Plasma 25-hydroxyvitamin D and not 1,25-dihydroxyvitamin D is associated with parathyroid adenoma secretion in primary hyperparathyroidism: a cross-sectional study

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Abstract

Background: Primary hyperparathyroidism (PHPT) is associated with reduced plasma 25-hydroxyvitamin D (P-25OHD) and usually increased plasma 1α,25-dihydroxyvitamin D (P-1,25(OH)2D). Parathyroid tissue expresses the vitamin D receptor and it is thought that circulating 1,25(OH)2D participate in the regulation of parathyroid cell proliferation, differentiation and secretion.

Aim: To investigate the relations between circulating levels of 1,25(OH)2D and 25OHD respectively and parathyroid adenoma weight (AW), plasma-parathyroid hormone (P-PTH) and PTH secretion expressed as P-PTH/AW.

Design: Cross-sectional study.

Material: One hundred and seventy-one consecutive hypercalcaemic caucasian patients aged 19–87 years (median 63, 84% females) with surgically proven parathyroid adenoma.

Results: A weak positive correlation was found between P-25OHD and P-1,25(OH)2D (r = 0.24, P < 0.005). AW depended on sex and body mass index. Following adjustment, it was correlated positively to P-PTH, calcium (Ca) and alkaline phosphatase (AP) and inversely to plasma phosphate in a multiple regression model. AW was not associated with vitamin D metabolites. Preoperative P-PTH correlated positively to plasma levels of Ca and AP, but inversely to phosphate and 25OHD (P < 0.001) levels. P-PTH was not associated with P-1,25(OH)2D (P = 0.65). The P-PTH:AW ratio correlated inversely to P-25OHD (P < 0.05), but showed no relations to plasma levels of Ca, phosphate or 1,25(OH)2D (P = 0.22).

Conclusion: In this material, low levels of 25OHD were related to higher levels of P-PTH and higher PTH:AW ratios in patients with PHPT suggesting that vitamin D deficiency increase PTH secretion activity. Neither PTH secretion nor AW was associated with circulating levels of 1,25(OH)2D.

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P-25OHD may participate in the regulation of parathyroid cell proliferation, differentiation and secretion. This is further corroborated by the finding of increased 25-hydroxyvitamin D₃ 1α-hydroxylase and reduced 25-hydroxyvitamin D₂ 24-hydroxylase expression in PAs (19). It has recently been documented that PHPT patients have reduced plasma levels of 25OHD independent of age, sex and season (20). Furthermore, based on sporadic P-25OHD measurements, it has been suggested that chronic vitamin D deficiency may accelerate PA growth and PTH secretion and thereby aggravate bone turnover and bone loss (14, 21, 22).

In a cross-sectional design, we investigated possible relations between the two above-mentioned vitamin D metabolites (P-1,25(OH)₂D and P-25OHD) and parathyroid adenoma weight (AW), plasma-parathyroid hormone (P-PTH) and PTH secretory activity respectively in 172 patients with surgically proven PA in order to disclose the pathophysiological importance of the two circulating metabolites for adenoma size and PTH secretion. PTH secretory activity was assessed as the ratio of P-PTH to AW taking potential confounders into account.

**Materials and methods**

**Materials**

Figure 1 depicts the study profile and Table 1 characterizes the included patients. From January 1994 to January 2003, we investigated a total of 357 consecutive caucasian patients with the suspicion of PHPT, because of hyperparathyroid hypercalcaemia. Hypercalcaemia was defined by elevated albumin adjusted plasma calcium (Ca) (>2.52 mmol/l) and hyperparathyroidism or high normal (upper one-third of reference range) of plasma intact parathyroid hormone (P-PTH > 5 pmol/l). The upper one-third of the normal range was included, since the normal range for P-PTH depends on vitamin D status in the reference population (23, 24). During the screening phase, we excluded ten patients because of suspicion of familial hypocalciuric hypercalcaemia due to a Ca/creatinine clearance rate <0.01 or a clinical significant mutation in the CaSR gene. Ten were excluded because of MEN1 (n = 7), MEN2a (n = 1) or isolated familial PHPT (n = 2). Among the remaining 337 PHPT patients, 249 underwent neck exploration and 197 (79%) had a PA removed. Parathyroid AW was available in 171 of the patients, all of whom were normocalcaemic postoperatively. These patients constitute the final material for the present investigation. The histological diagnosis was made from frozen section of the removed pathological parathyroid tissue. All removed parathyroid tissue was re-evaluated on paraffin-embedded sections by the same pathologist (F Melsen). The histological adenoma diagnosis was based on an enlarged gland with a confluence of dense parathyroid cells, no or sparse stromal and cyttoplasmatic fat, and often a rim of normal parathyroid tissue outside the adenoma (25). Among the remainder, 26 (10%) had hyperplasia and the histological classification was uncertain in 26 (10%) of the patients. Double adenomas were removed in four patients. The weights of the double adenomas were added in the analysis.

