World Journal of Surgery

Correlations between Vitamin D Status and Biochemical/Clinical and Pathological Parameters in Primary Hyperparathyroidism

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Abstract

Background: To determine the prevalence of vitamin D deficiency and the effects of vitamin D status on parathyroid adenoma weight, clinical and biochemical indices in patients with primary hyperparathyroidism (pHPT) were studied.

Methods: Eighty patients with pHPT who underwent surgical treatment and in whom the presence of parathyroid adenoma were confirmed histopathologically were studied retrospectively from recorded data files. Patients were divided into three groups: patients with 25-hydroxyvitamin D (25-OHD) concentrations < 15 ng/ml (group 1, n = 44), patients with 25-OHD concentrations 15–25 ng/ml (group 2, n = 9), and patients with 25-OHD concentrations > 26 ng/ml (group 3, n = 27). Serum calcium, phosphate, alkaline phosphatase, creatinine, and albumin levels and urinary calcium excretion were determined by auto-analyzer. Plasma 25-OHD and parathyroid hormone (PTH) levels were determined by immunoradiometric assay using commercially available kits.

Results: No statistically significant differences were observed with respect to serum calcium, phosphorus, albumin, and creatinine concentrations between these groups. Serum PTH, alkaline phosphatase concentrations, urinary calcium excretion, parathyroid adenoma weight, and postoperative sixth month PTH concentrations were significantly higher in group 1 patients than in group 2 and group 3 patients. Significant correlations were observed between parathyroid adenoma weight and serum 25-OHD concentrations ($r = -0.348$, $P = 0.020$); parathyroid adenoma weight and urinary calcium excretion ($r = 0.348$, $P = 0.021$). Multiple regression analysis revealed that parathyroid adenoma weight, serum 25-OHD, and preoperative PTH concentrations correlated independently and significantly with postoperative sixth month PTH concentrations.

Conclusions: Vitamin D deficiency leads to more severe bone disease, increased parathyroid tumor growth, and delayed postoperative recovery of parathyroid function in patients with primary hyperparathyroidism.

Primary hyperparathyroidism (pHPT) is a common endocrine disease.[1] That a tenfold increase in the incidence of the disease occurred after its association with nephrolithiasis was established 1934. The introduction of routine automated chemical analysis and intact parathyroid hormone (PTH) measurements has led to a large increase in the proportion of asymptomatic patients.[2]

The clinical presentation of pHPT is also influenced by a disturbance in vitamin D metabolism. A relationship is suggested between clinical severity of hyperparathyroid-

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ism and vitamin D deficiency.[3,4] Vitamin D deficiency may also effect the serum calcium concentrations in primary hyperparathyroidism, leading to normocalcemic forms of the disease.[3,5,6] The present study was designed to determine the prevalence of vitamin D deficiency among our patients with pHPT and to evaluate the effect of vitamin D status on clinical presentation, biochemical indices, parathyroid adenoma weight, and postoperative PTH levels in patients with primary hyperparathyroidism.

MATERIALS AND METHODS

Patients

From December 2000 through December 2004, we studied 80 patients with pHPT who underwent surgical treatment in whom the presence of parathyroid adenoma was confirmed histopathologically. Patients with increased serum creatinine concentrations and those with multiple endocrine neoplasia or multiglandular disease were excluded. None of the patients had bone cysts on plain radiograms of long bones. In all patients, serum calcium, phosphate, albumin, creatinine, alkaline phosphatase, PTH, 25-hydroxyvitamin D_3 (25-OHD) levels, and 24-hour urinary calcium excretion were measured preoperatively. Serum calcium concentration was adjusted for serum albumin. Of the 80 patients, 26 (28.7%) were normocalcemic at presentation (i.e., adjusted serum calcium concentrations lower than the upper limit of normal: < 10.5 mg/dl). In normocalcemic and hypercalcemic patients, indications for parathyroidectomy were established according to the NIH 1990 and 2002 guidelines.[2] Hypercalciuria/nephrolithiasis and a T score below –2.5 at any site (the lumbar spine, the hip, the distal third of the radius) on bone densitometry were the most commonly identified indications for parathyroidectomy in normocalcemic patients. The weight of the excised parathyroid adenoma was measured. Postoperative sixth month PTH levels of the patients when all of them were normocalcemic were also determined. The patients were divided into three groups according to their preoperative 25-OHD levels; group 1 (n = 44) patients, 25-OHD levels below 15 ng/ml, group 2 (n = 9), 25-OHD levels 15–25 ng/ml, and group 3 (n = 27), 25-OHD levels > 26 ng/ml. Among the patients who were normocalcemic at presentation, 10 were in group 1 (22.7%); 3, in group 2 (33.3%), and 13, in group 3 (48%). In preoperatively normocalcemic patients with 25-OHD levels < 15 ng/ml, we tried treatment with oral vitamin D, but this treatment led to an increase in serum calcium and/or urinary cal-

