CLINICAL STUDY

Histology and immunohistochemistry of the parathyroid glands in renal secondary hyperparathyroidism refractory to vitamin D or cinacalcet therapy

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Abstract

Background: Cinacalcet is a new effective treatment of secondary hyperparathyroidism (SHPT) in hemodialysis patients (HP), but the alterations of parathyroid gland (PTG) hyperplasia determined by cinacalcet and vitamin D have not been extensively investigated in humans.

Methods: We performed histological analyses of 94 PTGs removed from 25 HP who underwent parathyroidectomy (PTx) because of SHPT refractory to therapy with vitamin D alone (group A = 13 HP and 46 PTGs) or associated with cinacalcet (group B = 12 HP and 48 PTGs). The number, weight, the macroscopic cystic/hemorrhagic changes, and type of hyperplasia of PTG (nodular = NH, diffuse = DH) were assessed. In randomly selected HP of group A (4 HP and 14 PTGs) and group B (4 HP and 15 PTGs), the labeling index of cells positive to Ki-67 and TUNEL and the semiquantitative score of immunohistochemistry staining of vitamin D receptor, calcium-sensing receptor, and vascular endothelial growth factor- α (VEGF- α) were measured in the entire PTGs and in the areas with DH or NH.

Results: The number and weight of single and total PTG of each HP were similar in the two groups as well as the number of PTG with macroscopic cystic/hemorrhagic areas. TUNEL, Ki-67, and VEGF- α scores were higher in NH than in DH areas.

Conclusion: This observational study of a highly selected population of HP, submitted to PTx because SHPT refractory to therapy, shows that the macroscopic, microscopic, and immunochemistry characteristics of PTG in HP who received or did not receive cinacalcet before PTx did not differ significantly.

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Introduction

Secondary hyperparathyroidism (SHPT) amongst chronic hemodialysis patients (HP) is characterized by parathyroid gland (PTG) hyperplasia and excessive synthesis and secretion of intact parathyroid hormone (iPTH), resulting in unbalanced bone reabsorption, soft tissue and vascular calcification, and significantly increased risk for cardiovascular morbidity and mortality (1, 2, 3, 4). The hypersecretion of iPTH is associated with an increase in the PTG size, initially characterized by diffuse polyclonal hyperplasia (DH) and subsequently by nodular hyperplasia (NH) (5, 6, 7). SHPT sustained by NH is an irreversible condition usually refractory to conventional medical treatment or renal transplantation and it is classically defined as tertiary hyperparathyroidism (THPT). The failure of medical therapy is primarily due to progressive down-regulation (8, 9, 10) of both calcium-sensing receptors (CaSR) and vitamin D receptors (VDR) (4, 11, 12, 13).

Actually, it is widely acknowledged that the PTG volume (maximum diameter >10 mm or volume >500 mm³) negatively influences the response to calcitriol or vitamin D analog treatment (14, 15). In these cases, parathyroidectomy (PTx) may be indicated (16). Conversely, it remains to be determined whether cinacalcet may determine regression of PTG hyperplasia (2). Cinacalcet increases the sensitivity of CaSR to activation by extracellular Ca and suppresses the release of iPTH. The CaSR play a key role in the excessive cell proliferation in PTG hyperplasia (17, 18, 19, 20, 21, 22, 23). In humans, the regression of PTG hyperplasia has been reported rarely, in cases of spontaneous infarction of the glands (24, 25) and in cases of DH after kidney transplantation (26, 27).

Recently, ultrasonography (US) of the PTG has been performed in HP to examine the changes of PTG volumes after cinacalcet treatment. Some authors (28, 29, 30) reported a significant reduction in the PTG volume from baseline to the end of the follow-up.

Moreover, morphological changes in PTG during cinacalcet treatment, such as cystic degeneration and hypovascularization, were observed by US. Conversely, other authors (31, 32) failed to detect significant morphological changes after cinacalcet therapy.

To this date, it has not been fully elucidated whether patients with established NH can be controlled by cinacalcet therapy in the long term. Moreover, the hypothetical regression of the volume of PTG (reduction of cell proliferation and/or increase in cell apoptosis) in relation to the degree of glandular hyperplasia and to the type of treatment is still an argument for debate (33, 34).

