

Morphological Evaluation of Parathyroid Adenomas and Immunohistochemical Analysis of PCNA and Ki-67 Proliferation Markers

Paratiroid Adenomlarında Morfolojik Değerlendirme ve PCNA ile Ki-67 Proliferasyon Belirleyicileri Bakımından İmmünohistokimyasal İnceleme

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ABSTRACT

Objective: Parathyroid adenomas are the most common cause of primary hyperparathyroidism. Biological studies have shown that parathyroid adenomas are monoclonal proliferations. Up to date, five cell types have been identified in normal parathyroid tissues; chief cells, vacuolated chief cells, dark chief cells, oxyphil cells and transitional oxyphil cells. Most parathyroid adenomas are predominantly composed of chief cells.

In this study, we aimed to indicate the relationship between the predominant cell type in parathyroid adenomas and proliferating cell nuclear antigen, Ki-67 antigen, and serum parathormone levels and the gland weight.

Material and Method: 15 cases who had a diagnosis of parathyroid adenomas were included in the study. Histopathologically, the predominant cell type was determined in all the cases. Paraffin blocks were immunohistochemically stained with proliferating cell nuclear antigen and Ki-67.

Results: The average parathormone level of the cases was 239.52 ± 36.61 pg/ml before surgery. Mean gland weight was 1.69 ± 0.49 g. Two of the cases showed atypical adenoma characteristics. The predominant cell type was vacuolated chief cell. Immunohistochemical investigation showed that the mean average Ki-67 index value was $4.26 \pm 0.86\%$. The mean proliferating cell nuclear antigen index was $93.20 \pm 45.72/10^3$. There was a meaningful relationship between gland weights and serum parathormone levels. There was no meaningful relationship between predominant cell types and serum parathormone levels, proliferating cell nuclear antigen index, and Ki-67 index. The chief cell was identified as the predominant cell type.

Conclusion: It can be concluded that parathyroid adenomas come into existence as a result of neoplastic proliferation of chief cells, especially vacuolated chief cells.

Key Words: Parathyroid adenoma, PCNA, Antigen Ki-67, Immunohistochemistry

ÖZ

Amaç: Birincil hiperparatiroidiye en sık neden olan etken paratiroid adenomlarıdır. Biyolojik çalışmalar paratiroid adenomlarının monoklonal proliferasyonlar olduğunu göstermektedir. Normal paratiroid dokusunda 5 hücre tipi belirlenmiştir; esas hücre, berrak (vakuole) esas hücre, koyu esas hücre, oksifil hücre, transisyonel oksifil hücre. Çoğu paratiroid adenomu baskın olarak esas hücrelerden oluşmaktadır.

Bu çalışmada paratiroid adenomlarındaki baskın hücre tipi ile Prolifere hücre nükleer antijeni, Ki-67 antijeni, serum parathormon düzeyleri ve bez ağırlıkları arasındaki ilişkiyi belirlemeyi amaçladık.

Gereç ve Yöntem: 2007-2010 yılları arasında paratiroid adenomu sebebiyle opere edilmiş 15 olgu çalışmaya alındı. Olguların histopatolojik olarak baskın hücre tipi belirlendi. Parafin bloklara Prolifere hücre nükleer antijeni ve Ki-67 immünohistokimyasal boyaları uygulandı.

Bulgular: Hastaların tümü kadın olup ortalama yaş $58 \pm 13,36$ olarak saptandı. Operasyon öncesi ortalama parathormon düzeyleri $239,52 \pm 36,61$ pg/ml idi. Bez ağırlıkları ortalama $1,69 \pm 0,49$ gr idi. Olgulardan biri lipoadenomdu. İki tanesi atipik adenom özellikleri göstermekteydi. Olgulardan 8 tanesinde baskın hücre tipi "berrak esas hücre", 6 tanesinde "esas hücre", 1 tanesinde ise "oksifil hücre" idi. İmmünohistokimyasal incelemelerde Ki-67 indeksi ortalama değeri $\% 4,26 \pm 0,86$ olarak saptandı. Prolifere hücre nükleer antijeni indeksi ortalama $93,20 \pm 45,72/10^3$ olarak belirlendi. Bez ağırlıklarının serum parathormon düzeyleri üzerine anlamlı etkisi olduğu saptandı. Baskın olan hücre tiplerinin serum parathormon düzeyleri, Prolifere hücre nükleer antijeni indeksi, Ki-67 indeksi üzerine anlamlı etkisi olmadığı tespit edildi. Sonuç olarak çalışmamızda olguların çoğunda baskın hücre tipi esas hücre olarak saptanmıştır.