We collected preoperative data as part of a standardized protocol aimed at confirming the diagnosis and assessing possible complications. The database was accepted by the Danish Data Protection Agency.

**Methods**

We measured PA wet weight to the nearest milligram shortly after removal using an electronic scale.

We measured plasma and urinary Ca, albumin and creatinine by standard laboratory methods. We corrected total plasma Ca for individual variations in albumin by the equation,

\[
\text{adjusted plasma Ca (mmol/l)} = \frac{\text{plasma Ca total (mmol/l)} - 0.00 086 \times (6.50 - \text{plasma albumin (\mu mol/l)})}{1}
\]

We measured P-PTH by an IMMULITE automated analyser (Diagnostic Products Corporation, Los Angeles, CA, USA). The coefficient of variation (CV) in our lab. was less than 7%. We measured P-25OHD by an equilibrium RIA procedure (DiaSorin, Inc., Stillwater, MN, USA) with inter- and intraassay CV values of 13 and 10% respectively. P-1,25(OH)₂D was measured by RIA (Nichols Institute, San Clemente, CA, USA) with inter- and intraassay CV values of 11 and 11% respectively. Cross-reaction with 25OHD is 0.001%. We measured alkaline phosphatase (AP) spectrophotometrically using an automated instrument (Hitachi 917, Roche). The total CV was less than 8%.
Patient (Pt) creatinine clearance rate was calculated from the plasma creatinine and the 24-h renal excretion of creatinine. All measurements were accredited by DANAK (Skovlunde, Denmark).

The activity of the PA was estimated as the ratio of P-PTH to parathyroid AW, i.e. the P-PTH level obtained by a certain volume of PA tissue.

**Statistical analysis**

We expressed basic variables by their medians and ranges and assessed between group differences by the Mann–Whitney test. We used parametric statistics and multiple linear regressions following logarithmic transformation of data when necessary to assess relations between variables. The analysis was performed with backward stepwise estimation with a significance level for the removal of 0.05. We also used a hierarchic multiple linear regression model with preselected inclusion of either 25OHD or 1,25(OH)2D in order to compensate for colinearity between the two vitamin D metabolites. Statistical analyses were performed with STATA version 8.2 software (StataCorp., College Station, Texas, USA).

**Results**

**Baseline characteristics**

The clinical characteristics of the 171 patients with PHPT and PA are given in Table 1 together with locally established laboratory reference values. All patients were hypercalcaemic and 85% had elevated P-PTH compared with a normal reference population including vitamin D-insufficient, as well as vitamin D-sufficient individuals. All had P-PTH above 5 pmol/l. The PA patients had reduced P-25OHD compared with controls during both summer (72% \(P < 0.001\)) and winter (11%, \(P < 0.05\)). The percentage of patients with 25OHD below 50 and 75 nmol/l was 79 and 96% respectively compared with 35% \(P < 0.001\) and 60% \(P < 0.001\) of the controls. P-1,25(OH)2D was elevated in 29% \(P < 0.001\) and reduced in 3% \(P > 0.74\). Well, over half (56%) of the patients had elevated plasma AP (P-AP). The renal Ca excretion was increased in 56% and the endogenous creatinine clearance rate was reduced in 35%.

**Relation between vitamin D metabolites**

P-1,25(OH)2D correlated positively to plasma ln(25OHD) \((r = 0.24, P < 0.005; \text{Fig. 2})\). Overall, only 6% of the variation in P-1,25(OH)2D could be explained by variations in ln(P-25OHD).

**Parathyroid adenoma weight and vitamin D metabolites**

Table 2 explores the influence of age, sex, body mass index (BMI) and renal function on AW. Age did not influence AW. There was a significant effect of sex with a larger number of females with small PA. High BMI was associated with higher AW. AW was not related to renal function.

Table 3 gives unadjusted and multiple adjusted relationships between AW and biochemical variables related to Ca and vitamin D metabolism. Larger adenomas were positively associated with higher plasma levels of 25OHD and 1,25(OH)2D.
PTH, Ca and AP and lower levels of phosphate. In the unadjusted analysis, P-25OHD showed an inverse association with A W. However, when adjusted for covariates, the association became insignificant. A W was not related to plasma 1.25(OH)2D in any of the models used. Exclusion of three large PAs weighing 12.9, 25.5 and 26.5 g, or exclusion of three small adenoma (< 0.01 g), or exclusion of four double adenomas did not change these associations (data not shown).

