Turkish Journal of Endocrinology and Metabolism, (2006) 3 : 57-61 **ORIGINAL ARTICLE**

Proliferation Activity in Parathyroid Adenomas and Its Relation to the Clinical Parameters

Paratiroid Adenomlarında Proliferasyon Aktivitesi ve Klinik Parametrelerle İlişkisi

Hülya Ilıksu Gözü* **Aylin Gül Ege**** **Haluk Sargın*** **Fatih Erkal***** **Berkant Sönmez***** **Mehmet Sargın*** **Medine Murtezaoğlu**** **Cem Gezen****** **Turgay Erginer******* **Selahattin Vural******* **Nimet Karadayı****

Kartal Education and Research Hospital, Istanbul, Turkey
** Section of Endocrinology and Metabolism*
*** Department of Pathology*
**** Department of 1. Internal Medicine*
***** Department of 1.General Surgery*

Parathyroid adenomas are most frequently responsible for primary hyperparathyroidism. Ki-67 is an useful antigen which determines proliferation activity in adenoma, hyperplasia and carcinoma of the parathyroid glands. In recent studies, Ki-67 index has been reported as between 1.36% and 3.3% in parathyroid adenomas. In this study we aimed to demonstrate proliferation activity in parathyroid adenomas using Ki-67 antibody and to evaluate the relationship of this index to clinical findings. Sixteen patients who underwent surgery for primary hyperparathyroidism were enrolled in this study. All cases were females and mean age was 52.62±15.86 (range, 20-70) years. Mean serum calcium level was 12.12 ± 1.35 mg/dl, and plasma PTH level was 623.10 ± 505.20 pg/ml. Pathology revealed parathyroid adenomas in all cases. Ki-67 labeling index was calculated in each slide as the percentage of immunopositive nuclei by avidin-biotin-peroxidase method. The mean value of Ki-67 index was found as 2.48 ±2.61% (range, 0.01-10%) in adenomas and 0,17 ±0,50% (range, 0-0.5%) in peripheral tissues. Ki-67 index was significantly higher in adenomas than in peripheral tissues (p:0,001, Z:-3,517). No significant correlation was found between Ki-67 index and clinical findings. In conclusion; we demonstrated that Ki-67 index was found higher in parathyroid adenomas than in normal tissues as in the literature, but no significant correlation was observed between Ki-67 index and the clinical parameters. Cases with higher Ki-67 index should be closely followed-up and treated after surgery.

Keywords: Parathyroid adenomas, Ki-67 index

Primer hiperparatiroidizmden en sık paratiroid adenomları sorumludur. Ki-67, paratiroid adenom ve hiperplazilerinde proliferasyon aktivitesini belirleyen bir antijendir. Son yıllarda yapılan çalışmalarda paratiroid adenomlarında Ki-67 indeksi, %1.36-3.3 arasında değişen sıklıklarda saptanmıştır. Bu çalışmada; paratiroid adenomlarında proliferasyon aktivitesini göstermeyi ve bu aktivitenin klinik bulgular ile olan ilişkisini belirlemeyi amaçladık.

Primer hiperparatiroidizm nedeniyle opere edilmiş 16 olgu çalışmaya alındı. Olguların tümü kadın olup, yaş ortalamaları 52.62±15.86 (20-70 yaş) olarak saptandı. Ortalama serum kalsiyum düzeyi 12.12±1.35 mg/dl, plazma PTH düzeyi 623.10±505.20 pg/ml olarak tayin edildi. Patolojik inceleme tümünde paratiroid adenomu ile uyumluydu. Avidin-biotin-peroksidaz yöntemi ile uygulanan immunhistokimyasal inceleme ile, Ki-67 pozitif boyanan hücre sayısı bu alandaki hücre sayısına bölünerek, yüzdesi alındı. Ki-67 indeksi ortalama değeri adenomlarda %2.48 ±2.61 (0.01-10%), periferik dokuda ise %0,17 ±0,50 (0-0.5%) olarak bulundu. Adenomlarda Ki-67 indeksi periferik dokudan anlamlı olarak daha yüksek saptandı (p:0,001, Z:-3,517). Ki-67 indeksi ile klinik bulgular arasında anlamlı bir ilişki bulunmadı.

