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Original Article

Implications of MGMT methylation status in pituitary adenoma

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ABSTRACT

Background: Very little literature exists on frequency of MGMT methylation status in pituitary adenoma. We assessed the frequency of MGMT methylation and protein expression in pituitary adenoma and correlated with patients' age group and prognosis.
Methods: Tumor tissues from 30 patients with pituitary adenoma were evaluated for MGMT promoter methylation status by methylation specific PCR method. All tumors were also immunostained with MIB-1, anti-p53 and anti-MGMT antibodies.
Results: MGMT methylation status was noted in 40% of cases (7/20 non-functioning adenomas and 5/10 functioning adenomas). MGMT protein expression on IHC was noted in 72.2% of unmethylated tumors, while only 41.6% of methylated tumors demonstrated protein expression. The mean labeling index of MGMT protein was higher in unmethylated tumors as compared to the methylated group, though the difference was not statistically significant (18.6% vs 8.8%; $p = 0.131$). Tumor regrowth occurred in four unmethylated tumors as compared to none in methylated group. Even though there was no correlation between patient age and MGMT methylation status ($p = 0.823$), we noted that the frequency of methylation in middle age patients (between 30 and 59 yrs) was 64.7%, which was higher compared to other age groups.
Conclusion: This is the first study from India showing MGMT promoter methylation status with protein expression in pituitary adenoma. We noted that tumor regrowth was higher in unmethylated tumors.

Introduction

Microsurgery, either most commonly by trans-sphenoidal route, or sometimes by transcranial approach, is considered to be the standard treatment of non-functioning pituitary adenomas (NFPA). In view of their frequently large size at the time of diagnosis and their potentially invasive growth into parasellar structures, complete resection is not possible at all times, despite advancing technologies in surgery such as endoscopy, neuronavigation, and intraoperative magnetic resonance imaging (MRI) [4]. Since regrowth from adenoma remnants is variably observed (38–95%), additional treatments such as reoperation, stereotactic radiosurgery and fractionated radiotherapy may be required [6,21]. Despite multimodality treatment, a fraction of pituitary adenomas can progress and pose a significant therapeutic dilemma, as there are no pharmacologic treatment options available for non-functional pituitary adenoma. Therefore in such cases, need for alternative, clinically applicable therapeutic options becomes important.

Temozolomide (TMZ), an oral alkylating agent, is part of the routine therapy for glioblastoma (GBM) and it has been noted that there is improved survival in GBM with the combination of TMZ with radiotherapy [11]. Several clinical studies have demonstrated that methylation of the MGMT gene may have prognostic significance in glioblastoma, although the magnitude of effect varies across studies [14,18]. MGMT gene silencing has been associated with longer overall survival (OS) in patients with high grade gliomas (HGG) [8,9]. MGMT has ubiquitous expression in human tissue with increased expression in some gliomas [5].

Temozolomide (TMZ) also has been successfully used for the treatment of endocrine neoplasms [11]. TMZ has been proposed for the treatment of prolactin-producing and nonfunctioning, aggressive pituitary adenomas and carcinomas. A few published case reports of pituitary adenoma state clinical improvement, tumor shrinkage, and reduction of hormone secretory activity in most patients who were treated with TMZ. Consequently, the assessment of O^6-methylguanine DNA methyltransferase (MGMT) expression

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as a potential marker for adenoma responsiveness to TMZ treatment was proposed on the basis of two clinical cases. In the first case, an MGMT immunonegative tumor was identified to respond to TMZ; whereas, in the second case, high MGMT expression was associated with TMZ resistance [10].

The present study is the first of its kind in our country to assess the incidence of MGMT promoter methylation status along with protein expression in pituitary adenoma. This objective will enable us to define the incidence of MGMT methylation in pituitary adenoma and its correlation with MGMT protein expression. This data can potentially help in consideration of TMZ therapy for pituitary adenoma in future.

Methods

All patients of pituitary adenoma who underwent surgical decompression at the National Institute of Mental Health and Neurosciences, India, between January to December 2007 were considered for the study. After reviewing the blocks, the patients in whom adequate tissue was available for DNA isolation for MGMT methylation analysis were included for the study, which resulted in a cohort of 30 patients of pituitary adenoma. The clinical, endocrinological data were obtained from the case records. The histopathological slides were reviewed by the neuropathologist and the diagnosis was confirmed.

MGMT methylation analysis

DNA extraction

The paraffin blocks were retrieved from Department of Neuropathology. Genomic DNA was extracted from formalin-fixed paraffin-embedded [FFPE] sections using the QIAamp DNA FFPE Tissue Kit (Cat no.: 56404, Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The DNA concentration was estimated by spectrophotometry (Thermo Scientific NanoDrop™ 1000 Spectrophotometer). In most of the cases, the sections contained only tumor tissue. In a few cases, adjacent normal pituitary tissue was seen. This portion was removed from the paraffin block prior to DNA extraction to ensure that we obtained only the tumor tissue DNA.

