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ORIGINAL ARTICLE

MGMT immunoexpression in growth hormone-secreting pituitary adenomas and its correlation with Ki-67 labeling index and cytokeratin distribution pattern

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Abstract Recent publications suggest the utility of temozolomide (TMZ) in the management of aggressive pituitary adenomas and carcinomas, resistant to conventional treatments. The response to TMZ is inversely correlated with tumoral expression of O-6 methylguanine DNA methyl transferase (MGMT). Therefore, we aimed to assess MGMT immunoexpression in pure GH-secreting pituitary adenomas, in an effort to predict the likelihood of response to TMZ, and to correlate MGMT immunoexpression with Ki-67 LI and cytokeratin (CK) distribution pattern. Our material consisted of 36 GH-secreting pituitary adenomas (21 female,15 male, mean age 42.5 ± 10.5), operated at our center between 2003 and 2010. Immunostaining for MGMT, Ki-67, and CK was performed using avidin–biotin-peroxidase complex method. Immunoreactivity for MGMT and Ki-67 was evaluated microscopically and recorded as percentages of positive nuclear immunostaining. CK distribution pattern was also evaluated microscopically and assoreted into dot-like and nondot-like pattern subtypes. MGMT immunoexpression scored as 0 = none, 1 = <10%, 2 = <25%, 3 = <50%, and 4 = >50%. Staining for MGMT was <10% (score 1) in 30 (83.3%), 10–25% (score 2) in 3 (8.3%), 25–50% (score 3) in 2 (5.6%) and >50% (score 4) in 1 (2.8%) of the tumors, respectively. There was no correlation between Ki-67 LI and CK distribution pattern with MGMT immunoreactivity ($P > 0.05$). Data from the current study suggest a large proportion of GH-secreting adenomas, including those with dot-like CK distribution pattern and high Ki-67 LI, demonstrate negative/low MGMT immunoreactivity and could be treated with TMZ, if conventional treatment fails.

Keywords MGMT · Acromegaly · Chemotherapy · Temozolomide

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Introduction

Currently, multiple treatment options are available for the treatment of acromegaly including surgery, medical therapy, and radiotherapy (RT). Disease control is achieved with trans-sphenoidal adenomectomy in most patients with microadenomas but approximately 40–60% of those with macroadenomas are unlikely to be controlled with surgery alone [1]. About 10% of patients thought to be cured with surgery demonstrate significant tumor re-growth [2]. Treatment with somatostatin analogs (SSA) either as primary treatment or as second treatment option after trans-sphenoidal adenomectomy is effective in biochemical control and in reducing tumor size. Disease control can be attained in most of the patients treated with SSA, however, an approximately 10% of the patients were demonstrated to be resistant to SSA therapy, based on the biochemical results [3]. SSA were showed to induce a variable degree of tumor shrinkage in 30 and 48% of patients that received adjunctive or primary therapy, respectively [3]. Nevertheless, persistent tumor progression have been reported in up to 2.2% of the patients treated with SSA [2]. Although

pegvisomant is effective in cases with persistent elevated IGF-I levels despite treatment with maximal doses of SSA, persistent tumor enlargement also appears to occur in up to 2.9% of the patients treated with pegvisomant [2].

Although the characteristics of the minority of patients with treatment-resistant GH-secreting pituitary adenomas and the factors associated with aggressive tumor behavior are not well known, some morphologic features of the tumor including number of cytoplasmic granules, dot-like cytokeratin (CK) distribution pattern, and high Ki-67 labeling index (LI) have been demonstrated to be associated with aggressive tumor behavior, dural and cavernous sinus invasion, suprasellar extension, persistence of tumor after surgery, and lower response rate to SSA therapy [2, 4, 5].

Recently, temozolomide (TMZ) an alkylating cytostatic, has been administered successfully in the treatment of various pituitary adenomas and carcinomas, resistant to current conventional treatment modalities [6, 7]. The effect of TMZ is thought to be dependent on tumoral expression of *O*-6 methyl guanine DNA methyl transferase (MGMT). Studies suggest that, low expression of MGMT correlates with high likelihood of response to TMZ. Therefore, in the present study, we aimed to determine MGMT immunoexpression in pure GH-secreting pituitary adenomas, in an effort to predict the likelihood of response to TMZ, as well as its correlation with Ki-67 LI and CK distribution pattern.

