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Immunohistochemical expression of MGMT in gliomas

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Abstract: Glioma is one of the main tumors of the central nervous system occurring in the spinal cord or brain, and is the origin of glioma tumors. The most common site of glioma is the brain. Glioma accounts for 30% of all central nervous system tumors and 80% of malignant brain tumors. O6-methylguanine-DNA methyltransferase (MGMT) gene mutations have been identified in many human cancers including gliomas. This study aimed to evaluate the immune expression of MGMT in different gliomas and its relationship with different clinicopathological parameters. From January 2015 to January 2017, 97 cases of glioma were retrieved from the Rizgary Pathology Center in Erbil-Iraq. The tumors were typed and graded according to the 2007 World Health Organization Brain Tumor Classification. Immunohistochemical staining was done for MGMT using monoclonal Antibodies via the automated immunostainer technique. Positive MGMT immunoexpression was observed in 60 (61.9%) cases. Significantly high rates of MGMT immunoexpression were demonstrated among the 2 age groups: 45-54 years and 25-34 years (23.3% and 21.7% respectively). Most positive cases were observed among the supratentorial tumors (81.7%) and dominant grade IV gliomas (48.4%) with no significant association. Immunohistochemically, MGMT is frequently expressed in gliomas particularly in glioblastomas with bimodal age association.

Key words: Glioma; MGMT; Immunohistochemistry.

Introduction

Glioma is a tumor that grows in the brain and spinal cord. Glioma begins with the protective cells of glia surrounding nerve cells. Glial cells help these nerve cells function. Glioma affects your brain function and is life-threatening based on its location and growth rate. The type of glioma you have helps determine treatment and prognosis. Generally, treatment options for gliomas include surgery, radiation therapy, chemotherapy, targeted therapy, and experimental clinical trials. Gliomas, the most common type of primary brain tumors, have been categorized, according to the 2007-WHO classification, into four grades on the basis of clinicopathological and prognostic criteria from grade I gliomas with a slow growth pattern to grade IV or glioblastoma (1-5).

The O6-methylguanine-DNA methyltransferase (MGMT) gene at 10q26 is a key enzyme in the DNA repair base excision pathway. MGMT removes mutagenic and cytotoxic adducts from O6-guanine in DNA, which is the first point of attack for alkylated drugs used to treat glioblastoma. Hypermethylation of CpG islands located in the MGMT region is the main cause of MGMT loss of function in several tumor types (6).

The reported frequency of MGMT promoter hypermethylation in gliomas varies widely, ranging from 43%–93% in grade II gliomas to 35% - 73% in GBM. This considerable range of reported MGMT promoter hypermethylation frequencies is at least partly due to technical challenges (7-9). As far as prognosis is concerned; in GBMs, MGMT is regarded as an important prognostic and predictive factor in chemotherapy with Temozolomide (10-12), while in low-grade gliomas MGMT hypermethylation has been found to be associated with longer overall survival (8,13) and progression-free survival (7,8).

This study aimed to assess MGMT immunoexpression in different types of gliomas and its association with different clinicopathological parameters.

Materials and Methods

From January 2015 to January 2017, the MGMT immune expression of 97 reported glioma samples was collected, reviewed and studied. Retrieve samples from the Department of Histopathology at Rizgary Teaching Hospital in Erbil. Record clinical data including age, gender and tumor location. The paraffin-embedded block was cut to a thickness of three microns and stained with hematoxylin and eosin. Then classify and grade the tumors based on the 2007 WHO classification of CNS tumors (1). Ethical approval was obtained from the Ethics Committee of the author's University.

