by using Roti®-Mount Aqua (ROTH, Germany) followed by glass coverslip.

**Interpretation of immunohistochemistry staining and quality control**

Staining for MGMT protein was considered positive when a uniform nuclear staining was displayed and any staining that was restricted to the cytoplasm and granular nuclear reactivity was considered negative.(17) MGMT expression was evaluated by the percentage of stained tumor cells and scored as following: (18)

-Negative: without positive cells or <10%.

-weakly positive: positive cells of 10-24%.

-intermediately positive: positive cells of 25-50%.
-Strongly positive: positive cells >50%.

**Statistical analysis**

SPSS 16.0 (SPSS, Inc., Chicago, IL, USA) was used to analyze the data. Categorical data were presented as number and percentage. The comparison of number between different groups was done using Fisher exact test and chi square test. Fishers exact probability test was used to judge the relation between MGMT expression and gender and age for the patients with glioma. Spearmans correlation was taken to analyze the expression of MGMT in different grades of glioma. P value of <0.05 was considered significant, while if < 0.01 was considered highly significant.

**RESULTS:**

Out of 56 glioma cases and 28 controls, 24 (85.7%) cases of control group were of negative, while 4 (14.3%) cases were weakly positive and no any case with intermediate or strong score. Among 56 cases of glioma, 21 (37.5 %) cases of them were weakly positive score, and 13 (23.2%) cases were of negative score, 18 (32.1%) cases were of intermediate positive score, and 4 (7.1%) cases were strongly positive score. According to that, there is highly statistically significant difference in the immunohistochemical expression of MGMT between the control group and the disease group (p value <0.001) (Table 1).

**Table 1: Immunohistochemical expression score of the MGMT marker in glioma cases and control groups.**

| | | MGMT expression score | | | | |
|---|---|---|---|---|---|---|
| | | negative | Weakly positive +1 | Moderately positive +2 | Strongly positive +3 | |
| glioma | No. | 13 | 21 | 18 | 4 | |
| | % | 23.2% | 37.5% | 32.1% | 7.2% | |
| normal brain | No. | 24 | 4 | 0 | 0 | |
| | % | 85.7% | 14.3% | 0 | 0 | |
| Total | No. | 37 | 25 | 18 | 4 | |
| | % | 44.0 % | 29.8% | 21.4% | 4.8% | P value <0.001* |

Regarding the relation of MGMT immunohistochemical expression scoring and age, 5 (45.5%) cases of patients <10 years were negative expression, while all the cases between 10-20 years were positive (+1 and +2). Between ages 20-50 years the cases were distributed between negative and positive (+1 and +2) while only 1 case from 23 were strongly positive. Eight (57.1%) cases from 14 cases were of weakly positive (+1) and 4 (28.6%) cases were moderately positive (+2) and 1 case negative and 1 other strongly positive. According to that there is statistically highly significant relation between the expression of MGMT and the age of patient with glioma (p value of < 0.001) (Table 2).

**Table 2: Relation of MGMT expression to age of patient with gliomas**

| Age (years) of the patients with glioma | | MGMT expression score | | | | Total | |
|---|---|---|---|---|---|---|---|
| | | Negative 0 | Weakly positive +1 | Moderately positive +2 | Strongly positive +3 | | |
| <10 | No. | 5 | 2 | 2 | 2 | 11 | |
| | % | 45.5% | 18.2% | 18.2% | 18.2% | 100.0% | |
| 10-20 | No. | 0 | 3 | 3 | 0 | 6 | |
| | % | 0.0% | 50.0% | 50.0% | 0.0% | 100.0% | |
| 20-30 | No. | 3 | 1 | 2 | 0 | 6 | |
| | % | 50.0% | 16.7% | 33.3% | 0.0% | 100.0% | |
| 30-40 | No. | 1 | 3 | 4 | 0 | 8 | |
| | % | 12.5% | 37.5% | 50.0% | 0.0% | 100.0% | |
| 40-50 | No. | 3 | 4 | 3 | 1 | 11 | |
| | % | 27.3% | 36.4% | 27.3% | 9.1% | 100.0% | |
| 50-60 | No. | 0 | 7 | 1 | 0 | 8 | |
| | % | 0.0% | 87.5% | 12.5% | 0.0% | 100.0% | |
| >60 | No. | 1 | 1 | 3 | 1 | 6 | |
| | % | 16.7% | 16.7% | 50.0% | 16.7% | 100.0% | |
| Total | No. | 13 | 21 | 18 | 4 | 56 | P value <0.001 |
| | % | 23.2% | 37.5% | 32.1% | 7.1% | 100.0% | |

Regarding the relation of MGMT expression to the gender of patients with gliomas, 7 (53.8%) cases out of the 13 negative MGMT expression cases were females, while 6 cases (46.2%) out of the 13 negative MGMT expression cases were male. MGMT expression scored weakly positive in female (52.4%) more than in male (47.6%), while MGMT expression scored moderately and strongly positive expression equally between male and female. According to that there is no statistically significant correlation between the MGMT expression and the sex of the patient with glioma (p value 0.255) (Table 3).

