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Immunostaining Results of Growth Hormone Secreting Adenomas and Their Correlation with Laboratory Findings

Büyüme Hormonu Salgılayan Adenomlarda İmmünohistokimyasal İnceleme Sonuçları ile Laboratuvar Bulguları Arasındaki Korelasyon

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Abstract

Purpose: Depending on improvements in immunohistochemical and ultrastructural analyses, hormonal immunoreactivity are more commonly detected in pituitary adenomas. In this study, we investigated the correlation between immunohistochemical results and clinical and laboratory findings.
Material and Method: The records of 110 patients with acromegaly who were operated at our hospital were retrospectively evaluated. Of the patients, 43 were excluded because of missing data. All patients were clinically evaluated preoperatively and had pituitary hormone profile.
Results: Our study population was consisted of 42 females and 25 males. While there were 30 patients with growth hormone staining alone, plurihormonal staining was determined at the remaining 37 patients. Three of 14 patients were diagnosed with thyrotropin-secreting pituitary adenoma according to clinical and findings laboratory, preoperatively. Except these cases, there were no hormone-secreting adenomas even if positive cellular immunreactivity existed.
Discussion: Our data suggest that immunohistochemical results may not be correlated with the signs of hormone hypersecretion as evident by symptoms or laboratory results in patients with acromegaly.
Keywords: Acromegaly, immunohystochemistry, plurihormonality

Öz

Amaç: Hipofiz adenomlarının sınıflamasında daha sensitif immünohistokimyasal ve ultrastrüktürel tekniklerin kullanılması ile hormon immünreaktivite gösteren adenomların insidansında artış olduğu belirtilmektedir. Bu çalışmada akromegali nedeniyle opere edilen hastaların immünohistokimyasal inceleme sonuçları ile klinik ve laboratuvar bulguları arasındaki ilişkiyi araştırdık.
Gereç ve Yöntem: Hastanemizde akromegali nedeni ile opere edilen 110 hastanın verileri geriye dönük olarak tarandı. Kırk üç hastanın tetkikleri eksik olduğundan çalışma dışı bırakıldı. Tüm hastalar klinik ve laboratuvar olarak operasyon öncesi değerlendirilmiştir.
Bulgular: Hastaların 42'si kadın, 25'i erkekti. Otuz hastada sadece büyüme hormonu boyanması varken kalan 37 hastada plurihormonal boyanma saptandı. Tiroid uyarıcı hormon (TSH) boyanması olan 14 hastanın üçüne preoperatif olarak TSH salgılayan hipofiz adenomları tanısı konmuştu. Bu üç olgu dışında klinik ve laboratuvar olarak hormon sekrete eden adenom saptanmadı.
Tartışma: Çalışmamız akromegali hastalarında immünohistokimyasal olarak saptanan boyanma paternleri ile klinik ve laboratuvar bulguları arasında ilişki olmadığını gösterdi.
Anahtar kelimeler: Akromegali, immünohistokimya, plurihormonalite

Introduction

Tumors located in the sella turcica are mostly benign adenomas originating from the anterior pituitary (1). Formerly, pituitary tumors have been classified as either being acidophilic, basophilic or chromophobic. Pathological classification is now performed according to immunohistochemical (IHC) and ultrastructural analyses. Pituitary adenomas demonstrate immunoreactivity for growth hormone (GH) by 25-30%, prolactin (PRL) - by 11-26%, adrenocorticotrophic hormone (ACTH) -by 10-15%, follicle-stimulating hormone (FSH)- luteinizing hormone (LH) - by 10-15% and for thyroid-stimulating hormone (TSH) by 1% (2). About 25% of pituitary adenomas do not demonstrate any immunoreactivity. Utilizing more sensitive IHC techniques decreased the incidence of non-immunoreactive pituitary adenomas (3,4). Even though the hormone immunoreactivity is commonly observed in pituitary

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adenomas, this finding does not always correlate with the laboratory and clinical findings (5). In this study, we investigated the correlation between IHC results and clinical and laboratory findings in 67 patients who were operated due to GH-secreting adenoma.

