Original Paper

Analysis of α -Klotho, Fibroblast Growth Factor-, Vitamin-D and Calcium-Sensing Receptor in 70 Patients with Secondary Hyperparathyroidism

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Key Words

Secondary hyperparathyroidism • Klotho • VDR • CaSR • FGFR

Abstract

Background/Aims: Secondary hyperparathyroidism (sHPT) is known as a very common complication in patients with chronic kidney disease, and G-protein-coupled calcium-sensing receptor (CaSR), Vitamin D receptor (VDR) and Fibroblast growth factor receptor (FGFR)/Klotho complexes seem to be involved in its development. **Methods:** Hyperplastic parathyroid glands from 70 sHPT patients and normal parathyroid tissue from 7 patients were obtained during parathyroidectomy. Conventional morphological and immunohistochemical analysis of parathyroid glands was performed after dividing each slide in a 3x3 array. **Results:** The presence of lipocytes in the normal parathyroid gland and tissue architecture (nodal in patients with sHPT) allows for discrimination between normal parathyroid glands and parathyroid glands of patients with sHPT. Protein expression of Klotho, FGFR, CaSR and VDR was higher in the normal parathyroid glands compared to the sHPT group ($p < 0.001$, $p = 0.07$, $p = 0.01$ and $p = 0.001$). The variability of each protein expression within each tissue slide was high. Therefore correlations between the different immunohistochemical variables were analyzed for each of the nine fields and then analyzed for all patients. Using this analysis, a highly significant positive correlation could be found between the expression of FGFR and VDR ($p = 0.0004$). Interestingly, in terms

of VDR we found a shift to a more mixed nuclear/cytoplasmic staining in the HPT group compared to normal parathyroid gland cells, which showed solitary nuclear staining for VDR ($p > 0.05$). **Conclusions:** CaSR, VDR and an impaired Klotho-FGFR-axis seem to be the major players in the development of sHPT. Whether the detected correlation between FGFR and VDR and the shift to a more mixed nuclear/cytoplasmic staining of VDR will yield new insights into the pathogenesis of the disease has to be evaluated in further studies.

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Introduction

Secondary hyperparathyroidism (sHPT) is known as a very common complication in patients with chronic kidney disease. Reduced 1,25-dihydroxyvitamin D₃ production, increasing phosphate retention and a decrease in serum calcium contributes to the pathogenesis of sHPT [1]. New insights into the pathogenesis of sHPT were given by the description of the role of G-protein-coupled calcium-sensing receptor (CaSR), Vitamin D receptor (VDR) and Fibroblast growth factor receptor (FGFR)/Klotho complexes in the development of sHPT. In advanced sHPT, expression of CaSR and VDR is decreased in the parathyroid gland, with impaired Ca²⁺ sensitivity and basal PTH, affecting the pathogenesis of sHPT both quantitatively and functionally [2]. Recently, two pathways including α -klotho have been demonstrated in the regulation of PTH secretion. One is the fibroblast growth factor 23 (FGF23) α -klotho-FGF receptor (FGFR) pathway and the other is the α -klotho-dependent Na⁺/K⁺-ATPase pathway [3, 4]. Komaba et al. and Kumata et al. showed that the expression of klotho and FGFR was decreased in the parathyroid gland of patients with sHPT, supporting the theory of resistance to FGF23 in the development of sHPT [2, 5]. FGF23 is a protein, which binds in the presence of its co-receptor klotho to FGFR1, leading to phosphaturia and reduced synthesis of calcitriol by inhibiting the sodium-dependent transporter and 1 α -hydroxylase (CYP27B1) activities in the proximal tubule [6, 7]. Additionally, positive correlations were reported between α -klotho and FGFR and α -klotho and FGFR with CaSR [2].

To clarify the role of these players in the pathogenesis of sHPT, we established an expression pattern of morphological parameters and these newer factors in the parathyroid gland tissue of a large cohort of 70 patients with sHPT. Additionally, correlations between immunohistochemically findings and laboratory data were elucidated.

