

PROLIFERATION POTENTIAL IN GH-SECRETING ADENOMAS: MEASUREMENT BY MONOCLONAL ANTIBODY (MIB-1)

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SUMMARY

The growth fraction of 21 GH-producing macroadenomas was studied retrospectively by immunohistochemical analysis using the monoclonal antibody MIB-1 (Ki-67 equivalent). Ten patients presented with noninvasive macroadenoma, eleven had invasive macroadenoma. Among the invasive adenomas, 3 patients underwent subtotal removal, and they received postoperative radiation therapy. These patients were followed for 51.1 ± 40.7 months (ranged 4-120 months), but we have not observed tumor regrowth. The mean Ki-67 labeling index (LI) for noninvasive and invasive macroadenomas were 0.77 ± 0.41 %, and 1.1 ± 0.34 %, respectively. Although female patients had a higher mean Ki-67 LI than male patients (0.65 ± 1.74 % versus 0.23 ± 1.24 %, $p=0.028$), there was no significant difference in the mean Ki-67 LI values between invasive and noninvasive adenomas. As a result, the correlation of Ki-67 LI to age, hormonal state, tumor size and invasiveness did not reveal any statistically significant differences.

Key words: Ki-67 LI, GH-producing adenoma, invasiveness

INTRODUCTION

The clinical behavior of pituitary adenomas (PAs) is known to vary greatly, however, the events underlying this variability remain poorly understood. Whereas some of them are amenable to curative resection, others will progress relentlessly, often despite maximal surgical, and radiotherapeutic intervention. Some investigators have suggested that the Ki-67, PCNA, or p105 may be useful proliferation markers to predict invasive behavior and forewarn possible recurrence of a pituitary adenoma⁽¹⁻⁴⁾. In this study, we analyse the cellular proliferation of GH-PAs with the MIB-1 monoclonal antibody and compare these results with the behaviour of tumors as determined by the immunohistochemistry, radiological findings, and clinical course. Our primary goal was to determine if MIB-1 proliferating cell index (Ki-67 labeling index; Ki-67 LI) studies could be used to

predict tumor regrowth after incomplete tumor resection.

MATERIAL and METHODS

Among our acromegalic patients, 21 patients (10 female and 11 male, with a mean age of 38.8 ± 8.4 years, range 26-55 years) who underwent transsphenoidal surgical resection of GH-PAs at the same neurosurgery department were included in this study. All patients underwent endocrine testing before surgery. Mean GH (20.7 ± 15.7 ng/ml) and IGF-1 (601 ng/ml) levels were elevated. Their GH levels were not suppressed (<2 ng/ml by RIA) during OGTT. We have used the Hardy classification⁽⁵⁾, on the basis of CT and/or MR imaging findings for grading of the pituitary tumors. There were 10 noninvasive (grade 1 and 2) and 11 invasive (grade 3 and 4) adenomas. Only 3 of the 21

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patients with macroadenoma underwent subtotal removal, and they received postoperative radiation and bromocriptine therapies. Pituitary imagings were repeated postoperatively every year. Immunocytochemical analysis was performed on paraffin-embedded material using a monoclonal antibody (MIB-1) directed against a proliferation-associated nuclear antigen, Ki-67, to measure the growth fraction. Control specimens (nontumorous surrounding tissue) were also routinely processed. Specimens were deparaffinized and treated with heat in a water bath (preheat a coplin jar containing 10 mM citrate buffer, pH 6.0, as well as water bath to 95-99°C) prior to the immunohistochemical staining procedure. For greater adherence of tissue sections to glass slides, we used silanized slides (DAKO Code No.S3003). After thermal treatment, the jar with buffer and slides allowed to cool for 20 minutes at room temperature, then rinsed well with Tris buffer. Immunohistochemistry was performed using commercially-available kit (Ready-to-use, DAKO N-SE-RIES, Primary antibody and negative control, Code No. N1574, CA, USA). This antibody reacts with a nuclear antigen (Ki-67) present in proliferating human cells. The Ki-67 immunopositive nuclei stained dark brown color. Slides were lightly counterstained with methyl green, dehydrated, cleared, and mounted. Cell counts were obtained with the aid of a 10x10 square grid fitted into the eyepiece of the microscope. Vascular endothelial cells and nontumorous adenohypophysial cells were excluded. A minimum of 20 high-power fields were enumerated per specimen. An average of approximately 4200 nuclei were evaluated in each specimen. In each speci-

