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Original Article

Evaluation of the potential of the Ki67 index to predict tumor evolution in patients with pituitary adenoma

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Abstract: The aggressive course of a number of pituitary adenomas requires the investigation of potential predictors. This study aimed to investigate the proliferation marker Ki67 as a predictor of postoperative outcome in patients with pituitary adenoma regarding recurrence and regrowth of the tumor, using a Ki67 cut-off value of 3%. This retrospective study included 52 patients with pituitary adenoma who had undergone adenomectomy and had a pituitary image taken at least 1 year after surgery. Patients were divided according to Ki67 expression into high (≥3%) vs. low (<3%) levels of Ki67. The two groups were similar regarding the preoperative tumor invasion grade. The Ki67 index ranged from 0 to 30%; in 23 cases, Ki67 was ≥3%. The two groups were similar regarding tumor recurrence and regrowth: 4 cases (28%) of recurrence in the Ki67<3% group vs. none in the Ki67≥3% group (P=0.26); and 2 cases (13%) of regrowth in the Ki67<3% group vs. 7 cases (43%) in the Ki67≥3% group (P=0.11). A subgroup analysis was performed for nonfunctioning adenomas. Recurrence rates remained similar between groups (Ki67<3% group: 1 case [20%]; Ki67≥3% group: none; P>0.99), whereas regrowth rates were higher in the Ki67≥3% group (6 cases [67%] vs. 2 cases [17%] in the Ki67<3% group; P=0.03). The patient with the highest Ki67 index (30%) developed pituitary carcinoma. The results allow us to suggest the adoption of a stricter control of image monitoring in nonfunctioning adenomas with incomplete resection associated with a Ki67 index ≥3%.

Keywords: Pituitary adenoma, Ki67, regrowth, recurrence, predictor, behavior

Introduction

Although considered histologically benign, pituitary adenomas are invasive in 30 to 45% of cases, and a significant number of these tumors are considered aggressive based on recurrence during follow-up [1]. Ki67, a nuclear protein that reflects cell proliferation, is the most extensively studied tumor marker with potential predictive power for unfavorable treatment outcome. Since the 1980s, the relationship of Ki67 with tumor size and type, invasiveness, recurrence and malignancy has been investigated, often yielding conflicting results, except for a consistent positive association with tumor invasiveness [2-6]. The evidence to date has been insufficient to integrate Ki67 into follow-up flow charts for pituitary adenoma. The objective of this study was to investigate the presence of tumor regrowth/recurrence after surgery in a representative sample of patients with pituitary adenoma divided into patients with high vs. low levels of Ki67 according to the proposed cut-off value of 3%.

Patients and methods

The medical records of 52 patients from a tertiary neuroendocrinology center in southern Brazil, who had undergone pituitary adenectomy with determination of Ki67 index in the tumor sample, were retrospectively reviewed for clinical data, tumor functional class and size, preoperative imaging studies of the sella turcica, histological and immunohistochemical results, perioperative data, and additional treatments. Patients were eligible for inclusion if they had an image of the sella turcica that was taken at least 12 months after surgery. Patient follow-up ranged from 12 to 273 months. Regrowth of the adenoma was considered when postsurgical residual tumor showed

Table 1. Preoperative patient characteristics

	Ki67<3% (*n*=29)	Ki67≥3% (*n*=23)	*P*-value
Age (years)			
Median	43	46	0.99
Sex n (%)			
Female	16 (55%)	12 (52%)	>0.99
Duration of follow-up (months)			
Median	34	23	0.04
Range	12-252	12-273	
Type of adenoma			0.53
NFMA	17	12	
GH	10	8	
ACTH	2	3	
Macroadenomas *n* (%)	25 (87%)	20 (87%)	>0.99
Cavernous sinus invasion *n* (%)	9 (31%)	11 (48%)	0.26

NFMA: nonfunctioning macroadenomas; GH: growth hormone-secreting adenomas; ACTH: adrenocorticotropic hormone-secreting adenomas.

