Original Article

Expression of p53, Ki67, epidermal growth factor receptor, transforming growth-factorα, and p21 in primary and secondary hyperparathyroidism

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ABSTRACT

Background: Secondary hyperparathyroidism (SH) is major problem in chronic renal failure. There are studies to examine proliferation and apoptosis associated biomarkers expressions in parathyroid lesions to reveal specific features. In this study, we evaluated the expression of some growth factors and their receptors in parathyroid gland of patients with SH or primary hyperparathyroidism (PH). **Materials and Methods:** A total of 49 patients had been operated for PH and 26 for SH. Parathyroid tissue samples were evaluated histopathologically and immunohistochemically using antibodies to human p53, Kİ-67, anti-human p21, antitransforming growth factor (TGF) α, CPP32 (caspase 3), and epidermal growth factor receptor (EGFR). **Results:** Adenoma was higher in PH compared with SH as 48/49 and 3/26, respectively ($P = 0.000$). Parathyroid hyperplasia was found in 23/26 patients with SH and 1/49 patient with PH. In parathyroid tissue there were no difference between PH and SH for p53, Ki-67, caspase, EGFR expressions; while there were significantly difference for TGFα ($P = 0.047$) and borderline significant difference for p21 ($P = 0.06$) expressions. **Conclusion:** Adenoma was priority present in PH patients, hyperplasia was present in SH. There were no differences between primary and SH or adenoma and hyperplasia for expressions of cycline-dependent kinase inhibitor p21, p53, EGFR, Ki67, caspase; while TGFα expression was found to be different.

Key words: EGFR, hyperparathyroidism, Ki67, p53, p21, p21 hyperparathyroidism, TGFα

Introduction

Secondary hyperparathyroidism (SH) is one of the major problems of chronic renal failure (CRF). Phosphorus retention, disorders of metabolism and secretion of vitamin D, parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23), Mg, and other various factors play role in the pathogenesis of the SH. Pathological findings of the parathyroid gland in SH are diffuse or nodular hyperplasia, and adenoma. Adenoma and hyperplasia may also be detected in the primary hyperparathyroidism (PH). There are a lot of studies to examine proliferation and apoptosis associated biomarkers expressions in the parathyroid lesions to reveal specific features. In parathyroid hyperplasia down regulation of vitamin D receptor (VDR) and calcium sensitive receptor (CaSR) were reported,[1,2] whereas in highly proliferative parathyroid adenomas, VDR and CaSR were found markedly decreased.[3] In severe renal disease, VDR and CaSR decrease on parathyroid cells. In Asian Indians, the expression of both VDR and CASR was found reduced in symptomatic PH. These authors also found that cyclin D1 expression was greatly increased and correlated with adenoma weight, implying a potential role for CD1 in adenoma growth and differential clinical expression of PH.[4]

In parathyroid hyperplasia expression of transforming growth factor-α alpha (TGFα,) and its' epidermal growth

Access this article online	
Quick Response Code:	Website: www.ijem.in
	DOI: 10.4103/2230-8210.140265

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factor receptor (EGFR) were found increased.[5-7] Ki-67 plays role through all cell cycle (G1, S, and G2) except G0. It is used as proliferation marker.[8] P21 and p27 labeling index were found diminished significantly in both nodular hyperplasia and adenoma compared with that of diffuse hyperplasia and normal.[9] It was reported that p53, Bcl-2, and Bax spectrum were similar primary and secondary (i.e. renal failure) parathyroid alterations.[10] In this study, we aimed to evaluate the expression of proliferation and apoptosis biomarkers such as p53, Ki67, EGFR, TGFα, and p21 in parathyroid tissue in patients with primary and SH.

Materials and Methods

Parathyroid tissue samples obtained from patients undergoing parathyroid surgery because of primary or SH, were evaluated. Clinical data [age, sex, primary disease, disease period, dialysis period – if there's – and laboratory results including hemoglobin (Hb), hematocrit (hct), blood urea nitrogen (BUN), creatinine (Cr), sodium (Na), potassium (K), calcium (Ca), phosphorus (P), CaXP product, PTH, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, and albumin] were recorded. Patients undergoing dialysis were treated with calcitriol unless contraindicated. We did not use cinacalcet. Histopathologically, hematoxyline and eosin-stained slides were evaluated. Adenoma and hyperplasia differentiation was principally based on enlargement of the remaining glands. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue sections of 5 μm thick by using a manual Strept Avidin-Biotin complex immunoperoxidase procedure. For the procedure Monoclonal Mouse Antibodies against human p53 (Novocastra, NCL-L-P53-D07; diluted 1:80/60 min incubation), Kİ 67 (Dako, M7240; diluted 1:100/60 min incubation) anti-human p21 (Dako, M7202; diluted 1:100/60 min incubation), antiTGFα (BioGenex MU 377-UC; diluted 1:80/90 min incubation), CPP32 (caspase-3) (Novocastra, NCL-CPP32; diluted 1:100/45 min incubation), EGFR (Dako, M3563; diluted 1/150/90 min incubation) were used. For all antibodies tested, antigen retrieval treatment 15 min in 1 mmoL citrate buffer solution (Ph 6.0), using a microwave oven was performed and immune complexes were then detected with the (Dako, K 0690) and visualized by AEC. Slides were counterstained with Mayer's hematoxyline and mounted. For p53; colorectal carcinoma, for Ki 67; tonsil, for p21; skeletal muscle, for TGFα; breast carcinoma, for CPP32; tonsil, for EGFR; skin tissue was used as positive controls. Negative controls were obtained by omitting the primary antibody.