cium concentrations. Therefore preoperative treatment with vitamin D was unsuccessful for normocalcemic primary hyperparathyroid patients in association with vitamin D deficiency. After the operation, severe symptomatic hypocalcemia was treated with parenteral calcium and an oral 1,25-dihydroxy vitamin D_3 (1,25-OH D_3) preparation. After discharge, these patients were treated with oral calcium and 1,25-OH D_3 preparations at doses modified in relation to serum calcium concentration. At the 6th month postoperatively, all patients were normocalcemic, and 15 were still taking oral calcium and 1,25-OH D_3 preparations.

Laboratory Tests

After a 12-hour fasting period, blood samples were collected from patients. The blood samples were centrifuged at 1500 × g for 10 minutes and plasma was separated. Serum calcium, phosphate, alkaline phosphatase, creatinine and albumin levels, and urinary calcium excretion were determined by auto-analyzer (Cobas Integra 800, Roche Diagnostics, Basel, Switzerland). Plasma 25-OHD and PTH levels were determined by immunoradiometric assay using commercially available kits—the PTH-120 min-IRMA kit and the 25(OH)- vitamin D_3 RIA CT kit, respectively (BioSource Europe S.a., Nivelles, Belgium). Laboratory ranges for 25-OHD were 6–46 ng/ml.

Normal ranges of biochemical parameters were 8.5–10.5 mg/dl for serum calcium, 2.7–4.5 mg/dl for serum phosphate, 90–260 U/l for serum alkaline phosphatase, 0.6–1.5 mg/dl for serum creatinin, 3.5–5 g/dl for serum albumin, and 10–65 pg/ml for serum PTH concentrations.

Statistical Methods

Data were analyzed using SPSS 11.0 for Windows. Comparisons of data were done by analysis of variance (ANOVA) and the chi-squared test. Correlation and regression (simple and multiple) analyses were done by Pearson linear regression tests. Results were expressed as mean ± SD, and $P < 0.05$ was accepted as significant.

RESULTS

The mean age of the patients in the study group was 54 ± 13 years (Table 1). The female to male ratio was

Table 1.
Demographic and biochemical parameters of the patients (whole group)

Parameter	Mean ± SD	Range
Age (years)	54 ± 13	29–92
Female/male	75/5	—
Serum calcium (mg/dl)	11.2 ± 0.8	9.5–13.2
Serum phosphate (mg/dl)	2.2 ± 0.4	1.6–3.5
Alkaline phosphatase (U/l)	324 ± 255	171–1300
PTH pg/ml	436 ± 432	80–2578
25-OHD (ng/ml)	23 ± 20	5–80
Urinary calcium excretion (mg/day)	661 ± 216	352–1100
Albumin (g/dl)	3.7 ± 0.5	3–4.2
Serum creatinine (mg/dl)	0.9 ± 0.3	0.4–1.7
Parathyroid adenoma weight (mg)	1231 ± 930	110–4400
Postoperative PTH (pg/ml) (6th month)	54 ± 13	10–163

PTH: parathyroid hormone; 25-OHD: 25-hydroxyvitamin D.

15:1. The clinical and biochemical characteristics of the groups are summarized in Table 2. We divided patients into three groups according to the severity of vitamin D deficiency:

- group 1: patients with severe deficiency with 25-OHD levels < 15 ng/ml (n = 44)
- group 2: patients with moderate deficiency with 25-OHD levels 15–25 ng/ml (n = 9)
- group 3: patients with 25-OHD levels > 22 ng/ml (n = 27)

Group 1 patients were significantly older than patients in group 3.

