Simultaneous Expression Analysis of Vitamin D Receptor, Calcium Sensing Receptor, Cyclin D1 and PTH in Symptomatic Primary Hyperparathyroidism in Asian Indians

Shweta Varshney¹, Sanjay Kumar Bhadada¹*, Uma Nahar Saikia², Naresh Sachdeva¹, Arunanshu Behera³, Ashutosh Kumar Arya¹, Sadhna Sharma⁴, Anil Bhansali¹, Ambrish Mithal⁵, Sudhaker D. Rao⁶

Department of Endocrinology¹, Department of Histopathology², Department of General Surgery³, and Department of Biochemistry⁴ Postgraduate Institute of Medical Education and Research Chandigarh, Medanta The Medicity Hospital⁵, Gurgaon, India.

Bone & Mineral Research Laboratory⁶, Henry Ford Hospital, Detroit, USA.

Disclosure – Nothing to disclose

*Both authors contributed equally

Corresponding Author:
Dr. Sanjay Kumar Bhadada,
Associate Professor
Department of Endocrinology
Address: 4th floor, F-Block, Room no 2, PGIMER, Chandigarh, India-160012
Email id: bhadadask@rediffmail.com
Abstract

Background: To explore underlying molecular mechanisms in the pathogenesis of symptomatic sporadic primary hyperparathyroidism (PHPT).

Material & Methods: Forty one parathyroid adenomas from patients with symptomatic PHPT and 10 normal parathyroid glands either from patients with PHPT (n=3) or from euthyroid patients without PHPT during thyroid surgery (n=7) were analyzed for vitamin D receptor (VDR), calcium sensing receptor (CaSR), cyclin D1 (CD1) and parathyroid hormone (PTH) expressions. The protein expressions were assessed semi-quantitatively by immunohistochemistry, based on percentage of positive cells and staining intensity, and confirmed by qRT-PCR.

Results: Immunohistochemistry revealed significant reductions in VDR (both nuclear and cytoplasmic) and CaSR, and significant increases in CD1 and PTH expressions in adenomatous compared to normal parathyroid tissue. Consistent with immunohistochemistry findings, both VDR and CaSR mRNAs were reduced by 0.36 and 0.45 fold change (p<0.001), and CD1 and PTH mRNAs were increased by 9.4 and 17.4 fold change respectively (p<0.001) in adenomatous parathyroid tissue. PTH mRNA correlated with plasma PTH (r=0.864; p<0.001), but not with adenoma weight, while CD1 mRNA correlated with adenoma weight (r=0.715; p<0.001). There were no correlations between VDR and CaSR mRNA levels and serum Ca, plasma intact PTH or 25-hydroxyvitamin D levels. In addition there was no relationship either between the decreases in VDR and CaSR mRNA expressions or the increases in PTH and CD1 mRNA expressions.

Conclusions: The expression of both VDR and CaSR are reduced in symptomatic PHPT in Asian Indians. In addition, CD1 expression was greatly increased and correlated with adenoma weight, implying a potential role for CD1 in adenoma growth and differential clinical expression of PHPT.

Key terms: Parathyroid adenoma, VDR, CaSR, and CD1.
Introduction-

Primary hyperparathyroidism (PHPT) is a common sporadic endocrine disorder characterized by hypercalcemia with non-suppressed or elevated parathyroid hormone (PTH) levels. A single parathyroid adenoma is the most common cause of PHPT \(^1\), but the pathogenesis of these lesions is poorly understood. Altered calcium sensing receptor (CaSR) mediated control of PTH secretion with the consequent increased parathyroid cell proliferation is fundamental to the development of PHPT \(^2, 3\). In addition, 1,25-dihydroxyvitamin D, through its interactions with vitamin D receptor (VDR) in parathyroid cells modulates parathyroid function and growth \(^4-8\). Thus both VDR and CaSR play critical roles in the regulation of PTH synthesis and secretion, parathyroid cell proliferation, and maintenance of plasma calcium levels. Tumor-specific DNA rearrangements in a subset of parathyroid adenomas have shown over expression of CD1 leading to increasing mitotic rate and parathyroid adenoma development \(^9-11\).