**Table 3: Relation of MGMT immunohistochemical expression with gender of patient with glioma**

| MGMT expression score | | Sex | | Total | |
|---|---|---|---|---|---|
| | | Male | female | | |
| Negative 0 | No. | 6 | 7 | 13 | |
| | % | 46.2% | 53.8% | 100.0% | |
| Weakly positive +1 | No. | 10 | 11 | 21 | |
| | % | 47.6% | 52.4% | 100.0% | |
| Moderately positive +2 | No. | 9 | 9 | 18 | |
| | % | 50.0% | 50.0% | 100.0% | |
| Strongly positive +3 | No. | 2 | 2 | 4 | |
| | % | 50.0% | 50.0% | 100.0% | |
| Total | No. | 27 | 29 | 56 | P value =0.255 |
| | % | 48.2% | 51.8% | 100.0% | |

Regarding the relation between MGMT expression in semiquantitive scoring and histopathological type of glioma, Among the 26 cases of glioblastoma multiforme, 12(46.2%) and 9(34.6%) cases scored weakly positive and moderately positive respectively, while only 1(3.8%) case scored strongly positive. According to that there is no statistically significant relation between MGMT expression and the histopathological type of glioma (p value 0.065) (Table 4).

**Table 4: Relation of MGMT immunohistochemical expression to histopathological type of glioma.**

| Histopathological type of glioma | MGMT immunohistochemical expression score | | | | Total | |
|---|---|---|---|---|---|---|
| | Negative 0 | Weakly positive +1 | Moderately positive +2 | Strongly positive +3 | | |
| Anaplastic astrocytoma | 0 | 1 | 2 | 1 | 4 | |
| | 0.0% | 25.0% | 50.0% | 25.0% | | |
| Diffuse fibrillary astrocytoma | 3 | 3 | 3 | 0 | 9 | |
| | 33.3% | 33.3% | 33.3% | 0.0% | | |
| Ependymoma | 3 | 1 | 0 | 1 | 5 | |
| | 60.0% | 20.0% | 0.0% | 20.0% | | |
| Glioblastoma multiforme | 4 | 12 | 9 | 1 | 26 | |
| | 15.4% | 46.2% | 34.6% | 3.8% | | |
| Pilocytic astrocytoma | 2 | 3 | 3 | 0 | 8 | |
| | 25.0% | 37.5% | 37.5% | 0.0% | | |
| Others | 1 | 1 | 1 | 1 | 4 | |
| | 25.0% | 25.0% | 25.0% | 25.0% | | |
| Total | 13 | 21 | 18 | 4 | 56 | P value = 0.065 |
| | 23.2% | 37.5% | 32.1% | 7.1% | 100.0% | |

Regarding the relation of MGMT expression in semiquantitive scoring and the histological grade of glioma, Among the 56 cases, 13(23.2%) cases scored negative expression, 21(37.5%) of cases scored weakly positive, 18(32.1%) cases scored moderately positive, and only 4 cases scored strongly positive. According to above there is no statistically significant relation between MGMT expression and the histological grade of glioma (p value 0.082) (Table 5).

**Table 5: Relation of MGMT immunohistochemical expression with grade of gliomas.**

| Grade of glioma | MGMT immunohistochemical expression score | | | | Total | |
|---|---|---|---|---|---|---|
| | Negative 0 | Weakly positive+1 | Moderately positive +2 | Strongly positive +3 | | |
| I | 2 | 4 | 3 | 0 | 9 | |
| | 22.2% | 44.4% | 33.3% | 0.0% | | |
| II | 6 | 4 | 4 | 2 | 16 | |
| | 37.5% | 25.0% | 25.0% | 12.5% | | |
| III | 1 | 1 | 2 | 1 | 5 | |
| | 20.0% | 20.0% | 40.0% | 20.0% | | |
| IV | 4 | 12 | 9 | 1 | 26 | |
| | 15.4% | 46.2% | 34.6% | 3.8% | | |
| Total | 13 | 21 | 18 | 4 | 56 | P value = 0.082 |
| | 23.2% | 37.5% | 32.1% | 7.1% | | |