men, a Ki-67 labeling index (Ki-67 LI) was determined, expressed as the percentage of Ki-labeled nuclei. Using this method, the LI was determined to be the ratio of the Ki-67 labeled nuclear area to the total nuclear area. Such area-based measurement system have been shown to be reliably correlated with Lis determined by manual cell counts. Data obtained from this automated method were used only to validate the Lis manually obtained; statistical and subsequent analyses were performed on manually obtained cell counts only.

Tumor regrowth was diagnosed based on the radiological imaging.

The statistical significant differences was defined as a p-value <0.05 (Student's t-test).

RESULTS

All adenomas in this study were macroadenomas. The characteristics of 21 patients with GH-PA are summarized in Table 1.

Only four patients who had invasive macroadenomas required radiotherapy after subtotal resection of tumor, and dural invasion was proven histologically in only one. Posi-

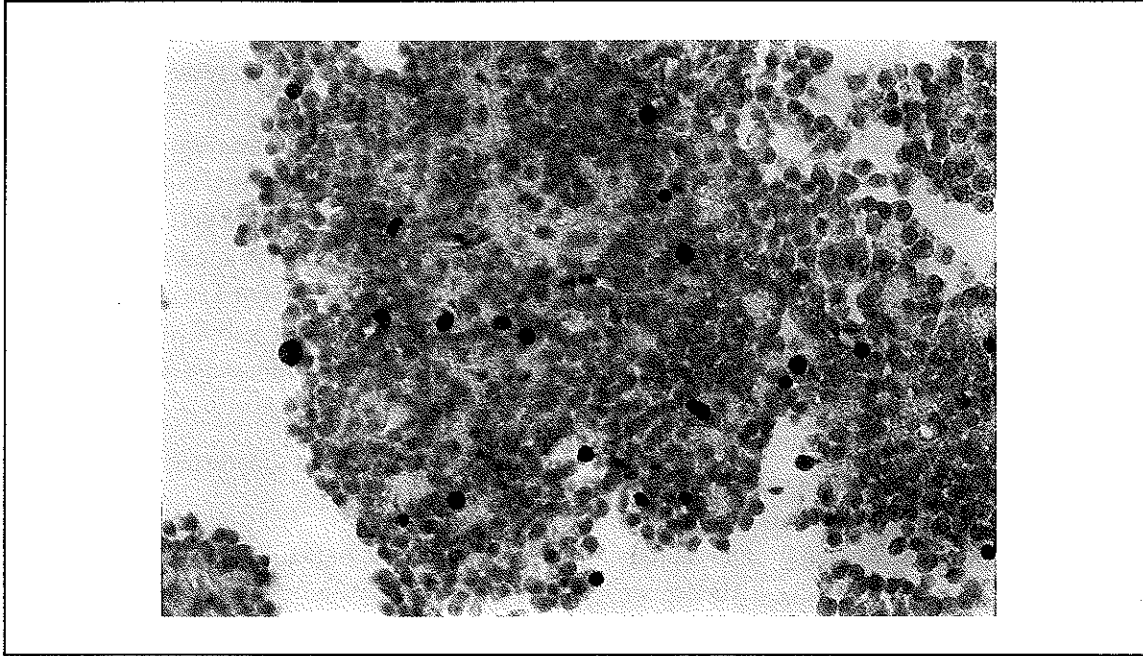
Table 1. Clinical results in 21 patients with Ki-67 LI (mean \pm SD)

