

MGMT immunoreactivity in adamantinomatous craniopharyngiomas

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Abstract At present, no effective medical treatment exists for recurrent and aggressive craniopharyngiomas that are resistant to conventional therapies, including surgery and adjuvant radiotherapy. Temozolomide is an alkylating chemotherapeutic agent used routinely in the management of high grade gliomas. The response to temozolomide is suggested to be dependent on the tumoral expression of O-6 methylguanine DNA methyltransferase (MGMT). Evidence supports that low MGMT immunoreactivity correlates with positive response to temozolomide. Therefore, we aimed to assess MGMT immunoreactivity in adamantinomatous craniopharyngiomas, in an effort to predict the likelihood of response to temozolomide. The MGMT immunostaining was performed on 23 adamantinomatous craniopharyngiomas operated at the Sisli Etfal Training and Research Hospital and identified by histological analysis. Paraffin embedded tissue sections were immunostained for MGMT and were evaluated semi-quantitatively. Of the 23 cases evaluated, 22 (96%) demonstrated negative (<10%) and 1 (4%) demonstrated low (10%) MGMT immunoreactivity. Data from this study suggest a high proportion of adamantinomatous craniopharyngiomas exhibit negative/

low MGMT immunoreactivity and could be treated with temozolomide, if conventional therapy fails.

Keywords Craniopharyngioma · Adamantinomatous · MGMT · Temozolomide · Chemotherapy

Introduction

Craniopharyngiomas are benign epithelial tumors, most of them are located in the sellar/parasellar region and arise from epithelial nests of the Rathke's pouch. According to World Health Organization (WHO) classification, craniopharyngiomas correspond histologically to tumoral grade I [1]. However, the growth characteristics of craniopharyngiomas show considerable variation and often they behave as aggressive tumors. Histologically, craniopharyngiomas are consisted of two subtypes, the adamantinomatous and the papillary, but a mixture of both types is frequently present. The adamantinomatous subtype is the most common form predominantly affecting young children but also all ages, whereas the papillary subtype is almost entirely seen in adults [2].

The management of craniopharyngioma remains challenging. Gross total resection or subtotal resection in combination with adjuvant conventional radiotherapy (RT) is currently the treatment of choice [2]. However, due to their large size, calcification and adherence to crucial surrounding neurovascular structures, complete resection is difficult. Therefore, they are associated with a high rate of recurrence. Treating recurrent tumors is more difficult compared to primary therapy and is associated with high rates of mortality and morbidity [3]. Currently, aside from repeat surgery and RT, treatment options are few in recurrent and aggressive cases [2]. For craniopharyngiomas

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that escape standard therapeutic approaches, the establishment of new alternative therapeutic options is important.

Temozolomide (TMZ) is an oral alkylating chemotherapeutic agent that is converted physiologically to 5-(3-methyltriazene-1-yl) imidazole-4-carboximide, has a cytotoxic effect by methylation of guanine at the *O*-6 position of DNA and inhibits DNA replication [4, 5]. Susceptibility of various tumors, including high grade gliomas and conventional treatment resistant pituitary adenomas and carcinomas, to temozolomide is well established [6–12].

O-6 methylguanine DNA methyltransferase (MGMT) is a DNA repair enzyme that alters the methylation state of DNA and removes the alkyl group adducts at the *O*-6 position of guanine, thereby counteracts TMZ's anti-neoplastic effect [12–14]. Immunohistochemical studies suggest that low MGMT immunoreactivity correlates with a high likelihood of response to TMZ therapy [7, 8, 11, 14, 15]. Therefore, we aimed to assess MGMT immunoreactivity in adamantinomatous craniopharyngiomas in an effort to anticipate the likelihood of response to TMZ. Our data may serve as a ground for considering whether TMZ could be used as an alternative treatment option for the cases of recurrent and aggressive adamantinomatous craniopharyngiomas that are resistant to conventional treatment modalities.

Materials and methods

For the current study, the database of the neurosurgery and pathology clinics of the Sisli Etfal Training and Research Hospital was searched for all cases of adamantinomatous craniopharyngioma. The initial diagnosis was based on clinical and radiological findings confirmed by histological evaluation. The paraffin embedded tissue sections of 23 patients, operated at our center during the interval 1993–2009 and diagnosed as adamantinomatous craniopharyngioma according to histological findings, were eligible for immunohistochemical analysis.

Immunohistochemistry (IHC) for MGMT was performed using avidin–biotin–peroxidase complex method. 3–5 μ m cut formalin-fixed, paraffin embedded tumor tissue specimens underwent heat induced antigen retrieval for 20 min in 0,01 M citrate buffer (PH 6,0) in microwave. Sections were incubated overnight with a mouse monoclonal anti MGMT antibody (Clone MT 3,1: Novus Biologicals, NB 100-692) at 1/50 dilution. Positive control consisted of tonsil with squamous metaplastic mucosa and basaloid cells with positive staining for MGMT. In addition, non-neoplastic endothelial cells and lymphocytes served as internal positive controls. MGMT immunoreactivity was evaluated on a multi-head light microscope

by to observer (C.T, H.Ö) and defined semi-quantitatively by visual impression according to the fraction of positive nuclear staining.

