Role of Ki-67 in acromegalic patients with hyperprolactinemia: Retrospective analysis in 61 Chinese Patients

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Abstract: To evaluate the specific characteristics in acromegalic patients with hyperprolactinemia by analyzing the differences between patients with different Ki-67 values. Between 2002 and 2010, a set of data on 61 patients undergoing transsphenoidal surgery was available at the Department of Neurosurgery, Provincial Hospital Affiliated to Shandong University. Patients were divided into Ki-67 ≥3% group and <3% group. A retrospective analysis of clinical, hormonal, immunohistochemical, and imaging was observed in all patients. There were no significant differences in age, gender, tumor size and apoplexy between the two groups. Time interval in Ki-67 ≥3% group was longer than <3% group (P=0.037). Patients in Ki-67 ≥3% group had a higher rate of invasiveness (P=0.048), higher incidences of diabetes mellitus (P=0.036), coarse facial features (P=0.048), large hands and feet (P=0.003), higher GH levels (P<0.05), higher diabetes insipidus rate (P<0.001), and more frequent recurrence (P=0.011) than Ki-67 <3% group. Patients with higher Ki-67 value harbored longer time interval, more aggressive tumors, more acromegaly manifestations, higher GH level, and higher recurrence than patients with lower Ki-67 value.

Keywords: Ki-67; acromegaly; hyperprolactinemia; transsphenoidal surgery; follow up.

INTRODUCTION

Ki-67 labeling index (LI) is one of the least complicated and most reliable methods of assessing the proliferative activity in human tissue (Thapar *et al*., 1996; Ahmed *et al*., 2012). Its expression has been measured immunohistochemically in a wide variety of tumor types, and typically conveyed as the percentage of positively stained tumor cells out of the total cells. Ki-67 estimation has been used to establish tumor grade (Nadler *et al*., 2013), measure treatment response, determine prognosis, and monitor for recurrent (Ramirez *et al*., 2012). Because of its wide utility, Ki-67 has become one of the most frequently assessed tumor biomarkers in use today.

Acromegaly is a disease that related to high levels of growth hormone (GH) and insulin-like growth factor-I (IGF-I), and is usually caused by pituitary adenomas (PA) (Killinger *et al*., 2012). However, about 16-27% of acromegalic patients have increased GH and prolactin (PRL) levels (Katznelson, 2010; Krzentowska-Korek *et al.*, 2011). Several studies have revealed the interpretation of Ki-67 LI in acromegalic patients (Kasuki *et al*., 2012; Dutta *et al*., 2012). However, the significance of the Ki-67 LI as a prognostic factor in acromegalic patients with hyperprolactinemia (adenomas secret both GH and PRL) remains undefined.

In this study, we aimed to investigate the possible prognostic value of the Ki-67 in predicting the clinical outcome of acromegalic patients with hyperprolactinemia who underwent transsphenoidal surgery for a GH- and PRL-secreting pituitary adenoma. We have therefore investigated the clinical, radiological, pathological characteristics, endocrine function, Ki-67 LI values, and the long time follow-up of a series of acromegalic patients with hyperprolactinemia to identify specific prognostic factors associated with the disease.

MATERIALS AND METHODS

Acromegalic patients with hyperprolactinemia admitted to the Department of Neurosurgery, Provincial Hospital Affiliated to Shandong University from 2002 to 2010 were analyzed. Patients were subdivided into a Ki-67 LI ≥3% group and a Ki-67 LI <3% group. The patients' ages, gender, past medical history, clinical presentation, hormone evaluation, images, immunohistochemical staining, and postoperative outcomes were obtained from clinical records. The time interval was defined from the onset of symptoms and signs to the date of diagnosis. The adenomas with other potential causes of elevation of GH and PRL (like drugs), missed follow-up appointments were excluded. All operations were performed by two experienced neurosurgeons. The study was approved by the Ethics Committee of the Provincial Hospital Affiliated to Shandong University. Informed consent was obtained from each individual participant.

The maximum tumor diameter was measured by preoperative magnetic resonance imaging (MRI). Macro adenomas were defined by maximum tumor diameters

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greater than or equal to 10mm and micro adenomas were defined by diameters less than 10mm. Cavernous sinus invasion by the tumor was classified by MRI according to Knosp *et al.* (2012). The normal range of GH was 0.12-7.79ng/ml. The normal range of PRL was 2.64-13.13ng/ml in males, 3.34-26.72ng/ml in premenopausal females and 2.74-19.64ng/ml in postmenopausal females. GH and PRL levels were re-evaluated at 3 days, 3 months and 12 months after operation. Surgically removed adenoma tissues were fixed in 10% formaldehyde and embedded in paraffin and examined by staining with corresponding antibodies (anti-TSH, anti-LH, anti-FSH, anti-ACTH, anti-GH and anti-PRL).