Sonuç olarak; paratiroid adenomlarında Ki-67 indeksi literatür ile uyumlu olarak adenomlarda normal dokudan yüksek olarak saptanmıştır. Ancak Ki-67 indeksi ile klinik parametreler arasında anlamlı ilişki bulunmamıştır. Ki-67 indeksi yüksek olan vakalar cerrahi sonrası daha yakından takip ve tedavi edilmelidir.

Anahtar kelimeler: Parathyroid adenom and Ki-67 indeks

Correspondence address:

Hülya Ilıksu Gözü
Kartal Education and Research Hospital
Section of Endocrinology and Metabolism
Istanbul, Turkey
E-mail : hulyagozu@yahoo.com

Introduction

Primary hyperparathyroidism is a common disorder characterized by inappropriate PTH secretion from the parathyroid glands with or without hypercalcemia (1). The most important and common cause of primary hyperparathyroidism is parathyroid adenomas (1, 2, 3). Parathyroid adenomas are monoclonal tumors, i.e., these tumors arise from a neoplastic growth of a single abnormal cell (3). Monoclonality suggests a pathogenic role of oncogenes or tumor suppressor genes in the development of tumor tissues (4)

There is a balance between cell proliferation and apoptosis in the tumor growth and development (5). High expression of the bcl-2 protein as a protooncogene and sporadic mutations in the p53 as a tumor suppressor gene contribute to tumor development by inhibiting the balance between the cell proliferation and apoptosis (4, 5).

On the other hand proliferation activity of the parathyroid tumors is evaluated by the expression of the markers of the Ki-67, Parathyroid Adenoma 1(PRAD1) oncogene and Proliferating Cell Nuclear Antigen (PCNA) (4, 5, 6). The Ki-67 is a proliferation marker which is expressed in all phases except G0 of the cell cycle (6, 7, 8, 9). The proliferating cell index in parathyroid adenomas as measured by Ki-67 antibody has been reported with varying frequency ranging from 1.36 % to 3.3 % in several studies (7, 10).

The aim of this study was to measure the expression of Ki-67 labeling index in the parathyroid adenomas and to correlate this index with the clinical parameters of the patients.

Materials and Methods

Sixteen patients undergoing parathyroid adenomectomy at Dr. Lutfi Kirdar Kartal Education and Research Hospital between January 2004 and December 2005 were enrolled for this study. Medical records of these patients were reviewed for the clinical parameters and paraffin blocks were retrieved for immunostaining.

Serum levels of intact PTH were measured by chemiluminescence immunoassay (normal levels; 10-65 pg/ml) (DPC, USA). Plasma levels of 25-0H Vit D3 were measured by RIA (normal levels; 8-40 ng/ml) (IDS, Bolden, UK).

Ki-67 antibody was used to identify proliferation activity. Formalin-fixed paraffin-embedded blocks were analyzed for expression of Ki-67 antigen. Paraffin blocks were cut into 3-µm sections and mounted on glass slides prepared with poly-L-lysine. Immunostaining was performed with using the avidin-biotin-peroxidase method. Tissues were deparaffinized in xylene and rehydrated in alcohol. Antigen retrieval was performed by microwaving the slides in 10 Mm citrate buffer, Ph 6, for 15 minutes. Endogenous peroxidase activity was blocked by incubating the slides in 1% hydrogen peroxidase. Then slides were incubated with Ki-67 antibody (Fremont, CA USA) for one hour. The regions with highest concentrations of MIB-1 positive nuclei were selected and evaluated with a high power magnification (X400) in both of parathyroid adenomas and residual rim of normal parathyroid tissue. On the basis of 1000 neoplastic nuclei, Ki-67 labeling index was calculated in each slide as the percentage of immunopositive nuclei.

Statistical Analysis

Computer-assisted data analysis was performed using SPSS for Windows 10.0 program. In addition descriptive statistical methods (mean and standard deviation), Wilcoxon signed test was used in the evaluation of parameters of the groups without a normal distribution. Correlation between Ki-67 labeling index and the clinical parameters was evaluated by Spearman's correlation analysis. The results were evaluated in 95% confidence interval and $P<0.05$ was considered as significant.