According to a study performed by Lau et al. the MGMT immunoreactivity scored as 1 = negative or limited to <10%, 2 = 10–25%, 3 = 25–50%, and 4 \geq 50%. Scores 1 and 2 represent low level MGMT expression, whereas scores 3 and 4 represent intermediate and high MGMT expression, respectively [8]. Clinical and radiological data were obtained through retrospective chart review. The age and gender of the patients, number of surgeries and frequency of recurrence was correlated with MGMT immunoreactivity. The project was approved by the local ethical board.

Results

The current study included 23 patients (13 male, 10 female), ranging in age between 5 and 61 years old ($26,3 \pm 16,9$ years old). Of the study population, 9 (39%) were children and 14 (61%) were adult patients. Among the 23 tumors, 13 were suprasellar, 8 were sellar/suprasellar and 2 were sellar according to radiological findings. The provided tissues for MGMT immunostaining were obtained at initial surgery in 16 (69%), and at surgeries performed following at least one recurrence in remaining 7 (31%) of the patients.

Overall 23 cases of adamantinomatous craniopharyngioma exhibited negative/low MGMT immunoreactivity. Staining for MGMT was limited to endothelium which served as positive internal control (Fig. 1a). Staining for MGMT was <10% (score 1) in 22 (96%) tumors and 10% (score 2) in 1 (4%) tumor (Fig. 1b, c). There is no correlation between age, gender, number of surgeries and frequency of recurrence with MGMT immunoreactivity. Patient characteristics and MGMT immunoreactivity levels are shown in Table 1.

Discussion

Craniopharyngiomas are rare, mainly sellar/suprasellar benign epithelial tumors arising from remnants of the Rathke's pouch and diagnosed during childhood and adult life.

Many therapeutic modalities have been tried so far with the goal of eradicating these tumors or to diminish their size. Currently, gross total resection or subtotal resection followed by RT is the treatment of choice. However, according to different studies, recurrence could occur in 0–62% of cases after gross total resection and in 25–100% of cases after subtotal resection [16]. This condition

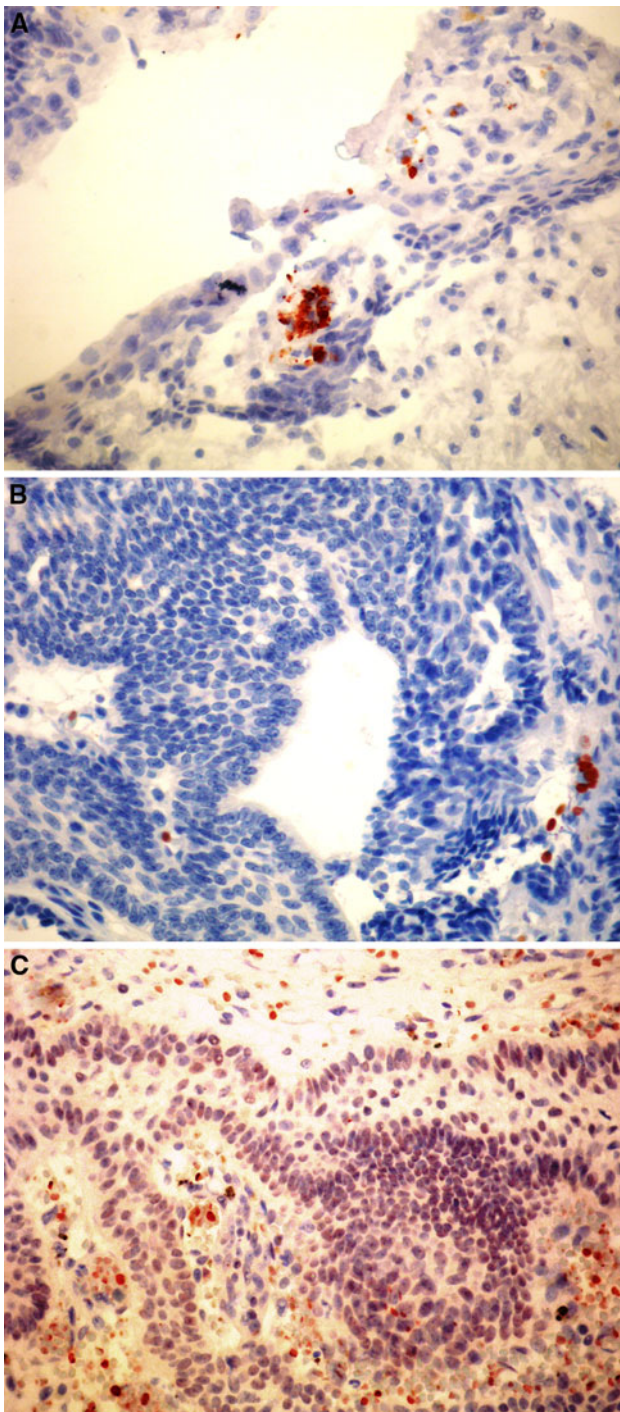


Fig. 1 MGMT immunohistochemistry. **a** and **b** negative MGMT staining (score 1) in adamantinomatous craniopharyngioma (case 23 and 5). The endothelial cells serve as internal positive control (**a**). **c** low MGMT staining (score 2) in adamantinomatous craniopharyngioma (case 2), the upper part of photomicrograph displays reactive gliosis with low MGMT immunoreactivity. **a–c** $\times 200$

requires further therapy such as repeat surgeries and/or RT. On the other hand, repeat surgeries are associated with a high failure rate of tumor control and carries the high risk of endocrine, hypothalamic and neuro-cognitive dysfunction

[17]. Recurrence could also occur in approximately 20% of patients after the use of RT for residual disease [18]. Nevertheless, RT is associated with less early morbidity but in long-term it is associated with serious complications, which is more important in children [19].

