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Ki-67 Labeling Index and Knosp Classification of Pituitary Adenomas

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Abstract

Pituitary adenomas (PAs) are common lesions that are reported to be the second most frequent primary brain tumor. The classification of pituitary tumors is frequently updated to optimize guidance for clinical treatment based on current knowledge. To date, the World Health Organization conducts periodic expert review consensus meetings and publishes the results. These include recommendations for the behavior of more aggressive, high Ki-67 index (>3%) pituitary. A high Knosp grade (>3) is also considered to exert a high risk of recurrence of pituitary tumors. We reported an inverse relationship between the Ki-67 labeling index and Knosp grade for functional pituitary adenomas and nonfunctional pituitary adenoma (Spearman correlation coefficient in functional pituitary adenomas $r=-0.59$, $p<0.001$ $n=75$ and $r=0.367$, $p<0.01$ $n=168$ in nonfunctional pituitary adenoma), and suggest that this results from early diagnosis and treatment before they become aggressive and recurrent. There were few articles analyzing the correlation of Ki-67 labeling index and Knosp classification of pituitary adenomas. Our study showed they were negatively correlated in functional pituitary adenomas and positively correlated in nonfunctional pituitary adenomas.

Keywords: Pituitary adenomas; Ki-67 labeling index; Knosp classification

Abbreviations: PAS: Pituitary Adenomas; PRL: Prolactinoma; ACTH: Adrenocorticotropic Hormone; GH: Growth Hormone; TSH: Thyroid-Stimulating Hormone; COR: Cortisol; NFPAs: Non-Functioning Pituitary Adenomas; FPAs: Functional Pas; DI: Diabetes Insipidus.

Introduction

The World Health Organization recently published the 2017 classification of pituitary adenomas. They emphasized that there may be some relationship between pituitary adenomas with a high Ki-67 index (>3%) and aggressive tumor behavior. The MIB-1 labeling index, using the MIB-1 monoclonal antibody that recognizes the cell cycle-specific antigen Ki-67, is correlated with increased growth rate and invasive growth [1,2]. Although most pituitary adenomas (PAs) are known to behave as benign tumors, efforts have been made to establish the clinical behavior of individual tumors, with emphasis on predicting those that might behave more aggressively. Here, The World Health Organization propose to define high-risk PAs with a high Ki-67 labeling index, frequent recurrence, resistance to conventional treatments and/or temozolomide, growing rapid, and

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invading into the cavernous sinus segment of the internal carotid artery. The Knosp classification categorizes tumor proximity to the cavernous sinus segment of the internal carotid artery, and thus is used to quantify invasion of the cavernous sinus. Grade 3 and 4 pituitary tumors are considered invasive [3]. Invasion into the cavernous sinus is the most common cause for incomplete resection, resulting in a higher recurrence rate [4]. High Knosp grade (>3) pituitary tumors, therefore, have a high risk of recurrence. In this study, invasive pituitary adenomas with Knosp Grades 0, 1, and 2 were regarded as low-grade, and Grades 3 and 4 adenomas were classified as high-grade invasive adenomas [4]. We aimed to investigate the relationship between the Ki-67 labeling index and Knosp classification grade for pituitary adenomas.

Materials and Methods

Subjects

We retrospectively analyzed the records of 247 surgical patients with pituitary adenoma in the saddle area who were admitted to the General Hospital of NingXia Medical University from January 2015 to February 2019. Inclusion criteria were as follows:

- Acceptance of the endoscopic endonasal resection technique.
- Confirmation of the functional status and permanent diagnosis for each tumor by hormone testing and histopathology, respectively.
- Pre-operative and post-operative pituitary magnetic resonance imaging (MRI): if an MRI was obtained from an outside institute, it was excluded from the postoperative MRI analysis.
- pre-operative and post-operative data for prolactinoma (PRL), adrenocorticotropic hormone (ACTH), growth hormone (GH), thyroid-stimulating hormone (TSH), and cortisol (COR) at 4 pm and 8 am.
- Postoperative pituitary MRI follow-up performed at the outpatient clinic at 3 months, 6 months and yearly thereafter (MRI at 1.5 T).

Pituitary adenomas from 247 patients (Table 1) were divided into 4 overlapping groups (Table 3). First, on the basis of distinct combinations of the Ki-67 labeling index and Knosp grade: Group 1: Ki-67 < 3%; Group2: Ki-67 > 3%; Second, Groups 3 and 4 comprised different types of PAs: clinically non-functioning pituitary adenomas (NFPAs), and clinically functional PAs (FPAs), respectively.