Sonuç: Paratiroid adenomlarının esas hücre, özellikle berrak esas hücrelerin neoplastik proliferasyonları sonucu meydana geldiği söylenebilir.

Anahtar Sözcükler: Paratiroid adenomu, PCNA, Ki-67 Antijeni, İmmünohistokimya

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INTRODUCTION

Parathyroid adenomas are the most common cause of primary hyperparathyroidism with a rate of 75-85% and are benign hypercellular neoplasms consisting of chief cells, oxyphil cells or a mixture of these cells (1). They are usually seen in only one gland and the rest of the glands can be normal or atrophic (1,2). They often develop from the lower parathyroid glands. They are macroscopically surrounded by a thin capsule and exhibit mild lobulation. Non-neoplastic parathyroid tissue is observed outside the capsule in some cases (3). Sometimes they may have pleomorphic and bizarre nuclei but vascular invasion, mitotic activity and metastasis are not observed. These cases are called 'atypical parathyroid adenoma' (1). They are called 'lipoadenoma' when glandular elements show solidarity with the population of mature adipocytes, and are called 'oxyphil parathyroid adenoma' when all or almost all of them are composed of oxyphil cells (4,5). A dominance of chief cells is usually observed in parathyroid adenomas (5,6). A small amount of oxyphilic cell groups can sometimes be observed.

Biological studies show that parathyroid adenomas are monoclonal proliferations and that these tumors arise from neoplastic proliferations of a single abnormal cell (2,7-9).

In our study, it was aimed to determine the relationship between proliferation activity and parathyroid hormone levels (PTH) and parathyroid cell types in parathyroid adenomas. The relationship between the predominant cell type, PCNA (Proliferating cell nuclear antigen) Labeling Index (LI), Ki-67 index and serum PTH levels in parathyroid adenomas was analyzed.

MATERIAL and METHOD

Fifteen cases who had underwent surgery for parathyroid adenoma between 2007-2010 at the Baskent University Istanbul Hospital were included in the study. Intra-operative pathological examination was performed for all the material. Weights were measured and recorded. All materials were diagnosed as parathyroid tissue during examination with light microscopy.

Tissue processing was performed to materials after the intra-operative pathological examination. Hematoxylin-eosin (H&E) staining was performed to sections prepared from paraffin blocks. All cases were consistent with parathyroid adenoma in the examination with light microscopy. The predominant cell types were determined by examining all the material. Sections prepared from paraffin blocks were immunohistochemically stained with Ki-67 (cloneMIB-1,

monoclonal mouse, DAKO) and PCNA (C 10 clone, mouse monoclonal, Novocastra) antibodies by using the peroxidase-antiperoxidase method. Tumor cells displaying positive nuclear staining among a total of 1000 cells in the areas where there was a maximum number of PCNA-positive cells in the lesions was expressed as PCNA LI. The most densely stained areas were chosen for Ki-67, 100 cells were counted and the percentage of positively stained nuclei was calculated. Ki-67 labeling index was expressed as the percent of positively stained cells.

Statistical analyses were performed with the GraphPad Prism 5.0 package program in this study. In addition to descriptive statistics (mean, standard deviation) used in the analysis of data, the Mann-Whitney U test was used in comparing paired groups and Spearman correlation analysis was used to analyze the relationships between groups. The results were considered significant at $p < 0.05$.

