Vitamin D Status Affects Osteopenia in Postmenopausal Patients with Primary Hyperparathyroidism

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Abstract. Controversy still exists about whether vitamin D status is related to the severity of primary hyperparathyroidism (pHPT), although vitamin D insufficiency is frequent in pHPT. The present study was therefore performed to examine the relationships between vitamin D status and various parameters in 30 postmenopausal pHPT patients. BMD values were measured by dual-energy x-ray absorptiometry at the lumbar spine (L2–4), femoral neck (FN) and distal one third of the radius (Rad 1/3). Serum levels of 25 hydroxy-vitamin D3 [25(OH)D] and 1,25-dihydroxy vitamin D3 [1,25(OH)2D3] were 15.8 ± 3.5 µg/l and 69.3 ± 33.3 ng/l in pHPT patients, respectively. Serum levels of calcium and PTH seemed to be negatively correlated to serum 25(OH)D levels, although the differences were not significant. However, when subjects with the highest serum PTH levels (PTH>1000 pg/ml) were excluded from the analysis, the correlation was significant between serum 25(OH)D levels and PTH, indicating that vitamin D status affects the severity of pHPT when severe cases were excluded. In addition, serum levels of 1,25(OH)2D3 were significantly and negatively correlated to serum 25(OH)D levels. On the other hand, serum levels of 25(OH)D were significantly and positively correlated to BMD (Z-score) at the lumbar spine, but not at the radius and femoral neck; however, serum 25(OH)D levels were not correlated to the levels of any bone metabolic indices measured. Moreover, serum levels of 25(OH)D were not related to urinary calcium and the tubular reabsorption rate of phosphorus, and they were similar in groups with and without renal stones. In conclusion, vitamin D status seemed to be related to the severity of disease in postmenopausal patients with pHPT. In particular, the relationship between serum 25(OH)D level and BMD at the lumbar spine was predominant.

Key words: Primary hyperparathyroidism, Vitamin D, Bone mineral density, Osteoporosis

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VITAMIN D insufficiency or deficiency is not rare in the elderly, and the importance of vitamin D status has been recognized in bone and mineral metabolism. Active vitamin D suppresses the secretion of PTH from the parathyroid gland in a negative feedback manner, and several studies revealed that serum levels of 25-hydroxy-vitamin D3 [25(OH)D], the most clinically reliable indicator of vitamin D insufficiency or deficiency, is inversely correlated to serum PTH levels [1–3]. Moreover, severe vitamin D deficiency causes osteomalacia. Increased PTH by vitamin D insufficiency may increase bone turnover and bone loss, and several studies indicated that vitamin D insufficiency is related to reduced bone mineral density (BMD) and increased fracture risk [1, 4, 5].

Primary hyperparathyroidism (pHPT) is a relatively common endocrine disorder that causes secondary osteoporosis. Patients with pHPT have reduced BMD, especially at the cortical bone [6, 7]. Several studies suggested that pHPT was associated with an increased risk of vertebral and forearm fractures, and a subsequent decrease of fracture risk after parathyroidectomy [8–12], although our recent cross-sectional study sug-
gested that the threshold of BMD for vertebral fractures was lower, especially at radial bone in female pHPT patients [13]. As for bone geometry, our previous study using peripheral quantitative computed tomography in female pHPT patients suggested that an excess of endogenous PTH stimulated periosteal bone formation, which might partly compensate for a decrease in bone strength induced by low BMD [14].

As for vitamin D insufficiency, several studies indicated that vitamin D insufficiency is related to the severity of pHPT [15–18]. Rao et al. [16] reported that suboptimal vitamin D nutrition stimulates parathyroid adenoma growth, although other reports did not confirm this [17, 19, 20]. Several reports suggested that vitamin D receptor polymorphisms are related to the severity of pHPT [21, 22]. Moreover, vitamin D repletion in patients with pHPT improves the state of hypercalcemia and bone turnover [18], although Boudou et al. reported that pre-surgery 25(OH)D was predictive of post-operative hypocalcemia in pHPT patients [23]. However, numerous points remain unclear about the effects of vitamin D insufficiency on the severity and bone metabolism of pHPT.

