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The role of hormonal status, morphological subtypes and proliferative marker Ki-67 labeling index on long-term outcomes in patients with acromegaly: a single tertiary center's experience

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ABSTRACT

Aims: Acromegaly is a rare disorder resulting from benign growth hormone secreting pituitary adenomas. Many factors affect long-term outcomes in acromegaly. In this study we aimed to investigate effects of hormonal status, morphological subtypes, immunohistochemical expression of pituitary hormones and Ki-67 labeling index on long-term outcomes in patients with acromegaly.

Methods:. We collected the medical and pathological records of sixty-four patients who underwent surgery for growth hormone (GH) secreting somatotroph tumors between 2005-2017.

Results: The remission rate after surgery was 48% (31/64) in all patients (33% for macroadenomas, 94% for microadenomas; p <0.001) with a median follow up of 48 months (12-198). There was no significant relationship between Ki-67 labeling index and remission status (p=0.140). The remission group were significantly older than the nonremission group [47 (21-67) vs 36 (18-56); p=0.012]. We found a statistically significant positive correlation between insulin-like growth factor 1 (IGF-1) levels and Ki-67 labeling index (r=+0.382, p=0.004). Also, there was a significant positive correlation between tumor size and GH (r=+0.368, p=0.027). There was no difference between densely and sparsely granulated adenomas in terms of surgical remission (p=0.866). In multivariate regression analysis, tumor size ≥ 10 mm (macroadenoma) was significant independent variable in predicting remission [95% CI [16.95 (1.92-142)]; p=0.011]. The baseline cortisol levels was correlated negatively with the Ki-67 labeling index (r=+0.293, p=0.02).

Conclusion: The Ki-67 labeling index was not associated with surgical remission in patients with acromegaly. However, the Ki-67 labeling index was higher in younger patients and those with larger adenomas.

Keywords: Acromegaly, Ki-67 labeling index, growth hormone, remission

INTRODUCTION

Acromegaly is a chronic disorder caused by growth hormon (GH) hypersecretion. Surgery is the primary therapeutic option for acromegalic patients, but in the vast majority of patients cure is not achieved by surgical treatment.[1,2] In transsphenoidal surgeries performed by experienced pituitary surgeons, remission is greater than 85% for microadenomas and 40-66% for macroadenomas.[3] In previous studies, higher initial GH levels, larger tumor size, histologic evidence of a sparsely granulated adenoma and local invasion findings have been associated with lower probability of biochemical remission after surgery.[4,5] Even if they have similar characteristics, different disease outcomes may be seen in patients with acromegaly. This has prompted clinicians to investigate other predictive factors that may affect long-term outcomes of acromegaly patients.

Ki-67 antigen is a nuclear protein expressed in all phases of cell cycle except G0 phase. The presence of Ki-67 antigen is measured by a monoclonal antibody called MIB-1.[6] Many studies showed that the fraction of Ki-67 positive tumor cells (Ki-67 labeling index) is often correlated with cellular proliferation and more aggressive behaviour in pituitary tumors.[7-10] However it is still controversial whether Ki-67 index is a definitive

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marker of prognosis in acromegaly patients. In this study, we aimed to evaluate the relationship between immunohistochemical expression of pituitary hormones and Ki-67 labeling index with remission.

METHODS

The study was carried out with the permission of Ankara University Faculty of Medicine Clinical Researches Ethics Committee (Date: 11.04.2016, Decision No: 07-296-16). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Our cohort was composed of 115 patients with acromegaly and among them 64 patients who underwent surgery between 2005 and 2017 and reevaluated pathological specimens were included in this study. The patients who had not undergone surgery for various reasons, the patients with insufficient laboratory data, and the patients who had lost in follow-up were excluded from the study. The biochemical diagnosis of acromegaly was based on the current criteria.[11] Patients were divided into microadenoma (<10 mm) and macroadenoma (≥10 mm) groups according to adenoma size. Transsphenoidal surgery was the preferred approach for all patients. The biochemical reevaluation was performed after 6-12 week by oral glucose tolerance test (OGTT) and serum insulin-like growth factor 1 (IGF-I) levels in all subjects. Remission criteria determined according to 2010 consensus report (Insulin like growth factor levels normal for age and sex, a postoperative random GH level of <1 ng/ml or GH level of <0.4 ng/ml after an oral glucose tolerance test). Demographic, clinical, laboratory and imaging data of all patients were collected. All tissue specimens were examined for anterior pituitary hormones (GH, prolactin, follicle stimulating hormone, luteinizing hormone, tyrotrophin stimulating hormone, adrenocorticotropic hormone) via immunohistochemistry. MIB-1 antibody was used to identify Ki-67 antigen. According to the World Health Organization (WHO) classification, adenomas were classified as sparsely or densely granulated adenomas.[12] Two exprienced pathologists (SK, EE) re-evaluated hematoxylin and eosin (H&E)-stained slides of the adenoma samples. For each case, blocks were determined to use for immunohistochemical evaluation. The Ki-67 labeling indexes of adenomas were performed (clone SP6, Cell Marque, 1:200 dilution) and the rate of Ki-67 were measured as the maximum percentage of positive nuclei by counting at least 1000 adenoma cells.