Results

Mean age of the patients was 52.62±15.86 (range, 20-70 yr**).** Primary hyperparathyroidism was diagnosed by high serum levels of Ca (mean; 12.12±1.35 mg/dl) and PTH (mean; 623.10±505.20 pg/ml) in all patients. Parathyroid adenomas were confirmed by the following methods: Technetium 99m-methoxy-isobutyl-isonitrile (99 m Tc-MIBI) scintigraphy and ultrasound examination of parathyroid glands.

Parathyroidectomy was performed in all patients. All adenomas were cervical other than an ectopic one. The diagnosis of parathyroid adenomas was established by histological analysis in all patients. The mean postoperative serum level of Ca and PTH were decreased to 8.77±1.18 mg/dl and 60.86±53.49 pg/ml in these patients, respectively. Demographic characteristics of the patients are summarized in Table 1.

The mean Ki-67 labeling index was found significantly higher in the parathyoid adenomas (2.48 ±2.61; range, 0.01-10%), than residual normal parathyroid tissues (0,17 ±0,50%; range, 0-0.5%) (p:0,001, Z:-3,517) (Table 2).

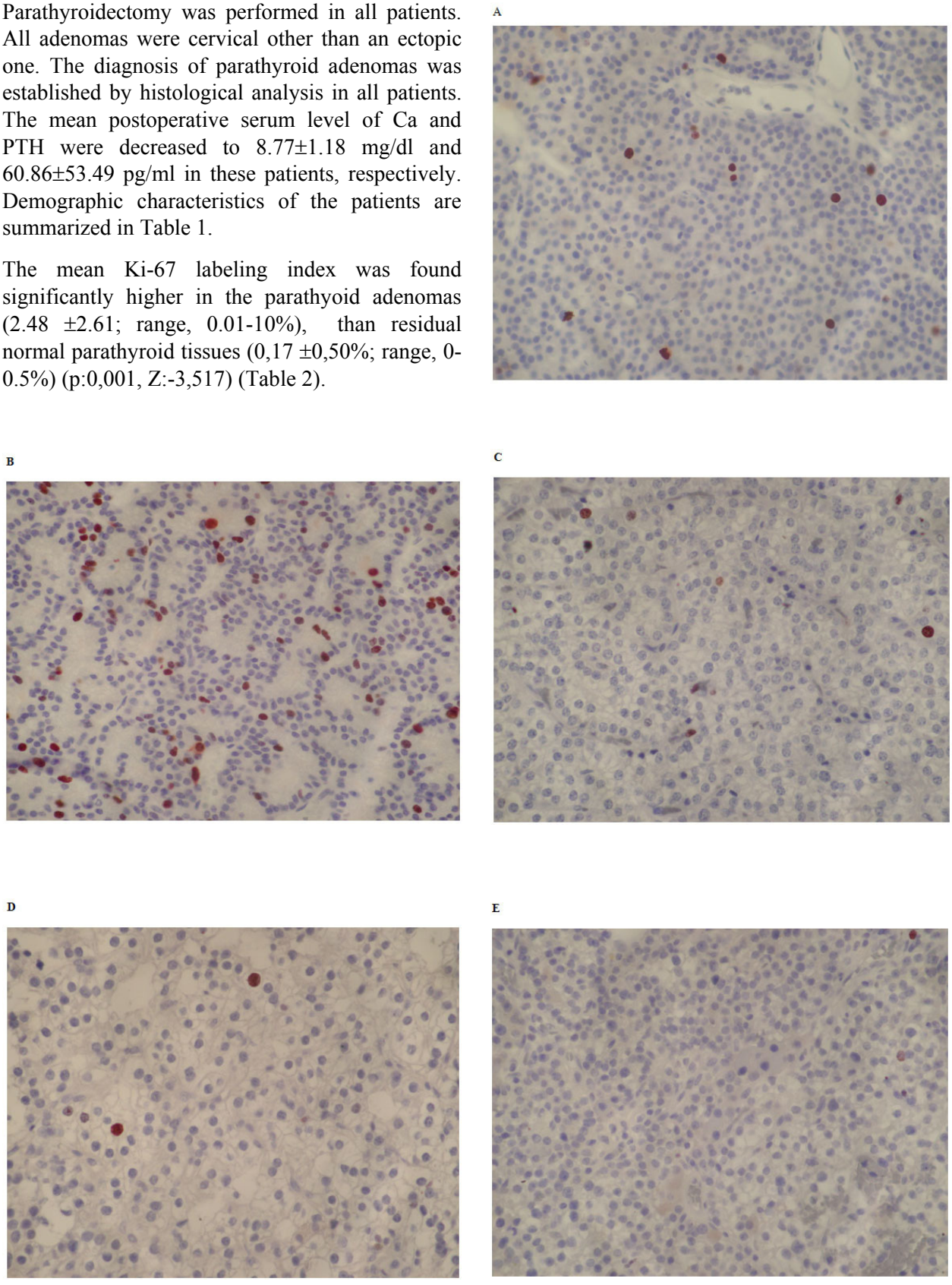

Figure 1. The samples of immunostaining for Ki-67 labelling index in parathyroid adenomas. **A, B, C, D and E:** The Ki-67 index in parathyroid adenomas 6%, 10%, 3%, 2% and 0.8%; respectively (original magnification, x400).

Table 1. Demographic characteristics of patients

	N	Mean	SD
Age (years)	16	52.62	15.86
Preoperative Ca (mg/dl)	16	12.12	1.35
Preoperative P (mg/dl)	15	2.39	0.62
Preoperative Alkaline Phospahatase (U/L)	16	884.62	1114.51
Preoperative PTH(pg/ml)	16	623.10	505.20
Ca in 24 hours urine (mg/24 hours)	11	346.85	147.20
25 OH Vit D3 (ng/ml)	13	19.48	13.02
Postoperative Ca (mg/dl)	16	8.77	1.18
Postoperative PTH (pg/ml)	16	60.86	53.49
Bone mineral density (L2-L4)	14	-2.36	1.88
Symptoms	**N**	**n**	**%**
Hypertension	16	7	43.8
Nefrolithiasis in history	16	4	25.0
Fractures in history	16	3	18.8

Table 2. Comparison of the Ki-67 index in the parathyroid adenomas and residual normal parathyroid tissues

	Median	Mean	SD	Values; p
