

The role of Ki-67 in women with a resistant prolactinoma: A retrospective analysis in 199 hospitalized patients over a period of 5 years

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Abstract: Proliferation-associated antigen Ki-67 is used for the histological evaluation of different tumors. Few studies have been conducted on women with a resistant prolactinoma. To better define the characteristics and to evaluate the differences between patients with different Ki-67 labeling index (LI), a retrospective study was designed to recruit 199 females with a resistant prolactinoma. The patients were divided into two groups, patients with Ki-67 LI \geq 3% and patients with Ki-67 LI $<$ 3%. Tumors in the LI \geq 3% group were also larger ($p=0.043$), had a higher rate of invasion ($p=0.014$), and were associated with more frequent polyuria and polydipsia ($p=0.008$) compared to the LI $<$ 3% group. The pre- and post-operative PRL levels in the LI \geq 3% group remained significantly higher compared to patients with LI $<$ 3% ($p<0.05$). The incidences of transient diabetes insipidus and hyponatremia in the LI \geq 3% group were also significantly higher ($p=0.037$, $p=0.041$). Additionally, the postoperative PRL normalization rate was lower in patients with LI \geq 3% compared with patients with LI $<$ 3% ($p=0.028$). The recurrence rate in the LI \geq 3% and LI $<$ 3% groups were 27.27% and 8.47%, respectively. In conclusion, high Ki-67 LI is predictive sign of a poor prognosis in women with resistant prolactinoma.

Keywords: Resistant prolactinomas, Ki-67, dopamine agonist, and women.

INTRODUCTION

Prolactinomas are one of the most frequent neuro-endocrine tumors, representing the clonal proliferation of pituitary cells (Kumar *et al.*, 2013). Prolactinomas often occur in females aged from 20 to 50 years and cause a variety of neurological and endocrine abnormalities (Gillam *et al.*, 2006). The treatment objectives are to eliminate the possible mass effects of the tumor and normalize possible hormonal and metabolic disturbances. Dopamine agonists (DAs) remain the cornerstone of therapy, and cabergoline is recommended as the first choice of therapy due to its high efficacy (Maiter *et al.*, 2012). However, drug intolerance and resistance are the main limitations for DA therapy (Brue *et al.*, 1992). The failure to attain biochemical control and/or reduction in tumor mass with DA therapy defined as a resistant prolactinoma (Molitch 2003). Kumar *et al.* (2013) described a 32-year-old lady evaluated for a resistant prolactinoma. Tamasauskas *et al.* (2012) suggested in their study that the remission was attained only in 45.5% of female patients who were treated with DA. Therefore, for the few prolactinoma patients with severe resistance to DA, surgery, especially trans-sphenoidal surgery, might turn into a more rational treatment strategy (Olafsdottir *et al.*, 2006).

Recently, many studies have focused on factors that may

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predict tumor behavior. Ki-67 is used for the histological evaluation of different tumors in order to assess the cell proliferation (Mastronardi *et al.*, 2001 and Schankin *et al.*, 2012), tumor grade, treatment response, and recurrence (Ramirez *et al.*, 2012). According to previous studies, in pituitary adenomas, the Ki-67 labeling index (LI) can be conflicting (Zemmoura *et al.*, 2013 and Sánchez-Tejada *et al.*, 2013).

At present, many studies have toward to patients with DA therapy, but relatively few have been conducted on prolactinoma in women with resistant to DA therapy. The limitation of previous reports concerning resistant prolactinomas is the lack of histopathologic analysis of the adenoma. As a prognostic factor, the importance of the Ki-67 LI in resistant prolactinomas still undefined. In order to evaluate the characteristics and investigate the possible role of Ki-67 in predicting outcomes, we performed a retrospective analysis in women seen in our institution with a resistant prolactinoma. For these reasons, data were gathered on the demographic parameters, clinical features, pathological characteristics, endocrine levels, and last follow up.

MATERIALS AND METHODS

Patients

From 2007 to 2011, 199 patients suffering from a prolactinoma underwent an operation in the Department

of Neurosurgery, Shandong Provincial Hospital. The inclusion criteria for the female patients were classic syndromes, an increased serum prolactin (PRL) level, positive immunohistochemical staining for PRL, a pituitary mass by magnetic resonance imaging (MRI), and resistance to DA therapy. All of the selected patients underwent surgery due to DA resistance. The patients were divided into the following two groups by Ki-67 LI value: patients with Ki-67 LI \geq 3% and patients with Ki-67 LI $<$ 3%. The medical records for the two groups of patients were reviewed for medical history. The period from the onset of symptoms to the date of diagnosis can be defined as the time interval. The Ethics Committee of the Shandong Provincial Hospital has approved this study. Informed consent was obtained from each participant.