Sex (F/M)	10/11
Age range (mean(SD) yr	26 to 55 (38.8 \pm 8.4)
Number of noninvasive tumor (grade 1 and 2)	10/21
Number of invasive tumor (grade 3 and 4)	11/21
Transsphenoidal surgery	21/21
Postoperative radiotherapy	4/21
Follow-up period (mean \pm SD) month	4.0 to 120.0 (51.1 \pm 40.7)
Ki-67 LI range (mean \pm SD) %	0.23 to 1.74 (0.93 \pm 0.40)
Noninvasive tumor	0.23 to 1.67 (0.77 \pm 0.40)*
Invasive tumor	0.56 to 1.74 (1.08 \pm 0.34)*
Female patients	0.65 to 1.74 (1.13 \pm 0.40)**
Male patients	0.23 to 1.24 (0.76 \pm 0.33)**

* (no significant difference).

** p=0.02)

Figure 1. Photograph showing immunohistologic staining of MIB-1 positive nuclei, darkly-stained nuclei (Anti Ki-67 x 310)



tivity for Ki-67 LI was confined to the cell nucleus, and positive nuclei were easily detectable in adenomas (Fig. 1).

In these GH-PAs, the Ki-67 LI ranged from 0.23 to 1.74%, with the mean Ki-67 LI of $0.93 \pm 0.4\%$. There were no significant differences in the mean Ki-67 LI between non-invasive ($0.77 \pm 0.41\%$) and invasive macroadenomas ($1.1 \pm 0.34\%$), however, female patients had a higher mean Ki-67 LI than male patients ($0.65 \pm 1.74\%$ versus $0.23 \pm 1.24\%$, $p=0.02$). In 20 patients (95%) no signs of dural infiltration were found. In a patient who had dura invasion, the Ki-67 LI was 1.74%. In all patient, there was no tumor regrowth during the follow-up period. As a result, no significant correlation was not found between the Ki-67 LI and age, hormonal state, tumor size and invasiveness. Control specimens were negative for the Ki-67 LI.

DISCUSSION

Although pituitary adenomas are usually be-

nign slow-growing tumors, some can display malignant behavior such as rapid regrowth, recurrence, and metastasis without obvious histological evidence of malignancy^(3,6). To determine the growth fraction of pituitary adenomas of various endocrine types and sizes, the monoclonal antibody MIB-1 was studied^(2,3,7-10). Kitz et al⁽¹⁰⁾ reported that the proliferation activity ranged from 0.20- to 2.42% in 24 acromegalic patients. Our results are in agreement with those of Kitz et al⁽¹⁰⁾. Shibuya et al⁽¹¹⁾ found that a Ki-67 LI over 1.5% was associated with rapid growth and an increased likelihood recurrence. But our data did not support this hypothesis. Losa et al⁽¹²⁾ reported that the Ki-67 LI was higher ($3.8 \pm 0.7\%$) in untreated acromegalic patients, and tumors with extrasellar extension had a higher Ki-67 LI ($4.0 \pm 0.7\%$) than intrasellar tumors ($1.7 \pm 0.3\%$), whereas sex, age, and preoperative GH levels had no significant effect on Ki-67 LI. On the other hand, microadenomas and invasive adenomas did not differ significantly in MIB-1 index, and there also was no significant relationship between MIB-1 in-