Subjects and Methods

Patients

The present study included 77 patients who underwent parathyroidectomy between 1st January 1995 and 30th April 2005 in our hospital. Patients were divided into following categories:

- 70 patients with secondary hyperparathyroidism (sHPT)
- 7 control patients with normal parathyroid gland.

Data collection included gender and age at time of surgery. Additionally, the following laboratory parameters were evaluated in all patients from the medical charts: leukocytes, thrombocytes, hemoglobin, glucose, urea, serum creatinine, calcium, phosphate and intact parathormone (iPTH) (baseline characteristics were shown in Table 1). Blood samples of the patients were obtained within one week prior to surgery. This study was performed in accordance with the principles of the Declaration of Helsinki and was approved by the local ethics committee of the University of Tuebingen. All patients provided written informed consent prior to enrolment in the study.

Table 1. Baseline characteristics and laboratory results of patients with sHPT and the control group

Variable	sHPT	Control group
n	70	7
Age (years ±SD)	49.4 ± 14.2	59.2 ± 16.8
Female/Male	36/34	4/3
RRT at time of surgery:		
HD	50	
PD	16	
NTx	4	
Preoperative medications:		
Vitamin D (n=23) (µg/day)	1.17 ± 0.83	
Active Vitamin D (n=19)(µg/day)	1.07 ± 0.87	
Phosphate binders		
Sevelamer (n=13) (mg/day)	3360 ± 1858	
Calciumacetate(n=26)(mg/day)	3852 ± 2087	
Algedrate (n=20) (mg/day)	3660 ± 1568	
Hb (g/dl ± SD [13–18])	12.4 ± 2.2	13.9 ± 1.6
Leukocytes (Giga/L ±SD [4.0–11.3])	7.3 ± 2.8	7.6 ± 1.4
Phosphate*** (mmol/l [0.68–1.68])	1.8 ± 0.6	1.06 ± 0.21
Calcium*** (mmol/l [1.90–2.70])	2.6 ± 0.2	2.18 ± 0.14
PTH*** (pmol/l [1.1–7.3])	132.3 ± 80.3	3.30 ± 2.28
Urea-N*** (mg/dl [10–25])	111.2 ± 50.3	28.8 ± 7.8
Creatinine*** (mg/dl [0.5–1.4])	7.7 ± 3.7	0.80 ± 0.13

RRT, Renal replacement therapy; HD, hemodialysis; PD, Peritoneal dialysis; NTx Transplantation; Hb, haemoglobin; PTH, parathyroid hormone; * p<0.05, ** p<0.01, *** p<0.001

Diagnosis

All patients with the diagnosis of sHPT in our study were patients on RRT (HD or PD) or had a functioning transplant. Diagnosis of sHPT was made, according to the K/DOQI clinical practice guidelines for bone metabolism and disease in chronic kidney disease [8]. All patients had untreatable sHPT under medical therapy including nonsuppressible serum PTH values, hyperphosphatemia or hypercalcemia and therefore parathyroidectomy was performed.

Tissue bank

Normal parathyroid tissue (n=7) was obtained in conjunction with thyroid surgery from patients with normal kidney function. Hyperplastic parathyroid glands were obtained from patients with sHPT who underwent parathyroidectomy. All biopsies were formalin-fixed and paraffin-embedded following routine protocols [9]. All slides were divided in a 3x3 array (9 fields) and all following analyses were done in each of the 9 fields. All patients had given their written informed consent concerning a scientific work-up of tissues taken during surgery and the locals ethic committee (University of Tuebingen) approved the study.