**P-PTH and vitamin D metabolites**

Table 4 gives unadjusted and multiple adjusted relationships between P-PTH and biochemical variables related to Ca and vitamin D metabolism. In the unadjusted analyses, P-PTH correlated positively to P-Ca and P-AP and inversely to plasma phosphate and P-25OHD. There was no significant correlation to P-1,25(OH)2D. Adjustment for age, sex, BMI and creatinine did not change these results. In a multiple backward regression analysis including age, sex, BMI, plasma Ca, plasma phosphate, plasma creatinine, P-25OHD and P-1,25(OH)2D, P-PTH depended (P<0.05) positively on plasma Ca and inversely on P-25OHD. The equation was

\[
\ln(P-\text{PTH}) = 5.71 \times \ln(\text{Ca}) - 0.36 \times \ln(25\text{OHD}) - 2.05, \quad R^2 = 0.51.
\]

**Parathyroid activity and vitamin D metabolites**

Parathyroid activity was estimated as the ratio of P-PTH to AW. Table 5 gives simple and multiple adjusted relationships between parathyroid activity and various variables related to Ca and vitamin D metabolism. In the unadjusted analysis, plasma phosphate correlated positively with the P-PTH:AW ratio. No relationship was found to plasma levels of Ca or vitamin D metabolites. In the adjusted analysis also including plasma creatinine, the association with plasma phosphate became insignificant. However, in this analysis, P-25OHD correlated inversely to the PTH/AW ratio (P<0.05). No relationship was found between P-1,25(OH)2D and PTH/AW in any of the analyses. In a multiple backward regression analysis including age, sex, BMI, plasma Ca, plasma phosphate, plasma creatinine, P-25OHD and P-1,25(OH)2D, parathyroid activity depended (P<0.05) positively on the female sex, inversely on BMI and P-25OHD. The equation was

\[
\ln(\text{PTH/adenoma weight}) = 0.607 \times (\text{sex (females)}) - 0.036 \times \text{BMI} - 0.317 \times \ln(P-25\text{OHD}) + 4.718, \quad R^2 = 0.13
\]

P-1,25(OH)2D neither correlates to P-PTH levels in this model nor in any hierarchic multiple regression analysis with a primary inclusion of P-1,25(OH)2D with or without exclusion of P-25OHD.

**Discussion**

The present cross-sectional study has shown that declining levels of 25OHD are related to increasing levels of P-PTH and vitamin D metabolites. Lower levels of 25OHD are associated with increased P-PTH levels, which in turn may affect parathyroid activity and vitamin D metabolism. The relationship between P-PTH and vitamin D metabolites is complex and influenced by various factors, including age, sex, BMI, and renal function. Further studies are needed to fully understand the mechanisms behind these associations.
Table 3 Simple linear regression and adjusted multiple regression analysis (partial correlation coefficients) of relations between parathyroid adenoma weight (AW) and various clinical indices of disease severity in patients with primary hyperparathyroidism (PHPT).

<table>
<thead>
<tr>
<th>AW (g) (ln)</th>
<th>Simple linear regression</th>
<th>Adjusted for age, sex, BMI and P-creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>r</td>
</tr>
<tr>
<td>P-PTH (pmol/l)</td>
<td>171</td>
<td>0.49</td>
</tr>
<tr>
<td>P-Ca (mmol/l)</td>
<td>171</td>
<td>0.48</td>
</tr>
<tr>
<td>P-phosphate (mmol/l)</td>
<td>161</td>
<td>-0.35</td>
</tr>
<tr>
<td>P-25OHD (mmol/l)</td>
<td>161</td>
<td>-0.17</td>
</tr>
<tr>
<td>P-1.25(OH)2D (pmol/l)</td>
<td>150</td>
<td>0.01</td>
</tr>
<tr>
<td>P-AP (U/l)</td>
<td>161</td>
<td>0.27</td>
</tr>
</tbody>
</table>

levels of P-PTH and higher PTH secretion activity (PTH:AW ratio) in patients with PHPT caused by PA. There was no relation between P-25OHD and AW after adjustment for age, sex, BMI and renal function. This suggests that vitamin D deficiency mainly increases PTH secretion activity in existing adenomas. Our findings could not reproduce an effect of vitamin D insufficiency on adenoma growth previously reported based on point estimates of P-25OHD (14). Neither PTH secretion nor AW was associated with circulating levels of 1,25(OH)2D.

The parathyroid glands express VDR and 1,25(OH)2D is thought to inhibit parathyroid tissue growth and secretion (11, 18) in part by increasing the expression of the CaSR (2, 12). The inhibitory effect of 1,25(OH)2D on PTH secretion is well established in patients with end-stage renal failure and secondary hyperparathyroidism (26). However, the present study has failed to show any relationship between P-1,25(OH)2D and adenoma size or secretion in PHPT patients.