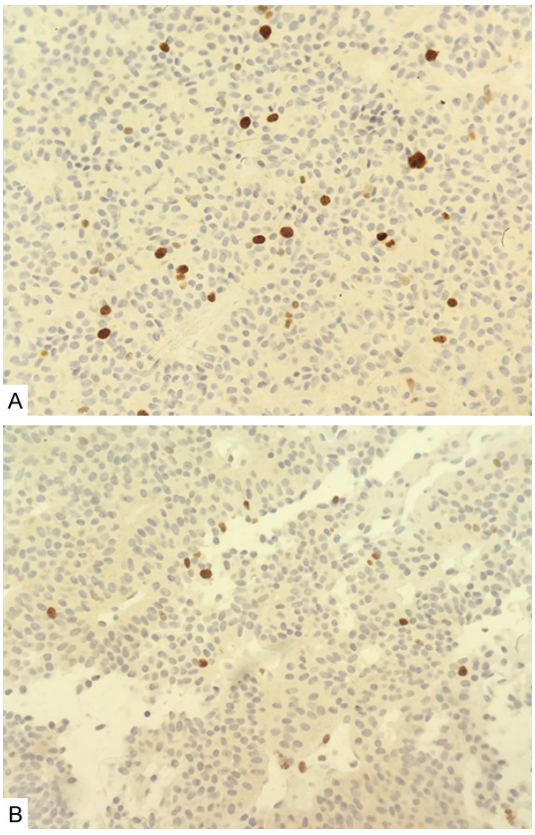

Figure 1. Nuclear expression of Ki67 in nonfunctioning macroadenomas (400×). A. Ki67≥3%; B. Ki67<3%.

increased volume during the follow up and recurrence was considered when a new lesion appeared after a complete surgical resection. The study was approved by the Research Ethics Committee of the institution and was conducted in compliance with the Declaration of Helsinki. Patient anonymity was preserved.

Tumors were classified according to size into microadenomas (<10 mm) or macroadenomas (≥10 mm). The sample included hormone negative and gonadotroph adenomas (nonfunctioning macroadenomas-NFMA), somatotroph adenomas (GH-secreting), and corticotroph adenomas (ACTH-secreting) [7]. Tumor invasion was defined as evidence of cavernous sinus invasion on imaging studies. Ki67 was assessed in formalin-fixed, paraffin-embedded tumor samples using the MIB-1 antibody. The sections were examined under a standard light microscope. Proliferative activity, was measured in areas of high density of labeled cells, at a magnification of 400×. Cells labeled for the antibody were counted and their fraction of the total number of cells was determined, with the resulting value being reported as percent. Patients were then divided into two groups: one group with Ki67≥3% (n=23) and one group with Ki67<3% (n=29). Quantitative data were expressed as mean and standard deviation. Variables with skewed distribution were expressed as median and interquartile range. Categorical data were expressed as counts and percentages. Quantitative data were compared using Student's t test or its nonparametric equivalent (Mann-Whitney U test). Fisher's exact test was used to compare categorical data. A $P<0.05$ was considered significant. Data were analyzed using SPSS, version 22.0.

Results

The sample consisted of 28 women and 24 men aged 20 to 75 years (43.5 ± 14.1 years) at the time of the first operation from which the material for Ki67 assessment was obtained. There were 29 cases of NFMA, 18 cases of GH-secreting adenomas (associated with acromegaly), and five cases of ACTH-secreting adenomas (Cushing disease). Of the total sample, seven were microadenomas. Considering all 52

Table 2. Postoperative outcome of patients

	Ki67<3% (*n*=29)	Ki67≥3% (*n*=23)	*P*-value
Residual tumor on first postoperative imaging study *n* (%)	15 (52%)	16 (70%)	0.26
Reintervention or radiotherapy after first operation *n* (%)	10 (34%)	10 (43%)	0.57
Tumor regrowth* *n* (%)	2 (13%)	7 (43%)	0.11
Tumor recurrence** *n* (%)	4 (28%)	0	0.26
Cavernous sinus invasion on last image *n* (%)	7 (24%)	10 (43%)	0.23

*Increased postoperative residual tumor: n=15 in the Ki67<3% group, and n=16 in the Ki67≥3% group. **Appearance of a new lesion: n=14 in the Ki67<3% group, and n=7 in the Ki67≥3% group.