Conventional morphological analysis of parathyroid glands

From each slide of the parathyroid glands (parathyroid glands of 7 patients with no parathyroid disorder and 70 patients with sHPT) hematoxylin and eosin staining was done for morphological analysis. Three types of parathyroid cells were discriminated:

- chief cells with an ambophil cytoplasm using the Fe staining Prussian method
- oxyphil or oncocytic cells with eosinophil cytoplasm and a large cytoplasm
- clear cells

The tissue architecture of the parathyroid gland was classified as nodular or diffuse as well as normal or degenerative (cystic alterations and / or hemorrhagia). Nuclei were assessed as either isomorph or anisomorph. The amount of lipocytes was graded as absent, very few (1-5%), few (5-25%) and moderate (>25%). In all cases an iron staining was done and classified as absent (0), faint (1), moderate (2) or strong (3).

Immunohistochemical staining

The antibodies used for immunostaining are listed in Table 2. Endogenous peroxidase blocking was done by Peroxidase Blocking Solution (S 2023, DAKO, Hamburg, Germany) for 8 minutes. The primary

Table 2. Characteristics of applied antibodies

Antibody used	source	dilution	pretreatment
Klotho-1	Goat antibody sc-22220, Santa Cruz	1:50	Steamer, pH6 (Dako S1699)
FGFR-1	Rabbit antibody F5421, Sigma	1:700	Steamer, pH9 (Dako S2367)
CaSR	Rabbit antibody SP4561P, Acris	1:200	Steamer, pH6 (Dako S1699)
VDR	Rat antibody SM5036P, Acris	1:100	Steamer, pH6 (Dako S1699)
Ki67	Mouse antibody M 7240, DAKO	1:100	Steamer, pH6 (Dako S1699)
CYP27B1	Rabbit antibody Sc-67261, SantaCruz	1:40	Steamer, pH9 (Dako S2367)

FGFR=fibroblast growth factor. CaSR=calcium-sensing receptor. VDR=vitamin D receptor

antibodies were diluted in antibody diluent (S 2022, DAKO, Hamburg, Germany) (see Table 2). For immunostaining we used a Techmate system (TechMate 500 Plus, DAKO, Hamburg, Germany). The staining method was a dextran-coated peroxidase coupled polymer system (Dako REAL™ EnVision™ Detektion Kit, Peroxidase/DAB+, Rabbit/Mouse, K 5007, DAKO, Hamburg, Germany). If goat antibodies were used, we applied an anti-goat IgG, IgM, IgA (H&L) (SAB1071, Open Biosystems, Huntsville, AL, USA) 1:500, diluted in a commercial buffer system (antibody diluent (S 2022, DAKO). If rat antibody was used, we applied rabbit anti-rat Ig (Z 0147, DAKO) 1:10.000 in antibody diluent (S 2022, DAKO). The final reaction product was produced by incubation in diaminobenzidine/H2O2 for 15 minutes followed by a short incubation in hemalaun for 1 minute.

Assessment of immunostaining results

All staining results, except Ki67 were assessed by the same system. Staining intensity was classified as negative (0), faint (1), moderate (2) and strong (3). The number of stained cells was expressed as proportion of immunostained parathyroid cells. As mentioned above, every tissue slide was divided in a 3x3 array (9 fields) and an IRS (score range from 0-300) of immune reaction was performed for each of the nine fields. Afterwards the median IRS of the 9 fields was calculated for each immunoreaction within each tissue slide. The distribution of staining scores was summarized as median and interquartile range (difference of 25% and 75% quantile). The correlation between the different immunohistochemical variables was analyzed for each of the nine fields and then correlated for all patients.

Additionally, the localization of the immune reaction was noticed: membranous, cytoplasmic, nuclear or mixed membranous/cytoplasmic, nuclear/cytoplasmic or nuclear/membranous. For the analysis of nuclear staining of Ki67, the percentage of proliferating cells was given as positive nuclear staining/100 parathyroid cells and the promille of positive nuclei was assessed.