Ki-67 index in adenomas (%)	2	2.48	2.61	p:0,001
Ki-67 index in normal tissues(%)	0	0,17	0,50	Z:-3,517

Table 3. Correlation of the Ki-67 index in the parathyroid adenomas with the clinical parameters of the patients

	Ki-67 index in parathyroid adenomas (%)	
	R	P
Age (years)	-0.127	0.639
Preoperative Ca (mg/dl)	-0.038	0.887
Preoperative P (mg/dl)	-0.269	0.332
Preoperativealkaline phosphatase (U/L)	0.429	0.097
Preoperative PTH (pg/ml)	0.312	0.240
Ca in 24 hours urine (mg/24 hours)	0.193	0.569
25 OH Vit D3 (ng/ml)	0.180	0.556
Postoperative Ca (mg/dl)	-0.395	0.130
Postoperative PTH (pg/ml)	0.183	0.497

There was no significant correlation between the Ki-67 index and age, plasma levels of 25 OH Vit-D3, serum levels of preoperative Ca, P, PTH, alkaline phosphatase and serum levels of post-operative Ca, PTH (Table 3).

Discussion

Many patients with primary hyperparathyroidism (PHPT) are diagnosed incidentally and considered as asymptomatic (11). The majority of them represent milder forms of hyperparathyroidism with minimal symptoms which do not progress during follow-up (12). It suggests that the parathyroid adenoma cell birth rate is very slow (13, 14). Parathyroid adenoma cell birth rate can be measured by tritiated thymidine labeling, by the prevelance of the mitotic karyotype or by the prevelance of labeling cells with the Ki-67 antibody (13).

Ki-67 antigen is a marker associated with proliferation, invasion and prognosis of neoplasms. MIB-1 detects this nuclear antigen expressed by proliferating cells during the entire cell cycle except G0 (15).

In the present study, using Ki-67 immunostaining, we noticed that the mean labeling index in parathyroid adenomas (2.48 ±2.61; range, 0.01-10%) was statistically higher than the mean labeling index in normal surrounding parathyroid tissues (0,17 ±0,50%; range, 0-0.5%) (p: 0.001, Z:-3,517). We found that all parathyroid adenomas were immunoreactive to Ki-67, while only in 4 out of 16 (25 %) showed positive results for Ki-67 in the surrounding normal parathyroid tissue.