Materials and methods

For the current study, the database of the endocrinology and pathology clinics of the Sisli Etfal Training and Research Hospital was searched for all cases of GH-secreting pituitary adenoma. The paraffin-embedded tissue section of 40 patients diagnosed as acromegaly and operated at our center during the interval 2003 through 2010 were eligible for immunohistochemical analysis. All paraffin-embedded tissue specimens were immunostained for GH, prolactin, ACTH, FSH, and LH, using standard immunohistochemical method and specimens that were immunopositive just for GH and negative for other pituitary hormones, included into the study. Paraffin-embedded tissue specimens of 36 patients were eligible for the study. Clinical and radiological data were obtained through chart review. The clinical and radiological data for 25 patients, being followed at our outpatient clinic, were available, and the remaining 11 patients had only been operated at our center.

Immunohistochemistry (IHC) for MGMT was performed using avidin–biotin-peroxidase complex method. 3–5 μm 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, Littleton, CO, USA) at 1/50 dilution. Positive control consisted of tonsil with squamous metaplastic mucosa and basaloid cells with positive staining for MGMT. Nonneoplastic endothelial cells and lymphocytes served as internal positive controls, in all tissue sections. Immunostaining for Ki-67 and CK was also performed using the same method. Sections were incubated overnight with a rabbit polyclonal antibody, MIB-1 (ScyTek Laboratories, Logan, Utah, USA) to determine Ki-67 immunoreactivity and with a prediluted mouse monoclonal anti CK antibody [Clone AE/1 (Acidic), ScyTek Laboratories, Logan, Utah, USA] to determine CK distribution pattern. MGMT, Ki-67, and CK immunoexpressions were evaluated on a multi-head light microscope by two observer (C.T, H.Ö) and defined semi quantitatively by visual impression.

MGMT immunoexpression scored as 1 = negative or limited to <10%, 2 = 10–25%, 3 = 25–50%, and 4 = >50%, according to the fraction of positive nuclear staining. Scores 1 and 2 represent low-level MGMT expression, whereas scores 3 and 4 represent intermediate and high MGMT expression, respectively [7]. CK distribution pattern was assorted into dot-like and nondot-like pattern subtypes. Dot-like pattern was defined by conspicuous intensive globular CK immunoreactivity in >70% of cells. The nondot-like pattern comprised all the remaining shapes. The age, gender, number of surgeries, status of remission under SSA therapy, cavernous sinus invasion, Ki-67 LI, and CK distribution pattern were correlated with MGMT immunoexpression. The study was approved by local ethical board.

Statistical analysis

Spearman correlation was used for correlation between age, Ki-67 LI, and CK distribution pattern with MGMT immunoreactivity. Mann–Whitney U test was performed for correlation between gender, number of surgeries, status of remission under SSA therapy, and cavernous sinus invasion with MGMT immunoreactivity. Statistical analysis of the data was performed using SPSS 10.0 software and a P value <0.05 was considered statistically significant.

Results

The current study included 36 patients (21 female, 15 male), ranging in age between 23 and 65 years old (42.5 ± 10.5 years old). According to presurgical MRI results, 23 (92%) of the 25 tumors were macroadenomas and 2 (8%) were microadenomas. Cavernous sinus invasion was present in 10 (40%) of the 25 patients. Six (24%) of the patients