RESULTS

All patients were female. The mean age was 58 (47-79) years. Localization was lower left parathyroid gland in 11, upper left in 2 and lower right in 2. The mean preoperative PTH levels of the patients was 239.52 ± 36.61 pg/ml. The mean weight of the glands was $1.69 (\pm 0.49)$ gr. One of the cases was lipoadenoma and two showed atypical parathyroid adenoma features on histopathological examination (Figure 1, 2). The microscopically predominant cell types were determined to be vacuolated chief cells in 8 cases, chief cells in 6 cases and oxyphilic cells in 1 case (Figure 3,4). The dark chief cell and transitional oxyphilic cell types were not found (Table I).

PCNA was positive in all cases on immunohistochemical examination and PCNA LI was determined as $93.20 \pm 45.72 / 103$. PCNA staining was not observed in normal parathyroid tissue outside the capsule. Ki-67 index in all cases was $4.26 \pm 0.86\%$.

PTH level in adenomas predominated by vacuolated chief cells was 254.76 ± 66.17 pg/ml while it was 228.78 ± 31.82 pg/ml in adenomas predominated by chief cells and no statistically significant difference was found between them ($p=0.754$). PCNA LI was determined to be $69.25 \pm 22.02/10^3$ in adenomas predominated by vacuolated chief cells and $138.83 \pm 113.72/10^3$ in adenomas predominated by chief cells ($p=0.796$). Ki-67 index was $4.25 \pm 1.03\%$ ($p=0,905$) in adenomas predominated by vacuolated chief cells and $4.28 \pm 1.52\%$ in adenomas predominated by chief cells. No significant difference was found between these values according to the predominant cell types (Figure 5).

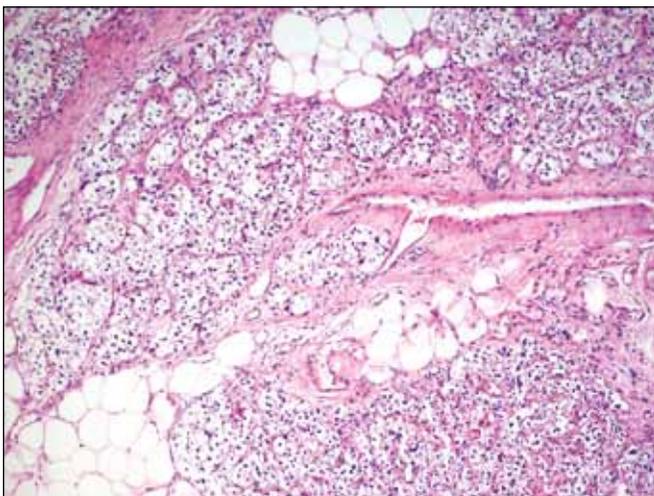


Figure 1: Lipoadenoma composed of vacuolated chief cells and mature adipocyte populations (H&E; x100).

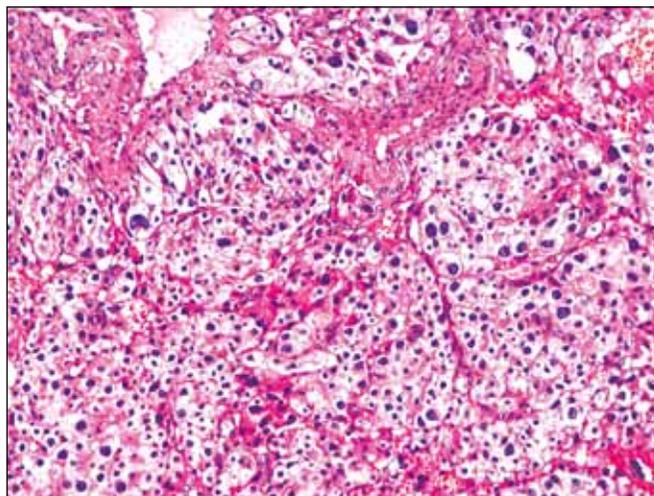


Figure 2: Atypical parathyroid adenoma; cells with pleomorphic nucleus, eosinophilic cytoplasm and oxyphil cell groups in between (H&E; x200).