All patients were followed up as outpatients 3 months after surgery and then at annual or biannual intervals. Recurrence was defined as reactivation of hormonal disease, radiological disease, or both, 12 months after surgery.

The Statistical Package for Social Sciences Version 16.0 (Chicago, IL, U.S.) was used to set up a database of the patients. Student's t-test, Mann-Whitney U test, Chi-square test, and Fisher's exact test were used for comparing the differences between groups. Statistical significance was established at the level of $P<0.05$.

RESULTS

Clinical features and characteristics

The clinical features and characteristics of the patients are presented in table 1. Analysis of age, gender, tumor size and apoplexy did not demonstrate significant differences between the two groups. Mean time interval in Ki-67 LI ≥ 3% group was longer compared to the <3% group. Tumors also described in the Ki-67 LI ≥3% group, also had a higher rate of invasiveness (P=0.048) than those in the Ki-67 LI <3% group.

MRI findings

Ki-67 LI <3% group included 4 (8.89%) micro adenomas and all of the patients were macro adenomas. However, there was no significant difference concerning micro adenomas or macro adenomas in the two groups (P=0.565).

Pre-operative clinical manifestations and complication

The incidences of diabetes mellitus, coarse facial features, large hands and feet were higher in Ki-67 LI ≥3% group than in the Ki-67 LI <3% group (table 2).

Endocrine function

The preoperative and post-operative (12 months after surgery) GH levels in the Ki-67 LI ≥3% group remained significantly higher than that in the Ki-67 LI <3% group ($P<0.05$) (table 3). Based on the postoperative hormone levels at 12 months, the normalization of GH, IGF1 and PRL were achieved in 68.75%, 75.00%, and 68.75% of patients with Ki-67 LI ≥3%. In Ki-67 LI <3% group, those were 86.67%, 86.67% and 77.78%, respectively. However, no significant difference concerning the normalization of GH, IGF1, or PRL was observed between the two groups.

Immunohistochemistry evaluation

The rates of positive staining in the two groups (Ki-67 ≥3% group vs Ki-67 <3% group) for GH were 81.25% vs 84.44% (P=0.713). And those for PRL were 87.50% vs 86.67% (P=1.000). Moreover, no significant correlations between the preoperative mean GH or PRL concentrations and the immunostaining-positive rates were observed ($r=+0.176$, $P>0.05$ and $r=+0.157$, $P>0.05$ respectively).

Follow up

The median duration to follow-up for the entire group was 35 months (range: 14-73). Based on the postoperative hormonal levels and radiological results as assessed at 12 months, five patients (31.25%) experienced recurrence in Ki-67 LI ≥3% patients, and 4.44% of Ki-67 LI <3% patients experienced recurrence. The rate of the recurrence in the Ki-67 LI ≥3% group was significantly higher than in the Ki-67 LI <3% group (P=0.011).

DISCUSSION

Plurihormonal PAs are even rarer usually having co-secretion of GH and PRL (Losa *et al*., 2002; Attanasio *et al.*, 2008). The presence of acromegaly with hyperprolactinaemia is relatively frequent and may be due to suprasellar extension of the adenoma (Fulton *et al*., 1990). The nuclear antigen Ki-67 is related to growth potential in many tumors. However, association of Ki-67 on the clinical relevance concerning the disease is still scarce. We aimed to investigate the possible prognostic value of the Ki-67 LI in predicting the clinical outcome of acromegalic patients with hyperprolactinemia as well as their response to after surgery. As a substantial proportion of acromegalic patients have co-existent hyperprolactinaemia, a retrospective endocrine and radiological study was undertaken to investigate the incidence and clinical features, imaging, histopathological characteristics, and long time follow-up in these patients quantified by the different Ki-67 LI values.