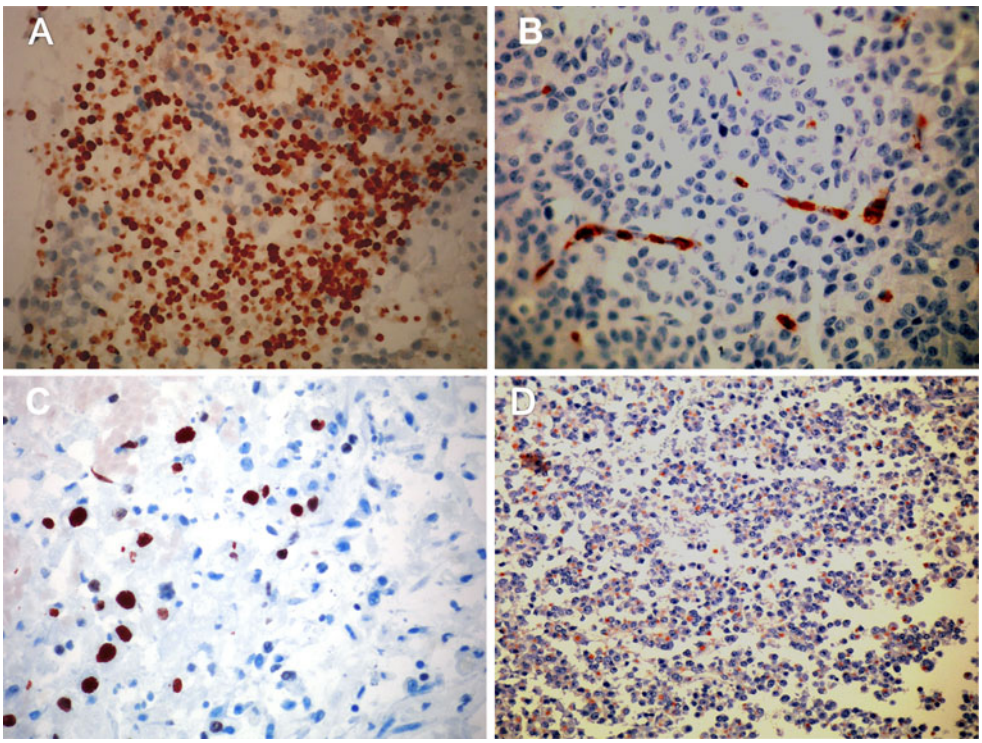

Fig. 1 MGMT, Ki-67, and CK immunohistochemistry. **a** A GH-secreting pituitary adenoma demonstrated high MGMT immunoexpression (60%, score 4), **b** negative MGMT immunoexpression in a GH-secreting pituitary adenoma (score 1), endothelial cells serve as internal positive control (case 1). **c** High Ki-67 LI (11%) and **d** dot-like CK distribution pattern in the same tumor (**a**, **b**, and **c** 200×, **d** 100×)

with available data had been operated for at least two times, and the remaining 19 (76%) patients had been operated on once. Four (16%) of the patients were cured at first surgery. Among the 21 (84%) patients treated with SSA, 7 (33.3%) were considered as controlled and 14 (66,7%) had active disease, according to current criteria used for defining disease status [8]. MGMT immunostaining was performed on tissue specimens obtained at first surgery in all patients. Staining for MGMT was <10% (score 1) in 30 (83.3%), 10–25% (score 2) in 3 (8.3%), 25–50% (score 3) in 2 (5.6%) and >50% (score 4) in 1 (2.8%) of the tumors, respectively. Tumor tissue specimens of four patients who underwent second surgery were also available and three of them were eligible for immunohistochemical analysis (cases 4, 12, and 21). The MGMT immunoexpression levels were not different between tissues obtained at first surgery and those obtained at second surgery [1% at first and 0% (score 1) at second surgery for case 4, 2% at first and 4% (score 1) at second surgery for case 12 and 5% at first and 5% (score 1) at second surgery for case 21, respectively]. Immunostaining for MGMT was positive for endothelial cells which served as internal positive control (Fig. 1a, b). Ki-67 LI was ≥4% in 12 (33.3%) and ≤3% in remaining 24 tumors (Fig. 1c). Thirteen (36%) tumors exhibited dot-like and 23 (64%) exhibited nondot-like CK distribution pattern (Fig. 1d). There was no correlation between gender, cavernous sinus invasion, number of surgeries, and status of remission under SSA therapy with MGMT immunoreactivity ($P > 0{,}05$). There was also no correlation between age, Ki-67-LI, and CK distribution pattern with MGMT immunoreactivity ($P > 0{,}05$). Patient charactaristics, Ki-67 LI, CK distribution pattern, and MGMT immunoexpression level of 25 patients with available follow-up data are given in Table 1.

Discussion

Trans-sphenoidal surgery is the most cost-effective and initial treatment option for GH-secreting pituitary adenomas [3], but the local and biochemical control is difficult to achieve with surgery alone in more than half of macroadenomas, including invasive and aggressive cases [2]. The majority of the cases that could not be controlled with surgery, respond to currently available medical therapies and RT. However, a small number of patients may escape these conventional treatments.