The main purpose of this study was to assess whether there are differences in the histology (i.e. weight and number of PTG, type of hyperplasia (NH vs DH), cystic/necrotic degeneration, and expression of the different cell types) and immunohistochemistry (TUNEL, Ki-67, vascular endothelial growth factor- α (VEGF- α), VDR, and CaSR) of PTG of HP affected by SHPT refractory to vitamin D or vitamin D associated with cinacalcet long-term therapy.

Materials and methods

Patients and study design

In this retrospective study, 25 consecutive HP with severe SHPT who underwent total (17 HP) or subtotal (8 HP) PTx at the Department of Surgery of the Catholic University of Rome from January 2004 to February 2011 were included. All patients were referred to our center from other dialysis units of our region. Indication for PTx was based on NKF-DOQI guidelines (1) (i.e. i) abnormalities in mineral metabolism (hypercalcemia or phosphatemia); ii) elevated iPTH levels (> 800 pg/ml); iii) nonresponsiveness to medical treatment; and iv) enlarged PTG (> 10 mm of diameter) detected by image diagnosis).

On the basis of preoperative treatment, the HP were divided into two groups: group A (13 HP and 46 PTGs) including HP who underwent vitamin D therapy (calcium supplements, phosphate binders, and active vitamin D analogs) without cinacalcet and group B (12 HP and 48 PTGs) including HP treated with vitamin D associated with cinacalcet.

Macroscopic characteristics of PTG

The number, weight, and maximum longitudinal diameter (MLD) of all PTGs removed in each patient were determined immediately after PTx in the operating room. During macroscopic examination of the longitudinal sections of PTG, the presence of foci of cystic/hemorrhagic necrosis was also determined (Fig. 1a) and then confirmed by histology (Fig. 1b).

Histology of PTG tissues

All histological specimens were fixed in 10% buffered formaldehyde, embedded in paraffin, and the 5 μ m-thick sections were stained with hematoxylin–eosin for the histological examination. The type of hyperplasia was classified as either DH (type 1) or NH (types 2–4) according to Tominaga *et al.* (4). The DH was defined as an increased number of parenchymal cells with normal lobular structures, and the NH was defined as at least one well-circumscribed, encapsulated, and virtually fat cell-free accumulation of parenchymal cells. Furthermore, the type of NH was classified as follows: early or micronodular (multiple microscopic nodules: the single-cell type tends to form nodules lacking fibrous bands (type 2)), macronodular (multiple macroscopic encapsulated nodules (type 3)), and single nodule or adenoma like (PTG hyperplasia characterized by a single markedly enlarged nodule of uniform parenchymal cell proliferation resembling adenoma and suppressing the adjacent parathyroid tissue (type 4)). The number of chief cell (CC), oxyphil cells (OC), and water cells (WC) was expressed as mean percentage of total cells detected

Figure 1 (a) Macroscopic examination of longitudinal section of PTG with cystic/hemorrhagic areas; and (b) histopathological image of hemorrhagic necrosis of PTG (hematoxylin and eosin stain). Full colour version of this figure available via <http://dx.doi.org/10.1530/EJE-12-0947>.

at a magnification of $\times 200$ in ten randomly selected fields of glandular section (five in areas with DH and five in areas with NH), which was evaluated in each PTG.

Immunohistochemistry

Fourteen PTGs of four randomly selected HP out of 13 HP of group A and 15 PTGs of 4 out of 12 HP of group B were studied by immunohistochemistry. According to the manufacturer's instructions and dilutions, immunostains for Ki-67 (nuclear protein expressed in proliferating cells) (Antibody (SP6) Novus Biologicals, Littleton, CO, USA), TUNEL (Mebstain Apoptosis kit II, Immunotech and Diagnostic System Laboratories, Marseille, France), VEGF- α (rabbit polyclonal IgG VEGF- α (A-20) Antibody:sc-152 Santa Cruz Biotechnology), VDR (Vitamin D Receptor Antibody (2F4) Novus Biologicals), and CaSR antibody (calcium-sensing receptor ab 27493, Abcam, Inc., Cambridge, MA, USA) were performed on the microtomic sections using the labeled streptavidin-biotin-peroxidase complex system (ABC). The tissues were incubated with commercial monoclonal antibodies after a prior microwave antigen retrieval (3-min passages in citrate buffer; pH 6.0) and the final results were revealed with 3,3'-diaminobenzidine.