SPSS software version 20 was used for statistical analysis. Simple descriptive statistics were expressed as mean±standard deviation for normally distribution variables, and as median values for non-normally distributed variables. The frequency distribution of categorical variables between subgroups was compared by the chi-square test. Numerical variables were compared by unpaired t tests or Mann Whitney U-test. Statistically significant results obtained from univariate analysis were submitted to multivariate logistic regression. Spearman correlation analysis was performed to evaluate the relationship between variables. P<0.05 was accepted as significant.

RESULTS

Patients' Characteristics

Sixty -four patients [Male:26 (41%); Female: 38 (59%)] who underwent surgery due to GH-secreting pituitary tumor were included in the study with a median follow up of 48 months (13-198). The median age of the patients at diagnosis was 44 (18-67) (**Table 1**).

The remission rate after surgery was 48% (31/64). Disease control was achieved with second-line treatments in 21 of 33 patients who were not in remission. Remission rates in patients with macroadenoma were lower than those with microadenoma (33% vs. 94%, p <0.001). There was no significant association between initial GH and insulin like growth factor (IGF-1) levels and remission (p=0.109 and p= 0.177). We also did not found a significant relationship between gender and remission status (46% for males and 50% for females; p=0.760) (**Table 2**). The age at diagnosis was significantly lower in patients with macroadenomas than with microadenomas [41 (18-67) vs 53 (23-64); p=0.012]. IGF-1 levels at diagnosis were significantly

Table 1. Baseline characteristics of patients

	Total (n=64)	Male (n=26)	Female (n=38)	P value
Follow-up time (months)	48(13-198)	48 (12-198)	48 (12-132)	0.544
Age at diagnosis (years)	44(18-67)	44 (23-67)	44 (18-63)	0.614
Macroadenoma	48 (75%)	19 (73%)	29 (76%)	0.821
Maximal diameter of adenoma (mm)	18 (6-42)	18 (7-28)	17 (6-42)	0.410
Initial IGF-1 (ng/ml)	949(244-4000)	1027 (435-4000)	882(244-2300)	0.031
Initial GH (ng/ml)	7 (0.30-58)	7.2 (0.60-58)	6.3 (0.30-49)	0.796
Ki-67 Labeling Index (%)	3.23±2.75	3.21±3.24	3.26±2.43	0.980
Prolactin immunostaining	21/64 (32%)	9/26 (34%)	12/38(31%)	0.712
Surgical Remission	31/64 (48%)	12/26 (46%)	19/38 (50%)	0.760

higher in male patients than in female [1027 (435-4000) ng/ml vs 882 (244-2300) ng/ml; p=0.031). IGF-1 level was significantly higher in patients with macroadenomas than in patients with microadenomas [1027 (244-4000) ng/ml vs 810 (579-1162) ng/ml; p=0.001).

Age at diagnosis was higher in the remission group than in the non-remission group, and the maximal adenoma diameter was smaller in the remission group [47 (21-64) vs 36 (18-56); p=0.012 and 11.62±5.14 mm vs 21.33±8.12 mm; p=0.002, respectively] (**Table 2**). In multivariate regression analysis, patients with tumor size lower than 10 mm (microadenoma) was found to be significant in predicting remission [95% CI [16.91 (1.92-142)]; p=0.011].

When the patients were divided into groups as those below 40 years old and those over 40 years old; the remission rate was significantly lower in the group of patients below 40 years of age (32% vs 63%; p=0.013). The incidence of macroadenomas was higher in the group below 40 years of age (91% vs 33%; p=0.022). We also found that the level of IGF-1 was significantly higher in the group of below 40 years of age [1338 (470-4000) ng/ml vs 849 (244-2699) ng/ml; p=0.008].