MRI

The diameter and invasion rate of tumor were defined by MRI. The maximum diameter of tumor greater than or equal to 10 mm defined as macroadenomas. However, the maximum diameter less than 10 mm defined as microadenomas. Invasion of cavernous sinus was defined according to Knosp *et al.* (1991).

Pathological methods

Surgically removed adenomas collected from patients were fixed in 10% formaldehyde and embedded in paraffin and examined by staining with the their corresponding antibodies (anti-PRL, anti-GH, anti-ACTH, and so on) via immunohistochemistry in accordance with the standard procedures. The Ki-67 antigen was measured by the MIB-1 antibody. The results were presented as the percentage of positive nuclei in at least 5 fields chosen randomly at $\times 400$ magnification.

Hormonal assessment

Serum PRL was measured using an immunoradiometric assay kit (Beckman Coulter, Inc., CA, U.S.). The inter-assay coefficients of variation were less than 15%. The intra-assay coefficients of variation were less than 10%. The normal range of PRL was 3.34-26.72ng/ml in premenopausal women, and the range was 2.74-19.64ng/ml in postmenopausal women.

Follow-up

The period range from the onset of symptoms to the last entry was defined as the follow-up time. When identified the definition of remission, patients with no clinical or hormonal symptom or no radiological remnants. The standard of recurrence was presented as reactivation of hormone, radiological disorder, or both in patients who had undergone tumor resection and had experienced at least 24 months of disease-free survival.

STATISTICAL ANALYSIS

The statistical analyses were performed by SPSS 16.0 (Chicago, IL, U.S.). The quantitative data were shown in

the means \pm standard deviation (SD). The qualitative data were shown in percentages. Student's t-test, Chi-squared test, Fisher's exact test, and One-Way ANOVA were used to compare the differences between different groups. And $p < 0.05$ was considered to be statistically significant.

RESULTS

Of the 199 patients with a resistant prolactinoma, 22 were in the Ki-67 LI \geq 3% group and 177 were in the Ki-67 LI $<$ 3% group. Table 1 discussed the clinical characteristics of the patients. In the Ki-67 LI \geq 3% group, the mean age was 31.00 \pm 7.51 (range: 21-43) years. The maximum diameter of the adenomas was 2.44 \pm 1.57 cm, with an invasion rate of 36.36%. In the Ki-67 LI $<$ 3% group, the mean patient age was 36.75 \pm 10.67 (range: 20-69) years. The maximum diameter was 1.96 \pm 0.96 cm, with an invasion rate of 14.12%. Patients in the Ki-67 LI \geq 3% group were younger than the patients in the Ki-67 LI $<$ 3% group ($p=0.015$). Tumors in the Ki-67 LI \geq 3% group also had a greater size ($p=0.043$) and higher rate of invasion ($p=0.014$) compared with the tumors in Ki-67 LI $<$ 3% group. The meaningful differences in the mean time interval and incidence of apoplexy were not observed between the two groups. Table 1 also shows the classification for the adenomas in the two groups. Two (9.09%) patients with LI \geq 3% had a microadenoma, and 20 (90.91%) had a macroadenoma. However, 58 (32.77%) patients with LI $<$ 3% had a microadenoma, and 119 (67.23%) had a macroadenoma. The differences were significant ($p=0.022$).

Tumor-specific clinical features and complications are presented in Table 2. The incidence of polyuria/polydipsia was higher in patients with Ki-67 LI \geq 3% compared with patients with Ki-67 LI $<$ 3% (13.64% vs. 2.26%, $p=0.008$). Additionally, galactorrhea occurred more frequently in the Ki-67 LI $<$ 3% group than in the Ki-67 LI \geq 3% group (87.57% vs. 63.64%, $p=0.031$).