Statistics

Statistical analysis was done using R (version 2.12.0). Data are reported as mean ± standard deviation (SD). IRS was calculated using median and range. To compare numeric data the non-parametric Kruskal Wallis test was used and nominal data were analyzed with Fisher's exact test. To find possible predictors of sHPT, univariate logistic regression was applied. Correlations were investigated by Spearman's rank correlation coefficient. Results were considered as significant with $p < 0.05$, as highly significant with $p < 0.01$, and as extremely significant with $p < 0.001$.

Results

In this study we investigated the parathyroid glands of 77 patients who underwent parathyroidectomy between 1st January 1995 and 30th April 2005, in our hospital.

Demographics

The mean age at diagnosis was 49.4 ± 14.2 in the sHPT and 59.2 ± 16.8 in the control group ($p > 0.05$). There was no predominance of gender and no predominant diagnosis concerning the underlying disease leading to chronic renal failure in the sHPT group. At time of surgery, 50 patients were on hemodialysis, 16 patients were on peritoneal dialysis

Fig. 1. Representative immunohistochemical findings in the parathyroid glands of patients with sHPT. A: Immunohistochemical staining of α -Klotho in the parathyroid glands. Klotho antigens were localized in the cytoplasm. B: Immunohistochemical staining of CaSR showing a mixed cytoplasmatic/membranous pattern. C: Immunohistochemical staining of VDR. D: Immunohistochemical staining of FGFR.

and 6 patients had a functioning transplant. As expected, there were statistically significant differences between the sHPT and the control group concerning the baseline characteristics. Phosphate, calcium, PTH, creatinine and urea levels were significantly elevated in the sHPT group compared to the control group (all $p < 0.0001$). The baseline characteristics of study patients are summarized in Table 1.

Morphology of the parathyroid gland

As assumed, the main cell type in the parathyroid glands of the healthy control patients is the chief cell. In 10 out of 70 patients with sHPT, oxyphil cells were the main cell type. No statistical differences could be detected between the sHPT and the control group concerning the numbers of chief cells ($p=0.36$).

Morphological analysis of the parathyroid glands of the healthy control patients showed a mainly diffuse tissue architecture. Using univariate logistic regression analysis, nodal tissue architecture was more often present in the sHPT group ($p=0.03$) as compared to the control group. Anisonucleosis and degenerative alterations were not observed in parathyroid glands of healthy control patients. Compared to the control group, an increasing number of tissue samples were showing anisonucleosis without reaching statistical significance ($p=0.63$). Iron staining, used as an indicator of cellular stress, degenerative tissue alteration or older hemorrhage was decreased in parathyroid glands of healthy control patients when compared to the sHPT group, but the difference was insignificant ($p>0.05$). Nevertheless, using the presence of lipocytes and tissue architecture (nodal in patients with sHPT) in the parathyroid gland, discrimination between normal parathyroid glands and parathyroid glands of patients with sHPT was possible ($p<0.001$).

Immunohistochemical staining of normal parathyroid glands and parathyroid glands of patients with sHPT

Klotho showed a significantly higher IRS in normal parathyroid glands compared as to the sHPT group ($p<0.001$). Analysis of the variability of klotho expression in the sHPT group showed a high variability (median klotho expression in the sHPT group was 10.0 with an interquartile range of 15). Representative immunohistochemical findings were shown in Figure 1 A-D. Immunostaining against FGFR showed no statistical differences between the control and the sHPT groups, but a trend towards a lower expression in the control group could be detected ($p=0.07$). Analogical to klotho expression, the variability of FGFR was high in the sHPT group (median FGFR expression was 33.8 with an interquartile range of 21.9).