Materials and Methods

Patients

This was a retrospective study including records of 110 patients who had been operated due to GH secreting adenoma between June 2010 and May 2013 at Ankara Numune Training and Research Hospital by highly experienced surgeons. Forty three patients without IHC examination and those with not accessible clinic evaluation results were excluded from the study. The levels of insulin like growth factor-1 (IGF-1), GH, FSH, estradiol or testosterone, free tri-iodotironin, free thyroxine, TSH and PRL were measured in the remaining 67 subjects. All the study participants received oral glucose tolerance test (OGTT)-GH and overnight dexamethasone suppression (DST) tests, preoperatively. The IHC battery included the use of antibodies to GH, PRL, ACTH, TSH, FSH and LH. Furthermore the Ki-67 index was determined for 32 pituitary adenoma samples and the presence of the p53 mutation was determined in 17 samples out of 67 patients. Statistical Package for Social Sciences for Windows (SPSS) version 18.0 was used to perform the statistical analyses for all the data obtained in this study.

Immunohistochemistry

All pituitary adenoma specimens were fixed in a 10% formalin solution and then were embedded in paraffin. Five micrometers thick serial sections were mounted on silanized slides. Each slide was stained with hematoxylin and eosin procedure. IHC was performed with primary antibodies against the GH, PRL, ACTH, TSH, LH, FSH, Ki-67 and p 53. Pituitary adenomas with patterned immunostaining in the cytoplasm were considered positive for the hormone question (Figure 1).

Results

A total of 67 patients met the criteria to participate in the research, and there were 42 females in the study. The mean age of the patients was 42.5±10.1 (23-63) years. The mean GH level was 29.97±29.54 (1-120) ng/mL and the mean IGF-1 level was 971.42±364 (237-1801) ng/mL. According to IHC examination, 66 patients had dense GH staining and 1 had sparse GH staining. While there were 30 patients with GH staining alone (monohormonal), plurihormonal staining was determined in the remaining 37 patients (Table 1). Dense PRL staining was observed in 28% of patients, and sparse PRL staining was found in 10% of patients. Regardless of the intensity of PRL staining, all patients with PRL positive adenomas had normal serum PRL levels and no clinical symptoms of elevated PRL. Dense ACTH staining was observed in five patients and four patients demonstrated weak ACTH staining. Preoperatively, 1 mg DST demonstrated levels below 1.8 mcg/dL and no clinical symptoms and findings related to Cushing's syndrome were identified in these patients. Dense TSH staining was found in 11 patients and sparse TSH staining was detected in three patients. Three of these patients were diagnosed with thyrotropinoma according to laboratory and clinical findings, preoperatively. Of the patients with thyrotropinoma, two demonstrated dense TSH staining and the other one demonstrated sparse staining. The remaining patients had normal preoperative thyroid function tests. Dense LH and FSH staining were found in three patients and weak immunostaining was identified in three patients. Preoperatively, serum LH, FSH, estradiol or testosterone levels were normal in patients with pituitary adenomas that stained positive for LH and FSH. The correlation between IHC results and clinical and laboratory findings is given in Table 2. The Ki-67 index was determined for 32 patients. In 21 patients, the Ki-67 index was ≤1 and 11 patients had a Ki-67 index score of ≥2. P53 mutation was found in five of the 17 patients who were examined for the presence of a p53 gene mutation.

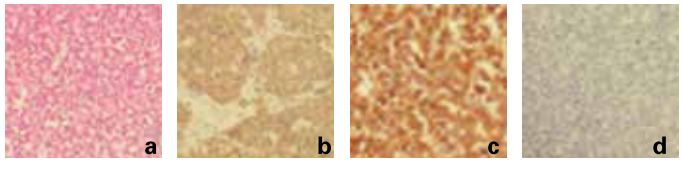

Figure 1. Immunohistochemical staining of pituitary hormons in adenomas. a) Nonfunctioning adenoma; round nüclei, large eosinophilic cytoplasm, uniform cells, H&E, x100, b) Growth hormone staining cells, growth hormone, x200, c) Cytoplasmic prolactin staining cells, prolactin, x200, d) 1% staining pattern with Ki-67, x200

Discussion

Although pituitary adenomas demonstrate immunoreactivity for various hormones, these findings are not always consistent with serum levels of relevant hormone and the development

Table 1. Immunohistochemical examination results of patients

	n (%)
Only GH	30 (44)
GH + PRL	13 (19)
GH + TSH	6 (9)
GH + ACTH	1 (2)
GH + FSH	1 (2)
Other combination	16 (24)
Plurihormonal	37 (56)