Table 3 presents the hormonal changes before and after surgery. The PRL levels in the Ki-67 LI \geq 3% patients decreased from 218.23 \pm 170.10nmol/L before surgery to 87.33 \pm 66.03nmol/L at 3 days, 88.95 \pm 68.46nmol/L at 3 months, and 94.32 \pm 70.74nmol/L at 1 year after surgery. The levels of PRL in the Ki-67 LI $<$ 3% patients decreased from 151.14 \pm 126.95ng/ml before surgery to 57.39 \pm 47.97ng/ml at 3 days, 63.54 \pm 51.24ng/ml at 3 months, and 62.41 \pm 52.36ng/ml at 1 year after surgery. The pre- and post-operative levels of PRL in the Ki-67 LI \geq 3% group all remained significantly higher than in the Ki-67 LI $<$ 3% group ($p < 0.05$). According to the postoperative PRL levels as assessed at 3 months, 40.91% patients were found to have a normalized level in the Ki-67 LI \geq 3% group and 64.97% in the Ki-67 LI $<$ 3% group. Thus, the PRL normalization rate in the Ki-67 LI \geq 3% group was obviously lower than in the Ki-67 LI $<$ 3% group ($p=0.028$).

The mean duration of hospitalization was 5 days (range: 3-14 days). In addition, 90% were discharged by day 5. There was no mortality or other morbidity in the patients. Fig. 1 presents the post-operative complications. Transient diabetes insipidus (DI) was observed in 11 (50.00%) patients with Ki-67 LI \geq 3% and in 50 (28.25%) patients with Ki-67 LI $<$ 3%. The incidence of transient DI in the Ki-67 LI \geq 3% group was significantly higher than in the Ki-67 LI $<$ 3% group ($p=0.037$). Hyponatremia occurred in 18.18% of patients in Ki-67 LI \geq 3%. The incidence of hyponatremia in the Ki-67 LI $<$ 3% group was 5.08%. Thus, there was an evident difference in hyponatremia between different groups ($p=0.041$). Two patients occurred cerebrospinal fluid (CSF) leaks and had been treated by lumbar continuous drainage.

The median duration of the long-term follow-up period was 31 months (range: 12-78) months. The recurrence rates for patients with Ki-67 LI \geq 3% and Ki-67 LI $<$ 3% were 27.27% and 8.47%, respectively. The recurrence rate in the Ki-67 LI \geq 3% group was higher than the Ki-67 LI $<$ 3% group ($p=0.016$).

DISCUSSION

Similar to many other endocrine disorders, prolactinomas are more common in women, and the proportion of women to men is 10:1 (Ciccarelli *et al.*, 2005). In other words, in men, prolactinomas are relatively rare according to clinical data. The first-line treatment is DA treatment, but surgical resection is indeed indicated in few patients with severe intolerance or resistance to DA treatment. Few patients do not respond satisfactorily to pharmacological treatment. In assessing the level of proliferative activity in human tissue, Ki-67 LI is the least complicated and most reliable way. Ki-67 LI can provide information about the cellular proliferation rate, which is useful for determining the long-term prognosis (McCormick *et al.*, 2002). Clinical data are available concerning Ki-67 in resistant prolactinomas in women. In this report, in order to identify the role of Ki-67 in

resistant prolactinomas in women, we carried out the 5-year research of 199 female patients.

Recently, the pharmacological resistance still remains poorly understood concerning the biological function (Vasilev *et al.*, 2011). Empirically, a failure to normalize the PRL level and/or reduce tumor volume by at least 50% could be defined as the resistance prolactinomas (Molitch 2005). Especially, less than 10% of patients do not undergo the normalization of PRL in response to a DA. Resistant prolactinomas have increased the incidence of angiogenesis and lead to cellular proliferation, invasiveness. According to Molitch, D2 receptor gene transcription and inhibitory G protein may lead to the resistance (Molitch 2008). DA-resistant prolactinomas tend to be large and invasive. The management of DA-resistant prolactinomas includes surgery and alternative medical therapies.

This observational retrospective study based on the clinical medical records of inpatients found that Ki-67 plays an important role in women with a resistant prolactinoma. Our study reported that the resistant prolactinoma patients with a higher Ki-67 level were younger and had bigger tumors, higher invasion rates, more pre- and post-operative complications, higher pre- and post-operative PRL levels, a lower PRL normalization rate, and higher recurrence rate than patients with a lower Ki-67 level.