Immunohistochemical technique

Another 3 micron thick slides from the tumor were dewaxed and rehydrated. Antigen recovery was performed by autoclaving at 97 °C for 20 minutes using an antigen recovery solution (citrate buffer 10 mmol/L, pH 6.0). The sections were allowed to cool at room temperature, and then washed three times in phosphate buffered saline (PBS) for 3 minutes each time. Endogenous peroxidase activity was blocked by immersing the sections in a 3% hydrogen peroxide blocker (Dako) for 10

minutes, and washed with 3 kinds of PBS. After this initial processing step, the sections were incubated with anti-human MGMT primary antibody (monoclonal, 1: 200-1: 500; clone 0537, Novusbio, USA) overnight at room temperature. It was then incubated with the ultrasensitive non-biotin HRP detection system for 35 minutes. Finally, the sections were counterstained with hematoxylin, dehydrated and fixed.

MGMT protein is expressed in cell nuclei. Mutations in its gene give a nuclear protein overexpression in tumor cells, in addition, other non-tumor cells (e.g., endothelial and inflammatory cells) stained, serving as positive internal controls. GBM tissue with high MGMT protein expression was used as a positive control. Negative control was performed by the omission of the primary antibody (14).

Scoring

According to Capper et al. (14) Lechapt-Zalcman et al. (15) and Younis et al. (16) who found that the best cut off value of MGMT protein expression was 15%; so >15% positively stained nuclei considered immunopositive and ≤15% positively stained nuclei considered immunonegative.

Statistical analysis

Data were interpreted in forms of frequencies and percentages. A Chi-square test and Fisher exact test were used to associate the MGMT status and the different study variables. Statistical analysis was performed by using Statistical Package for Social Sciences (SPSS) version 20. P-value of less than 0.05 was considered significant.

Results

Ninety-seven cases of different types of gliomas were included in this study. Patients' age ranged from 3 months to 84 years with a mean of 37.08±20 years and a median of 38 years; 59 cases were males and 38 were females with a male to female ratio of 1: 0.64.

Positive MGMT staining was observed in 60 (61.9%) cases and the highest frequency was observed among 45-54 years and 25-34 years' age groups (23.3% and 21.7% respectively) and the association of MGMT expression with age groups was statistically significant (p-value 0.006).

Out of 38 females, 22 cases (36.7%) were positive and out of 59 males, 38 cases (63.3%) were positive for MGMT, however, the association of MGMT expression with gender was statistically not significant (p-value 0.519).

Seventy-nine cases were supratentorial located; of these, 49 cases were positive for MGMT immunoexpression. While the remaining 18 cases were infratentorial and 11 of them were positive and the association of MGMT expression with tumor location was statistically not significant (p-value 0.943).

Regarding the tumor type, astrocytic tumors were the predominant type forming 84 (86.6%) cases; including 44 glioblastomas (GM) (including one gliosarcoma), 18

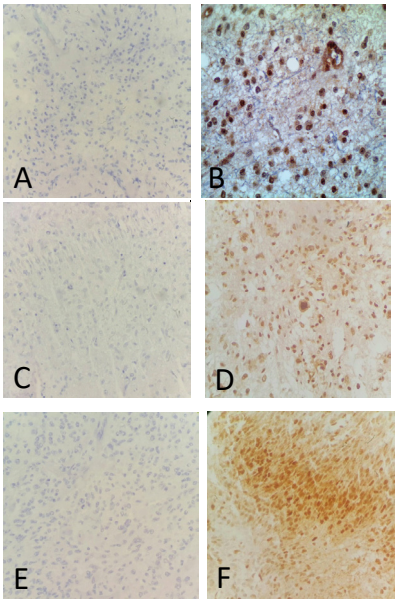

Figure 1. Immunohistochemical expression of MGMT in different grades of astrocytoma. A: Diffuse astrocytoma, WHO grade II negatively stained and B: positively stained. C: Anaplastic astrocytoma, WHO grade III negatively stained and D: positively stained. E: Glioblastoma, WHO grade IV, negatively stained, and F: positively stained (A- F, ×400).

Table 1. MGMT status with tumor type.