Abnormalities in VDR\(^{12-14}\), CaSR\(^{14-18}\) and CD1\(^{19-23}\) expression have been reported in the cases probably having mild form of PHPT, parathyroid cancers, and in renal secondary hyperparathyroidism. However, very little is known about such abnormalities in adenomas from patients with symptomatic sporadic PHPT\(^{24-26}\). Presentation of PHPT in Asian Indian is symptomatic unlike the mostly asymptomatic nature of presentation in the West\(^{24}\). Accordingly, we examined the expression of VDR, CaSR, CD1, and PTH using immunohistochemistry and confirmed with mRNA analyses in a cohort of parathyroid adenomas from Asian Indian patients with symptomatic PHPT.

Material and methods-

**Patient Description and Tissue Collection:** The study was conducted at the Post Graduate Institute of Medical Education and Research, Chandigarh, India, from January 2008 to December 2010. After approval by the institutional ethics committee, informed written consent was obtained from each patient. The diagnosis of PHPT was confirmed in each patient by the presence of hypercalcemia and elevated
PTH levels, and verified at surgery. Patients with parathyroid hyperplasia, secondary hyperparathyroidism and multiple endocrine neoplasia syndromes were excluded.

Histopathologically confirmed parathyroid adenomas (n=41) were collected immediately in RNA-Later and stored at -80°C until isolation of total RNA. Paraffin blocks of each tumor were retrieved from the Department of Histopathology. Normal parathyroid tissue (n=10) was obtained either from the PHPT patients (n=3) or from patients undergoing thyroid surgery for euthyroid goiters in whom parathyroid gland was inadvertently removed.

**Assay Methods:** Preoperative serum calcium, adjusted for serum albumin, (reference range (RR), 8.6-10.2 mg/dL), alkaline phosphatase (RR, 40-129 U/L), and creatinine (RR, 0.5-1.2 mg/dl) were measured by auto-analyzer (Modular P 800: Roche Diagnostics, Germany). Plasma intact PTH (RR, 15-65 pg/ml) and 25-hydroxyvitamin D (RR, 11.1-42.9 ng/ml) levels were measured by electro-chemiluminisence immunoassay (ELECSYS-2010, Roche Diagnostics, Germany) as per the manufacturer’s instructions.

**Histopathology:** After surgical excision, the specimens were weighed and fixed in 10% buffered formalin and embedded in paraffin for routine processing and paraffin blocking. A 3-5µm thick section was cut from each paraffin embedded block stained with hematoxylin and eosin (H&E) and assessed by an expert parathyroid histopathologist (UNS) to confirm the diagnosis of parathyroid adenoma as determined by an encapsulated tumor with very little fat associated with hypercellularity. Images were captured using a Leica DMR microscope (Leica, Germany) and a monochrome photometrics CCD camera (Photometrics, USA).

**Immunohistochemistry:** Representative paraffin blocks were retrieved and immunostained as previously reported (12, 22), and incubated with peroxidase labeled secondary antibody (rabbit/mouse, Dako Denmark). At a magnification 20X and 40X, approximately 500 cells were counted at 4 different locations for each section. Results were scored semi-quantitatively based on percentage of positive cells and staining intensity. Detailed information on material and methods for immunostaining are shown in
Supplementary Table-1, and the scoring criteria for stain intensity of each antibody are in Supplementary Table-2.

**RNA Extraction and cDNA Synthesis:** Briefly, each tissue sample was crushed in 1 ml of TRI Reagent (Sigma-Aldrich, USA). After chloroform addition, the aqueous and organic phases were separated by centrifugation. The aqueous (upper) phase was removed from each tube and transferred to a clean 1.5 ml microcentrifuge tube, where total RNA was precipitated by isopropanol. The RNA pellet was washed with 75% ethanol, allowed to dry, and dissolved in sterile water and stored at -20°C until analysis. The 260/280 nm ratio of RNA (interval 1.9-2.1) was determined by Biophotometer plus (Eppendorf, Germany). The integrity was confirmed by denaturing gel electrophoresis, whereby sharp 28S and 18S bands were demonstrated. The cDNA was synthesized from 6 µg of total RNA by cDNA synthesis Kit (Fermentas, Life Sciences, USA), according to the manufacturer’s protocol.