Immunohistochemical evaluation of all antibodies was performed by using a double evaluating system calculated by multiplying the intensity of staining (0, negative; 1, weak; 2, moderate; and 3, strong) with the percentage of stained cells in a low power field (1, 1%−10%; 2, 11%-50%; 3, 51%-75%; and 4, >75%). The immunohistochemical staining score was recorded.

Statistically, SPSS-X14th program were used and $P < 0.05$ was accepted significant.

Results

Totally, 75 patients whose parathyroid gland was removed were taken to the study. A total of 3 of the 26 patients with SH were operated in 6 months, following renal transplantation.

All patients' clinical and laboratory findings are summarized in Table 1. A total of 48 of the 49 PH patients had adenoma, 23 of the 26 SH patients had hyperplasia. There was no difference from the point of diabetes mellitus (DM) and hypertension (HT) between the both groups of the PH and SH patients. Presence or absence of chronic glomerulonephritis, autosomal dominant polycystic renal disease, urinary system stone disease, DM, and HT were not found significant in adenoma and hyperplasia groups. The results of expression of p21, p53, Ki67, capes, TGFα on parathyroid tissue samples according to primary or SH are presented on Table 2.

Discussion

SH is a frequent complication of CRF and is characterized with parathyroid hyperplasia, increase of the expression and secretion of PTH.[11,12] Persistence of hypocalcemia, hyperphosphatemia, and deficiency of vitamin D stimulates PTH secretion and parathyroid gland hyperplasia. High serum levels of PTH causes bone lesions, systemic and cardiovascular complications which increase morbidity and mortality.[13,14] In CRF patients, active form of vitamin D (calcitriol) inhibits expression of PTH gene and stops growth of gland. So, calcitriol is an alternative therapy for SH.[12] By the progression of renal failure, resistance of vitamin D and parathyroid cell proliferation developed.[15]

Turnover of normal parathyroid cell cycle is slow[16], but in CRF parathyroid cell proliferation increases by mitogenic stimuli of hypocalcemia, calcitriol deficiency, and retention of phosphorus.[15,17] Progression of renal disease causes parathyroid hyperplasia. Similar to other studies, we detected adenoma in PH, hyperplasia in SH.[10]

Table 1: The biochemical parameters of patients with primary and secondary hyperparathyroidism

Parameters	Primary hyperparathyroidism	Secondary hyperparathyroidism	*P*
Age	54.2±12.2	43.9±13.3	0.001
Number	49	26	
Hb (13.6-17.2 g/dL)	12.2±2.3	11.1±1.4	0.016
Hct (39.5-50.3%)	37.3±5.9	33.4±4.1	0.006
WBC (4500-10300μL)	7910±3708	8160±2980	
Platelet (156-373 μL)	277960±88375	275040±123315	
BUN (5-20 mg/dL)	122.10±17.32	54.28±31.16	0
Creatinine (0,6-1 mg/dL)	1.91±2.68	7.64±3.93	0
Calcium (8,4-9,7 mg/dL)	11.61±3.10	10.37±2.03	0.001
Phosphorus (2,7-4,5 mg/dL)	3.24±1.70	5.20±1.95	0
CaXP (mg^2/dL^2)	38.75±21.97	51.53±18.55	0.001
ALP (IU/L)	441.92±4398	763.2±505.27	0.007
AST (0-31 IU/L)	23.03±12.98	20.19±15.17	
ALT (IU/L)	20.58±11.58	21.35±22.16	
PTH (12-88 pg/ml)	496.3±571	1899±1305	0

PTH: Parathyroid hormone, ALT: Alanine aminotransferase, ALP: alkaline phosphatase, AST: Aspartate aminotransferase,BUN: Blood urea nitrogen, Hct: Hematocrit

Table 2: The immunohistochemical staining score for p21, p53, Ki67, caspas, TGFα expression on parathyroid tissue samples according to primary or secondary hyperparathyroidism

	Primary hyperparathyroidism	Secondary hyperparathyroidism	*P*
P21	3.65±7.42	1.74±4.22	0.06
P53	0.19±0.79	0.26±0.68	0.282
Ki67	0.85±0.85	1.43±1.47	0.106
Caspas	1.44±1.72	1.17±1.85	0.269
TGFα	0.60±1.21	1.22±1.62	0.047

TGF: Antitransforming growth factor

It was reported that downregulation of VDR and CaSR causes parathyroid hyperplasia.[1,2] In highly proliferative parathyroid adenomas, VDR and CaSR were found markedly decreased.[3] Varshney *et al.*,[4] reported that the expression of both VDR and CASR were found reduced in symptomatic PH in Asian Indians. These authors also found that cyclin D1 expression was greatly increased and correlated with adenoma weight, implying a potential role for CD1 in adenoma growth, and differential clinical expression of PH. According to Latus *et al.*,[18] CaSR, VDR, and an impaired Klotho-FGFR-axis seem to be the major players in the development of SH.