## DISCUSSION:

MGMT is ubiquitously expressed in human cells of different origin. Many studies, showed that there is significant variation of MGMT activity in different human tissues, the same tissue of different individuals and neoplastic versus non-neoplastic tissues[15] MGMT has been observed that its detection was mainly nuclear in hepatocellular, brain, gastric, ovarian, biliary tract, and breast carcinomas with only very few sections positively immunostained in cytoplasm. In human gliomas, a wide range of MGMT activity was noted, with higher activity than in adjacent brain tissue collected at surgery[9]

Current study showed that there is highly statistically significant difference in the MGMT expression between the control group and the case group (p value <0.001). This is parallel to Chinese study performed by Li Q. *et al* during 2017, also parallel to a German study performed by Christmann during 2011; in which there was significant difference in MGMT expression in control group compared to glioma patients

group; it was higher in glioma.[15,18] The elevated MGMT activity in human primary brain tumors likely reflects the need in proliferating cells for greater capacity to repair O6-alkylguanine adducts before replication.[15]

According to current study, pilocytic astrocytomas (WHO grade I) showed positive MGMT expression in 75% of cases and negative expression in 25% of cases; and this disagreed with a study performed by Horbinski *et al.* which showed that MGMT was expressed in 12.5% of pilocytic glioma cases; and it was negative in 87.5% of cases. [19] This may be attributed to wide difference sample size between the 2 studies; in which Horbinski examined 147 cases while in current study only 8 cases of pilocytic glioma. Diffuse fibrillary astrocytoma (WHO grade II) in the current study showed 33.3% of cases with negative MGMT expression, 33.3% of cases with weakly positive expression, and 33.3% of cases with moderate positive expression. This study didn't agree with Chinese study by Hu during 2013 which MGMT expression was negative in 51.8% of cases.[20] Anaplastic astrocytoma (WHO grade III) in this study showed that all cases revealed positive MGMT expression, and this disagrees with a study by Brell in 2005 where positive MGMT expression was reported in 54.9% of cases.[21] In this study, 46.2% of glioblastoma multiforme (WHO grade IV) cases had positive MGMT expression of score +1; 34.6% of cases had score +2, and score +3 has only one case, while 15.9% of cases were negative MGMT expression. This was parallel to British study by Rodriguez 2008 and Japanese study by Ogura 2015 [22,23] but it differs with Iranian study by Afsharnezhad in 2018 who found 70% of glioblastoma cases were negative.[24] It was showed that the difference between positive expression of MGMT and WHO grade of glioma had no statistical significance with p value of 0.082. A study performed by Capper et al. in 2007 showed that expression of MGMT was significantly decreased with increasing the WHO grade of glioma with a p value of 0.01.[25] while study by Li in 2017 found that MGMT expression was not significantly different among all tumor grades with a p value of > 0.05 [26] and this variation in MGMT expression between different grades of glioma might be due to the changes in the rate of synthesis and/or stability of the MGMT protein, but not by synthesis of a protein that is functionally inactive or less efficient in 06-methylguanine repair, or due to the changes in transcriptional activity of the MGMT gene.[27]

Regarding the relation of MGMT expression categorical scoring and age during the current study, among 56 of glioma, there was a statistically significant relation between the immunohistochemical expression of MGMT score and increase of the age of patient with glioma with p value of <0.001. A Swiss study performed by Capper 2008 showed a positive significant correlation between the patient's age and MGMT expression with a p value of 0.046.[28] while an Indian study by Pandith 2018 found no significant difference in MGMT expression in different age groups in patients with glioma ($p > 0.05$).[17] Also other study by Li 2017 showed no correlation between MGMT expression and increasing the age of patient.[18] The age dependence of MGMT might reflect processes associated with the physical and functional maturation of the CNS during life.[28]

Concerning the gender of the patients with glioma; current study showed no statistically significant relation between the immunohistochemical expression and the gender of patient with glioma with p value of 0. 255. This is parallel to studies by Brell in 2005, Kimura in 2014; and Ryken in 2011. [21,29,30]

Regarding the location of the glioma, this study showed no statistically significant relation between the immunohistochemical expression and the location of glioma with correlation p value of 0.147, this study agreed Italian study by Mellai in 2009.[31]

In conclusion MGMT revealed a highly significant difference in its expression among brain tumors (glioma) compared to control group which may reflects the need in proliferating cells for greater capacity to repair O6- alkylguanine adducts before replication. Also, MGMT expression revealed a significant correlation with age which may reflect processes associated with the physical and functional maturation of the CNS during life. While gender and tumor site showed no statistical significance in MGMT expression. Grading of tumor and histopathological type also revealed no statistical significance with MGMT expression which may indicate that it has no relation to tumor progression.