**Gene Expression Analysis:** The relative expression of VDR, CaSR, CD1 and PTH in parathyroid adenoma cells was determined using quantitative real-time PCR (qRT-PCR) by SYBR Green I dye method (Fermentas, Life Science, USA) on Light Cycler 480 Real-Time PCR System (Roche Diagnostics) as per the manufacturer’s recommendations. All samples were amplified in duplicate; non-template reactions were included as a negative control. Throughout qRT-PCR analysis, product identities were confirmed by melting curve analysis (Supplementary Fig-1).

Primers were designed by using a web based application (www.ncbi.nlm.nih.gov/tools/primer-blast/; Supplementary Table-3). All reactions were carried out in a 96-well optical reaction plate. One µl of template cDNA (equivalent to 300 ng of total RNA) were added to 25 µl of PCR reaction mixture containing 0.5 µM each forward and reverse primers. The pre-incubation was done at 95°C for 10 min, followed by 50 cycles of PCR amplification (95°C, 15 sec; variable annealing temperature; 72°C, 20 sec), melting at 70°C, for 1 min and final step was extended to 10 min at 72°C. To confirm specificity of the primers, gel electrophoresis of the PCR products revealed one distinct band for each transcript. Finally,
sequencing of the amplified product was performed (Applied Biosystems, USA) to confirm the correct transcript. Amplification efficiencies of the targets and reference were found to be approximately equal. So analysis of the relative gene expression data was done by the $2^{\Delta\Delta C_T}$ method to produce the data as fold change up or down regulation. In the fold change analysis, crossing point value (Cp Value) was determined as the number of cycles required for fluorescent signal to cross the threshold. The Cp values of both the calibrator (Control) and the sample of interest were normalized to the corresponding mean value of an endogenous housekeeping gene, GAPDH.

**Statistical Analyses:** All analyses were performed using SPSS (10.01, Chicago, USA). The nonparametric Kolmogorov-Smirnov test was used to assess differences in all the study parameters between normal and adenomatous parathyroid samples. Values are presented as mean ± SD. Mann-Whitney, Chi-Square and t-test were performed as appropriate to compare the data between normal and adenomatous tissue. The Spearman’s rho correlation test was applied for correlation analyses. The percentage of positive cells and staining intensity were compared using the Wilcoxon signed rank test. A p value of <0.05 was considered statistically significant.

**Results:**

**Clinical Characteristics:** Of the 55 consecutive patients who underwent parathyroidectomy during the study period, 41 patients (74.5%) had single adenomas, 12 (21.8%) had hyperplasia and 2 (3.6%) had carcinoma. All the 41 parathyroid adenomas were included for detailed molecular studies. The mean age was 41.5 ± 13.3 years (range 11-67 years) with a male to female ratio of 1:3.2. The most common presenting manifestation was bone pain in 22 (52%) followed by renal stones in 17 (41%), fractures in 15 (36%), nephrocalcinosis and gall stones in 7 (17%) each, and pancreatitis in 5 (12%) patients. The relevant baseline biochemical measurements are shown in Table-1.

**Vitamin D Receptor (VDR) Expression:** Thirteen (32%) of the parathyroid adenomas showed only cytoplasmic immuno-reactivity for VDR while 26 (63%) showed both nuclear and cytoplasmic positivity.
and only 2 (5%) adenomas showed exclusive nuclear positivity. The mean proportion of adenomatous cells positive for VDR nuclear stain was 4% (median: 2%; range 0-40%) compared to 75% (median: 75; range 60-90%) in normal parathyroid tissue cells (p<0.05, figure 1A). The mean proportion of adenomatous cells positive for cytoplasmic VDR stain was 29% (median: 30; range 0-70%) compared to 84% (median 85: range 70-100%) in normal parathyroid cells (p<0.05; Figure 1A). Staining intensity was 1+ in 17 (42%), 2+ in 22 (54%) and 3+ in 1 (2%) adenomas and one adenoma (2%) had neither nuclear nor cytoplasmic positivity. All normal parathyroid cells showed 3+ staining intensity (Figure-2), with a relative reduction in VDR expression of about 71% in nuclear and 55% in cytoplasmic stain (p<0.05) in all the adenoma cells.

In qRT-PCR analysis, VDR mRNA expression was also reduced in 93% of adenoma cells with a fold change value of 0.36 ± 0.29 compared to normal parathyroid tissue cells (p<0.001; Figure-3A). The frequency of VDR under-expression was not correlated with indices of disease (albumin adjusted calcium, intact PTH, 25-hydroxyvitamin D or adenoma weight).