Differences in some other expression molecules have also been described in parathyroid hyperplasia. Increased expression of TGFα and its' EGFR are the most important ones.[5-7] In our study, TGFα showed no significant difference between PH and SH patients ($P = 0.077$), but it was significantly different between adenoma and hyperplasia groups ($P = 0.047$). TGFα was found higher in SH patients (23 of 26 patients). EGFR was not found increased. Gogusev *et al.*,[19] found strongly expression of TGFα mRNA and protein in parathyroid endocrine cells of all six cases with primary parathyroid adenoma and nearly all cases of secondary hyperplasia, in contrast to absence of expression in normal control parathyroid tissue.

Ki-67 antigen is a 345 and 397 kDa weight nuclear "nonhistone" biomolecule complex. Ki-67 is associated with proliferation and plays role through all cell cycle (G1, S, and G2) except G0. It is used as proliferation marker.[8] Cyclin-dependent kinase (CDK) family is a group of protein that acts with cyclin proteins during cell proliferation. This role of CDK family is inhibited by CDK inhibitors and prevents progression of the cell cycle.[20,21] p21 and p27 are CDK inhibitors. In our study, there were no significant differences from the point of these three proteins between PH and SH or adenoma and hyperplasia. P21 and p27 labeling index was found diminished in nodular hyperplasia and adenoma compared with control group and diffuse hyperplasia.[9] According to some authors; the reduced expression of p21 and p27, in a VDR-dependent manner, is a major pathogenetic factor for a nodular parathyroid gland growth.[9,10]

P53 is a tumor suppressor gene located on chromosome 17p13 and codes p53 protein, known as "gatekeeper" of the cell.[22] P53 is an "encode" phosphoprotein which plays role in cell proliferation, apoptosis and differentiation, and acts in arrest of cell cycle, gene transcription and DNA repair.[22] P53 is an inhibitor on cell proliferation, so induces p21 expression. When p53 mutation occurs, during cell cycle p53 cannot be activated. Control of apoptosis gets lost and uncontrolled cell proliferation becomes as a result.[23] In our study, there was no significant difference between PH and SH, or adenoma and hyperplasia. In another study, no significant differences in the p53, bcl-2 and Bax spectrum were found in primary and secondary parathyroid alterations.[10] P53 immunoexpression was not found useful in differentiating between the histopathological parathyroid subgroups.[24]

In SH group, VDR density was found diminished in nodular hyperplastic parathyroid gland and this was associated with diminished expression of p21 and p27. In our study, we could not evaluate VDR density. Calcitriol decreases parathyroid cell proliferation on c-myc gene expression which modules transition from G1 to S phase.[25] Decrease in serum calcitriol levels or deficiency of effect on parathyroid cell is frequently seen in uremic patients. This causes loss of inhibition of c-myc expression, so cell cycle progresses. Calcitriol activates p21 gene by VDR-dependent way and p53-independent way.[26] On the other side, Cozzolino *et al*.,[27] have showed uremic rats that 1,25 vitamin D and its' lesser calcemic analog 19-nor-1,25 vitamin D prevents hyperphosphatemia and parathyroid hyperplasia, this effect develops with decreased ki67 levels which causes increase of p21 expression. This condition makes to think that calcitriol plays role in parathyroid cell proliferation. In our study, there was no significant difference from the point of ki67, p21 between SH patients who received vitamin D therapy and PH patients who did not receive vitamin D therapy. Vitamin D therapy may affect p21 and ki67 expression in SH patients.

In our study, adenoma was priority present in PH patients, hyperplasia was present in SH. Our patients with SH were treated with vitamin D to which affects low VDR levels, but we did not give therapy for CaSR. This might be the probable reason for high levels of TGFα in SH patients. p21, p53, ki67, caspase, and EGFR expression were similar in primary and secondary HPT patients. Only TGFα expression showed significant difference. However, EGFR expression was similar.

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Cite this article as: Paydas S, Acikalim A, Kaya B, Bicer BH, Ulker M, Demircan O, *et al*. Expression of p53, Ki67, epidermal growth factor receptor, transforming growth-factorα, and p21 in primary and secondary hyperparathyroidism. Indian J Endocr Metab 2014;18:826-30.

Source of Support: This study was funded by Cukurova University Research Project, **Conflict of Interest:** None declared.