Bisulfite modification

The bisulfite modification of isolated DNA was performed using the EZ DNA Methylation kit (Zymo Research, Orange, CA, USA; D5001) as described by the manufacturer. This reaction selectively deaminates unmethylated cytosine residues resulting in a conversion to uracil, whereas 5-methyl cytosine residues are not modified.

Methylation-specific PCR (MSP)

The methylation status of the MGMT gene was determined by the method of Nested, gel-based MSP, as follows: DNA methylation patterns in the CpG island of the MGMT gene is determined by chemical modification of unmethylated, but not the methylated, cytosines to uracil and subsequent PCR using primers specific for either methylated (*m_MGMT*) or the modified unmethylated (*u_MGMT*) DNA.

The modified DNA was subjected to stage-1 PCR of 35 cycles, further the product of 1st stage was used as a template for the 2nd stage PCR. The specific primers for *m_MGMT*: 5′-TTTCGACGTTCGTAGGTTTTCGC-3′ (forward primer) and 5′-GCACTCTTCCGAAAACGAAACG-3′ (reverse primer) and *u_MGMT*: 5′-TTTGTGTTTTGATGTTTGTAGGTTTTTGT-3′ (forward primer) and 5′-AACTCCACACTCTTCCAAAAACAAAACA-3′ (reverse primer) recognize the methylated and unmethylated sequences, respectively. The amplified product of *m_MGMT* and *u_MGMT* yields 81 and 93 bp, respectively.

The products were visualized on 4% agarose gels to determine the MGMT methylation status. The valid results of all clinical samples will be classified as methylated or non-methylated depending on the presence or absence of a band for *m_MGMT*. DNA from normal lymphocytes was used as unmethylated control. Glioblastoma tissue that had earlier shown methylated band was used as a positive control for methylated alleles.

Immunohistochemistry

Paraffin sections of thickness 5 μm were collected on silane-coated slides and subjected to immunohistochemistry using monoclonal antibodies: MIB-1 (Ki-67-specific monoclonal antibody; DAKO, Denmark, Dilution – 1:50), p53 (Dako, Denmark; Clone – DO7, Dilution – 1:200), and MGMT (Millipore, USA; Dilution – 1:50) After preliminary standard processing steps, the sections were incubated with the primary antibody for 2 h followed by secondary antibody; super-sensitive non-biotin HRP Detection Kit (Biogenex) for 1 h. Chromogenic substrate used was 3,3′-diaminobenzidine (Sigma, Germany). Sections were counterstained with hematoxylin. Standard positive controls were used for MIB-1 staining. For MGMT and p53 staining, glioblastoma sections that previously showed strong immunoreactivity for each of these markers, respectively, were used as positive controls. A negative control slide in which the primary antibody is excluded was incorporated with each batch of slides. The staining pattern was nuclear for all markers and immunolabeling was scored by a visual semi-quantitative method and the labeling index (LI) was calculated as the percentage positivity after counting 1000 tumor cells.

Results

The present study included 30 patients with pituitary adenoma who have undergone surgery at a tertiary neurosurgical center in the year 2007. The subjects included 12 women and 18 men. Among these, 20 (66.7%) were non-functioning adenomas and 10 (33.3%) were functioning adenomas. The functioning adenomas included five GH-secreting, three PRL-secreting and two mixed adenomas (one mixed GH- and PRL-secreting adenoma and one PRL- and TSH-secreting adenoma). The age of the patients ranged from 16 to 67 years (mean = 39.1 ± 14.5 yrs). Five patients presented with pituitary apoplexy. The patients were treated either by transcranial or transsphenoidal route based on the tumor extent on imaging. One patient expired in the peri-operative period. All the three prolactinomas were treated with adjuvant chemotherapy with bromocriptine or cabergoline. Radiotherapy was advised in six patients for the residual tumor after initial surgery. None of the patients had been treated with temozolomide.

MGMT methylation status (Fig. 1)

MGMT methylation was found in 40% (12/30) pituitary adenoma patients (Fig. 1). Of these, MGMT methylation was found in 7 of 20 non-functioning adenomas (35%) and 5 of 10 functioning adenomas (50%). Of these functioning adenomas, MGMT methylation occurred in 1/3 PRL-secreting adenoma and 3 out of 5 GH-secreting adenoma and one out of two cases of the mixed adenoma in the study. No association was noted between MGMT methylation status and functioning status of pituitary adenomas ($p = 0.758$).

The correlation of MGMT methylation status with age was done using Mann–Whitney *U*-test, which did not demonstrate any significant association ($p = 0.823$). Interestingly, on stratifying patients