**Calcium Sensing Receptor (CaSR) Expression:** All the parathyroid adenomas showed membranous positivity similar to normal parathyroid tissues, but the mean proportion of positive cells was 56.3 ± 15.9% compared to 78 ± 10.3% in normal parathyroid tissue cells (p<0.05, figure-1B). Staining intensity was 1+ in 19 (46%), 2+ in 15 (37%) and 3+ in 7 (17%) adenomas. All the normal parathyroid tissue cells showed 3+ membranous positivity (figure-4).

The CaSR mRNA expression was reduced in 90% of the adenoma cells with a mean fold change of 0.45 ± 0.35 compared to normal parathyroid tissue cells (p<0.001; Figure-3A). There was no significant relationship of CaSR mRNA expression to disease indices (corrected calcium, intact PTH, 25-hydroxyvitamin D or adenoma weight).

**Cyclin D1 (CD1) Expression:** All the parathyroid adenomas showed cytoplasmic positivity, while normal parathyroid tissues showed intense nuclear as well as cytoplasmic positivity. The mean proportion
of positive cells was 76.2 ± 11.4% compared to 66.5±13.1% in normal parathyroid tissues (p<0.05; Figure-1B). Stain intensity was 1+ in 18 (44%), 2+ in 14 (34%) and 3+ in 9 (22%) adenomas (Figure-5). Normal parathyroid tissue samples showed 2+ to 3+ staining positivity for CD1.

The CD1 mRNA expression was increased in 85.4% of the adenomatous compared to normal parathyroid tissue cells with a mean fold change of 9.45 ± 8.86 (p<0.001; Figure-3B). The frequency of CD1 over-expression was related to the adenoma weight (r=0.715; p<0.001; Figure-6) but not with albumin adjusted calcium, intact PTH, or 25-hydroxyvitamin D.

**Parathyroid Hormone (PTH) Expression:** All the parathyroid adenoma cells showed diffuse cytoplasmic positivity in 83.3 ± 10.2% cells compared to 64.5 ± 11.2% of normal parathyroid tissues (p<0.05). Stain intensity was 1+ in 15 (37%), 2+ in 17 (41%) and 3+ in 9 (22%) adenomas. Staining intensity of PTH in normal parathyroid tissue samples were 2+ to 3+.

An increased expression of PTH mRNA was seen in all the parathyroid adenoma cells with variable fold change. The mean PTH mRNA fold change was 17.36 ± 14.6 (range 1.7 - 45.2 folds) in adenomatous compared to normal parathyroid tissue cells (p<0.001; Figure-1B). In addition, the PTH analyses in the tissues served as “internal controls”, thus validating our other observations in this study. The PTH mRNA over expression was related to intact PTH (r=0.864; p<0.001) but not with albumin adjusted calcium, 25-hydroxyvitamin D or adenoma weight.

**Discussion:**

This is the comprehensive study of relevant candidate gene expressions in parathyroid adenomas with symptomatic PHPT, as commonly seen in India. We found significantly reduced VDR and CaSR and increased CD1 and PTH expressions in parathyroid adenomas compared to normal parathyroid tissue. In addition, we demonstrated a positive relationship between CD1 over expression and adenoma weight. The major clinical manifestation in our patient group was bone pain and more than half of our patients
had bone involvement. Our previous study\textsuperscript{24} and a recent publication from China\textsuperscript{28} have also shown that
PHPT is still symptomatic with bone manifestations in this part of the world.

The down-regulation of VDR expression in parathyroid adenomas in patients with symptomatic
PHPT is similar to previous report of probably mild PHPT patients\textsuperscript{12}. Because the normal parathyroid
tissues in our samples showed both nuclear and cytoplasmic positivity, we separately assessed nuclear
and cytoplasmic VDR expression and found decreased expression of VDR at both cellular locations in
parathyroid adenomas. This is the first time such separate analysis has been performed as there is no
mention of cytoplasmic VDR assessment in previous studies\textsuperscript{13, 14} presumably considered as non-specific
staining\textsuperscript{12}. Nuclear localization of VDR with subsequent binding to vitamin D response elements
(VDREs) in target genes is essential for vitamin D biologic activity. The balance between the cellular
compartments (nuclear/cytoplasmic) is probably relevant to the regulatory actions of vitamin D. More
pronounced cytoplasmic VDR staining could indicate an “escape” of parathyroid adenoma cells from
homeostatic surveillance and growth control, especially if the regulatory mechanisms depended on ligand
activated receptor activity\textsuperscript{29}. Such alterations in cytoplasmic and nuclear VDR staining have also been
reported in other neoplasms\textsuperscript{30-32}.