The present study was therefore performed to examine the relationships between the status of vitamin D insufficiency and various indices including BMD in 30 postmenopausal pHPT patients.

Materials and Methods

Subjects

Thirty Japanese female patients diagnosed with pHPT in Kobe University Hospital participated in this study. All patients were postmenopausal. In all patients, abnormal parathyroid gland enlargement was successfully identified by at least two imaging techniques among ultrasonography, computed tomography, magnetic resonance imaging, or technetium sestamibi scintigraphy, and the biochemical data were compatible with pHPT. Patients whose diagnosis was ambiguous were excluded from the study. Moreover, familial hypocalciuric hypercalcemia was excluded, based on the low calcium/creatinine clearance ratio. The pHPT patients with drugs or other systemic diseases affecting bone metabolism were excluded from the study. Patients with pHPT were not separated by disease severity. The study was cross-sectional and approved by the ethical review board of Kobe University Hospital. All subjects agreed to participate in the study and gave informed consent.

Biochemical measurements

Serum and urinary chemistry determinations were performed by standard automated techniques. Serum chemistry was performed in daily routine assays. Urine was collected as the second void morning urine. Serum concentrations of intact PTH were measured by immunoradiometric assay (Allegro Intact PTH IRMA kit; Nichols Institute Diagnostics, San Juan Capistrano, CA; normal range, 10–65 ng/l) [24]. Serum levels of osteocalcin (OCN) or bone-type alkaline phosphatase (BAP) and urinary levels of deoxypyridinoline (Dpd) and aminoterminal telopeptide of type I collagen (NTX) were measured as previously described [24]. Serum 1,25(OH)_2D3 levels were measured using a radioreceptor assay kit, as previously described [25]. Serum 25(OH)D levels were measured by competitive protein binding assay, as previously described in detail [26].

BMD measurements by DXA

BMD values were measured by dual-energy x-ray absorptiometry (DXA) using QDR-2000 (Hologic Inc., Waltham, MA) at the lumbar spine (L_2–4), femoral neck (FN) and distal one third of the radius (Rad 1/3). Vertebrae with vertebral fractures or overt osteoarthrosis were excluded from BMD analysis, because these factors may increase BMD through artifacts. BMD was automatically calculated from the bone area (cm^2) and bone mineral content (BMC) (g) and expressed absolutely in g/cm^2. The Z-score is the number of SD by which a given measurement differs from the mean for a sex-, age-, and race-matched reference population. The coefficients of variation (precision) of measurements of the lumbar spine, femoral neck and radius were 0.9, 1.7 and 1.9%, respectively.

Statistical analysis

All data are expressed as the mean ± SD for each index. Statistical analyses were carried out using a computer program StatView IV (Abacus Concepts, Inc., Berkeley, CA). Comparisons of each group were made with the non-parametric Mann-Whitney U-test. Sim-
ple regression analyses were performed to assess the linear relationship between several parameters, and then Pearson’s correlation coefficients were calculated. Multiple regression analysis was performed to determine serum 25(OH)D or 1,25(OH)\textsubscript{2}D\textsubscript{3} levels were independently and significantly associated with BMD scores when BMI or PTH was considered. P values <0.05 were considered significant.

Results

Background data

Baseline indices are shown in Table 1 in 30 postmenopausal patients with pHPT. Five patients were diagnosed as having one or more vertebral fractures by X-ray examination. Five patients had renal stones when pHPT was diagnosed. Body height, weight and body mass index were 150.3 ± 6.0 cm, 50.9 ± 9.0 kg, and 22.5 ± 3.5 kg/m\textsuperscript{2}, respectively. Serum levels of calcium and PTH were elevated, which was compatible with pHPT. BMD and the Z-score (age-matched BMD) were lower in pHPT patients at the lumbar spine, femoral neck and distal radius. The extent of reduced BMD was predominant in the radius, and BMD at the lumbar spine and femoral neck was relatively preserved. Serum levels of non-specific alkaline phosphatase (ALP) were elevated. Serum levels of BAP and OCN were 59.3 ± 65.2 IU/l and 21.4 ± 30.0 µg/l, respectively. Urinary levels of Dpd and NTX were 13.1 ± 14.2 pmol/mol.Cr and 198.5 ± 246.2 µmol.BCE/mol.Cr, respectively. These data indicate that bone turnover was increased in pHPT patients.