In our results, the mean time interval in Ki-67 LI ≥3% group was longer than in the <3% group. The long time interval from the onset of symptoms to initiation of treatment may be caused by a delay. Patients' delays in seeking medical help may constitute most part of this delay. In agreement with Rahimi-Rad *et al.* (2013), rapid diagnosis and treatment may reduce the mortality and morbidity. Additionally, we found tumors described in the Ki-67 LI ≥3% group, also had a higher rate of invasiveness than those in the Ki-67 LI <3% group. We agreed with some authors that reported a positive correlation between pituitary tumor Ki-67 expression and invasiveness (Thapar *et al*., 1996; Fusco *et al*., 2008). Blevins *et al*. (1998) also suggested the invasive adenomas would express greater amounts of Ki-67, compared to non-invasive. We also found that no statistical differences were observed in the two groups in relation to age, gender, mean maximal diameter of adenomas and apoplexy. Blevins *et al*. (1998) also found no significant relationship between Ki-67 and pituitary tumors, such as tumor size and its invasiveness potential. However, Mastronardi *et al*. (2002) was able to find

Table 1: Clinical features and characteristics of patients in the two groups

Variables		Ki67≥3% (n=16)	Ki67<3% (n=45)	P
Mean age (years)		33.19±13.64	33.78±11.47	0.867
Mean time interval (months)		53.31±73.79	23.74±19.97	0.037
Ki67 (%)		5.56±4.10	0.40±0.58	<0.001
Tumor size (cm)		3.03±1.31	2.67±1.44	0.388
Sex	Male	5(31.25%)	18(40%)	0.535
	Female	11(68.75%)	27(60%)	
Invasiveness	+	11(68.75%)	18(40%)	0.048
	-	5(31.25%)	27(60%)	
Apoplexy	+	0(0%)	8(17.78%)	0.097
	-	16(100%)	37(82.22%)	

Data were expressed as mean +SD ($\overline{X} \pm S$) or n (%).

Table 2: Pre-operative clinical manifestations and complications in the two groups

	Ki67≥3% (n=16)	Ki67<3% (n=45)	P
Headache/dizziness	9(56.25%)	20(44.44%)	0.417
Coarse facial features	11(68.75%)	18(40.00%)	0.048
Large hands and feet	13(81.25%)	17(37.78%)	0.003
Fatigue	1(6.25%)	1(2.22%)	0.459
Distension and numbness	3(18.75%)	2(4.44%)	0.108
Visual impairment	7(43.75%)	20(44.44%)	0.962
Visual field defects	5(31.25%)	17(37.78%)	0.640
Menstrual disorders	6/11(54.55%)	14/27(51.85%)	0.880
Galactorrhea	7(43.75%)	17(37.78%)	0.674
Sexual dysfunction	1/5(20.00%)	2/18(11.11%)	0.539
Polyuria/polydipsia	2(12.50%)	2(4.44%)	0.279
Hypertension	1(6.25%)	2(4.44%)	1.000
Diabetes mellitus	4(25.00%)	2(4.44%)	0.036

Data were expressed as n and %.

Table 3: The levels of GH and PRL in the two groups

		Before surgery	3 days After surgery	3 months after surgery	12 months after Surgery	P
Ki6≥3% (n= 16)	PRL (ng/ml)	151.00±99.70	35.23±23.35	36.21±22.89	38.95±24.31	<0.001
	GH (ng/ml)	37.34±15.83*	18.22±11.47	17.25±12.57	19.85±10.34*	<0.001
Ki67<3% (n=45)	PRL (ng/ml)	128.34±87.24	30.34±19.00	33.57±20.85	32.51±16.53	<0.001
	GH (ng/ml)	20.54±16.33	11.68±12.26	11.54±12.58	12.35±11.42	0.005

Data were expressed as mean +SD ($\overline{X} \pm S$). '*' indicates the significant difference between the two groups at the same time point of GH level (P<0.05). P: Comparative analysis in Ki67≥3% group or Ki67<3%group before surgery and 3 days after surgery.

tumors of older patients had greater Ki-67 positivity than tumors of younger ones.

The most common pre-operative complications in the two groups were large hands and feet, coarse facial features, headache and dizziness, menstrual disorders, visual impairment, and galactorrhea. These complications are the main clinical findings of acromegalic patients with hyperprolactinemia (Wang *et al*., 2012). However, the incidences of diabetes mellitus, coarse facial features, large hands and feet were higher in Ki-67 LI ≥3% group than in the Ki-67 LI <3% group. Hypersecretion of GH and/or PRL has a variety of manifestations as it affects many organs. Diagnosis of acromegalic patients with hyperprolactinemia is a challenge even for very experienced endocrinologists, often requiring long time and uneasy dynamic hormonal investigations. Additionally, proliferative potentials are analyzed by Ki-67 staining index (Nakashima *et al*., 2009). We also found that patients with a higher Ki-67 value often harboured more acromegalic features, i.e. coarse facial features and large hands and feet compared to other patients. Therefore, considering the longer interval from the onset of symptoms to the date of diagnosis, the more physical features of acromegaly, and the more aggressive of tumors, we may conclude that the proliferation activity is proposed as a possible pathogenetic mechanism.