Ki-67 labeling index and Knosp classification correlation analysis	r_s	P
	0.037	0.563

Table 1: Ki-67 labeling index and Knosp classification correlation analysis (n=247).

Ki-67 labeling index and Knosp grade

PAs were classified into clinically nonfunctioning pituitary adenomas (NFPAs), and clinically PRL, ACTH, GH, or TSH according to their pituitary hormone (Table 2). Patients who developed abnormal hormone concentrations during follow-up after surgery and required medical treatment were categorized with recurrent disease. Postoperative persistent or recurrent disease was defined by elevated plasma cortisol or urinary free cortisol, elevated insulin-like growth factor-1, insufficiently suppressed GH during the oral glucose tolerance test, or a random GH > 1.0 ng/ml; elevated PRL, elevated free thyroxine (T4) and TSH levels, and radiologically verified residual tumor postoperatively controlled at the outpatient clinic. These indicators were measured at 4 weeks, 3 months, 6 months and longer, depending on tumor growth, at least once a year [2].

Antibodies/Clones	Dilution
Anti-PRL Polyclonal	1:2000
Anti-GH Polyclonal	1:2000
Anti-FSH C10	1:50
Anti-LH C93	1:50
Anti-ACTH O2A3	1:100
Anti-TSH 42	1:50
p53 DO7	1:100
Ki-67 MIB 1	1:100

Table 2: Classification of PAs based on pituitary hormone.

Surgical technique

The primary surgical technique has been transsphenoidal surgery since Hardy introduced surgical microscopes in 1962. Further development of this technique has improved the gross total resection rate [5]. In our database, all surgical procedures were performed by the same 4 neurosurgeons, which were experienced and performed the procedures routinely throughout this period. After induction of general anesthesia, a standard microscope (Storz, Germany) transsphenoidal microsurgical technique was used in all patients. For access, the right nostril was typically selected unless this corridor was extremely narrow. Adenomas were removed using blunt curettes. After adenoma removal, hemostasis was achieved with temporary placement of Spongostan® and, if necessary, Surgiflo® (Johnson & Johnson Medical NV, Zaventem, Belgium) or FloSeal (Baxter Healthcare

Corporation, LA, USA). The dura was closed using artificial dura mater (TianXinFu Medical Appliance, Beijing) and in some cases the seller floor was reconstructed using autologous fat. The nasal septum was repositioned and fixed with nasal packing for 12-24 h. If cerebrospinal leakage was noted, autologous fat grafts were performed. Additionally, rhinoliquorrhea and diabetes insipidus (DI) are common complications [5].

Postoperative pathological and immunohistochemical analysis

The diagnosis was based on the postoperative pathological and immunohistochemical analysis. For histopathological evaluation, sections were stained with the routine hematoxylin and eosin method. Additional paraffin sections from each tumor were immunostained using primary antibodies against the following pituitary hormones to diagnose PRL-, ACTH-, GH-, or TSH-secreting adenomas.

Knosp classification

Knosp classification is the graded invasion of the cavernous sinus based on tumor incursions into medial or lateral intercarotid lines (Figure 1-5). Every pre-operative and post-operative pituitary MRI was graded by 2 experienced neurosurgeons.

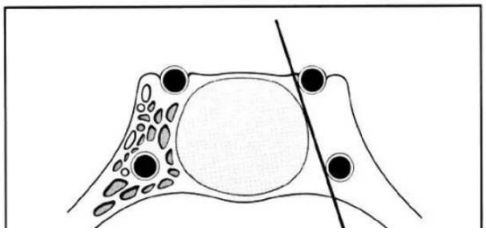

Figure 1: (Normal type): Cavernous sinus was in normal shape, with enhancement of cavernous sinus venous plexus, and the tumor did not exceed the internal tangential line of c2-c4 vascular diameter.

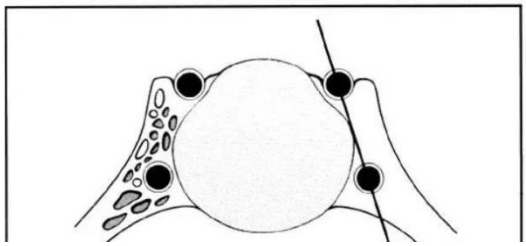

Figure 2: When the tumor exceeded the cutoff line of c2-c4 vascular diameter, but did not exceed the Central Line of c2-c4 vascular diameter, the venous plexus in the medial cavernous sinus disappeared.