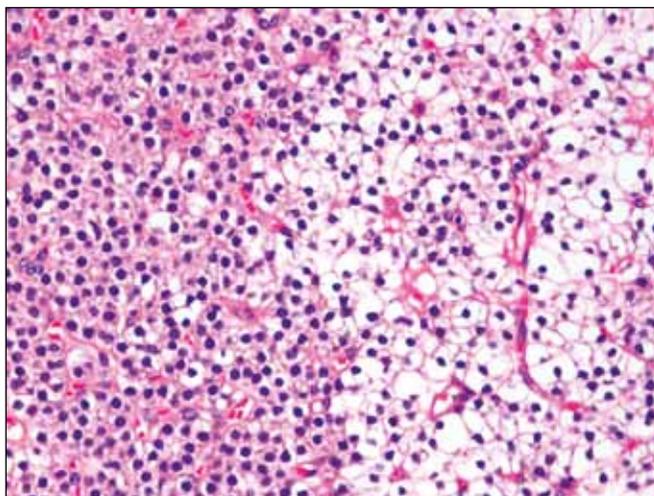


Figure 3: Chief cells (left side) and vacuolated chief cells with vacuolated cytoplasm (right side) (H&E; x200).

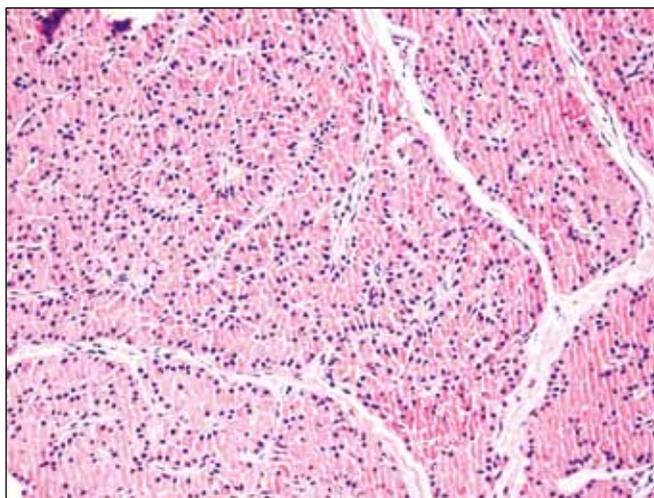


Figure 4: Oxyphil cells with large eosinophilic cytoplasm and peripheral nucleus (H&E; x100).

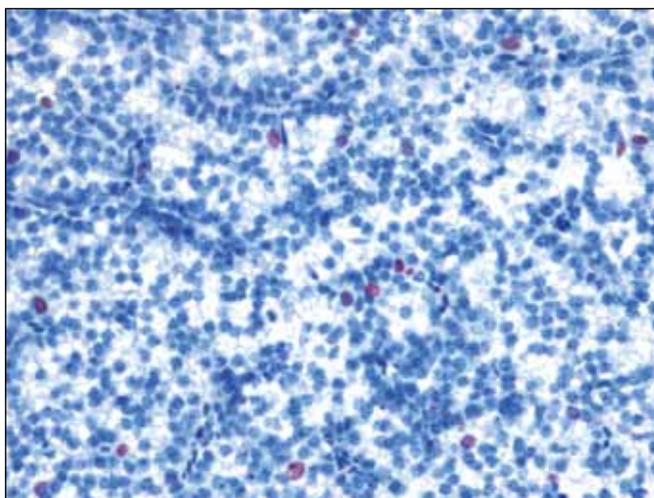


Figure 5: Ki-67 positive cells with dark red nucleus in parathyroid adenoma (x400).

The weight of the glands were found to have a significant effect on PTH levels with PTH levels increasing as the weight of the gland increased (Spearman $r=0.5821$; $p=0.02$). The highest PCNA LI values were found in two cases showing atypical parathyroid adenoma morphology with one predominated by vacuolated chief cells and the other one with chief cells (Figure 6).

No correlation was found between PCNA LI and serum PTH levels ($r=-0,087$; $p=0,756$) (Figure 7).