Plurihormonality of PA can be defined as the ability of an adenoma to express more than one pituitary hormone. Elevated preoperative GH and PRL levels were detected in acromegalic patients with hyperprolactinemia. Our study confirmed that patients with higher levels of Ki-67 also had higher GH levels. The application of immunohistochemistry to diagnose surgically removed PA revealed that a great number of PAs are in fact plurihormonal. In our results, no significant correlations between the preoperative mean GH or PRL concentrations and the immunostaining-positive rates were observed. Asano *et al*. (1996) has also referred that Ki-67 values are related to serum levels of GH and PRL in patients with prolactinoma and acromegaly.

Recurrence is a common issue bothering pituitary surgeons. Our analysis also revealed the possible correlation between Ki-67 and tumor recurrence. The rate of recurrence in the Ki-67 LI ≥3% group was significantly higher than in the Ki-67 LI <3% group. The Ki-67 may predict clinical outcome in postsurgical management of acromegalic patients. It also seems to predict a higher risk of tumor recurrence. Schreiber *et al*. (1999) also showed positive relationship between Ki-67 with tumor recurrence. PAs are heterogeneous in invasiveness, and recurrence. In the recurrent patients, we also found increasing hormone levels, which indicated the increasing hormone level was a more useful factor for predicting the recurrence.

Plurihormonal adenomas may be caused by the distinction between polyclonal diffusion and monoclonal expansion. Acromegalic patients disclose adenomas that secrete GH or mixed adenomas, composed of two different types of cells, GH- and PRL-secreting cells, or adenomas secreting both, GH and PRL, in the same cell (Kovacs and Horvath., 1986). More convincing evidence-based hypothesis has to be provided to explain current findings. Due to the common origin of GH- and PRL-secreting cells, the presence of lesions secreting both hormones and the need for understanding the intrinsic cell factors that may be involved in cell proliferation, we studied the presence of Ki-67 in a series of GH- and PRL-secreting tumors. Ki-67 LI has been demonstrated to be a reliable tool in the measurement of tissue proliferation (Zhao *et al.*, 1999). The discrepancies among this and other studies might be the result of heterogeneity in patients' samples and type of tumors, as well as definitions of surgical removal, and tumor invasiveness.

In conclusion, our study suggested that patients with higher Ki-67 LI harbour longer time interval, more aggressive tumors, more acromegaly manifestations, higher GH level and higher recurrence rate compared to the other group. The information would provide new insight to current knowledge about tumor biology, and could help in understanding the Ki-67 values of plurihormonal tumors. Future studies are necessary to clarify the risk of developing acromegaly in patients with hyperprolactinaemia.

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REFERENCES

Ahmed NY, Ismail AT and Kareem TS (2012). A clinicopathologic study of Ki-67 proliferation index in colorectal carcinoma. *Saudi Med. J*., **33**(8): 841-845.

Asano K, Tajika Y, Huang MC and Takakura K (1996). The relation between cell-proliferation activity and secretion in pituitary adenoma: a review of 63 cases. *No To Shinkei.*, **48**(6): 534-539.

Attanasio R, Montini M, Valota M, Cortesi L, Barbò R, Biroli F, Tonnarelli F, Albizzi M, Testa RM and Pagani G (2008). An audit of treatment outcome in acromegalic patients attending our center at Bergamo. *Italy Pituitary*, **11**(1): 1-11.

Blevins Jr LS, Verity DK and Allen G (1998). Aggressive pituitary tumors. *Oncology (Williston Park)*, **12**(9): 1307-1312.

Dutta P, Bhansali A, Vaiphei A, Dutta U, Ravi Kumar P, Masoodi S, Mukherjee KK, Varma A and Kochhar R (2012). Colonic neoplasia in acromegaly: Increased proliferation or deceased apoptosis? *Pituitary*, **15**(2):

166-173.

Fulton JD, Shand J, Ritchie D and McGhee J (1990). Hyperostosis frontalis interna, acromegaly and hyperprolactinaemia. *Postgrad. Med. J.*, **66**(771): 16-19.