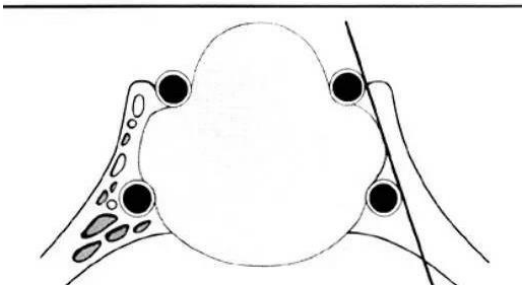

Figure 3: When the tumor exceeds the Central Line of c2-c4 vascular diameter, but does not exceed the external tangential line of c2-c4 vascular diameter, the upper or lower venous plexus of the cavernous sinus may disappear.

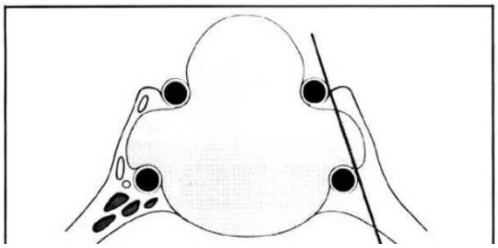

Figure 4: When the tumor exceeded the circumscribed line of c2-c4 vascular diameter, the medial, upper and/or lower venous plexus of the cavernous sinus disappeared, as did the lateral venous plexus.

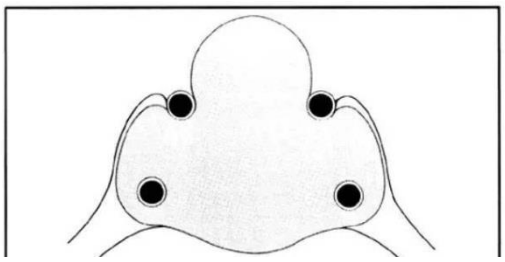

Figure 5: The internal carotid artery in the cavernous sinus segment was completely encapsulated, resulting in internal diameter stenosis, disappearance of venous plexus in each part, and spherical outward expansion and protrusion of the superior wall and outer wall of the cavernous sinus.

Statistical analyses

The data are presented as means and ranges for not continuous variables, we used Spearman Rank Correlations. All data analysis documented here was performed using IBM SPSS (Version 17.0 ©Copyright IBM

Corporation and others1989.2013.). Statistical tests to assess differences between groups and associations between variables, included analyses of variance. First, we take Kolmogorov-Smirnova of 4 groups. Then an assessment of the relationship between the Ki-67 labeling index and Knosp classification grade between the 4 groups was tested with Spearman correlation coefficient. P-values<0.05 were considered significant.

Ethical considerations

This study was approved by the ethics committee of our Institution (EC Nr: 1008/2014) and was performed in accordance with the principles of the Declaration of Helsinki. Tumor specimens for immunohistological analyses were obtained during operative procedures intended to cure or limit the extension of the disorders, and informed consent was obtained from all patients for further histopathological examination of these samples.

Nonfunctional Pituitary Adenomas

Initial symptom of nonfunctional pituitary adenomas may be headache before it becomes compression symptoms. Conservative treatment is the first treatment options before compression symptoms coming up. MRI is usually examined 3 or 6 months and later, depending on symptoms, at least once a year. There will be more time for nonfunctional pituitary adenomas to grow. Therefore, conservative treatment might lower gross total resection rate of nonfunctional pituitary adenomas with cavernous sinus invasion. When nonfunctional pituitary tumors are often large enough to cause symptoms like related to compression of the optic apparatus, characteristically resulting in a bitemporal hemianopia, patients would be aware to seek medical advice. Patients with nonfunctional pituitary tumors tend to seek medical attention delayed because particularly nonfunctional pituitary tumors are typically benign and slow growing, it would take many years to grow before the symptoms of compression develop. So nonfunctional pituitary adenomas with gradual symptom development that may delay diagnosis until they are invasive. However, some NFPAs were not diagnosed due to the small size without compression symptoms. There are a lot of influences factors may act on the size of nonfunctional pituitary adenomas cell proliferation is one of them. Cells with high proliferation rate might promote the growing of NFPAs with compression symptoms. It could explain the positive correlation of NFPAs between Ki-67 labeling index and Knosp classification. Patients may also experience hypopituitarism secondary to compression of the normal pituitary gland. In contrast, patients with hormone-secreting adenomas experience noticeable symptoms such as amenorrhea that prompt them to seek medical attention and were diagnosed sooner.