Reduced CaSR expression was also found in adenomas compared to normal parathyroid tissue
cells and the magnitude of reduction was similar to previous reports on patients probably having mild
forms of PHPT\textsuperscript{12, 14-18}. Decreased CaSR expression in parathyroid adenomas would be consistent with a
less efficient control of PTH synthesis and secretion by calcium. The CaSR gene has VDREs in the 5’-
flanking region and is regulated by binding 1,25-dihydroxyvitamin D and VDR complex to VDREs\textsuperscript{33, 34};
thus the reduction in CaSR could be a consequence of reduced VDR. Previously it was suggested that
reduced CaSR expression could be the initiating event in parathyroid tumorigenesis\textsuperscript{35}. However, it seems
more likely that CaSR reduction is secondary to reduction in VDR expression since we found a greater
magnitude of reduction in VDR compared to CaSR expression. This could be explained by inhibition of
gene transcription, less stable mRNA or post-translational modification or gene silencing by hyper-
methylation in the promoter region as suggested by previous studies\textsuperscript{12, 13, 18}. It seems unlikely that hypercalcemia or elevated serum PTH, or adenomatous transformation itself due to increased proliferative activity are responsible for VDR and CaSR down-regulation\textsuperscript{12, 13, 18} since we found no correlation between adenoma weight and preoperative serum calcium, PTH or 25-hydroxyvitamin D levels in our patients.

Interestingly CD1 immunostaining was seen exclusively in the cytoplasm in all the samples without nuclear staining. In contrast, previous studies have observed both nuclear, and to a lesser degree, cytoplasmic staining\textsuperscript{19-23}. Over expression of CD1 both in nuclear and cytoplasmic compartments has been reported in many human malignancies. CD1 plays an important role in proliferation and differentiation, and a shift between nucleus and cytoplasm is necessary to regulate smooth passage across different phases of the cell cycle. Cytoplasmic staining for CD1 occurs during G\textsubscript{1} to S transition of the cell cycle, while nuclear staining is visualized only in G\textsubscript{1} phase\textsuperscript{36}. CD1 expression is lowest during S phase\textsuperscript{37}, perhaps explaining why we observed low intensities of CD1 in most of the adenomas comparing to normal parathyroid tissue.

We found over expression of CD1mRNA in 85\% of the parathyroid adenomas in comparison to 20-40\% reported previously\textsuperscript{19-23}. Of the four genes (VDR, CaSR, CD1 and PTH) analyzed in this study, only CD1 expression was significantly associated with parathyroid adenoma weight. Also, the increase in CD1 expression was numerically related to PTH level, but did not quite reach statistical significance. This rather robust over expression of CD1 could conceivably be related to the larger tumors, higher PTH levels, and more severe clinical expression of the disease in this part of the world. Since we did not systematically examine the “upstream” gene expressions (as was reported in a previous study\textsuperscript{22}) either in this or our previous study\textsuperscript{12}, the potential role of over expression of CD1 in parathyroid adenoma size or differential expression of the disease requires further investigation.

There were certain limitations such as relatively small sample size, lack of comparable data from patients with mild PHPT, a phenotype rarely seen in India, smaller number of normal
parathyroid tissue (n=10), and our inability to measure serum 1,25-dihydroxyvitamin D levels for lack of resources. Nevertheless, the strength our study is the simultaneous analysis of 4 relevant genes and demonstration of a similar magnitude of reductions in VDR and CaSR in moderate severe PHPT just as in the cases reported probably having mild form of PHPT\(^{12}\). Our study results also suggest that the mechanisms underlying parathyroid tumorigenesis and their growth behavior are probably different.