Tumor type	MGMT		Total no. (%)	P-value
	Positive No. (%)	Negative No. (%)		
Pilocytic astrocytoma	9 (15.0%)	6 (16.2%)	15(15.5%)	P 0.593* * Fisher's Exact Test
Subependymal gaint cell astrocytoma	0 (0.0%)	1 (2.7%)	1(1%)	
Diffuse astrocytoma	9 (15.0%)	9 (24.3%)	18(18.6%)	
Oligodendroglioma	1 (1.7%)	1 (2.7%)	2(2.1%)	
Oligoastrocytoma	1 (1.7%)	0 (0.0%)	1(1%)	
Pleomorphic xanthoastrocytoma	1 (1.7%)	1 (2.7%)	2(2.1%)	
Ependymoma	3 (5.0%)	1 (2.7%)	4(4.1%)	
Anaplastic astrocytoma	2 (3.3%)	2 (5.4%)	4(4.1%)	
Anaplastic oligodendroglioma	1 (1.7%)	1 (2.7%)	2(2.1%)	
Anaplastic ependymoma	4 (6.7%)	0 (0.0%)	4(4.1%)	
Glioblastomamultiforme	28 (46.7%)	15 (40.5%)	43(44.3%)	
Gliosarcoma	1 (1.7%)	0 (0.0%)	1(1%)	
Total	60 (61.9%)	37 (38.1%)	97(100%)	

Table 2. MGMT immunoexpression in gliomas in other studies.

Study(year)	Location	Sample (n)	MGMT positive (n)	MGMT positive (%)
Current study (2018)	Erbil-Iraq	97	60	61.8
Brell et al (20)	Spain	93	51	54.8
Nakasu et al (21)	Japan	28	19	67.8
Christmann et al.(22)	Germany	59	30	50.8
Groenendijk et al. (23)	Netherlands	70	39	56.0
Mellai al. (24)	Italy	275	104	37.8
Hu et al. (25)	China	152	84	55.3
Ogura et al. (26)	Japan	312	126	40.4
Gepp et al. (27)	Brazil	39	24	61.5
Megova et al. (28)	Czech	143	75	52.0
Pandith et al. (29)	India	61	36	60.0

Table 3. MGMT immunoexpression in GBM in other studies.

Study (year)	Location	Sample (n)	MGMT positive (n)	MGMT positive (%)
Current study (2018)	Erbil-Iraq	44	29	48.4
Shah et al (30)	USA	24	10	42.0
Lotfi et al (34)	Iran	50	15	30.0
Mellai al.(24)	Germany	180	61	33.9
Wang et al. (35)	China	78	43	55.1
Ogura et al (26)	Japan	165	76	46.0
Younis et al (16)	Egypt	73	35	47.9
Pan et al. (36)	China	59	27	45.76
Megova et al. (28)	Czech	90	43	48.0
Anvari et al. (32)	Iran	78	19	24.4

diffuse astrocytomas (DA), 15 pilocytic astrocytomas (PA), and 4 anaplastic astrocytomas (AA), also there were 4 ependymomas and 4 anaplastic ependymomas, in addition, there were 4 oligodendroglial tumors and one oligoastrocytoma. Among astrocytomas; GBM showed the highest MGMT expression (48.4%) followed by DA and PA (15.0% each), at the same time 3 out of 4 cases of ependymomas and all 4 cases of anaplastic ependymomas were positive for MGMT expression but the association of MGMT expression with tumor type was statistically not significant (p-value 0.593), Table 1.

Concerning the tumor grade, the predominant grade was grade IV representing 44 cases followed by grade II; 27 cases then grade I; 16 cases and lastly grade III; only 10 cases. The highest MGMT expression was among grade IV tumors forming (48.4%) of the positive cases, followed by grade II (25.0%) than grade I (15.0%), and lastly, grade III (11.6%). The association between MGMT expression and grade of gliomas was statistically not significant (p-value 0.598).

Representative pictures of MGMT immunoexpression in different grades of astrocytomas (DA, AA, and GBM) are shown in figure 1.