DISCUSSION

Five cell types have been identified in normal parathyroid tissues; chief cells, vacuolated chief cells, dark chief cells, oxyphil cells and transitional oxyphil cells. Dark chief cells

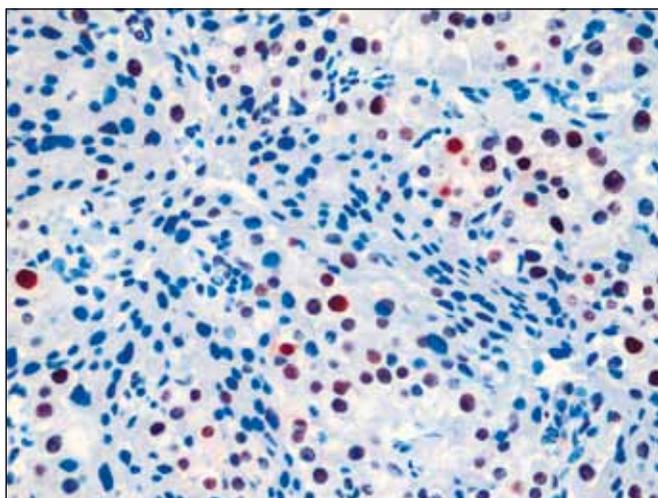


Figure 6: PCNA immunoreactivity in atypical parathyroid adenoma case (x400).

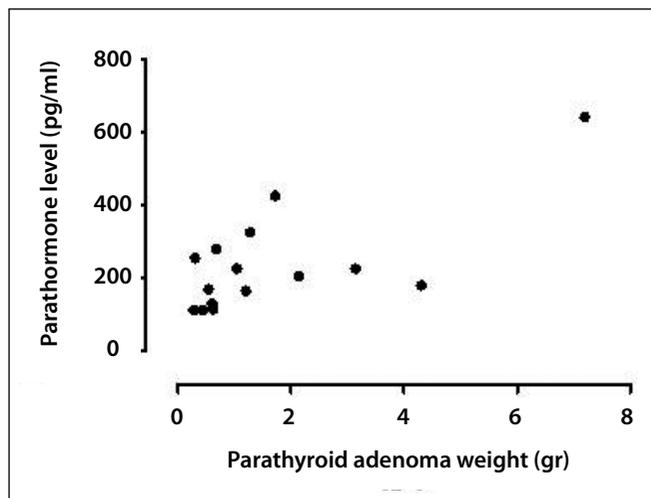


Figure 7: The relationship between weight of parathyroid adenoma and serum parathormone levels.

Table I: Clinical, morphological and immunohistochemical characteristics

CASE	AGE	WEIGHT(gr)	CELL	PCNA LI	Ki-67	PTH (pg/ml)
1	54	1.2	CC-VCC	24	7	166.3
2	50	2.14	VCC-CC	14	4	206.4
3	79	0.3	CC-VCC	10	1	256.3
4	63	1.04	VCC	160	7	227.7 (LA)
5	27	4.3	OC-CC	11	1	182.1
6	62	7.20	VCC-OC	491	1	643.7 (AA)
7	61	0.28	VCC	74	2	114.2
8	53	0.68	CC	30	2	282
9	77	0.54	VCC-OC	30	9	170.7
10	47	3.14	CC-VCC	19	1	227.9
11	50	1.28	CC-VCC	707	11	326.7 (AA)
12	73	0.62	VCC-CC	157	1	116.2
13	61	1.72	VCC	11	6	427.2
14	66	0.44	CC-VCC	43	7	113.5
15	48	0,60	VCC-CC	17	4	132

CC: Chief Cell, VCC: Vacuolated Chief Cell, OC: Oxyphil Cell, LA: Lipoadenoma, AA: Atypical Parathyroid Adenoma, PTH: Parathormon

have a more narrow cytoplasm and darker nucleus than normal chief cells. Vacuolated chief cells are smaller than normal chief cell, have a peripherally located nucleus and a clear, large, vacuolated cytoplasm. Oxyphilic cells are large and have an eosinophilic cytoplasm. Transitional oxyphilic cells are smaller than classical oxyphilic cells and have a cytoplasm lighter in color (7).