GH: Growth hormone, PRL: Prolactin, TSH: Thyroid stimulating hormone, ACTH: Adrenocorticotrophic hormone, FSH: Follicle stimulating hormone

Table 2. The association of immunohistochemical results and clinical and laboratory findings

Immunohistochemical staining results	n	Clinical confirmation	Laboratory confirmation
PRL +	26	No	No
TSH +	14	3	3
ACTH +	9	No	No
FSH-LH +	6	No	No

PRL: Prolactin, TSH: Thyroid stimulating hormone, ACTH: Adrenocorticotrophic hormone, FSH: Follicle stimulating hormone, LH: Luteinizing hormone

of clinical symptoms. These tumors are clinically classified as nonfunctional adenomas (NFA). It has been suggested that these tumors do not cause any hormone-associated clinical disorder either due to inactive hormone expression or due to defect in secretion despite normal hormone expression. It has also been hypothesized that NFAs catabolize the hormones in lysosomes (5). In our study, there were no clinical symptoms of Cushing's syndrome or elevated PRL in patients with pituitary adenomas that stained positive for ACTH and PRL, respectively. Three of the 14 patients with TSH-positive staining were diagnosed with TSHoma preoperatively. Wang et al. (6) investigated 279 patients with acromegaly and found no significant correlations between hormone levels and adenoma immunostaining. Furthermore, in another study by Bălinişteanu et al. (7) no correlation was found between conventional features and the presence of hormone immunostaining. Pituitary adenomas exhibit varying growth rates, different capacities to invade adjacent tissues, and abilities to reoccur. Various histological parameters and IHC indicators have been emphasized for understanding this different biological behavior, especially Ki-67 index and presence of p53 mutation (8). According to the World Health Organization classification, Ki-67 index is a major indicator of prognosis for patients with pituitary adenoma. An adenoma with Ki-67 index of above 3% or presence of p53 mutation is referred as atypical adenoma (9). Saeger et al. (10) reported that 2.7% of patients had atypical adenomas in a study including 451 patients with pituitary adenomas. In our study, 3.3% of the patients had atypical adenomas, consistent with the literature. Controversial results have been reported regarding the relationship between Ki-67 index and pituitary adenoma recurrence and aggressiveness. While some reports advocate that the Ki-67 proliferative index is only associated with growth capacity alone, other studies demonstrate that Ki-67 proliferative index is correlated with tumor aggressiveness and recurrenceb (11). In our study, there was no relationship between hormonal staining and Ki-67 index or the presence of a p53 mutation. Mori et al. (12) reported that 55% of growth hormone-producing adenomas expressed other hormones as well. In our study, we found that 56% of the pituitary adenomas demonstrated immunostaining for at least 2 hormones, which is similar to the findings in the literature. Higher recurrence rate of pituitary adenomas are associated with elevated Ki-67 index values in adenomas expressing multiple hormones (13). However, Wang et al. (6) reported that there was no relationship between adenoma plurihormonality and prognosis. We observed similar rates of Ki-67 index and presence of p53 mutation between plurihormonal and monohormonal groups. To our knowledge, this was the first study evaluating the association between immunostaining and laboratory and clinic findings in patients with GH-secreting adenomas in the Turkish population.

Conclusion

Our data suggests that IHC results may not be associated with hormone hypersecretion and laboratory findings in patients with GH-secreting adenomas. We also indicated that hormonal staining may not be associated with atypical adenomas in patients with acromegaly.

Ethics

Ethics Committee Approval: Based on pathology reports retrospectively reviewed article. Was not given any identification data. So patient have not been taken in the consent form.
Peer-review: External and Internal peer-reviewed.

Authorship Contributions

Concept: Mazhar Müslüm Tuna, Ersen Karakılıç, Design: Mazhar Müslüm Tuna, Mehtap Navdar Başaran, Data Collection or Processing: Mazhar Müslüm Tuna, Berçem Ayçiçek Doğan, Narin Nasıroğlu İmga, Immunohistochemical Analysis: Tuba Ünal, Analysis or Interpretation: Ayşe Arduç, Yasemin Tütüncü, Literature Search: Dilek Berker, Serdar Güler, Writing: Mazhar Müslüm Tuna.
Conflict of Interest: No conflict of interest was declared by the authors.
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