Recent reports suggest the efficacy of TMZ in the treatment of conventional treatment-resistant pituitary adenomas and carcinomas [6, 7]. TMZ is an orally available cytostatic-alkylating agent that readily crosses the blood–brain barrier.

Table 1 Patient charactaristics, Ki-67 LI, CK distribution pattern and MGMT immunoexpression level in GH-secreting pituitary adenomas (present study)

Case No.	Age/ gender	MGMT immuno expression (%)	CK distribution pattern	Ki-67 LI (%)	Size of tumor on MRI	Cavernous sinus invasion on MRI	No of surgeries	Cure after first surgery	Medical treatment	RT	GNRS	Remission status under SSA therapy
1	23/M	1	DP	11	MA	No	1	No	SSA	NA	NA	No
2	29/F	3	DP	5	MA	Yes	1	No	SSA/DA	NA	Yes	No
3	41/F	5	DP	8	MA	No	1	No	SSA/DA	NA	NA	No
4	50/M	1	DP	1	MA	No	2	No	NA	Yes	NA	Yes
5	42/M	1	NDP	4	MicA	No	1	Yes	NA	NA	NA	–
6	65/F	4	NDP	1	MA	No	1	No	SSA	NA	NA	No
7	48/F	10	NDP	1	MA	No	1	Yes	NA	NA	NA	–
8	33/M	1	NDP	2	MA	Yes	1	No	SSA	NA	NA	No
9	60/M	1	NDP	1	MicA	No	1	Yes	NA	NA	NA	–
10	46/F	1	NDP	1	MA	No	1	No	SSA	NA	NA	Yes
11	27/F	1	DP	5	MA	Yes	2	No	SSA	NA	NA	No
12	44/F	2	NDP	4	MA	No	2	No	NA	NA	NA	Yes
13	56/M	20	DP	8	MA	Yes	3	No	SSA	NA	NA	No
14	42/F	5	NDP	3	MA	Yes	1	No	SSA	NA	NA	No
15	36/F	7	NDP	7	MA	Yes	1	No	SSA	NA	NA	Yes
16	31/M	1	NDP	3	MA	No	1	No	SSA	NA	NA	No
17	46/F	20	NDP	1	MA	No	1	No	SSA	NA	NA	No
18	65/M	4	NDP	1	MA	No	1	Yes	NA	NA	NA	Yes
19	49/F	60	NDP	5	MA	Yes	1	No	SSA	NA	NA	No
20	56/F	7	NDP	1	MA	No	1	Yes	NA	NA	NA	–
21	31/M	5	DP	1	MA	No	2	No	SSA	NA	NA	No
22	30/F	3	DP	1	MA	Yes	1	No	SSA	NA	Yes	No
23	45/F	1	DP	3	MA	Yes	1	No	SSA	NA	NA	Yes
24	37/F	8	DP	5	MA	Yes	1	No	SSA	Yes	Yes	No
25	44/M	2	NDP	1	MA	No	2	No	SSA	No	No	Yes

Abbreviations: *CK* cytokeratin, *DP* dot-like pattern, *NDP* nondot-like pattern, *RT* radiotherapy, *GNRS* gamma knife radiosurgery, *MA* macroadenoma, *MicA* microadenoma, *SSA* somatostatin analogs, *DA* dopamin agonists, *NA* not applied

It is not cell-cycle specific and can inhibit all stages of tumor cell growth. Therefore, it is a suitable drug for slow-growing tumors [9].