Hormone-Secreting Adenomas

There are two primary reasons why patients with hormone-secreting adenomas seek medical advice: first, hormonal symptoms such as amenorrhea-galactorrhea syndrome and/or acromegaly. Second, compression symptoms such as hypopituitarism, vision field impairment, and/or ophthalmoplegia. In such cases, dopamine agonists like cabergoline and bromocriptine are well established first-line therapies to reverse the increases in serum prolactin that lead to amenorrhea. Hormone-secreting adenomas may be resected at smaller sizes than nonfunctional pituitary tumors clinically. Because hormonal symptoms is noticed earlier than compression symptoms so there is no enough time for hormone-secreting adenomas to grow as nonfunctional pituitary adenomas, leading to higher average Knosp grades for the latter. It's not the usual way of thinking about FPAs could not grow big enough with compression symptoms. Clinically, before compression symptoms coming out, hormonal symptoms are noticed earlier. Such as amenorrhea, healthy women have regular periods every month. Once successive two months did not come menstruation, most of them would seek medical advice. Compared with compression symptoms, there is less time for FPAs to grow big enough with compression symptoms. The same with everything else FPAs. This may account for our study showing mean Ki-67 labeling index and Knosp classification were negatively correlated in functional pituitary adenomas. However, our sample sizes were relatively small, reducing the power of our statistical analyses to support this conclusion fully. This apparent paradox would be an important topic for future studies. We therefore maintain that a higher Ki-67 labeling index may manifest in tumors that produce more hormone, thus causing more obvious symptoms and earlier treatment, explaining the negative correlation between Ki-67 labeling index of hormone-secreting adenomas and Knosp classification. However, the WHO classification does not offer an accurate association between the histopathological findings and the clinical behavior of the tumor [6]. In most published studies, the Ki-67 proliferation index was correlated with hormonal profile, size, and degree of tumor invasion. Variable expression of the Ki-67 antigen, observed in different types of pituitary adenomas, suggests the implication of pituitary hormones in the tumor proliferation potential. Bălinişteanu provided the evidence that a higher Ki-67 value is associated with PRL-secreting pituitary adenomas, mainly prolactinomas and mixed GH/PRL-secreting adenomas [7]. The highest Ki-67

labeling index was found in prolactinomas [8]. In addition, other studies of prolactinomas have demonstrated that a higher Ki-67 index is related to higher prolactin levels and larger macroprolactinomas [9-11]. On account of our limited study, we formed 4 groups:

1. Prolactinoma with Ki-67 labeling index>3%.
2. Prolactinoma with Ki-67 labeling index<3%.
3. Growth hormone adenomas with Ki-67 labeling index>3%.
4. Growth hormone adenomas with Ki-67 labeling index<3%.

Variance analysis was performed to analyze the serum hormone level of the same type of hormone-secreting adenomas with different Ki-67 labeling indexes. We observed a difference that did not reach statistical significance. The increased Ki-67 proliferation index supports the role of Ki-67 proliferation index as a predictive marker for invasive tumors. Based on previous studies, we can conclude that in prolactinomas and mixed GH/PRL-secreting adenomas with high Ki-67 labeling index, serum hormone levels are higher and symptoms manifest sooner than for low Ki-67 labeling index tumors. Patients visit doctors earlier in the progression of their disease, and their pituitary adenomas are excised invasion of the cavernous sinus, leading to low Knosp grades. This might explain why the Ki-67 labeling index and Knosp classification were negatively correlated in functional pituitary adenomas and positive correlated in nonfunctional pituitary adenomas.

Limitations and Strengths

We selected patients on the basis of the surgical procedure alone and were forced to exclude some cases due to absence of either a preoperative MRI scan or records of the surgical procedure. Our inclusion criteria resulted in a highly diverse but representative group of patients with PAs. Furthermore, all patients were operated by the same four neurosurgeons at the same center. There were few articles analyzing the correlation of Ki-67 labeling index and Knosp classification of pituitary adenomas. Our study showed they were negatively correlated.

Results

The level of Ki-67 expression did not show any significant relationship with sex and age. There was statistical significance in the 3 groups: the Ki-67 labeling index and Knosp grade for functional pituitary adenomas and nonfunctional pituitary adenoma (Spearman correlation coefficient in functional pituitary adenomas r=-0.59, p<0.001 n=75 and r=0.367, p<0.01 n=168 in nonfunctional pituitary adenoma), and the Ki-67 labeling index>3% r=0.35, p<0.01 n=56.