The most common symptom at diagnosis was acral enlargement (91%; 58/64). Other common symptoms were headache (32%), excessive sweating (27%), loss of vision (27%). There were no correlation between remission status and presentation signs such as acral enlargement, amenorhea, visual disturbances, headache, perspiration, libido loss. Panhypopituitarism was seen in 16% of patients postsurgically. The most common isolated hormone deficiency was gonadotropin deficiency (17%).

Relationship between Hormonal Status, Tumoral Characteristics and Out-Comes

Although not statistically significant the patients with remission had lower Ki-67 index than in patients without remission (2.69±2.22 vs 3.58 ±3.22; p=0.140). When the patients were classified according to their Ki-67 index as Ki-67<3 (Group 1) and Ki-67 ≥3 (Group 2), remission rate was higher in group 1 (58% vs 35%; p= 0.072) but this result did not reach statistical significance. Ki-67 index was almost the same between female and male groups (3.26±2.43 vs 3.21±3.24; p=0.980) (**Table 3**).

Table 3. Ki-67 labeling index according to clinical and laboratory parameters in patients.

	Ki-67 < 3 (n=36)	Ki-67 ≥ 3 (n=28)	P value
Sex (male/female)	16/20	10/18	0.480
Age at diagnosis	46 (22-67)	41 (18-60)	0.041
Macroadenoma	23 (63%)	25 (89%)	0.023
Cortisol (mcg/dl)	13.51±6.42	9.49±3.75	0.004

Macroadenomas were more common in group 2 than group 1 (89% vs 63%; p=0.023) and Ki-67 index was significantly higher in patient with macroadenoma (3.56±3 vs 2.07±1.65; p=0.024). When the granulation patterns were evaluated, 62% of the patients had dense granulated adenoma and 38% had sparsely granulated adenoma. No difference was found between densely and sparsely granulated adenomas in terms of Ki-67 labeling index values (3.23±2.56 vs 3.38±2; p=0.632). Also, we did not find any significant difference in remission status between dense or sparsely granulated adenomas (P=0.866).

Immunohistochemical examination showed that 27 patients (42%) had GH staining alone (monohormonal), 20 patients had plurihormonal staining (40%). We did not found any relationship between remission status and prolactin, adrenocorticotropic hormone (ACTH), Follicle stimulating hormone (FSH), Luteinizing hormone (LH) expression or multihormonal expression in our patients. No significant difference was found in terms of Ki-67 labeling index and remission status in mixed GH/Prolactin tumors.

Table 2. Comparison of clinical and laboratory characteristics of patients according to remission status.

	Remission (n=31)	Non-remission (n=33)	P value
Age at diagnosis	47 (21-67)	36 (18-56)	0.012
Sex (Female/Male)	19/12	19/14	0.760
Macroadenoma	16 (51%)	32 (96%)	<0.001
Adenoma diameter (mm)	11.62±5.14	21.33±8.12	0.002
Ki-67 labeling index (%)	2.69 ± 2.22	3.58 ± 3.22	0.140
Ki-67<3 / Ki-67≥3	21/10	15/18	0.072
IGF-1 (ng/ml)	850 (435-2699)	1050 (244-4000)	0.109
GH (ng/ml)	6.60 (0.6-49)	12.4 (0.3-59)	0.177
LH (mIU/ml)	12.20 (7.40-17.10)	4.75 (2.6-6.9)	0.005
FSH (mIU/ml)	10 (0.10-113)	5.7 (0.50-81)	0.019
ACTH (pg/ml)	29.5 (12-101)	46 (11-107)	0.085
Prolactin (ng/ml)	9.1 (2.4-48)	13 (2-100)	0.548
TSH (mIU/L)	1.34 ± 1.1	1.24 ± 1.01	0.791
Cortisol (mcg/dl)	12 ± 5.3	11.8 ± 6.1	0.909

The median IGF-1 level at diagnosis in group 1 was significantly lower than group 2 [817 (244-2699) ng/ml vs 1239 (540-4000) ng/ml; p=0.002]. The age at diagnosis in group 1 was significantly higher than group 2 [46 (22-67) vs 41 (18-60); p=0.041)]. In correlation analysis, there was a statistically significant positive correlation between IGF-1 levels and Ki-67 labeling index (r=0.382, p=0.004). Also, there was a significant positive correlation between tumor size and GH (r=0.368, p=0.027). There was no correlation between Ki-67 labeling index and tumor size (p=0.109). Furthermore basal cortisol level was significantly higher in group 1 than those group 2 (13.51±6.42 vs 9.49±3.75; p=0.004) (**Table 3**). There was also an inverse correlation between cortisol level and absolute Ki-67 values (r=-0.301, p=0.017). In multivariate regression analysis, younger age at diagnosis and lower cortisol levels were found as independent variables predicting the patients with Ki 67≥ 3 (p=0.019 and p=0.019, respectively) (**Table 4**).