The demographic characteristics of our study population showed that prolactinoma females with a higher Ki-67 level were younger, which was in agreement with previous research (Pizarro *et al.*, 2004 and Ferreira *et al.*, 2005). The Ki-67 index may depend on the age to a small extent, in order to influence the proliferative behavior. Studies also have shown that female patients with a higher Ki-67 level have bigger tumors and a higher invasion rate compared to patients with a lower Ki-67 level. These data are consistent with the results described by several authors that Ki-67 correlates with the tumor

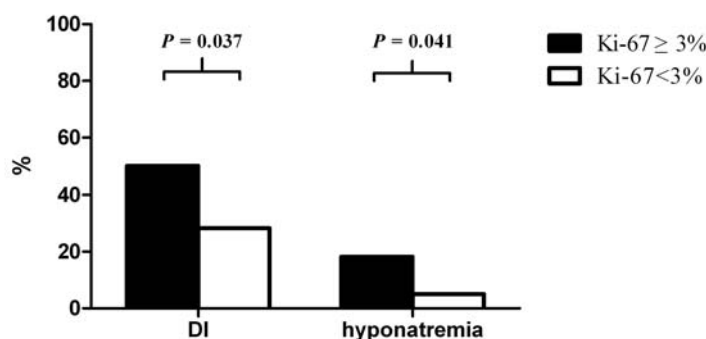


Fig. 1: Post-operative complications in the Ki-67 LI \geq 3% and Ki-67 $<$ 3% groups. The data are expressed as percentages (%). p: Comparative analysis between the groups.

size and invasiveness (Chacko *et al.*, 2010 and Matsuyama 2012). Consistent with previous evidence (Vroonen *et al.*, 2012), we found that a large majority of patients had a macroadenoma. Our study suggested that hormonal resistance may be associated with large adenomas. A cut-off of 3% of Ki-67 has been showed to predict the function of invasion, and higher levels revealing more invasiveness (Metz *et al.*, 2012). Nevertheless, Ma *et al.* (2002) failed to define an association between tumor invasiveness and Ki-67 values. These discrepancies might be due to different sample size, gender differences, and the adenoma growth fraction. DAs are effective in permitting PRL normalization at a higher rate for microadenomas than macroadenomas (Di Sarno *et al.*, 2001 and Colao *et al.*, 2011). Thus, the majority of patients had macroadenomas in our present study.

Symptoms and signs of prolactinoma (elevated serum prolactin, galactorrhea, amenorrhea, headache and visual changes) may be the sequence of symptomatic hyperprolactinemia. Moreover, laboratory findings revealed that the pre- and post-operative PRL levels in patients with Ki-67 LI \geq 3% remained higher than those in patients with Ki-67 LI<3%. Generally, our finding

revealed that higher PRL levels may result in higher incidences of prolactinoma-related effects (Schlechte 2003). The increased degree of PRL may prevent the pituitary gland to release luteinizing hormone and follicle-stimulating hormone. These changes may lead to menstrual irregularities, such as amenorrhea (Schlechte 2003) in women. Menstrual disorders can be due to an invasive prolactinoma. Similar to other neuroendocrine tumors, the use of Ki-67 to identify a tumor is very important. In our results, the most common clinical symptom encountered in the two groups was menstrual disorders. Polyuria/polydipsia was more associated with a higher Ki-67 level in women with a resistant prolactinoma.

Webster *et al.* (1994) and Delgrane *et al.* (1997) paid their attention to the patients with unnormalised PRL levels for cabergoline treatment, which depend on a dose-response study of macroadenomas. In our results, the postoperative PRL level as assessed at 3 months indicated that a higher Ki-67 LI was more likely to show normalization in endocrine level. Surgery helped us to reduce the tumor mass. However, the postoperative PRL normalization was acquired in few cases in LI \geq 3% group. These data are in disagreement with the data described by (Vasilev *et al.*,

Table 1: Clinical characteristics of the patients

Variables	Ki-67 LI \geq 3% (n=22)	Ki-67 LI<3% (n=177)	p
Mean age (years)	31.00 \pm 7.51	36.75 \pm 10.67	0.015
Mean time interval (months)	50.55 \pm 65.96	51.16 \pm 47.74	0.956
Ki-67 LI (%)	5.91 \pm 2.33	0.13 \pm 0.40	<0.001
Tumor size (cm)	2.44 \pm 1.57	1.96 \pm 0.96	0.043
Microadenomas	2(9.09%)	58(32.77%)	0.022
Macroadenomas	20(90.91%)	119(67.23%)	0.022
Invasion			
+	8(36.36%)	25(14.12%)	0.014
-	14(63.64%)	152(85.88%)	
Apoplexy			
+	6(27.27%)	32(18.08%)	0.386
-	16(72.73%)	145(81.92%)	

The quantitative data are expressed as the means \pm SD. The qualitative data are expressed as n and %. P < 0.05 is considered to be statistically significant. The symbol – indicates that the characteristic in question was not observed. The symbol + indicates that the characteristic in question was observed.