One explanation for the lack of effect of 1,25(OH)2D on PTH secretion in our study could be that the expression of VDR and CaSR is reduced in PA cells (13–15) in combination with the sparse increase in P-1,25(OH)2D observed in PHPT. The possible partial resistance of PA cells to 1,25(OH)2D is supported by in vitro studies showing that 1,25(OH)2D inhibits cell proliferation and apoptosis in secondary but not in PHPT, suggesting that 1,25(OH)2D may reduce gland mass in the former but not in the latter condition (11). However, in a recent case report, Kinoshita et al. (27) showed that ectopic 1,25(OH)2D production in sarcoidosis may reduce P-PTH in a patient with PHPT.

Table 4 Simple linear regression and adjusted multiple regression analysis (partial correlation coefficients) of relations between plasma-parathyroid hormone (P-PTH) and various indices of calcium (Ca) and vitamin D metabolism in primary hyperparathyroidism (PHPT).

<table>
<thead>
<tr>
<th>P-PTH (pmol/l) (ln)</th>
<th>Simple linear regression</th>
<th>Adjusted for age, sex, BMI and P-creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>r</td>
</tr>
<tr>
<td>P-Ca (mmol/l)</td>
<td>171</td>
<td>0.62</td>
</tr>
<tr>
<td>P-phosphate (mmol/l)</td>
<td>161</td>
<td>-0.24</td>
</tr>
<tr>
<td>P-25OHD (mmol/l)</td>
<td>161</td>
<td>-0.31</td>
</tr>
<tr>
<td>P-1.25(OH)2D (pmol/l)</td>
<td>150</td>
<td>-0.14</td>
</tr>
<tr>
<td>P-AP (U/l)</td>
<td>160</td>
<td>0.53</td>
</tr>
</tbody>
</table>
Table 5 Simple linear regression and adjusted multiple regression analysis (partial correlation coefficients) of relations between plasma-parathyroid hormone (P-PTH)/parathyroid adenoma weight (AW) and various indices of calcium (Ca) and vitamin D metabolism in primary hyperparathyroidism (PHPT).

<table>
<thead>
<tr>
<th></th>
<th>Simple linear regression</th>
<th>Adjusted for age, sex and BMI, creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>r</td>
</tr>
<tr>
<td>P-Ca (mmol/l)</td>
<td>171</td>
<td>-0.06</td>
</tr>
<tr>
<td>P-phosphate (mmol/l)</td>
<td>161</td>
<td>0.20</td>
</tr>
<tr>
<td>P-25OHD (mmol/l)</td>
<td>161</td>
<td>-0.04</td>
</tr>
<tr>
<td>P-1,25(OH)2D (pmol/l)</td>
<td>150</td>
<td>-0.11</td>
</tr>
</tbody>
</table>

The observed inverse relationship between P-25OHD and adenoma in the univariate linear regression is in accordance with the results reported by Rao et al. (14). However, after adjustment for age, sex and BMI, there was no significant relation between 25OHD and AW. This may be explained in several ways. P-25OHD is a point estimate and may not reflect individual average vitamin D status during adenoma growth. Furthermore, the observed positive effects of BMI on adenoma size, which is in accordance with a recent metaanalysis (34) showing that body weight and BMI are increased by 0.3 s.d. (95% CI 0.19–0.40) in PHPT compared with normocalcaemic controls, may interact. At present, it is unknown whether PHPT leads to increased body weight through stimulation of lipogenesis (35) or obesity facilitates the development of hyperparathyroidism through deposition and catabolism of vitamin D metabolites in the adipose tissue (36–38). The finding that the increase in body weight appears to antedate the development of hypercalcaemia favours the last mechanism (39). In both situations, adiposity and adenoma development may build up over time inducing a positive relationship between the variables in a cross-sectional study.

Our study supports the concept that treatment with vitamin D may have some benefits in the preoperative management of PHPT patients and, perhaps in combination with an antiresorptive agent, may be valuable as long-term treatment in unoperated patients. However, large-scale randomized trials are warranted to assess possible benefits and risks of such a treatment.

In conclusion, this study showed that in PHPT plasma levels of 1,25(OH)2D correlates positively to P-25OHD. Furthermore, decreased levels of P-25OHD are related to increased levels of P-PTH and higher PTH:AW ratios suggesting that vitamin D deficiency increase PTH secretion activity without demonstrable effect on adenoma growth. Neither PTH secretion nor AW was associated with circulating levels of 1,25(OH)2D.
References


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