CaSR and VDR were significantly higher expressed in the control group compared to the sHPT group ($p=0.01$ and $p=0.001$). In terms of the variability of CaSR and VDR

Fig. 2. Correlations between the different protein expressions in the parathyroid glands of 70 patients with sHPT. Ki67 was used as proliferation marker.

immunostaining, a high variability could be detected (median CaSR expression was 70 with an interquartile range of 40, median VDR expression was 27.5 with an interquartile range of 26.9) *Cyp27B1* showed no significant differences in expression as measured by the immunoreactive score index between the control and the sHPT group ($p=0.8$). In the sHPT group there were statistically significant positive correlations between Klotho and CaSR ($R=0.34$, $p=0.007$), FGFR and VDR ($R=0.25$, $p=0.03$). Correlations between the proteins and protein expressions in the different groups were shown in Figure 2 and Figure 3.

Additionally, we investigated the protein expression in nodal versus diffuse areas of the parathyroid gland. A statistically significant negative correlation between CaSR and VDR ($R=-0.287$, $p=0.006$) could be detected in diffuse areas of the parathyroid gland of patients with sHPT. Apart from that, no correlation between protein expression and tissue architecture (diffuse or nodal) could be found.

Furthermore, the localization of the proteins was investigated. Klotho and FGFR antigens were localized in the cytoplasm, whereas a mixed cytoplasmic/membranous pattern was found for CaSR. No differences between the control and sHPT group concerning localization of the described cellular proteins could be detected ($p>0.05$). Ki 67 and VDR

Fig. 3. Protein expression of Klotho, FGFR, CaSR and VDR of the parathyroid gland in the sHPT compared to the control group (CG). IRS, Immunoreactive score; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Fig. 4. A: In a first step, the protein expressions in the parathyroid glands of 70 patients with sHPT were calculated for each of the nine fields. Then correlations between the protein expressions were calculated between the corresponding fields of each slide and secondly correlated in all patients. **B:** Immunohistochemical stainings against FGFR, VDR and CaSR within the same area of the tissue slide.

were localized in the nuclei. Interestingly, in terms of VDR we found a shift to a more mixed nuclear/cytoplasmic staining in the HPT group compared to normal parathyroid gland cells, which showed solitary nuclear staining for VDR ($p > 0.05$).

Due to the high variability of the protein expression in the tissue slides of the parathyroid glands, additional analyses were performed. Each slide was divided in a 3x3 array and the protein expression was measured in each of the nine fields. Afterwards, correlations between all protein expressions were calculated within each corresponding field and all patients (Figure 4).

Table 3. Correlations between immunohistochemical results and laboratory values

	calcium	phosphat	creatinine	urea	iPTH
Klotho	R=0.063, P=0.62	R=0.0003, P=0.32	R= -0.019, P=0.68	R=0.138, P=0.35	R=0.236, P=0.10
FGFR	R=0.137, P=0.26	R=0.240, P=0.07	R=0.214, P= 0.08	R=0.073, P=0.61	R= 0.152, P= 0.26
VDR	R=0.232, P=0.05	R=0.301, P=0.02	R=0.254, P=0.04	R=0.162, P=0.25	R=0.022, P=0.28
CaSR	R=0.135, P=0.26	R=0.089, P=0.50	R=0.111, P=0.36	R=0.06, P=0.68	R=0.136, P=0.98

Statistical significant correlations (p<0.05) were marked in bold.

Using this analysis, a highly significant positive correlation could be found between the expression of FGFR and VDR (p=0.0004). No other statistically significant correlation could be detected between the other protein expressions in the corresponding fields in all patients.

Proliferation rate in parathyroid glands in the different groups

The proliferation rate in the parathyroid glands was analyzed using Ki67 antigen. There were no differences between the different cell types regarding proliferation rate. The overall proliferation rate of the normal parathyroid gland was $0.1 \text{ o/oo} \pm 0.1$. Compared to the sHPT group significantly less proliferating cells could be detected in the normal parathyroid glands (p<0.05).

Correlations between protein expression, laboratory parameters and time on Renal replacement therapy (RRT)

In patients with sHPT, a significant positive correlation could be detected between VDR expression, phosphate and creatinine (p=0.02 and 0.04). A trend was seen between the expression of VDR and calcium concentrations (p=0.05). Additionally, there was a positive correlation between the expression of FGFR, phosphate and creatinine, without reaching statistical significance, but a trend could be detected (p=0.07 and 0.08) (Table 3).