Quantitative evaluation of positive cells

According to Wada *et al.* (13), the cells were considered Ki-67 positive when a dark brown staining of the nucleus was observed; on the other hand, cells were considered TUNEL positive when black nuclei, corresponding to a marked condensation of nuclear chromatin, were observed (Fig. 2a and b). Areas of obvious necrosis were excluded from counting. The mean number of Ki-67- and TUNEL-positive cells was counted using a $\times 40$ objective lens under a light microscope in ten selected high-power fields showing ~ 200 –500 cells per field. The mean labeling index (LI%) was determined as the number of positive cells per 100 counted cells. The ten fields were randomly selected as follows: i) for diffuse and single nodule (adenoma like) glands, five fields around the periphery and five in the center fields of each gland were selected, and ii) for early and multi-nodular glands, five fields around the areas of DH and five in the areas containing NH were selected.

The VEGF- α , VDR, and CaSR staining (Fig. 2c, d, and e) was semiquantitatively assessed according to a scale previously defined by Martins *et al.* (35): 0 (0 cells

Figure 2 Representative findings of immunohistochemical staining of parathyroid tissue: (a) Ki-67 (the arrows indicate the Ki-67-positive cells); (b) TUNEL assay (the arrows indicate positive cells); (c) VEGF- α , vascular endothelial growth factor- α ; and (d) VDR, vitamin D receptor; (e) CaSR, calcium-sensing receptor. Full colour version of this figure available via <http://dx.doi.org/10.1530/EJE-12-0947>.

stained), 1 (<25% cells with mild staining), 2 (25–74% cells with moderate staining), and 3 (>75% cells with intense staining). Repeat recounts for Ki-67 and TUNEL, VEGF- α , VDR, and CaSR conducted by the same examiner in all PTGs showed variability of LI% and scores $\leq \pm 5\%$ from the original counts.

Statistical analysis

Data were analyzed by MedCalc Software (Belgium Version 12 – for Microsoft Windows XP). Continuous variables were expressed as mean $\pm$ s.d. and categorical and interval variables as frequencies or median and interquartile range. The appropriate test was used when comparing the groups' means (*t*-test, ANOVA-Student–Newman–Keuls *post hoc* comparison) or median (independent Mann–Whitney *U* test and paired Wilcoxon test) and frequencies (Fisher's exact test or χ^2 test, Kruskal–Wallis). The correlations between two variables were calculated by parametric test (Pearson's correlation test) or nonparametric test (Spearman's coefficient of correlation). Multiple linear regressions with backward stepwise procedure were also performed to study the relationship between Ki-67, tunnel, VEGF- α , VDR, and CaSR and clinical and laboratory parameters. Covariates introduced in the models were variables significantly correlated at the univariate analysis. $P < 0.05$ was considered statistically significant.

Results

The demographic, clinical, and laboratory characteristics of HP of groups A and B are shown in Table 1. Preoperative serum Ca levels were significantly lower in group B than in group A (10.1 ± 0.6 and 9.2 ± 0.9 mg/dl respectively; $P < 0.016$) and the length of treatment was higher in group B than in group A (18.1 ± 5.4 and 13.2 ± 4.1 months respectively; $P < 0.0001$).

The macroscopic characteristics of the 94 PTGs removed in 25 HP (46 PTGs in group A and 48 PTGs in group B) are shown in Table 2. The total number, the MLD, and the weight of single and total PTGs of each HP were similar in the two groups as well as the number of PTGs with macroscopic cystic/hemorrhagic areas. The MLD and the weight of the PTG with hemorrhagic areas were higher than the PTGs without cystic/hemorrhagic areas (17.1 ± 4.4 and 11.8 ± 5.3 mm respectively, $P < 0.002$; and 2.1 ± 0.9 and 1.0 ± 0.7 g respectively, $P < 0.0001$). The logistic regression coefficients of a model including weight, type of hyperplasia of PTGs, and type and duration of medical treatment showed that the weight of PTG (coefficient 1.34, s.e.m. 0.49, $P < 0.006$) only predicted the presence of cystic/hemorrhagic areas.