Table 2: Clinical features

	Ki-67 LI \geq 3% (n=22)	Ki-67 LI<3% (n=177)	p
Headache/dizziness	10(45.45%)	68(38.42%)	0.524
Vomiting	2(9.09%)	12(6.78%)	0.657
Visual impairment	8(36.36%)	46(25.99%)	0.302
Visual field defects	4(18.18%)	50(28.25%)	0.317
Menstrual disorders	19(86.36%)	140(79.10%)	0.577
Galactorrhea	14(63.64%)	155(87.57%)	0.008
Polyuria/polydipsia	3(13.64%)	4(2.26%)	0.031

The data are expressed as n and %. P<0.05 was considered to be statistically significant.

Table 3: Hormone levels for PRL before and after surgery

	Ki-67 LI \geq 3% (n=22)	Ki-67 LI $<$ 3% (n=177)	p ^a
Before surgery	218.23 \pm 170.10	151.14 \pm 126.95	0.026
3 days after surgery	87.33 \pm 66.03	57.39 \pm 47.97	0.009
3 months after surgery	88.95 \pm 68.46	63.54 \pm 51.24	0.036
1 year after surgery	94.32 \pm 70.74	62.41 \pm 52.36	0.010
p ^b	<0.001	<0.001	
p ^c	0.689	0.495	

The data are expressed as the mean \pm SD. p<0.05 was considered to be statistically significant.

p^a Analysis between the two groups at the same time point.

p^b Analysis before surgery and 3 days after surgery.

p^c Analysis between different time points after surgery.

2011). In addition, diabetes insipidus and hyponatremia, which result from disturbances in osmoregulation, are well known complications in sella area postoperatively. The incidences of transient DI and hyponatremia in patients with Ki-67 LI \geq 3% were significantly higher than in patients with Ki-67 LI $<$ 3%.

Ki-67 was found to be a useful marker for resistant prolactinoma recurrence. Our overall recurrence rate of 10.55% is higher than the previously reported recurrence rates of 0.8% and 6% (Shao *et al.*, 2013 and Ikeda *et al.*, 2013). The recurrence rates in patients with Ki-67 LI \geq 3% and Ki-67 LI $<$ 3% were 27.27% and 8.47%, respectively. Recurrence was more frequent in the Ki-67 LI \geq 3% group. The present findings showed a correlation between recurrence and Ki-67 values. Ki-67 is a well-known factor in predicting recurrence after surgery. Matuyama *et al.* (2012) set a Ki-67 cut-off of 3% for predicting the risk of progression and regrowth for residual tumors. However, there is still some different results in the literature concerning on the recurrence. Especially, some authors have found no correlation between Ki-67 level and the recurrence (Dubois *et al.*, 2007 and Fusco *et al.*, 2008 and Marko *et al.*, 2012). Our present study reveals that Ki-67 LI may play an important prognostic role although the reported discrepancies. Ki-67 is useful in evaluating the level of proliferation in different tumors. The most important point is that it could provide relevant information for the long-term prognosis.

CONCLUSION

In conclusion, our study provides the vital evidence that higher Ki-67 value is associated with worse outcome in women with resistant prolactinomas. Our study also suggests that patients with higher Ki-67 value often have bigger tumor size, more invasive tumor, more pre- and post-operative features and manifestations, higher PRL level, and higher recurrence rate. The information could provide new insight into the current knowledge about tumor biology and could help us in understanding the Ki-67 values in women with resistant prolactinomas. A high Ki-67 is predictive of a poor prognosis and resistance to

adjuvant therapy in this group of patients. Resistant prolactinomas retain a majority therapeutic challenge in women. Future studies are necessary to clarify the diagnosis and treatment of prolactinomas in women.

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