Vitamin D status in pHPT patients

Mean serum levels of 25(OH)D and 1,25-dihydroxy vitamin D\textsubscript{3} [1,25(OH)\textsubscript{2}D\textsubscript{3}] were 15.8 ± 3.5 µg/l and 69.3 ± 33.3 ng/l in pHPT patients, respectively. When patients with pHPT were separated into three groups based on serum 25(OH)D levels, the distribution of 25(OH)D levels was as in Fig. 1. The number of patients with putative vitamin D insufficiency (25(OH)D <20 µg/l) was relatively high (27/30).

Relationship between serum 25(OH)D levels and various indices in pHPT patients

The correlation coefficients between serum levels of 25(OH)D and various parameters are shown in Table 2. Serum 25(OH)D levels were not related to age or body size indices. Moreover, they were not correlated to urinary calcium excretion and the phosphorus reab-

Table 1. Baseline indices of pHPT patients

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>65.0 ± 9.0</th>
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<tbody>
<tr>
<td>Renal stone (n of patients)</td>
<td>5/30</td>
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<tr>
<td>S-Ca (mmol/l)</td>
<td>2.78 ± 0.30</td>
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<tr>
<td>S-P (mmol/l)</td>
<td>0.96 ± 0.20</td>
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<tr>
<td>ALP (IU/l)</td>
<td>475 ± 404</td>
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<tr>
<td>intact PTH (ng/l)</td>
<td>284 ± 395</td>
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<tr>
<td>S-Cr (mg/l)</td>
<td>0.040 ± 0.050</td>
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<tr>
<td>U-Ca/U-Cr</td>
<td>0.294 ± 0.127</td>
</tr>
<tr>
<td>L2–4 BMD (g/cm\textsuperscript{2})</td>
<td>0.727 ± 0.215</td>
</tr>
<tr>
<td>L2–4 (Z-score)</td>
<td>–0.521 ± 1.686</td>
</tr>
<tr>
<td>FN BMD (g/cm\textsuperscript{2})</td>
<td>0.545 ± 0.119</td>
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<tr>
<td>FN (Z-score)</td>
<td>–0.487 ± 1.042</td>
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<tr>
<td>R1/3 BMD (g/cm\textsuperscript{2})</td>
<td>0.462 ± 0.118</td>
</tr>
<tr>
<td>R1/3 (Z-score)</td>
<td>–1.049 ± 2.501</td>
</tr>
</tbody>
</table>

Table 2. Relationships between serum 25(OH)D levels and various indices in pHPT patients

<table>
<thead>
<tr>
<th>r</th>
<th>p</th>
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<tbody>
<tr>
<td>Age</td>
<td>0.140</td>
</tr>
<tr>
<td>Height</td>
<td>0.118</td>
</tr>
<tr>
<td>Body weight</td>
<td>0.267</td>
</tr>
<tr>
<td>BMI</td>
<td>0.249</td>
</tr>
<tr>
<td>S-P</td>
<td>0.329</td>
</tr>
<tr>
<td>ALP</td>
<td>–0.417</td>
</tr>
<tr>
<td>%TRP</td>
<td>0.006</td>
</tr>
<tr>
<td>U-Ca/U-Cr</td>
<td>–0.045</td>
</tr>
</tbody>
</table>