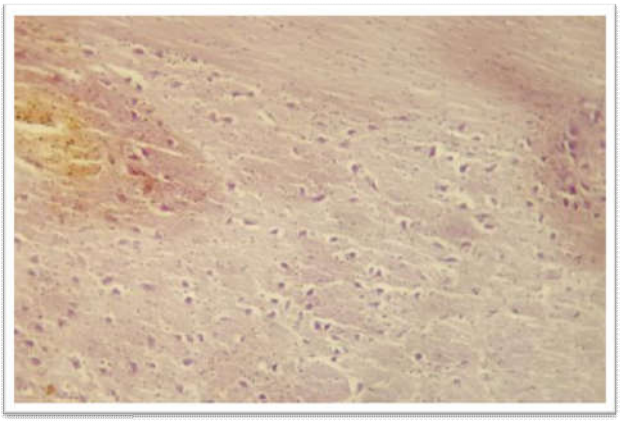

**Figure 1: Normal brain tissue showing negative immunohistochemical nuclear staining of MGMT (40X)**

**Figure 2: Pilocytic astrocytoma showing brown immunohistochemical nuclear staining of MGMT (red arrows) of 40% of cells ( score+2) (40X)**

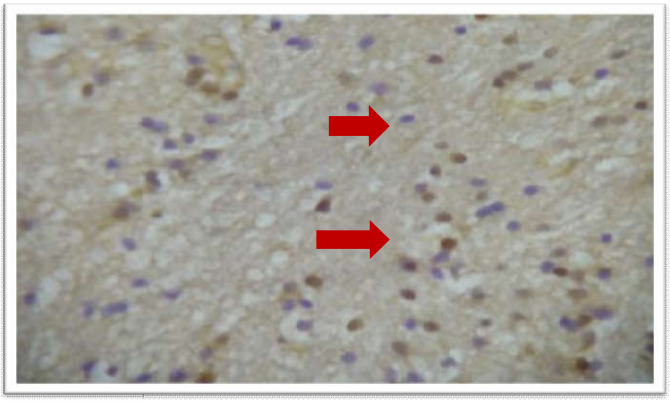

**Figure 3: Diffuse Fibrillary Astrocytoma showing brown immunohistochemical nuclear staining of MGMT (red arrows) (40X)**

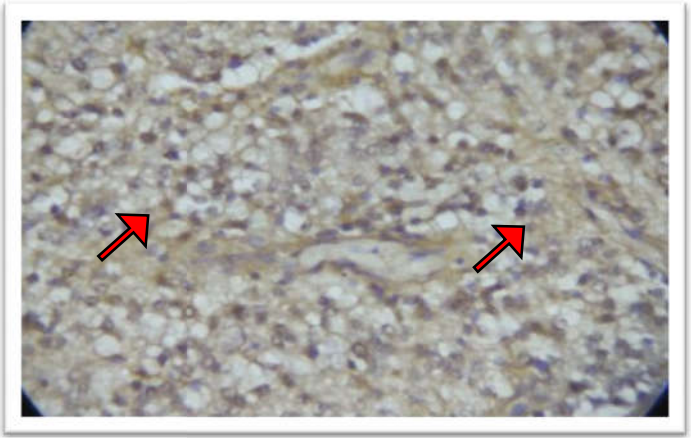

**Figure 4: Anaplastic astrocytoma showing brown immunohistochemical nuclear staining of MGMT (red arrow) of 35% of cells (40X)**