No statistically significant differences were observed with respect to serum calcium, phosphorus, albumin, and creatinine concentrations between these groups. However preoperative serum PTH, alkaline phosphatase concentrations, urinary calcium excretion, parathyroid adenoma weight and postoperative sixth month PTH concentrations were significantly higher in group 1 patients than in their group 2 and group 3 counterparts (Table 2). No statistically significant differences were observed between group 2 and 3 with respect to these variables. The number of the normocalcemic patients was significantly higher in group 3 than in group 1. Severe symptomatic hypocalcemia developed more commonly in group 1 patients compared with group 2 and 3, leading to significantly prolonged hospitalization in group 1 patients, as expected. No statistically significant difference was observed between group 2 and 3 patients with respect to duration of hospitalization.

Significant correlations were observed between the age of the patients and serum 25-OHD concentrations ($r = -0.374$, $P = 0.012$); age and urinary calcium excretion ($r = 0.376$, $P = 0.012$), parathyroid adenoma weight and serum 25-OHD concentrations ($r = -0.348$, $P = 0.020$), and parathyroid adenoma weight and urinary calcium excretion ($r = 0.348$, $P = 0.021$) (Table 3).

Postoperative sixth month blood tests revealed that all patients were normocalcemic. Serum PTH concentrations were significantly and positively correlated with parathyroid adenoma weight ($r = 0.720$, $P < 0.001$), preoperative alkaline phosphatase concentrations ($r = 0.379$, $P = 0.001$), and preoperative PTH concentrations ($r = 0.378$, $P = 0.001$) (preoperative urinary calcium excretion ($r = 0.281$, $P = 0.012$); there was a negative correlation between serum PTH and serum 25-OHD concentrations ($r = -0.599$, $P < 0.001$) (Table 4). Multiple regression analysis revealed that parathyroid adenoma weight, serum 25-OHD levels, and preoperative PTH concentration correlated independently and significantly with postoperative sixth month PTH concentrations (Table 5).

DISCUSSION

Primary hyperparathyroidism and vitamin D insufficiency are relatively common disorders. A previous study indicated a 2.2% prevalence rate of coexistent primary hyperparathyroidism and vitamin D insufficiency in a 229 consecutive patient population.[7] Silverberg et al.,[4] indicated that in 66 (53 %) of 124 patients with mild hyperparathyroidism the levels for 25-OHD were lower than 20 ng/ml. More than 30% of different groups of subjects in Turkey have vitamin D insufficiency.[8,9] Previous investigations indicated that vitamin D deficiency leads to more severe clinical forms of primary hyperparathyroidism and may have positive effects on parathyroid tumor growth.[3,10]

Vitamin D via its receptor has essential actions on parathyroid cells, inhibiting PTH secretion and parathyroid cell proliferation. Vitamin D deficiency and/or reduced expression of vitamin D receptor on parathyroid cells are reported to be associated with development of both secondary and primary hyperparathyroidism.[11,12] Carling et al.,[12] reported decreased vitamin D receptor mRNA levels in parathyroid adenomas and hyperplasias compared with normal glands. In addition, vitamin D receptor polymorphism is reported to be associated with an increased risk of parathyroid adenoma, influencing the level of receptor mRNA expression.[11] Therefore impaired

Table 2.

Comparison of biochemical findings between group 1 (*i.e.*, serum 25-OHD concentration < 15 ng/ml), group 2 (*i.e.*, serum 25-OHD concentration 15–25 ng/ml), group 3 (*i.e.*, serum 25-OHD concentration > 26 ng/ml)