As shown in Table 3, 17 PTGs (18.1%) showed DH and 77 (81.9%) showed NH. The percentage of PTGs with DH and NH was similar in groups A and B. The MLD and the weight were significantly lower in PTGs with DH than in PTGs with NH (7.2 ± 3.9 vs 13.1 ± 5.1 mm, $P < 0.001$, and 0.48 ± 0.49 vs 1.27 ± 1.1 g, $P < 0.005$ respectively). In addition, the percentage of the constituent cells of PTGs as well as the OC:CC ratio was as similar in group A and in group B.

The immunostaining scores of VEGF- α , VDR, and CaSR and the LI% of TUNEL and Ki-67 corresponding to the DH and NH areas of PTG are shown in Tables 4 and 5. Overall, either in group A or in group B, the TUNEL and Ki-67 LI% and VEGF scores were significantly higher in NH than in DH areas. Conversely, VDR and CaSR scores were similar in DH and NH areas. In DH and NH areas of PTG of group B, with respect to group A, the VEGF score was lower but not statistically significant (χ^2 for trend respectively 0.007 and 0.032).

Linear multivariate regressions showed that: i) the TUNEL LI% index was directly correlated with Ki-67 ($P = 0.001$), VEGF- α ($P = 0.010$), Ca serum concentrations ($P = 0.002$), and iPTH serum levels ($P = 0.002$) and indirectly correlated with alkaline phosphatase

Table 1 Clinical, biochemical, and therapeutic characteristics of patients with SHPT refractory to conventional therapy without (group A) and with cinacalcet (group B). Data are presented as mean $\pm$ S.D.

	All patients	Group A	Group B	P value
<i>n</i>	25	13	12	
Age (years)	51.1 $\pm$ 17.2	45.5 $\pm$ 18.3	57.2 $\pm$ 14.1	0.088
F/M	7/18	4/9	3/9	
Dialysis duration (years)	4.1 $\pm$ 2.2	4.2 $\pm$ 2.5	3.9 $\pm$ 1.8	0.724
Ca (mg/dl)	9.7 $\pm$ 0.8	10.1 $\pm$ 0.6	9.2 $\pm$ 0.9	0.016
Pi (mg/dl)	6.6 $\pm$ 1.7	6.5 $\pm$ 1.6	6.7 $\pm$ 1.9	0.721
ALP (IU/l)	478.7 $\pm$ 520.4	457.9 $\pm$ 468.4	501.5 $\pm$ 594.4	0.846
iPTH (pg/ml)	1156 $\pm$ 536	1004 $\pm$ 602	1274 $\pm$ 448	0.294
Total therapy (months)	15.9 $\pm$ 5.4	13.2 $\pm$ 4.1	18.1 $\pm$ 5.4	0.001
Dose calcitriol (μ g/week)		2.9 $\pm$ 0.5	3.3 $\pm$ 0.9	0.243
Dose cinacalcet (mg/week)			73.8 $\pm$ 12.8	

Pi, inorganic phosphorus; ALP, alkaline phosphatase activity; iPTH, intact parathyroid hormone serum concentrations.

Table 2 Macroscopic characteristics of parathyroid gland (PTG).