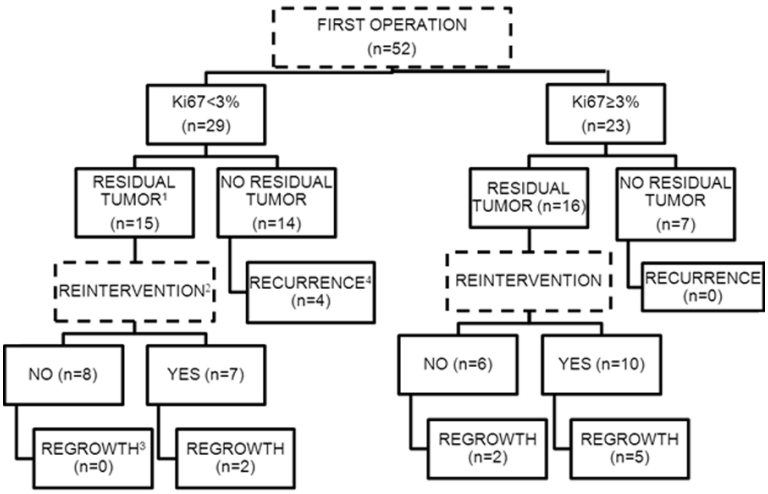

Figure 2. Postoperative outcome in the total sample. 1 Residual tumor: residual lesion after first operation; 2 Reintervention: surgery ou radiotherapy; 3 Regrowth: increased postoperative residual tumor; 4 Recurrence: appearance of a new lesion.

cases, the Ki67 index ranged from 0 to 30%. The median Ki67 was 2.0% in the NFMA group, 2.7% in the GH-secreting group and 4.5% in the ACTH-secreting group. In 23 cases, Ki67 was ≥3%; in five of these cases, Ki67 was >10%. Patients were divided into two groups according to Ki67 expression (<3% or ≥3%), as shown in **Table 1** and **Figure 1**.

When the Ki67<3% and Ki67≥3% groups were compared, there was no significant difference in the frequency of the variables analyzed, although the percentages of the outcomes of interest were higher in the group with higher Ki67 expression (**Table 2**). The median follow-up from surgery to last assessment was 11 months longer in the Ki67<3% group than in the Ki67≥3% group.

Figure 2 shows the postoperative outcome in the total sample. In the Ki67<3% group (n=29), 14 (48%) patients had no postoperative residual tumor; four of these patients developed tumor recurrence during follow-up. Of the patients with recurrence, two underwent surgical reintervention and one received radiotherapy; only one of these patients had no evidence of residual tumor on the last imaging study. Of 15 (52%) patients with postoperative residual tumor, seven required surgical reintervention and/or radiotherapy. Of the seven patients, three had no evidence of residual tumor on the last imaging study. In the Ki67≥3% group (n=23), seven (30%) patients had no postoperative residual tumor; after 18 months, none had tumor recurrence. Of 16 (70%) patients with residual tumor, 10 required surgical reintervention and/or radiotherapy.

Considering only patients with NFMA (n=29), with similar follow-up between groups, there was no significant difference between the Ki67<3% and Ki67≥3% groups in tumor invasion on the last imaging study. The results for tumor invasion, recurrence, and regrowth are shown in **Table 3** and **Figure 3**. Tumor regrowth was significantly higher in the Ki67≥3% group. The relative risk of tumor regrowth in the presence of residual tumor in NFMA was 4.0 (95% CI 1.04-15.38).