The proliferating cell index in parathyroid adenomas have measured with varying frequency ranging from 1.36% to 3.3 % in several studies (7, 10). It was demonstrated that parathyroid adenomas have a statistically higher index than surrounding normal parathyroid tissue in these studies (4, 7, 10, 16). Hadar et al. were found that Ki-67 was stained in more than half of the parathyroid adenomas while none of the residual rims of normal parathyroid tissue (4). The mean Ki-67 index was reported as 0.08% in normal parathyroid glands and 3.28 % in adenomas in another study (7). Ki-67 index expressed as percentage positive cell nuclei, was found statistically higher in adenomas (1.36 ± 0.62 %) than in normal suppressed glandular tissue (0.03 +/- 0.02 %) in a study reported by Karak et al. (10).

In agreement with the literature, the mean Ki-67 index in adenomas was found to be 2.48 ± 2.61% and it was also found higher in adenomas than in normal surrounding tissue in the present study.

Proliferation activity was found as an additional useful parameter for evaluating parathyroid tumors. Tumor marker of Ki-67 was found useful in distinguishing malignant from benign lesions in another studies (17, 18, 19). Parathyroid carcinoma might be expected in those tumors with a Ki-67 labeling index greater than 6% (7). The Ki-67 labeling index was found significantly higher in parathyroid carcinomas compared to adenomas (7.1 ± 1.0% vs 2.4 ± 0.2%) (17). Although analysis of the proliferation marker showed that Ki-67 index was found more intense in malignant cases, it is not suitable for definitive differentiation between benign and malignant tumors because of the considerable overlap between groups of tumors. However, these cases with more intense Ki-67 staining should be followed more closely (20). Although Ki-67 labeling indexes in two adenomas were measured as 6 and 10%, they were not classified as parathyroid carcinom in the present study.

In the present study, no significant correlation was demonstrated between the mean Ki-67 index and age, plasma levels of 25 OH Vit-D3, serum levels of preoperative Ca, P, PTH, alkaline phosphatase and serum levels of postoperative Ca, PTH. Naccarato et al. found no significant differences in serum levels of calcium, intact PTH and tumor size between MIB-1-positive and MIB-1-negative adenomas (21). Again no significant correlation was reported between Ki-67 index and patients age, gland weight, total plasma Ca and intact PTH in the another study (14). The correlation between Ki-67 index and clinical parameters has not been evaluated in the other studies in the literature.

In conclusion Ki-67 might be helpful in the diagnosis of histologically difficult parathyroid lesions. High Ki-67 index does not always indicate parathyroid carcinomas, but cases with high Ki-67 index should be followed up and treated more closely and carefully. In parathyroid adenomas, correlation of Ki-67 index with clinical parameters needs to be evaluated prospectively in studies with larger groups of patients.