	All patients	Group A	Group B	P value
Patients and (PTG)	25 (94)	13 (46)	12 (48)	
MLD (mm) of PTG mean $\pm$ s.d.	12.1 $\pm$ 5.4	11.2 $\pm$ 5.5	12.9 $\pm$ 5.2	0.142
Weight (g) of PTG mean $\pm$ s.d.	1.1 $\pm$ 1.0	1.0 $\pm$ 1.4	1.2 $\pm$ 0.8	0.504
No. of PTG for patient median (IQR)	4 (3–4)	4 (3–4)	4 (3.5–4)	0.453
Weight of largest PTG	1.9 $\pm$ 0.8	1.8 $\pm$ 0.8	2.1 $\pm$ 0.7	0.517
Total weight PTG mean $\pm$ s.d.	4.4 $\pm$ 2.0	4.0 $\pm$ 2.1	4.4 $\pm$ 1.8	0.330
PTG with hemorrhagic areas n (%)	16 (17)	6 (13)	9 (19)	0.576
MLD with hemorrhagic areas	17.1 $\pm$ 4.4*	18.3 $\pm$ 5.1	16.5 $\pm$ 4.2	0.002*
MLD without hemorrhagic areas	11.8 $\pm$ 5.3*	11.5 $\pm$ 5.1	12.1 $\pm$ 5.6	
Weight with hemorrhagic areas	2.1 $\pm$ 0.9 [†]	2.9 $\pm$ 0.4	1.9 $\pm$ 0.9	0.001 [†]
Weight without hemorrhagic areas	1.0 $\pm$ 0.7 [†]	0.9 $\pm$ 0.6	1.0 $\pm$ 0.7	

MLD, maximum longitudinal diameter; IQR, interquartile range. *Indicates significant difference ($P = 0.002$) in the MLD with and without hemorrhagic mass; [†]indicates significant difference ($P = 0.001$) in weight with and without hemorrhagic mass.

activity (ALP) ($P = 0.033$; $R^2 = 0.663$); ii) the Ki-67 LI% was directly correlated with TUNEL ($P < 0.05$), serum inorganic phosphorus (Pi) concentrations ($P = 0.039$), and ALP ($P = 0.007$; $R^2 = 0.440$); iii) the VEGF- α score was directly correlated with Pi ($P = 0.003$; $R^2 = 0.315$); and iv) the VDR score was directly correlated with Pi ($P < 0.001$) and indirectly correlated with Ca ($P = 0.001$) and iPTH ($P = 0.008$; $R^2 = 0.663$). The small size of the sample did not allow to compare the immunohistochemical expression of the different markers in all types of hyperplasia.

Discussion

This study shows that the histology and immunohistochemistry of PTG of HP who received or did not receive cinacalcet before PTx did not differ significantly. In particular, the proliferating (Ki-67) and apoptotic cells (TUNEL) and the expression of VEGF- α , VDR, and

CaSR were overall similar in the PTGs of the HP of two groups. To the best of our knowledge, this is the first study that extensively evaluates the histology of PTG as well as the expression of makers of cell proliferation, apoptosis, and angiogenesis of PTG of HP who received or did not receive cinacalcet before PTx.

Indeed, data on the effect of cinacalcet on the pathology of human PTG in HP with SHPT are very few (33, 34, 36). Lomonte *et al.* did not find significant differences in the number, weight, and percentage of NH in the PTG of patients who received calcitriol and cinacalcet with respect to those who received calcitriol only. With regard to the expression of the different cell types, the author reported that, despite the short-term therapy (for 3.3 ± 1.8 months), the OC:CC ratio was significantly higher in patients treated with cinacalcet. Sumida *et al.* (34) also reported that the percentage of OC area to the total area was significantly higher in the cinacalcet group (duration of treatment 16.1 ± 6.8 months) compared with the conventional group. In this

Table 3 Microscopic characteristics of PTG of patients with SHPT refractory to conventional therapy without (group A) and with cinacalcet (group B).

	All patients	Group A	Group B	P value
Patients and PTG (n)	25 (94)	13 (46)	12 (48)	
Diffuse hyperplasia (DH) PTG (%)	17 (18.1)	11 (23.9)	6 (12.5)	0.242
Nodular hyperplasia (NH) PTG (%)	77 (81.9)	35 (76.1)	42 (87.5)	
Early (micronodular)	50 (53.2)	24 (52.2)	26 (54.1)	0.286
Macronodular	11 (11.7)	3 (6.5)	8 (16.7)	
Single nodule (adenoma-like)	16 (17)	8 (17.4)	8 (16.7)	
MLD (mm) PTG with DH	7.2 $\pm$ 3.9*	8.1 $\pm$ 4.6	5.7 $\pm$ 1.2	0.001*
MLD (mm) PTG with NH	13.1 $\pm$ 5.1*	12.2 $\pm$ 5.4	13.9 $\pm$ 4.7	
Weight (g) PTG with DH	0.48 $\pm$ 0.49 [†]	0.54 $\pm$ 0.53	0.31 $\pm$ 0.07	0.005 [†]
Weight (g) PTG with NH	1.27 $\pm$ 1.1 [†]	1.29 $\pm$ 1.53	1.26 $\pm$ 0.78	
Cellularity (% of total cells)				
Chief cells = CC median (IQR)	70 (40–90)	65 (40–90)	70 (60–88)	0.102
Oxyphil cells = OC	20 (10–32)	20 (10–40)	20 (10–20)	0.208
Water clear cells = WC	10 (0–11.5)	1 (0–11.5)	12 (0–11.5)	0.724
Oxyphil cells:chief cells ratio	0.25 (0.11–0.67)	0.25 (0.12–0.67)	0.43 (0.11–1)	0.187