Regarding patients with GH-secreting adenomas, 70% of patients in the Ki67<3% group and 75% of patients in the Ki67≥3% required drug treatment at the last assessment, with no significant difference between the groups (P> 0.99). Regarding patients with Cushing disease (all five cases were microadenomas and three

Table 3. Postoperative outcome in nonfunctioning adenomas

	Ki67<3% (*n*=17)	Ki67≥3% (*n*=12)	*P*-value
NFMA with invasion on last imaging study, *n* (%)	5 (29%)	7 (58%)	0.14
Tumor regrowth*, *n* (%)	2 (17%)	6 (67%)	0.03
Tumor recurrence**, *n* (%)	1 (20%)	0	>0.99
Duration of follow-up (months) Median	34	32.5	0.25

NFMA: nonfunctioning macroadenomas. *Increased postoperative residual tumor: n=12 in the Ki67<3% group, and n=9 in the Ki67≥3% group. **Appearance of a new lesion: n=5 in the Ki67<3% group, and n=3 in the Ki67≥3% group.

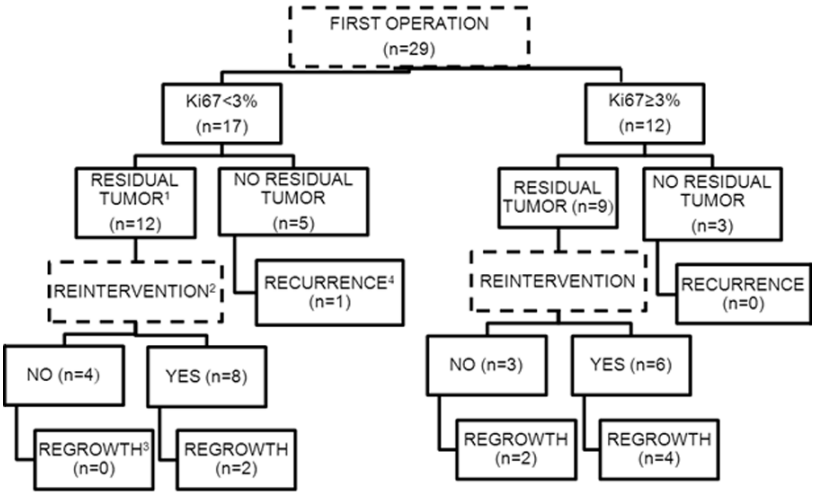

Figure 3. Postoperative outcome in nonfunctioning macroadenomas. 1 Residual tumor: residual lesion after first operation; 2 Reintervention: surgery or radiotherapy; 3 Regrowth: increased postoperative residual tumor; 4 Recurrence: appearance of a new lesion.

of them were in the Ki67≥3% group), all patients were in remission at the last assessment.

During follow-up, one patient with GH-secreting adenoma developed pituitary carcinoma, with a central nervous system metastatic lesion in the right frontal lobe. This occurred 28 years after the first pituitary operation and 5 years after reintervention, when the patient was evaluated for the first time for Ki67 expression, which was 30% at the time of assessment.

Discussion

In the vast majority of cases, pituitary adenomas have a Ki67 index of 1 to 2%, while values greater than 3% are uncommon [8]. In the present sample, Ki67 ranged from 0 to 30%, values close to those described by Salehi et al [9], less than 1% to 23%, and by Padrão [10], between 0 and 36.9%. Also, there were proportionally more tumors with Ki67≥3% (44%) in the present study than in the reports of Thapar et al [2], 36%, Padrão [10], 29%, and Magagna-Poveda et al [11], 12%. Consistent with previous reports, tumor size [6, 12-14] and presence or absence of hormonal hypersecretion [8, 15] produced no difference in Ki67 expression.

Several Ki67 cut-off values have been proposed to differentiate aggressiveness in pituitary adenomas, ranging from 1.5% [16] to 4% [17]. The proposed cut-off value of 3% used in the present study was initially suggested to differentiate between invasive and noninvasive adenomas, with 97.3% specificity and 72.7% sensitivity [2]. More recently, Righi et al [18] associated the threshold labeling index of 3% with a specificity of 89.5% and a sensitivity of 53.8% to evaluate tumor recurrence [18]. The threshold of 3% is the most commonly agreed-upon cut-off value to define tumors of uncertain behavior [1].