In conclusion, we have demonstrated reduced VDR and CaSR expression and increased CD1 and PTH by immunohistochemistry, and confirmed with the respective mRNA analyses in parathyroid adenomas. In addition, we found that over expression of CD1 correlated with the weight of parathyroid adenoma. Further investigations of the pathway of these genes with larger sample size could help better understand the pathogenesis and progression of disease in patients with severe PHPT and unravel potential new therapeutic targets.
Declaration of interest: The authors declare that there is no conflict of interest

Funding: This work was partially supported by a grant from the Indian Council of Medical Research (ICMR), New Delhi India (IRIS ID No. 2009-04680).

Acknowledgments: We thank Dr Rajesh Khadgawat, Additional Professor, Department of Endocrinology, All India Institute of Medical Sciences (AIIMS), New Delhi, and Dr K Rajagopal from the Institute of Microbial Technology (IMTECH), Chandigarh, for their helpful discussions.
References:


7. DeLuca HF & Zierold C. Mechanisms and functions of vitamin D. *Nutr Rev* 1998 **56** S4-10; discussion S 54-75.


Legends -

Figure 1: Bar diagrams representing immunostaining positive cells indicating (A) VDR expression in nucleus and cytoplasm (B) CaSR, CD1 and PTH expression in parathyroid normal and adenoma tissues respectively. All values are expressed as mean±SD, * p<0.05.

Figure 2: Microphotographs showing localization of VDR expression in tissue sections of parathyroid gland. (1) Normal, (2), (3) and (4) represent intensity score in adenoma as 1+, 2+ and 3+ respectively. Fig.2 (2) is in 10X and other figures are in 20X magnification.

Figure 3: Bar diagrams showing qRT-PCR analysis of (A) VDR and CaSR, (B) CD1 and PTH expression in normal parathyroid glands and adenomas.* p<0.001.

Figure 4: Microphotographs showing CaSR expression in tissue sections of parathyroid gland. (1) Normal, (2) (3) and (4) represent intensity score in adenoma as 1+, 2+ and 3+ respectively. Fig.4 (2) is in 10X and other figures are in 20X magnification.

Figure 5: Microphotographs showing CD1 expression in tissue sections of parathyroid gland. (1) Normal, (2) (3) and (4) represent intensity score in adenoma as 1+, 2+ and 3+ respectively. Fig.5 (1) is in 10X and other figures are in 20X magnification.

Figure 6: Correlation of CD1 mRNA with weight of parathyroid adenomas.
Table 1: Relevant Biochemical measurements of the PHPT patients and controls.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adenoma (n=41)</th>
<th>Control† (n=10)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected calcium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(8.6-10.2 mg/dl)</td>
<td>12.1 (1.8)</td>
<td>9.2 (0.4)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>11.7</td>
<td>9.2</td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(40-129 U/L)</td>
<td>336.0 (347.1)</td>
<td>80.3 (29.3)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>238</td>
<td>75.5</td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(0.5-1.2 mg/dl)</td>
<td>1.3 (1)</td>
<td>0.9 (0.7)†</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Intact PTH (pg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(15-65 pg/ml)</td>
<td>678.5 (727.7)</td>
<td>24.5 (11.7)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>275</td>
<td>21.0</td>
<td></td>
</tr>
<tr>
<td>25-hydroxyvitamin D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(11.1-42.9 ng/ml)</td>
<td>24.5 (19.1)</td>
<td>23.9 (8.4)</td>
<td>P=0.5</td>
</tr>
<tr>
<td></td>
<td>19.4</td>
<td>23.03</td>
<td></td>
</tr>
<tr>
<td>Adenoma weight (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.5*(3.2)</td>
<td>0.5 (0.1)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>3.29# (3.2)</td>
<td>0.5 (3.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data expressed as mean (SD) and median,*Arithmetic mean, †Geometric mean

†Seven normal parathyroid glands obtained from euthyroid surgery and 3 from patients undergoing single parathyroid adenoma removal.

† from the 3 PHPT patient only
Figure 1:

(A) 

(B) 

- Nuclear Immunostain positive cells (%) 
- Cytoplasmic 

- CaSR 
- CD1 
- PTH 

- Normal 
- Adenoma
Figure 3:

(A) Relative expression of VDR and CaSR in normal and adenoma tissues.

(B) Relative expression of CD1 and PTH in normal and adenoma tissues.
Figure 4:
Figure 5:
Figure 6:

\[ y = 2.0983x + 0.5009 \]

\[ R^2 = 0.5931 \]