Intratumoral bleomycin has been proposed as an alternative to surgery or RT for predominantly cystic craniopharyngiomas. It has a limited role in the management of these tumors and just provide short-term control. Furthermore, serious damage to surrounding brain structures has been exhibited after the leakage of bleomycin from inside of the tumor [19]. Progression of disease required re-operation within 1 year of treatment has also been reported [20].

As an alternative to intracystic bleomycin, intracystic interferon-alpha-2a (IFN- α) has been used in the treatment of predominantly cystic craniopharyngiomas. According to a recent study conducted by Cavalheiro et al. tumor control was achieved in 47 patients treated with intracystic IFN- α [21]. However, in 13 patients the tumor continued to grow and 8 patients developed new different pituitary hormone deficiencies after treatment [21]. A rapid increase in the size of cyst has also been reported in 2 patients, after the commencement of intracystic IFN- α [19]. Like intracystic bleomycin, intracystic IFN- α may provide short term control of predominantly cystic craniopharyngiomas, but long term effect is yet unknown. IFN- α has a minimal effect when administered systemically. Moreover, tumor enlargement has been observed in most patients after discontinuation of the drug [2].

Systemic chemotherapy has been applied in very small number of patients with aggressive craniopharyngioma. Bremer et al. treated a case of cystic craniopharyngioma with systemic chemotherapy using the combination of vincristine, carmustine and procarbazine, resulting in tumor shrinkage and clinical improvement [22]. Lippens et al. reported four cases of adamantinomatous craniopharyngioma with multiple recurrences after several surgical procedures and RT. These patients were treated with the combination of doxorubicin and lomustin. In follow-up, they observed complete remission in one, stable disease in two, and cystic relapse in one of the patients [23]. However, to date, TMZ has not been administered in the management of conventional treatment resistant craniopharyngioma of any subtype. The advantage of TMZ is that it is not cell-cycle specific, so it can inhibit all stages of tumor cell growth. Thus, it is suitable for slow-growing tumors [24].

In the present study, 23 cases of adamantinomatous craniopharyngioma assessed for MGMT immunoreactivity. Of the 23 tumors evaluated, 22 (96%) demonstrated negative (<10%) and 1 (4%) demonstrated low (10%) MGMT immunoreactivity. There was no correlation between age,

Table 1 Patient characteristics and MGMT immunorexpression level in adamantinomatous craniopharyngioma (present study)

Case	Patient age (years)/gender	Tumor site	MGMT immunorexpression result (%)	Recurrence/disease outcome ^a
1	15/F	Sellar/suprasellar	1	No
2	12/M	Suprasellar (3. vent)	10	Yes, 2. surgery
3	61/M	Suprasellar	4	Yes, 2. surgery
4	39/M	Suprasellar	1	Yes, 4 recurrences, 5 surgeries, radiotherapy
5	12/F	Sellar/suprasellar	1	Died soon after the 1. surgery
6	43/F	Suprasellar	5	Died soon after the 1. surgery
7	22/F	Sellar	5	No
8	30/M	Sellar/suprasellar	7	Yes, 2 recurrences, 3 surgeries, radiotherapy
9	56/F	Suprasellar	5	No
10	9/M	Suprasellar	4	No
11	14/F	Sellar/suprasellar	6	No
12	18/M	Suprasellar	1	No
13	5/M	Suprasellar	3	No
14	23/F	Sellar/suprasellar	5	No
15	6/M	Suprasellar	2	No
16	23/M	Suprasellar	4	No
17	23/F	Sellar/suprasellar	7	Yes, 2. surgery
18	23/M	Sellar/suprasellar	4	Yes, 2. surgery
19	13/M	Suprasellar	5	No
20	56/F	Suprasellar	1	Yes, 2. surgery, died soon after the 2. surgery
21	15/M	Sellar	5	No
22	42/F	Sellar/suprasellar	5	No
23	44/M	Suprasellar	3	No

^a All surviving patients are followed for recurrence

gender, number of surgeries and frequency of recurrence with MGMT immunoreactivity. Thus, our findings suggest that adamantinomatous craniopharyngiomas may be susceptible to TMZ therapy.

In summary, the present study suggests a high proportion of adamantinomatous craniopharyngiomas express a low level of MGMT. These findings provide a ground for the assessment of TMZ's efficacy in clinical trials as an alternative agent in this rare tumor subtype, if conventional therapy, including repeat surgeries and RT, fails.

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