Statistically significant differences have been found in Ki67 values when comparing invasive vs. noninvasive adenomas. As early as 1987, Landolt et al [19] found a correlation between the Ki67 index and the invasive potential of pituitary adenomas [19]. Subsequent studies have supported this finding [2-6, 9, 20]. Others, however, have not yielded the same results [15, 21-23]. In the present study, the frequency of invasive tumors was higher in the Ki67≥3% group both on the preoperative imaging study (48% vs. 31%) and on the last imaging study (43% vs. 24%), but these values did not reach a statistically significant difference between groups. We have published a study that involved 139 adenomas positive for Ki67 [5]. In this previous study, the Ki67 index was significantly dif-

ferent between adenomas associated and not associated with local invasion (2.01 ± 3.15 for invasive tumors vs. 1.12 ± 1.87 for noninvasive tumors, P=0.02), suggesting that the current statistical result might have been dependent on the sample size.

Magagna-Poveda et al [11] reported 100% disease-free survival (absence of new lesion formation on imaging studies) in the Ki67<3% group vs. 30% in the Ki67≥3% group after 15 months of follow-up [11]. The present results do not support this observation, since tumor recurrence was similar between the two groups. However, it is important to note that patients with Ki67<3% who had recurrence were followed for a longer period.

Regarding tumor regrowth, studies have demonstrated that tumors that regrow from residual tumors have increased Ki67 expression [11, 18, 24-30]. Recent results show that a Ki67 index ≥2% is associated with 75% sensitivity and 90% specificity to predict tumor recurrence and regrowth, indicating that this cut-off value is an independent predictor of such behavior [31]. In the current series, the Ki67≥3% group had more cases of regrowth. Although not statistically significant in the total sample, this difference was significant in the subgroup analysis of nonfunctioning adenomas. Ekramullah et al [24] found a correlation between the Ki67 index and tumor regrowth in 33 patients with nonfunctioning adenomas within 5 years of follow-up (P<0.01). Ramírez et al [28], in nonfunctioning adenomas, showed that a Ki67 index >2% had an odds ratio of 5.09 for tumor recurrence (P<0.002), with a median follow-up of 32.5 months. We found no association between the Ki67 index ≥3% and tumor invasion on the last imaging study in nonfunctioning adenomas within 3 years of follow-up.

When GH-secreting adenomas were analyzed separately, the frequency of patients requiring adjuvant drug treatment was similar between the Ki67<3% and Ki67≥3% groups. This result differs from those of previous studies that have found a positive correlation between the Ki67 index and hormonal recurrence, presence of residual tumor after surgery, and poorer control with somatostatin analogs in patients with acromegaly [6, 32]. A comparison of data in patients with Cushing disease is not feasible due to the absence of postoperative residual tumor and biochemical cure in all cases.

Thapar et al [2] suggest that a Ki67 index >10% should raise suspicion of the malignant potential of the tumor, a suggestion that is supported by Trouillas [1]. In the present study, one of the five patients with a Ki67 index >10%, the case of GH-secreting adenoma with a Ki67 index of 30%, developed pituitary carcinoma 5 years after surgical intervention. It is noteworthy that this patient had at least 23 years of pituitary disease progression before undergoing the operation that was associated the Ki67 index of 30%.

There is no current definition about the superiority of incomplete resection or high proliferation ki67 index in predicting recurrence. However, when added, these variables suggest a worse prognosis. The authors note that the results of this study are limited by the size of the sample and by the incomplete spectrum of the type of adenomas evaluated.

In conclusion, in the present study, the Ki67 index lacked the statistical power to determine a significant difference in the occurrence of tumor recurrence or regrowth in the total sample. However, it was predictive of regrowth in nonfunctioning adenomas. Adding to the previously described association between Ki67 and aggressive tumor behavior, the current results support the suggestion that more attention should be paid to a Ki67 index ≥3%. Therefore, we recommend that at least the interval between control imaging studies should be reduced, especially in nonfunctioning macroadenomas with incomplete resection.

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Informed consent was waived due to the noninterventional design of the study and retrospective nature of data collection. To ensure confidentiality, all investigators signed a data use agreement for a limited data set.

Disclosure of conflict of interest

None.

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