Recently, MGMT immunoreactivity of pituitary adenomas was found to be correlated inversely with therapeutic response to TMZ [6, 7]. MGMT is a DNA repair protein that removes the alkyl group adducts at the *O*-6 position of guanin bases, by transferring the alkyl group to a sulfur group of cysteine within its sequence, and induces resistance to TMZ [10, 11]. Studies demonstrated inhibition of cell proliferation, induction of apoptotic cell death, and morphologic changes including significant decrease in Ki-67 LI in pituitary tumor cells exposed to TMZ [12, 13]. To date, almost 30 cases of conventional treatment-resistant, aggressive pituitary adenomas and carcinomas have been treated with TMZ, with various degrees of response [6, 7, 14–16]. Immunohistochemical analysis demonstrated low MGMT immunoreactivity in most of the TMZ responsive cases [6, 7, 16]. McCormack et al. and Morin et al. reported failure of TMZ to attain disease control in two cases of conventional treatment-resistant GH-secreting pituitary adenomas [17, 18]. However, in the case reported by McCormack et al. the tumor exhibited high MGMT immunoreactivity and in case reported by Morin et al. MGMT immunostaining was not performed on tumor tissue specimens. Therefore, the lack of response to TMZ may be due to high MGMT expression of the tumor. McCormack et al. indicated that prolactinomas are accounted for >60% of pituitary adenomas with low MGMT immunorectivity [17]. This result may be due to inclusion of a small number of GH-secreting adenomas into their study. In the present study, we found that the majority of GH-secreting adenomas are also associated with negative/low-level MGMT expression.

Although the standard method for assessment of MGMT is the identification of promoter methylation by polymerase chain reaction, McCormack et al. demonstrated that MGMT promoter methylation is associated with low immunohistochemical expression of MGMT [17]. Therefore, assessment of MGMT status using IHC is a useful technique for detection of MGMT in pituitary tumors.

Studies suggest that young age, high GH levels, and larger tumor size at diagnosis, morphologic characteristics of the tumor, including dot-like CK distribution pattern and high Ki-67 LI are associated with aggressive tumor behavior [2].

The nuclear antigen Ki-67 is related to growth potential in many human tumors, and is a major prognostic indicator for pituitary adenomas in the recent WHO classification of pituitary tumors, in which, adenomas with Ki-67 LI more than 3% are classified as atypical adenomas [19, 20].

Ki-67 is expressed during all nonG0 phase cell-cycle except for the early G1 phase. The estimation of Ki-67 LI using MIB-1 is a useful method for determining the proliferative activity of pituitary adenomas, because it remains stable in paraffin tissue sections [21]. The close relationship between Ki-67 LI with cavernous sinus invasion, persistence of tumor after surgery, and lower response rate to SSA therapy is well established [4, 5].

Pure GH-secreting pituitary adenomas are structurally classified into two distinct subtypes: densely granulated (DG) and sparsely granulated (SG) adenomas [22]. SG adenomas could be identified by IHC as dot-like CK distribution pattern versus perinuclear distribution pattern for DG adenomas [23]. Each of these adenoma subtypes demonstrate distinct clinical and biochemical features. Dot-like CK distribution pattern in GH-secreting adenoma is demonstrated to be associated with aggressive growth, higher incidence of suprasellar extension, cavernous sinus invasion, and lower response rate to SSA therapy [22–25]. In the present study, 12 (33.3%) of 36 GH-secreting adenomas had Ki-67 LI more than 3% and 13 (36%) exhibited dot-like CK distribution pattern. Although the number of adenomas with high MGMT immunoexpression were low, we found no significant correlation between Ki-67 LI and CK distribution pattern with MGMT immunoreactivity. These findings suggest that GH-secreting adenomas with high Ki-67 LI as well as those with dot-like CK distribution pattern may be susceptible to TMZ therapy.

In recent two studies, Raverot et al. and Bush et al. found no significant relation between MGMT immunoexpression with response to TMZ [14, 15]. In their studies, three pituitary carcinoma with low MGMT expression have not responded to TMZ, whereas four tumors (one carcinoma and three aggressive adenomas) with moderate/high MGMT immunoreactivity have responded to TMZ. However, in the study of Bush et al. the best response to TMZ has been achieved just in tumors with low MGMT immunoreactivity [14]. Similarly, in the study performed by Raverot et al. all responder tumors demonstrated low/moderate MGMT immunorectivity [15]. On the contrary, a more recent prospective study performed by Losa et al. showed that negative staining for MGMT seems likely to predict a high likelihood of response to TMZ [16].

In summary, the current study suggests a large proportion of GH-secreting adenomas, including those with dot-like CK distribution pattern and high Ki-67 LI, demonstrate negative/low MGMT immunoreactivity and could be treated with TMZ, if conventional therapy fails.

Conflict of interest None.

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