*Indicates significant difference ($P = 0.001$) between the MLD with DH and the MLD with NH; [†]indicates significant difference ($P = 0.005$) in weight with DH and the weight with NH.

Table 4 Labeling index of immunostaining of PTG of patients with SHPT refractory to vitamin D therapy without (group A) and with cinacalcet (group B).

	All patients	Group A	Group B	P value*
Ki-67 (mean $\pm$ s.d.)				
DH	1.0 $\pm$ 0.9	1.2 $\pm$ 0.8	0.8 $\pm$ 0.9	0.249
NH	2.2 $\pm$ 1.5	2.6 $\pm$ 1.4	1.8 $\pm$ 1.4	0.128
P value	0.001	0.002	0.007	
TUNEL (mean $\pm$ s.d.)				
DH	0.8 $\pm$ 0.9	1.0 $\pm$ 1.4	0.85 $\pm$ 0.69	0.731
NH	1.6 $\pm$ 1.5	1.5 $\pm$ 1.8	1.7 $\pm$ 1.3	0.745
P value	0.009	0.183	0.015	

*P values comparing groups A and B. Ki-67, nuclear protein expressed in proliferating cells; DH, areas of PTG with diffuse hyperplasia; NH, areas of PTG with nodular hyperplasia.

study, we did not observe an increase in OC:CC ratio in the cinacalcet group compared with the vitamin D group. However, the increase in the OC:CC ratio observed after short- or long-term cinacalcet therapy did not translate into pharmacological control of SHPT. Certainly, the OC are not simply deactivated CC (36, 37, 38, 39, 40, 41). However, additional studies are required to define the effects of vitamin D and cinacalcet on the expression and role of the OC in the pathophysiology of PTG.

With regard to macroscopic characteristics of PTG, Sumida *et al.* (34) reported that, in the PTG of patients who received cinacalcet with respect to those treated with vitamin D, the weight of the maximal PTG and the weight ratio of maximal-to-minimal PTGs were significantly higher; there were no significant differences in either cystic or hemorrhagic score. In this study, although the length of treatment was greater in the cinacalcet group (18.1 ± 5.4 months) than in the conventional group, the number, the MLD, and the weight of PTGs were similar in both groups of patients. This observation is against the hypothesis that cinacalcet may decrease the size and volume of PTG in refractory SHPT sustained by advanced NH or

adenoma-like hyperplasia. Nevertheless, this study does not allow to determine whether a regression of PTG with DH is possible after cinacalcet therapy. Actually, this hypothesis has been first based on the data obtained in experimental studies (18, 19, 20, 21, 22, 23) and then by the US evaluation of PTG (28, 29, 30, 31, 32).

With regard to experimental studies, Mizobuchi *et al.* (19) demonstrated that high concentrations of calcimimetics determine apoptosis in parathyroid cells from 5/6 subtotal nephrectomized uremic rats *in vitro*. Conversely, others (18, 20) have failed to detect apoptosis in parathyroid cells *in vivo*. Finally, Chin *et al.* (20) demonstrated the possibility of regression of PTG size, but the reduction in PTG volume was attributed to a decrease in cell volume but not in cell number. Noteworthy, it is well known that experimental models of SHPT do not completely resemble the characteristics of human SHPT due to the lack of NH.