Furthermore we investigated whether protein expressions correlates with time on RRT. No correlation could be detected between time on RRT (in months) to time point of surgery (FGFR: R=0.0007, p=0.82; Klotho: R=0.002, p=0.71; VDR: R=0.001, p=0.80; CaSR: R=0.003, p=0.63).

Discussion

In the present study, we investigated the expression pattern in 77 surgically excised parathyroid glands from patients with sHPT (70 patients) and a control group (7 patients) concerning morphology, CaSR, VDR and FGFR/Klotho complexes. Additionally, detailed analyses of the correlation between these markers among each other, and with laboratory data, were performed in this large cohort of sHPT patients.

In a first step, a detailed morphological analysis (cell types, tissue architecture, cellular stress, degenerative alterations) of the parathyroid glands was performed. Microscopically, normal parathyroid glands showed the constitution of parenchymal cells, with chief cells as main cell type. These findings are compatible with previous reports [10] Parathyroid glands in our cohort of patients with sHPT showed less chief cells, without reaching statistical significance. As expected, nodal tissue architecture was significantly more often present in the sHPT group as compared to the control group. Nodular hyperplasia consisting of nodules of various sizes was often observed in the early and advanced stages of sHPT [10-12]. In summary, from the morphological point of view, only the presence of lipocytes and nodal tissue architecture allows a differentiation between a normal parathyroid gland and parathyroid glands from patients with sHPT.

Secondly, we investigated in detail the expression of all presumably involved proteins in the pathogenesis of sHPT and performed analyses of correlations between the proteins

among each other and between laboratory findings. In our study, a decreased protein expression could be detected regarding klotho, CaSR and VDR in the parathyroid glands in the cohort of patients with sHPT, when compared to a control group. In contrast, no differences could be detected regarding expression of Cyp27B1 and FGFR between both groups. These results are in keeping with previous studies from Komaba et al. and Kumata et al., who investigated klotho and FGFR expression in the parathyroid glands of 23 and 12 patients. Actually, the mechanisms of interaction between FGF23 and CYP27B1 resulting in inhibition of CYP27B1 and leading to phosphaturia and reduced synthesis of calcitriol were reported recently [6, 7]. FGF23 may play an important role in hyperparathyroidism's pathogenesis, because FGF23 decreases renal 1- α -hydroxylase activity [13] and will oppose the action of PTH to increase the level of 1,25(OH) $_2$ D. FGF23 elevations in chronic kidney disease may contribute to the inability to maintain calcitriol levels as kidney function decreases. Because Klotho is abundantly expressed in the parathyroid, it also is relevant to ask whether FGF23 directly affects parathyroid function. It has been demonstrated that FGF-23 increased the expression of growth factors in parathyroid [14]. These observations were recently confirmed in bovine parathyroid cells and rat parathyroid glands [4, 15]. FGF-23 decreased PTH mRNA expression and decreased PTH secretion. It is interesting that in contrast to the effects on 1- α -hydroxylase in kidney, FGF-23 increased parathyroid 1- α -hydroxylase expression within 3 h of exposure, and it was concluded that FGF-23 is a negative regulator of PTH mRNA expression and secretion in vitro [15]. In vitro results suggested that FGF-23 regulates PTH gene expression by its effects on the mitogen-activated protein kinase pathway and the actions on this pathway was confirmed in vivo using an extracellular signal-regulated kinase inhibitor [4].