Table 4. Predicting factors for Ki67 labeling index ≥3

	P	Odds ratio	95% confidence interval	
			Lower	Upper
Lower cortisol	0.019	1.16	1.02	1.32
Younger age	0.019	1.06	1.01	1.12

DISCUSSION

In this study, 48% of the patients with acromegaly achieved remission and 51% of them had macroadenomas. The Ki-67 labeling index was not associated with remission status. The younger age at diagnosis, the lower baseline cortisol value and the larger tumor size were significantly associated with Ki-67 labeling index in patients with acromegaly. We also found that smaller tumor size, the older age at diagnosis were associated with surgical remission in patients with acromegaly. In the literature the remission rate after surgery alone is reported to be 27 and 80%. Our results were compatible with the literature.[13]

Whether the Ki-67 labeling index is a factor in surgical remission is controversial. To the best of our knowledge there were very limited studies showing that high Ki-67 labeling index values affect surgical remission. Fusco et al.[5] reported that Ki-67 labeling index is significantly higher in surgically not cured patients. They claimed that Ki-67 labeling index is associated with a better clinical outcome in patients with acromegaly. However, some other studies, like ours did not find any significant association.[14,15]

Jaffrain et al.[16] (2002) reported that Ki-67 labeling index decreased with increasing age and tumor volume but other studies claim the opposite of this. Similar to our study, Mohseni et al.[17] found higher Ki-67 levels in younger patients. According to Pinto et al.,[18] Fusco et al.[5] and Mastronardi et al.[19] Ki-67 expression was independent from the tumor size. In a recent study by Alimohamadi et al.[6] Ki-67 level was not significantly different according to tumor size. In our study, Ki-67 labeling index values were significantly higher in the macroadenoma group (p=0.027).

The relationship between histological subtype and postsurgical surgical cure is a controversial topic in the literature. While some studies have demonstrated lower surgical remission rates for sparsely granulated adenomas compared to densely granulated adenomas, in others remission rates did not differ by histological subtypes.[14,20-22] The frequency of histologic subtypes of adenomas in our cohort (62% densely ; 38% sparsely) is almost consistent with the range of frequencies reported in the literature.[15,23,24] Also, no difference was found in terms of remission status according to histological subtypes.

In our study low cortisol levels were associated with higher Ki-67 labeling index. Such a relationship has not been shown in previous studies. In a recent study evaluating the postoperative outcomes of 348 functional pituitary tumors, higher MIB-1/Ki-67 labeling index and preoperative low cortisol axis were shown to be associated with suboptimal outcomes.[25] This finding in our study can be attributed to the inhibitory effect of cortisol on cell proliferation, but this relationship cannot be clearly stated with the current study findings.[26,27]

According to previous studies younger age at diagnosis, higher pretreatment IGF-1 and GH levels and larger tumor size are associated with a lower probability of biochemical remission after surgery.[14] In accordance with previos studies, in our study, younger age at diagnosis and larger tumor size were associated with lower remission rates. Fernandez Rodriguez et al.[28] identified the main factors determining the prognosis of acromegaly and proposed risk score for each factors. In line with this scoring system we found that larger tumor size was independent risk factor in determining remission. Preoperative GH and IGF-1 levels have been associated with surgical remission in many studies but we did not find any association with remission as in the studies of Yıldırım et al.[29] and Shirvani et al.[30]

There are several limitations of this study. First, our study was retrospective in nature. Second, our study had small sample size therefore some results may not have reached statistical significance. Third, the pathological examination of immunohistochemical parameters such as mitotic index, p53 positivity other

than Ki-67 index could increase the strength of the study. More detailed classification of invasiveness according to radiologic apperance such as Knosp classicifation would have allowed to assess the parasellar invasion status. Also it would be better to evaluate the low cortisol levels with ACTH test for corticotroph function.

CONCLUSION

This study suggest tumor size is an important parameter for predicting remission. Younger age and lower cortisol level are associated with higher proliferation index in GH-secreting pituitary tumors. There is no significant relationship between Ki-67 labeling index and surgical remission.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara University Faculty of Medicine Clinical Researches Ethics Committee (Date: 11.04.2016, Decision No: 07-296-16).

Informed Consent: Written informed consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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