Parameter	Group 1 n = 44	Group 2 n = 9	Group 3 n = 27
Age (years)	58 ± 12[a]	55 ± 12[a]	47.9 ± 13[b]
Serum calcium (mg/dl)	11.3 ± 0.8 [a]	10.9 ± 0.5 [a]	11.3 ± 0.9 [a]
Normocalcemic patients (n)*	10	3 [a]	13 [b,c]
Serum phosphate (mg/dl)	2.2 ± 0.4 [a]	2.2 ± 0.3 [a]	2.2 ± 0.3 [a]
Alkaline phosphatase (IU/l)	423 ± 256 [a]	203 ± 100 [b]	201 ± 83 [b]
PTH pg/ml	540 ± 468 [a]	279 ± 119 [b]	239 ± 149 [b]
25-OHD (ng/ml)	9.2 ± 3.6 [a]	23 ± 11 [b]	46 ± 18 [c]
Urinary calcium excretion (mg/dl)	711 ± 203 [a]	544 ± 118 [b]	528 ± 100 [b]
Albumin (g/dl)	3.6 ± 0.5 [a]	3.9 ± 0.6 [a]	3.7 ± 0.4 [a]
Serum creatinine (mg/dl)	0.9 ± 0.3 [a]	1.2 ± 0.3 [a]	0.9 ± 0.3 [a]
Parathyroid gland weight (mg)	1778 ± 899 [a]	615 ± 478 [b]	448 ± 242 [b]
Duration of hospitalization (day)	5 ± 1.5 [a]	2.8 ± 1.2 [b]	2.3 ± 0.5 [b]
Postoperative PTH (pg/ml) 6th month	71 ± 25 [a]	35 ± 19 [b]	28 ± 12 [b]

Values with different letters have significance according to ANOVA test.
*chi-squared: [a]$P > 0.05$, [b]$P < 0.05$, compared to group 1; [c]$P > 0.05$, compared to group 2.

Table 3.
Significant correlations between study parameters

Variable	r	P
PAW/25-OHD	−0.348	0.020
PAW/calcium excretion	0.348	0.021
Age/25-OHD	−0.374	0.012
Age/urinary calcium excretion	0.376	0.012

Table 4.
Significant correlations between postoperative PTH concentrations and study parameters

Variable	r	P
PGW	0.720	0.0001
Alkaline phosphatase	0.379	0.001
Preoperative PTH	0.378	0.001
25-OHD	−0.599	0.0001
Urinary calcium excretion	0.281	0.012

PGW: parathyroid glang weight

effects of active vitamin D, either by vitamin D deficiency or decreased receptor activity, can play a role for increased PTH secretion and cell proliferation in hyperparathyroidism. Rao *et al.*,[3] indicated a significant negative correlation between serum 25-OHD (the best parameter of vitamin D nutritional status) and parathyroid gland weight (the best parameter of parathyroid tumor cell number). In contrast Silverberg *et al.*,[4] and Yamashita *et al.*,[13] did not find such a significant relationship between parathyroid weight and 25-OHD status. Alternatively, low vitamin 25-OHD concentration reduces the calcemic response to PTH independent of 1,25 OHD deficiency and may lead to normocalcemic forms of the disease, thereby creating confusion and delay in diagnosis.[3]

In our study, all patients had histopathologically proven parathyroid adenoma other than parathyroid hyperplasia. This study group represents an appropriate model in which to study the effects of vitamin D insufficiency on parathyroid tumor weight and clinical parameters. In our series, 55% of the patients had 25-OHD concentrations

< 15 ng/ml. Our patients with lower vitamin D concentrations (group 1) had biochemical parameters indicating more severe disease. Serum alkaline phosphatase, PTH concentrations, and urinary calcium excretion were significantly higher in those patients, implying severe skeletal involvement and increased parathyroid tumor cell burden. It was previously shown that parathyroid tumor growth eventually stops when there is sufficient number of parathyroid cells to increase the PTH to achieve the new set-point for plasma calcium.[14] Predictably, parathyroid adenoma weight was significantly higher in patients with low vitamin D concentrations. Our results add additional clues to the studies pointing out the deleterious effects of vitamin D deficiency on disease severity and parathyroid tumor growth in primary hyperparathyroidism.[3,10,15] No significant difference was observed between serum calcium concentrations in group 1 and 2 patients. Twenty-six of 80 patients were normocalcemic at presentation according to the adjusted serum calcium concentrations. Interestingly, normocalcemia

Table 5.
Multiple linear-regression analysis between postoperative PTH levels and other variables in patients with pHPT

Dependent variable	Independent variable	Standard coefficients	T	P
Postoperative PTH	PGW	0.486	5.210	0.0001
	25-OHD	−0.230	−2.389	0.019
	Preoperative PTH	0.187	2.469	0.016