With regard to US, some authors reported that the PTG volume reduced significantly (28, 29, 30), areas of cystic degeneration appeared, and glandular vascularization decreased (29) after cinacalcet therapy with consequent reduced functioning PTG mass. Indeed, in agreement with Sumida *et al.*, we found that the number of PTG with macroscopic hemorrhagic or cystic areas was similar in the two groups of HP and that only the weight of PTG predicted the presence of hemorrhagic areas. In effect, very rarely smaller PTG (MLD < 1 cm and weight < 1 g) showed cystic/hemorrhagic areas. Noteworthy, we evaluated only the macroscopic hemorrhagic degeneration of PTG because we believe that only these alterations may be detected by US.

On the basis of the data of some authors showing a reduced vascularization in PTG of patients receiving cinacalcet, and on the basis of evidence of increased angiogenesis in pathological PTG compared with normal glands (42, 43, 44, 45), we also measured the expression of VEGF- α at the PTG level. VEGF- α plays a

Table 5 Scores for immunostaining of PTG of patients with SHPT refractory to vitamin D therapy without (group A) and with cinacalcet (group B).

	All patients				Group A				Group B				P value*
	0	1	2	3	0	1	2	3	0	1	2	3	
VEGF (scores)													0.008[†]
DH	5	10	7	1	0	4	5	1	5	6	2	0	0.059
NH	0	7	7	9	0	1	3	6	0	6	4	3	0.077
VDR (scores)													0.358[†]
DH	7	14	2	0	4	4	2	0	3	10	0	0	0.111
NH	8	15	0	0	5	5	0	0	3	10	0	0	0.613
CaSR (scores)													0.389[†]
DH	1	10	6	6	0	4	4	2	1	6	2	4	0.497
NH	4	7	8	4	2	3	3	2	2	4	5	2	0.968

*P values comparing groups A and B; [†]P values comparing all patients with DH and NH. VDR, vitamin D receptor; CaSR, calcium-sensing receptor; VEGF- α , vascular endothelial growth factor- α ; DH, areas of PTG with diffuse hyperplasia; NH, areas of PTG with nodular hyperplasia.

key role in both physiological and pathological angiogenesis through the proliferation/migration of endothelial cells and increasing endothelial permeability. Moreover, VEGF- α is also an anti-apoptotic factor promoting the survival of endothelial cells in neo-formed vessels (42). Indeed, we found a lower expression of VEGF- α in both areas of PTG with DH and NH of group B than in those of HP of group A, although the difference was not statistically significant due to the small sample size. Although this finding is very interesting, our retrospective and observational study does not allow to establish a causative role of cinacalcet therapy in the reduced expression of VEGF- α . Recent studies have shown that several angiogenic growth factors are produced and secreted by normal endocrine cells and are increased in pathological states of glands, including inflammation, hyperplasia, and neoplasia. However, in hyperplastic PTG due to SHPT, the increase in angiogenesis and apoptosis may occur simultaneously (44, 46). Although it has been hypothesized that a reduction of the size and volume of the PTG may result from a decreased cell proliferation and/or an increased apoptosis (2), this study shows that the LI of proliferating (Ki-67) and apoptotic cells (TUNEL) in PTG did not differ significantly between SHPT patients who received or did not receive cinacalcet before PTx.

This study has some limitations. First, the study is observational and reports associations. Secondly, the number of HP included in the study was relatively small and all patients were affected by THPT. The patients submitted to PTx represent a highly selected population. Indeed, the PTG had a relatively large size and most had NH (87.5%). Thirdly, there was not a control group of patients without history of treatment with either vitamin D or cinacalcet. However, it is very difficult to have such patients to be included in cross-sectional studies because all HP with SHPT who undergo PTx usually have a history of treatment with vitamin D and/or cinacalcet. Fourthly, there was an unbalance in the duration of treatments between vitamin D and cinacalcet and this might have potentially affected the results. However, the longer duration of treatment was with cinacalcet.

In conclusion, the present retrospective and observational study shows that the macroscopic, microscopic, and immunochemistry characteristics of PTG of patients with advanced degree of parathyroid hyperplasia who received or did not receive cinacalcet long term before PTx did not differ significantly. However, the study does not allow to adequately clarify the hypothetical effects of cinacalcet on smaller PTG, which are usually detectable in SHPT responders to medical treatment (47).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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