Fusco A, Zatelli MC, Bianchi A, Cimino V, Tilaro L, Veltri F, Angelini F, Lauriola L, Vellone V, Doglietto F, Ambrosio MR, Maira G, Giustina A, degli Uberti EC, Pontecorvi A and De Marinis L (2008). Prognostic significance of the Ki-67 labeling index in growth hormone-secreting pituitary adenomas. *J. Clin. Endocrinol. Metab.*, **93**(7): 2746-2750.

Kasuki L, Neto LV, Takiya CM and Gadelha MR (2012). Growth of an aggressive tumor during pregnancy in an acromegalic patient. *Endocr. J.*, **59**(4): 313-319.

Katznelson L (2010). Approach to the patient with persistent acromegaly after pituitary surgery. *J Clin Endocrinol Metab*, **95**(9): 4114-4123.

Killinger Z, Kužma M, Sterančáková L and Payer J (2012). Osteoarticular Changes in Acromegaly. *Int J Endocrinol*, **2012**: 839282.

Knosp E, Steiner E, Kitz K and Matula C (1993). Pituitary adenomas with invasion of the cavernous sinus space: A magnetic resonance imaging classification compared with surgical findings, *Neurosurgery*, **33**(4): 610-618.

Kovacs K and Horvath E (1986). Pathology of growth hormone-producing tumors of the human pituitary. *Semin Diag. Path*, **3**(1): 18-33.

Krzentowska-Korek A, Gołkowski F, Bałdys-Waligórska A and Hubalewska-Dydejczyk A (2011). Efficacy and complications of neurosurgical treatment of acromegaly. *Pituitary*, **14**(2): 157-162.

Losa M, Mortini P, Barzaghi R, Gioia L and Giovanelli L (2002). Surgical treatment of prolactin-secreting pituitary adenomas: Early results and long-term outcome. *J. Clin. Endocrinol. Metab.*, **87**(7): 3180-3186.

Mastronardi L, Guiducci A, Puzzilli F and Maira G (2002). Anterior pituitary adenomas in patients aged more than 65 years: Analysis of growth fraction (using the MIB-1 monoclonal antibody) and of clinical features in comparison to younger patients. *Clin. Neurol. Neurosurg.*, **104**(1): 44-48.

Nadler A, Cukier M, Rowsell C, Kamali S, Feinberg Y, Singh S and Law CH (2013). Ki-67 is a reliable pathological grading marker for neuroendocrine tumors. *Virchows Arch.*, **462**(5): 501-505.

Nakashima M, Takano K and Matsuno A (2009). Analyses of factors influencing the acute effect of octreotide in growth hormone-secreting adenomas. *Endocr. J.*, 56(2): 295-304.

Rahimi-Rad MH, Rahimi-Rad S and Zarrin S (2013). Delays in diagnosis and treatment of venous thromboembolism in a developing country setting. *Tuberk. Toraks.*, **61**(2): 96-102.

Ramirez C, Cheng C, Vargas G, Asa SL, Ezzat S, González B, Cabrera L, Guinto G and Mercado M (2012). Expression of Ki-67, PTTG1, FGFR4 and SSTR 2, 3 and 5 in nonfunctioning pituitary adenomas: A high throughput TMA, immunohistochemical study. *J. Clin. Endocrinol. Metab.*, **97**(5): 1745-1751.

Schreiber S, Seager W and Ludecke DK (1999). Proliferation markers in different types of clinically non-secreting pituitary adenomas. *Pituitary*, **1**(3-4): 213-220.

Thapar K, Kovaks K, Scheithauer BW, Stefaneanu L, Horvath E, Pernicone PJ, Murray D and Jr Laws ER (1996). Proliferative activity and invasiveness among pituitary adenomas and carcinomas: An analysis using the MIB-1 antibody. *Neurosurgery*, **38**(1): 99-107.

Wang M, Mou CZ, Jiang M, Han LZ, Fan SQ, Huan C, Qu X, Han T, Qu YM and Xu GM (2012). The characteristics of acromegalic patients with hyperprolactinemia and the differences in patients with merely GH-secreting adenomas: Clinical analysis of 279 cases. *Eur. J. Endocrinol.*, **166**(5): 797-802.

Zhao D, Tomono Y and Nose T (1999). Expression of P27kip1 and Ki-67 in pituitary adenomas: An investigation of marker of adenoma invasiveness. *Acta. Neurochir. (Wien)*, **141**(2): 187-192.