PGW: parathyroid glang weight

was more frequent in patients with 25-OHD concentrations > 26 ng/ml (group 3). That there were significantly increased numbers of normocalcemic patients in group 3 compared with vitamin D–deficient patients may reflect the presence of more severe disease in the vitamin D–deficient group. Normocalcemic forms of primary hyperparathyroidism unrelated to vitamin D deficiency have been described elsewhere. Silverberg and Bilezikian[6] hypothesized that vitamin D sufficiency in normocalcemic patients represents the earliest manifestation or preclinical phase of primary hyperparathyroidism. Maruani et al.,[5] identified 34 normocalcemic patients from among 178 patients with primary hyperparathyroidism. Serum 25-OHD concentrations were similar in the normocalcemic and hypercalcemic patients. In their study, normocalcemic and hypercalcemic patients were matched for serum PTH concentrations, age, and sex. When compared with PTH, age and sex-matched patients with hypercalcemia, normocalcemic patients with primary hyperparathyroidism had lower fasting urine calcium excretion and tubular calcium reabsorption, as well as lower values of markers of bone turnover and plasma 1,25-OHD and higher values of renal phosphate threshold. Therefore they suggested that normocalcemic primary hyperparathyroidism represents the bone and kidney resistance to the biological actions of PTH.

Alternatively, a typical biochemical profile, i.e., hypercalcemia in association with increased PTH concentration, can be obscured by coexisting vitamin D deficiency. Typical biochemical profile can be evident during/after vitamin D replacement therapy. Relatively older patients in group 1 in our study may indicate an increased frequency of vitamin D deficiency in older patients or, alternatively, may simply reflect the delay in diagnosis in patients with coexistent primary hyperparathyroidism and vitamin D deficiency. Age was significantly and negatively correlated with vitamin D status in our patients.

In our study, parathyroid adenoma weight correlated significantly and negatively with 25-OHD concentrations and positively with urinary calcium excretion. Rao et al.,[10] also indicated significant negative correlation and similar regression slopes between parathyroid adenoma weight and serum 25-OHD concentrations in Asian Indians and in whites and blacks in the United states. The significant correlation between parathyroid adenoma weight and urinary calcium excretion may reflect the effect of parathyroid adenoma mass on the severity of the disease.

Vitamin D deficiency may negatively influence the postoperative recovery of parathyroid function. In our patients, significantly prolonged hospitalization for postoperative severe hypocalcemia was observed in group 1 patients. Postoperative sixth month PTH concentrations when the operated patients were all normocalcemic were significantly higher and correlated significantly and independently with parathyroid adenoma weight. Dhillon et al.,[16] indicated elevated serum PTH levels in eucalcemic patients after parathyroidectomy for primary hyperparathyroidism. In their study, patients with renal dysfunction and osteomalacia were excluded. They suggested that, after secondary causes of hyperparathyroidism are excluded, a temporary resistance of the kidneys to PTH-mediated 1-alpha hydroxylation of 25-OHD may explain their finding. In our study group, ongoing unresolved vitamin D deficiency and/or more severe disease may contribute to increased PTH concentrations postoperatively. Unfortunately, postoperative 25-OHD concentration values were not available for all patients in our study.

We suggest that vitamin D deficiency in association with primary hyperparathyroidism influence both disease presentation and follow-up after parathyroidectomy. Kantorovich et al.,[7] indicated that bone mineral density increases in patients with coexistent vitamin D insufficiency and primary hyperparathyroidism, even in the short term after vitamin D replacement therapy despite persistence of hyperparathyroid state. In their study, vitamin D supplementation led to progressive hypercalcemia in one patient and hypercalciuria in three others. In our patients preoperative treatment with vitamin D led to an increase in serum and/or urine calcium concentrations. According to our results, patients with both vitamin D deficiency and primary hyperparathyroidism can be treated effectively and safely for vitamin D deficiency after parathyroidectomy.

We conclude that vitamin D deficiency leads to more severe bone disease, increased parathyroid tumor growth and delayed postoperative recovery in parathyroid func-

tion in patients with primary hyperparathyroidism from vitamin D deficient areas.

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