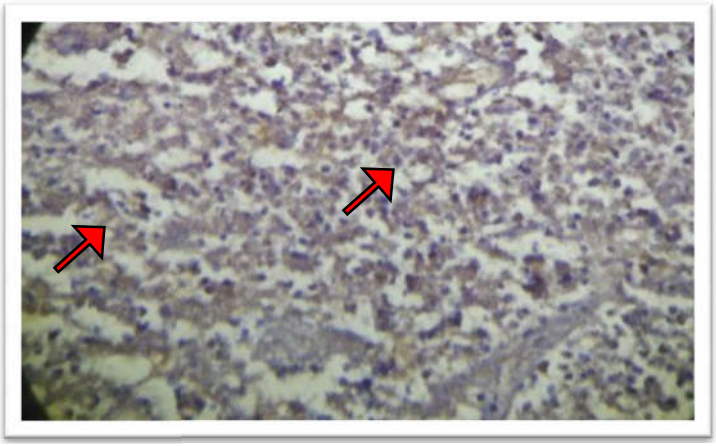

**Figure 5: Glioblastoma multiforme showing brown immunohistochemical nuclear staining of MGMT (red arrow) of 45% of cells (20X)**

## REFERENCES:


1. Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. ActaZ Neuropathol. 2016;131:803–20.
2. Banan R, Hartmann C. The new WHO 2016 classification of brain tumors—what neurosurgeons need to know. Acta Neurochirurgica. 2017.
3. Ostrom QT, Gittleman H, Farah P, Ondracek A, Chen Y, Wolinsky Y, et al. CBTRUS statistical report: Primary brain and central nervous system tumors diagnosed in the United States in 2006 - 2010. J Neurooncol. 2013;15:788–96.
4. Board IC. Annual Report Iraqi Cancer Registry. 2015;
5. Cancer Council Australia. Adult gliomas (astrocytomas and oligodendrogliomas): a guide for patients, their families and carers. 2011; Available from: zhttp://www.cancer.org.au/content/pdf/HealthProfessionals/ClinicalGuidelines/Adult_Glioma_Consumer_Guide_FINAL_bookmarked.pdf
6. Barnholtz-Sloan JS, Ostrom QT, Cote D. Epidemiology of Brain Tumors. Neurologic Clinics. 2018.
7. Baldi I, Engelhardt J, Bonnet C, Bauchet L, Berteaud E, Grüber A, et al. Épidémiologie Des Méningiomes. Neurochirurgie [Internet]. 2018;64:5–14. Available from: https://doi.org/10.1016/j.neuchi.2014.05.006
8. Schmitt AM, Pavel M, Rudolph T, Dawson H, Blank A, Komminoth P, et al. Prognostic and predictive roles of MGMT protein expression and promoter methylation in sporadic pancreatic neuroendocrine neoplasms. Neuroendocrinology. 2014;100:35–44.