Furthermore, a positive correlation between α -klotho and CaSR could be detected [2, 5]. Interestingly, we could not detect a different protein expression between diffuse or nodular areas of the parathyroid gland, excluding an inverse correlation between CasR and VDR in diffuse areas of the parathyroid gland. Additionally, we investigated the proliferative marker Ki67 in our cohort. In contrast to the study of Komaba et al., yet in line with the results of Kumata et al., we found a negative correlation between Ki67, Klotho and FGFR, which did not reach statistical significance. It should be mentioned though, that both FGFR and α -klotho were associated with parathyroid cell proliferation. As suggested by Kumata et al., it could be possible, that the decreased expression of α -klotho and FGRF presents a cellular response to hyperproliferative activity without being causally related to the mechanism of the development of sHPT [2].

During analysis of the different immunostainings, we found a highly variant expression of the proteins within the same tissue slides (variability of the immune reactions was extremely high between each of the 9 fields within one sample). Using an detailed "per field"-analysis a highly significant positive correlation of between FGFR and VDR could be detected. It is well accepted, that the production of FGF23 is stimulated by phosphate retention and elevated FGF23 levels should enhance the phosphate control in renal failure [16]. In normal parathyroid glands, PTH secretion was decreased and both, VDR and CasR was increased by FGF23 [17]. In contrast, Canalejo et al. [17] showed in uremic rats, that there was a very low expression of the FGF23 receptor 1 and the co-receptor klotho in uremic hyperplastic parathyroid glands. Furthermore, uremic parathyroid glands showed a decreased expression of VDR, and administration of FGF23 did not increase VDR expression in parathyroid glands from uremic rats. This might explain the lack of response to FGF23 in the parathyroid tissue. It could be speculated, that the strong positive correlation of VDR and FGFR in a detailed "nine-field" analysis points to the importance of the FGF/VDR-axis in the regulating mechanisms of the development of sHPT.

Another finding in this study was the shift to a more mixed nuclear/cytoplasmic staining of VDR in the sHPT group compared to normal parathyroid gland cells, but without reaching statistical significance. Parathyroid cells showed solitary nuclear staining for VDR. It is well accepted, that VDR transits from the cytoplasm to the nucleus after ligand binding, where it regulates gene expression. Recently, it has been shown that a cytosolic pool of VDR can

also rapidly activate an intracellular signaling cascade in colon cancer cells (in response to $1,25(\text{OH})_2\text{D}$ treatment), thus enhancing transcriptional regulation by nuclear VDR [18].

In a third step, we correlated the immunohistological stainings with laboratory parameters and time on RRT in the sHPT group. In our study population, a positive correlation could only be detected between VDR, creatinine and phosphate. Björklund et al. [19] reported in their study of patients with primary hyperparathyroidism a strong negative association between serum calcium and klotho expression. We could not find a correlation between klotho expression and serum calcium in our patients with sHPT, although klotho regulates serum calcium concentrations directly at the level of the kidney and indirectly in the parathyroid gland [19-21]. Remarkably, no correlation was found between time on RRT and protein expression.

From the clinical point of view, the obviously involved proteins in parathyroid function were less expressed in sHPT while overall proliferation was increased. The obvious explanation is the loss of the differentiation during the evolution of sHPT and the gland starts to proliferate which eventually leads to an autonomous HPT. At this point, the patient cannot be managed with drug therapy any more and may require surgery. Whether medical treatment with Vitamin D, active Vitamin D or calcimimetics might influence different protein expression leading to treatment failure is still unclear. Remarkably, due to time period, none of the patients in our study received calcimimetics.

Conclusion

A detailed analysis of immunostainings of parathyroid gland tissue samples is presented. To our knowledge, this is one of the largest single center study of patients with sHPT. Undoubtedly, CaSR, VDR and an impaired Klotho-FGFR-axis seem to be important to the understanding of the development of sHPT. It is noteworthy, that the variability of protein expression in the parathyroid is extremely high. Whether the detected correlation between FGFR and VDR and the shift to a more mixed nuclear/cytoplasmic staining of VDR in the sHPT group could give new insights into the pathogenesis of the disease is yet unknown.

Conflict of Interests

None.

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