dex and serum GH levels (7). So, there are many conflicting results between Ki-67 LI and pituitary tumor characteristics. Our study demonstrates that invasive and non-invasive GH-PAs have similar Ki-67 LI, but female patients have higher Ki-67-LI than male patients. In some studies, a comparison of Ki-67 LI with sex did not show any significant association (6,8,10,13). We were not able to find any statistically significant difference in the proliferation rate of GH-PAs with regard to GH production, which is in contrast to the observation of Landolt (6), who found a significantly higher percentage (range 0.1-3.7%) of immunoreactivity in GH-secreting adenomas. Knosp et al (8) reported that no significant difference was present between the proliferation index and hormonal states of the pituitary adenomas, as we report. However, they found that the number of Ki-67 immunoreactive cells was higher in the group of dural infiltration compared to non-invasive pituitary adenoma. We had only one case with dura invasion. In regards to Ki-67 LI, it is possible to demonstrate different proliferation activities ranging from 0.23 to 1.74%, which indicates that there exists no biologically uniform group of GH-PAs. Invasiveness among pituitary adenomas continues to be a vaguely understood phenomenon, of which the biological basis remains obscure. We do not know as yet what the critical value of proliferation activity should be to justify aggressive postoperative therapy. So, regrowth of residual tumors can only be demonstrated by repeated postoperative CT or MRI scans. Other indicators of potential prognostic significance include growth factors, receptor expression, and alterations of oncogens and tumor suppressor genes can be added to the immunohistochemical analysis. In conclu-

sion, Ki-67 LI may provide more helpful information for prediction of the patient's prognosis, but factors other than proliferative activity may determine invasive potential.

REFERENCES

1. Fitzgibbons PL, Appley AJ, Turner RR, et al: Flow cytometric analysis of pituitary tumors: correlation of nuclear antigen p105 and DNA content with clinical behavior. *Cancer* 1988; 62:1556.
2. Hsu DW, Hakim F, Biller BMK, et al: Significance of proliferating cell nuclear antigen index in predicting pituitary adenoma recurrence. *J Neurosurgery* 1993; 78:753 (1993).
3. Knops E, Kitz K, Perneczky A: Proliferation activity in pituitary adenomas: Measurement by monoclonal antibody Ki-67. *Neurosurgery* 1989; 25:927.
4. Thapar K, Kovacs K, Scheithauer BW, et al: Proliferative activity and invasiveness among pituitary adenomas and carcinomas: An analysis using the MIB-1 antibody. *Neurosurgery* 1996; 38:99.
5. Hardy J: Transsphenoidal microsurgery treatment of pituitary tumors. In recent advances in the diagnosis and treatment of pituitary tumors vs.(ed. J.A. Linfoot), Raven Press, New York, 2000; 375.
6. Landolt AM, Shibata T, Kleihues P: Growth rate of human pituitary adenomas. *J Neurosurgery* 1987; 67:803.
7. Yonezawa K, Tamaki N, Kokunai T: Clinical features and growth fractions of pituitary adenomas. *Surg Neurol* 1997; 48:494.
8. Knops E, Kitz K, Perneczky A: Proliferation activity in pituitary adenomas: Measurement by monoclonal antibody Ki-67. *Neurosurgery* 1989; 25:927.
9. Abe T, Sanno N, Osamura YR, Matsumoto K: Proliferative potential in pituitary adenomas: measurement by monoclonal antibody MIB-1. *Acta Neurochir* 139:613 (1997).
10. Kitz K, Knosp E, Koos WTh, Korn A: Proliferation in pituitary adenomas: Measurement by MAb KI 67. *Acta Neurosurg* 1991; 53:60.
11. Shibuya M, Saito F, Miwa T, et al : Histochemical study of pituitary adenomas with Ki-67 and anti-DNA polymerase (monoclonal antibodies, and nucleolar organizer region counts. *Acta Neuropathol* 1992; 84:178 .
12. Losa M, Ciccarelli E, Mortini P, et al: Effects of octreotide treatment on the proliferation and apoptotik index of GH-secreting pituitary adenomas *J Clin Endocrinol Metab* 2001; 86:5194.
13. Landolt AM, Shibata T, Kleihues P, Tuncdogan E: Growth of pituitary adenomas: Facts and speculations. *Adv Biosciences* 1988; 69: 53.