9. Kdolsky RK, Gibbons DF, Kwasny O, Schabus R, Plenk H. J, Zeman P, et al. ABSTRACTS. Am J Sports Med. 2016;
10. Baran K, Yang M, Dillon CP, Samson LL, Green DR. The proline rich domain of p53 is dispensable for MGMT-dependent DNA repair and cell survival following alkylation damage. 2017;24:1925–36. Available from: http://dx.doi.org/10.1038/cdd.2017.116
11. Fahrer J, Kaina B. Review O 6 -methylguanine-DNA methyltransferase in the defense against N -nitroso compounds and colorectal cancer. 2013;34:2435–42.
12. Fu D, Calvo JA, Samson LD. Balancing repair and tolerance of DNA damage caused by alkylating agents. 2012;12(February).
13. Christmann M, Kaina B. O 6-methylguanine-DNA methyltransferase (MGMT): Impact on cancer risk in response to tobacco smoke. Mutat Res - Fundam Mol Mech Mutagen [Internet]. 2012;736:64–74. Available from: http://dx.doi.org/10.1016/j.mrfmmm.2011.06.004
14. Christmann M, Nagel G, Horn S, Krahn U, Wiewrodt D, Sommer C, et al. MGMT activity, promoter methylation and immunohistochemistry of pretreatment and recurrent malignant gliomas: A comparative study on astrocytoma and glioblastoma. Int J Cancer. 2010;127:2106–18.
15. Christmann M, Verbeek B, Roos WP, Kaina B. Biochimica et Biophysica Acta O 6 -Methylguanine-DNA methyltransferase ( MGMT ) in normal tissues and tumors : Enzyme activity , promoter methylation and immunohistochemistry. BBA - Rev Cancer [Internet]. 2011;1816:179–90. Available from: http://dx.doi.org/10.1016/j.bbcan.2011.06.002
16. Pollack IF, Hamilton RL, Sobol RW, Burnham J, Yates AJ, Holmes EJ, et al. O6-Methylguanine-DNA methyltransferase expression strongly correlates with outcome in childhood malignant gliomas: Results from the CCG-945 cohort. J Clin Oncol. 2006;24:3431–7.
17. Pandith AA, Qasim I, Zahoor W, Shah P, Bhat AR, Sanadhya D, et al. Concordant association validates MGMT methylation and protein expression as favorable prognostic factors in glioma patients on alkylating chemotherapy (Temozolomide). Sci Rep [Internet]. 2018;8:1–11. Available from: http://dx.doi.org/10.1038/s41598-018-25169-2
18. Li Q, Guo J, Wang W, Wang D. Relationship between MGMT gene expression and treatment effectiveness and prognosis in glioma. Oncol Lett. 2017;14:229–33.
19. Horbinski C, Hamilton RL, Lovell C, Burnham J, Pollack IF. Impact of morphology, MIB-1, p53 and MGMT on outcome in pilocytic astrocytomas. Brain Pathol. 2010;
20. Hu X, Miao W, Zou Y, Zhang W, Zhang Y, Liu H. Expression of p53, epidermal growth factor receptor, Ki-67 and O6-methylguanine-DNA methyltransferase in human gliomas. Oncol Lett. 2013;
21. Brell M, Tortosa A, Verger E, Gil JM, Viñolas N, Villá S, et al. Prognostic Significance of O 6 -Methylguanine-DNA Methyltransferase Determined by Promoter Hypermethylation and Immunohistochemical Expression in Anaplastic Gliomas Determined by Promoter Hypermethylation and Immunohistochemical. Clin cancer Res. 2005;11:5167–74.
22. Rodriguez FJ, Thibodeau SN, Jenkins RB, Schowalter K V., Caron BL, O'Neill BP, et al. MGMT Immunohistochemical Expression and Promoter Methylation in Human Glioblastoma. Appl Immunohistochem Mol Morphol [Internet]. 2007;PAP:59–65. Available from: https://insights.ovid.com/crossref?an=00129039-900000000-99992
23. Ogura R, Tsukamoto Y, Natsumeda M, Isogawa M, Aoki H, Kobayashi T, et al. Immunohistochemical profiles of IDH1, MGMT and P53: Practical significance for prognostication of patients with diffuse gliomas. Neuropathology. 2015;
24. Lotfi M, Afsharnezhad S, Raziee HR, Ghaffarzadegan K, Sharif S, Shamsara J, et al. Immunohistochemical assessment of MGMT expression and p53 mutation in glioblastoma multiforme. Tumori. 2011;
25. Capper D, Mittelbronn M, Meyermann R, Schittenhelm J. Pitfalls in the assessment of MGMT expression and in its correlation with survival in diffuse astrocytomas: Proposal of a feasible immunohistochemical approach. Acta Neuropathol. 2008;115:249–59.
26. Li X, Wei J, Liu Y, Li P, Fan L, Wang Y, et al. Primary Astrocytic Tumours and Paired Recurrences have Similar Biological

Features in IDH1, TP53 and TERTp Mutation and MGMT, ATRX Loss. Sci Rep. 2017;

**27.** Preuss I, Eberhagen I, Haas S, Eibl RH, Kaufmann M, Minckwitz G Von, et al. O6-methylguanine-DNA methyltransferase activity in breast and brain tumors. Int J Cancer. 1995;61:321–6.

**28.** Bobola MS, Berger MS, Ellenbogen RG, Roberts TS, Geyer JR, Silber JR. O6-methylguanine-DNA methyltransferase in pediatric primary brain tumors: Relation to patient and tumor characteristics. Clin Cancer Res. 2001;

**29.** Shah N, Lin B, Sibenaller Z, Ryken T, Lee H, Yoon JG, et al. Comprehensive analysis of MGMT promoter methylation: Correlation with MGMT expression and clinical response in GBM. PLoS One. 2011;6.

**30.** Miyazaki M, Nishihara H, Terasaka S, Kobayashi H, Yamaguchi S, Ito T, et al. Immunohistochemical evaluation of O 6 - methylguanine DNA methyltransferase ( MGMT ) expression in 117 cases of glioblastoma. 2014;(November 2013):268–76.

**31.** Mellai M, Caldera V, Annovazzi L, Chiò A, Lanotte M, Cassoni P, et al. MGMT promoter hypermethylation in a series of 104 glioblastomas. Cancer Genomics and Proteomics. 2009;6:219–27.