Discussion

Ki-67 is considered biomarker of aggressive tumor behavior in the World Health Organization classification of pituitary adenomas. It seems that increased proliferation rate is associated with a more aggressive behavior in pituitary adenomas, with low proliferation rates observed in noninvasive tumors [4]. Some studies have focused on which the simultaneous expression of the p53 and Ki-67 markers in relation to tumor invasiveness and aggressiveness, and tumor progression and/or recurrence of pituitary adenomas [5]. Thapar et al. reported that hormone-secreting adenomas had a significantly higher mean Ki-67 labeling index (3.25%) than non-functioning adenomas (2.06%) [8]. Other case series studies focusing on prolactinomas have demonstrated that a higher Ki-67 index is related to higher prolactin levels and larger macroprolactinomas. In 2010, Pawlikowski et al. suggested that plurihormonal adenomas, especially ACTH-secreting tumors, have a higher Ki-67 index relative to that of monohormonal tumors [12]. A high Ki-67 index in ACTH-secreting adenomas was found in another study as well. In the case of gonadotropin-secreting and null cell adenomas, other studies showed low levels of the Ki-67 proliferation marker. A study performed on a relatively low number of pituitary carcinoma samples concluded that the mean Ki-67 index was 2.6% for primary tumors and 11% for metastatic tumors. In 1991, Kitz et al showed a significantly higher Ki-67 in invasive than in noninvasive adenomas [13]. Mastronardi et al reported that Ki-67 index was higher in functioning than in nonfunctioning tumors, particularly in ACTH adenomas [14]. Also, Honegger et al. concluded that expression of the Ki-67 antigen is positively correlated with the growth velocity of pituitary adenomas, while invasive behavior is independent of the Ki-67 antigen [15]. Chang et al. identified cavernous sinus invasion as a stronger predictor of recurrence, but recommended extreme care in adjunctive postoperative radiotherapy [16]. In a multivariate analysis, sex and parasellar extension of the tumor were the best predictors of persistent disease [6].

We analyzed the relationship between the proliferation rate and invasive behavior of pituitary adenomas using the Ki-67 labeling index and Knosp classification. Firstly, Kolmogorov-Smirnova be used to tested our data that p-valve of 0.001 showed our data was not normal distribution. Secondly, Spearman correlation coefficient were recorded to make a conclusion that p-value of 0.067 and r of 0.113 (Table 1) had no

statistical significance. Subsequently, we divided 247 patients into 4 groups (Table 3).

Ki-67 labeling index and Knosp classification correlation analysis	rs	P
	-0.007	0.927
ki67 index>3% (n=56)		
Ki-67 labeling index and Knosp classification correlation analysis	rs	P
	0.35	<0.01
Non-functioning adenomas(n=168)		
Ki-67 labeling index and Knosp classification correlation analysis	rs	P
	0.367	<0.01
Functional pituitary adenomas(n=75)		
Ki-67 labeling index and Knosp classification correlation analysis	rs	P
	-0.59	<0.001

Table 3: Ki-67 index<3% (n=191).

In the next, we divided Group2 (ki67 index>3%) into 2 subgroups, Subgroup1 ki67>3knosp<3 and Subgroup2 ki67>3knosp>3 (Table 4).

Ki-67 labeling index and Knosp classification correlation analysis	rs	P
	0.108	0.767
ki67 index>3% and Knosp grade>3 (n=46)		
Ki-67 labeling index and Knosp classification correlation analysis	rs	P
	0.324	0.028

Table 4: ki67 index>3% and Knosp grade<3 (n=10).

Spearman correlation coefficient were recorded to make a conclusion that p-value of 0.028 and r of 0.324 (subgroup2) had statistical significance. That conform increasing growth rate may have positive correlation with invasive growth. High Ki-67 proliferation index may be a predictive marker for invasive tumors. Next the Ki-67 labeling index and Knosp classification were analyzed in NFPAs and FPAs respectively.

Conclusion

We have suggested that the negative correlation between the Ki-67 labeling index and Knosp grade in FPAs is a clinical manifestation rather than evidence that high proliferation index is associated with low invasive behavior of tumors. The early clinical hormonal symptoms of functional pituitary adenomas that are associated with high Ki-67 labeling, allow less time for tumors to grow. In addition, it is challenging to manage the functional pituitary tumors since they tend to recur quickly following initial treatment and are generally unresponsive to therapy. We recommend that patients with symptoms of hormonal imbalances seek advice in outpatient clinics as soon as possible to increase the frequency of procedures to successfully arrest the invasive behavior of functional pituitary adenomas. If patients with clinical hormonal symptoms rapidly deteriorate in a short time, we should consider whether the value of Ki-67 labeling is high. Delayed treatment may come up with a poor prognosis and limited therapeutic options. Early treatment may improve gross total resection rate, with beneficial therapeutic effects. For NFPAs, improving gross total resection rate to avoid recurrence is the key of treatment. When postoperative immunohistochemistry showed that nonfunctional pituitary adenoma with high Ki-67 labeling index, increase the frequency of re-examination was recommended. The high Ki-67 labeling index in tissue samples suggests the need for careful clinical and radiological follow-up.

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