Shannon et al. have investigated the secretory cycle of chief cells ultrastructurally and found that large cells are in the

rest phase while dark chief cells are in the vesiculation phase. These findings demonstrate that dark chief cells have higher hormone secretion. The proliferative activity of oxyphilic cells was determined to be the same as or higher than chief cells (10).

Immunohistochemical PCNA staining of adenomas, secondary hyperthyroidism cases and normal parathyroid tissue cells in studies showed PCNA LI to be highest in adenomas (7,11). It was highest in vacuolated chief cells and lowest

in dark chief cells according to the cell types. Dark chief cells have the most active hormone secretion but are a cell type in the G0 phase of the proliferation cycle and have low PCNA LI. Oxyphilic cells have a high proliferation index similar to vacuolated chief cells. Transitional oxyphilic cells have higher PCNA LI values than classical oxyphilic cells (7).

PCNA LI was determined to be $70 \pm 68/10^3$ in chief cells and $14 \pm 0/10^3$ in transitional oxyphilic cells while staining was not observed in other cell types in a study conducted by Yamaguchi et al. They found a few PCNA positive cells in normal parathyroid tissue outside the capsule (7). PCNA positive cells were not observed in normal parathyroid tissue in studies by Larian et al. and Loda et al. and in our study (2,11).

No correlation was observed between PCNA LI and serum PTH levels according to cell types in our study. This also suggests that there is no relationship between hormone secretion and the proliferation activity of cells. However, this was not considered statistically significant due to low number of our cases and the lack of dark chief cell and transitional oxyphilic cell types.

PCNA LI was 45.8 ± 33.1 in a study of 12 cases and a correlation was found between PCNA LI and serum PTH levels (12). No correlation was found between PCNA LI and serum PTH levels in our study. The highest PCNA LI level was observed in atypical adenomas and the serum parathyroid hormone levels was also higher in these two cases. However, finding high serum parathyroid hormone levels in another case that was also determined to have very low PCNA LI supports the lack of a relationship between proliferation activity and hormone secretion of cells and suggests that atypical parathyroid adenomas should be addressed in a different category.

One of our patients showed lipoadenoma features. Parathyroid lipoadenomas are rare in the literature. Histologically, more than 50% of the lesion is composed of adipocytes. Clinically, they show characteristics similar to classical parathyroid adenomas (4). The mean serum PTH level was 239.52 ± 36.61 in our case. PCNA LI and Ki-67 index were above the mean value of the study. Histopathologically, they were composed of adipocyte populations as well as vacuolated chief cells.

A significant relationship was found between the Ki-67 index and serum PTH levels in a study carried out by Gozu et al. (13). Ki-67 expression in carcinoma is significantly higher than in adenoma. Ki-67 proliferation index was found to be 2% in adenoma and 25% in carcinoma in

studies. This also shows that Ki-67 can be helpful in lesions that are difficult to differentiate between adenoma and carcinoma (1,14).

Observing higher Ki-67 expression in adenoma than normal parathyroid tissue indicates that adenomas are clonal proliferations (8).

Clonal analyses have shown that parathyroid adenomas are monoclonal (2,8,9). All adenomas were determined to be monoclonal and normal parathyroid tissue to be polyclonal with the phosphoglycerate kinase gene in studies of Larian et al. (2).

A study has found positive correlation between the weight of the parathyroid adenoma and serum Ca^{++} concentrations but no relation was determined with serum parathyroid hormone levels (15). Similar to our study, many studies have determined a positive correlation between the weight of the adenoma and serum PTH levels (16-18).

In conclusion, the predominant cell type was determined to be the chief cell in the majority of cases in our study. It can be said that the majority of parathyroid adenomas arise from neoplastic proliferations of vacuolated chief cells. Larger series are required to determine the relationship between cell types and serum PTH levels.

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