Discussion

Brain and other CNS tumors rank the fifth among the top ten cancers in Iraq following breast and lung cancer, leukemia and bladder cancer (17). MGMT is one of the promising biomarkers in glioma with its ubiquitous DNA repair enzyme (18). Nowadays, MGMT has been firmly established as a marker in patients diagnosed with gliomas, at the level of both clinical trials and also in routine clinical management (19).

The present study, as per our knowledge; is the first of its kind in our region and Iraq to detect MGMT immunoexpression in gliomas by using an antibody specific for MGMT mutation. In the current study, MGMT was expressed in 60 (61.8%) cases of gliomas; similar figures have been reported by other studies as shown in Table 2.

The highest frequency of MGMT immunoexpression was between 45-54 and 25-34 years' age groups (23.3% and 21.7% respectively). This is could be explained by the fact that majority of MGMT positive cases were in grade IV and grade II gliomas which usually occur in the above-mentioned age groups, in agreement with our study; Capper et al. (14), Shah et al. (30) and Molennar et al. (31) also found a significant association between MGMT expression and age.

Although statistically not significant, MGMT expression was observed more among males (63.3%) than females (36.7%). Other studies also found male predominance and a higher rate of MGMT expression in males without statistically significant association (16, 28, 30), however, Anvari et al. (32) although found more MGMT expression in males than females in contrast to our study the association was statistically significant. The majority of MGMT positive cases were supratentorial, tumours; a finding which was observed in other studies (27, 33) and could be explained by the fact that most of the cases of low and high-grade astrocytomas which showed MGMT expression were supratentorial in location. As far as histological types of gliomas are concerned, GBM cases demonstrated the highest frequency of MGMT expression (48.4%); our figure was within the range that has been reported by others as shown in Table 3.

Concerning DA; MGMT expression was positive in 15.0% of the cases which is lower than figures repor-

ted by Capper et al. (14), Mellai et al. (24), Ogura et al. (26) and Nakasu et al. (37) who reported 25.6%, 38.4%, 42.0% and 67.85% positive cases respectively, at the same time regarding PA; there were 15% positive cases, while Mellai et al. (24) and Sippl et al. (38) reported 30.0% and 44.5% positive cases respectively. This lower MGMT expression in DA and PA in our study is probably due to technical aspects (the type of antibody, method of MGMT scoring) or due to genetic differences or environmental factors.

A matter of concern in our study is that we observed MGMT expression in 7 out of 8 cases of ependymal tumors (in 3 ependymomas, WHO grade II and 4 anaplastic ependymomas, WHO grade III) which was in agreement with Sardi et al. (39) who found all their 9 cases showed MGMT expression, while Gramatzki et al. (40) reported 25.0% (3 out of 12) positive cases, but in contrast to that; Mellai et al. (24) observed no expression in all their 12 cases, this variability needs further evaluation as MGMT is extensively assessed in astrocytomas, particularly in GBM but the number of cases and studies on MGMT expression in ependymal tumors are still limited.

Regarding tumor grade; grade IV gliomas (GBM) showed the highest MGMT expression (48.4%) that was within the range reported by others as previously clarified in Table 2. In grade II tumors 25.0% of the cases showed MGMT expression; however, Ogura et al. (26), Hu et al. (25), Mellai et al. (24) and Nakasu et al. (37) reported higher figures (30.4%, 48.2%, 49.0% and 67.8% respectively), in grade I gliomas Mellai et al. (24) and HU et al. (25) reported 30.0% and 33.3% MGMT expression respectively which were higher than ours (15.0%) also in grade III gliomas higher figures reported by other researchers (24, 25). As we previously explained there's a considerable range of MGMT immunoexpression in gliomas in different studies and probably due to technical problems and variable genetic and environmental factors.

In the end, it can be said that the genetic genes of cancer are very large and complex (41-44), and new technologies such as genome editing (45) may be helpful in this regard. Additionally, RNA expression analysis in tissues strengths the immunohistochemistry techniques (46).

Our study revealed that MGMT is frequently expressed in different types of gliomas, particularly in GBM and its significantly associated with the patient's age.

Conflict of interest

The author declares no conflict of interest.

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