References

1. Colognesi A, de Tullio D, Messina F, Ferrocci G, Stano R, Azzena G. Primary hyperparathyroidism related to a parathyroid adenoma: the dramatic clinical evolution of a misdiagnosed patient and its surgical solution. *Minerva Chir* **61**: 51-6, 2006.
2. Hamidi S, Soltani A, Hedayat A, Kamalian N. Primary hyperparathyroidism: a review of 177 cases. *Med Sci Monit* **12**: CR86-9, 2006.
3. Silverberg SJ, Blezikian JP. Clinical presentation of primary hyperparathyroidism in the United States. (Ed: Bilezikian JP, Marcus R, Levine MA). The Parathyroids. Basic and Clinical Concepts 2. edition. Academic Press, San Diego, 2001, 331-347.
4. Hadar T, Shvero J, Yaniv E, Ram Eduard, Shvili I, Koren R. Expression of p53, Ki-67 and Bcl-2 in parathyroid adenoma and residual normal tissue. *Pathol Onco Res* **11**: 45-49, 2005.
5. Thomopoulou GE, Tseleni-Balafouta S, Lazaris AC, Koutseleni H, Kavantzas N, Davaris PS. Immunohisto-chemical detection of cell cycle regulators, Fhit protein and apoptotic cells in parathyroid lesions. *Eur J Endocr* **148**: 81-87, 2003.
6. Kameyama K, Takami H, Umemura S, Osamura YR, Wada N, Sugino K, Mimura T, Ito KI. PCNA and Ki-67 as prognostic markers in human parathyroid carcinomas. *Ann Surg Onco* **7**: 301-304, 2000.
7. Abbona GC, Papotti M, Gasparri G, Bussolati G. Proli-ferative activity in parathyroid tumors as detected by Ki – 67 immunostaining. *Hum Pathol* **26**: 135-138, 1995.
8. Dervan PA, Magee HM, Buckley C, Carney DN. Proliferating cell nuclear antigen counts in formalin-fixed paraffin-embedded tissue correlate with Ki-67 in fresh tissue *Am J Clin Pathol* **97**: S21-28, 1992.
9. Loda M, Lipman J, Cukor B, Bur M, Kwan P, DeLellis RA. Nodular foci in parathyroid adenomas and hyperplasias: an immunohistochemical analysis of proliferative activity. *Hum Pathol* **25:** 1050-1056, 1994.
10. Karak AK, Sarkar C, Chumber S, Tandon N. MIB-1 proliferative index in parathyroid adenoma & hyperplasia. *Indian J Med Res* **105**: 235-8, 1997.
11. Kouvaraki MA, Greer M, Sharma S, Beery D, Armand R, Lee JE, Evans DB, Perrier ND.Indications for operative intervention in patients with asymptomatic primary hyper-parathyroidism: practice patterns of endocrine surgery. *Surgery* **139**: 527-34, 2006.
12. Sivula A, Pelkonen R. Long-term health risk of primary hyperparathyroidism: the effect of surgery**.** *Ann Med* **28**: 95-100, 1996.
13. Parfitt AM, Wang Q, Palnitkar S. Rates of cell proli-feration in adenomatous, suppressed and normal para-thyroid tissue: implications for pathogenesis. *J Clin Endo-crinol Metab* **83**: 863-869, 1998.

14. Parfitt AM, Willgoss D, Jacobi J, Lloyd HM. Cell kinetics in parathyroid adenomas: evidence for decline in rates of cell birth and tumor growth, assuming clonal origin. *Clin Endocrinol* **35**: 151- 157, 1991.

15. Gerdes J. Ki-67 and other proliferation markers useful for immunohistological diagnostic and prognostic evaluations in human malignancies. *Semin Cancer Biol* **1**: 199-206, 1990.

16. Wang W, Johansson H, Kvasnicka T, et al Detection of apoptotic cells and expression of Ki-67 antigen, Bcl-2 , p53 oncoproteins in human parathyroid adenoma. *APMIS* **104:** 789-796, 1996.

17. Lloyd RV, Carney JA, Ferreiro JA, Jin L, Thompson GB, Van Heerden JA, Grant CS, Wollan PC. Immunohistochemical Analysis of the cell cycle-associated antigens Ki-67 and retinoblastoma protein in parathyroid carcinomas and adenomas. *Endocrine Pathol* **6:** 279-287, 1995.

18. Vargas MP, Vargas HI, Kleiner DE, Merino MJ. The role of prognostic markers (Mıb-1, RB, and bcl-2) in the diagnosis of parathyroid tumors. *Mod Pathol* **10**: 12-7, 1997.

19. Erickson LA, Jin L, Wollan P, Thompson GB, van Heerden JA, Lloyd RV. Parathyroid hyperplasia, adenomas, and carcinomas: differential expression of p27Kip1 protein. *Am J Surg Pathol* **23**: 288-95, 1999.

20. Farnebo F, Auer G, Farnebo LO, Teh BT, Twigg S, Aspenblad U, Thompson NW, Grimelius L, Larsson C, Sandelin K. Evaluation of retinoblastoma and Ki-67 immunostaining as diagnostic markers of benign and malignant parathyroid disease. *World J Surg* **23**: 68-74, 1999.

21. Naccarato AG, Marcocci C, Miccoli P. Bcl-2, p53 and MIB-1 expression in normal and neoplastic parathyroid tissue. *J Endocrinol Invest* **21**: 136-141, 1998.