* p<0.05
sorption rate, although they significantly correlated to serum levels of ALP. As shown in Fig. 2, serum levels of calcium and PTH seemed to be negatively correlated to serum 25(OH)D levels, although the differences were not significant. However, when three subjects with the highest serum PTH level (PTH>1000 ng/l) were excluded from the analysis, the correlation was significant between serum 25(OH)D levels and PTH. The correlation coefficients and p-values of relationships between serum 25(OH)D levels and serum levels of calcium and PTH were \( r = -0.356, p = 0.0744 \) and \( r = -0.510, p = 0.0070 \), respectively. These findings indicated that vitamin D status affects the severity of pHPT when severe cases are excluded. Serum levels of 1,25(OH)_2D_3 were significantly and negatively correlated with serum 25(OH)D levels (Fig. 2).

Relationship between vitamin D state and BMD or bone metabolic indices in pHPT patients

We then compared the correlation coefficients between serum levels of 25(OH)D and BMD or bone metabolic indices in pHPT patients. As shown in Fig. 3, serum levels of 25(OH)D were significantly correlated to BMD (Z-score) at the lumbar spine, but not at the radius and femoral neck. Since 25(OH)D levels were related to 1,25(OH)_2D_3 levels, the correlation between serum levels of 1,25(OH)_2D_3 and BMD was examined. The correlation coefficients and p-values of relationships between serum 1,25(OH)_2D_3 levels and BMD (Z-score) at the lumbar spine, radius and femoral neck were \( r = -0.452, p = 0.0256 \), \( r = -0.416, p = 0.0422 \) and \( r = -0.241, p = 0.2946 \), respectively. The correlation coefficients and p-values of relationships between serum PTH levels and BMD (Z-score) at the lumbar spine, radius and femoral neck were \( r = -0.178, p = 0.3635 \), \( r = -0.337, p = 0.0855 \), \( r = -0.368, p = 0.0700 \), respectively. In multiple regression analysis, the relationships between serum levels of 25(OH)D levels and BMD (Z-score) at the lumbar spine, radius and femoral neck were not significant (p = 0.0657, 0.6202 and 0.8140, respectively), when serum 25(OH)D and PTH levels were employed as independent variables. Moreover, the relationship between serum levels of 1,25(OH)_2D_3 and BMD (Z-score) was significant only at the lumbar spine, but not at the radius and femoral neck (p = 0.0299, p = 0.0640, 0.1733, respectively), when serum 1,25(OH)_2D_3 and PTH levels were

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**Fig. 2.** Relationships between serum 25(OH)D levels and calcium metabolic indices in pHPT patients. Simple regression analysis was performed to assess the linear relationships between serum 25(OH)D levels and serum levels of calcium, PTH or 1,25(OH)_2D_3 in pHPT patients. Pearson’s correlation coefficients were calculated. p<0.05 was considered statistically significant.
employed as independent variables. These findings suggest that the vitamin D state affects BMD at the lumbar spine partly through PTH; however, serum 25(OH)D levels were not correlated to the levels of any bone metabolic indices measured (Table 3).

**Comparison of serum 25(OH)D levels with and without renal stones in pHPT patients**

We compared serum 25(OH)D levels of pHPT patients with or without renal stones at the diagnosis of pHPT. As shown in Fig. 4, serum levels of 25(OH)D were similar in groups with and without renal stones.

**Discussion**

If vitamin D status is not sufficient in pHPT patients, the clinical presentation of pHPT may be influenced by vitamin D status. In the present study, serum levels of 25(OH)D were less than 20 ng/ml in more than 80% of patients with pHPT. This frequency was compatible with previous evidence [20, 27]. Silverberg et al. reported that serum 25(OH)D levels were lower than 20 ng/ml in 53% of 124 patients with mild pHPT [20]. Bussey and Bruder also reported that 40 and 37% had
vitamin D deficiency and insufficiency in pHPT patients, respectively [27]. Several studies suggested that vitamin D insufficiency is related with the severity of pHPT [16, 28]. Serum levels of 25(OH)D were inversely related to parathyroid gland weight in the study of 148 pHPT patients [16]. In that study, gland weight and serum levels of PTH, ALP and calcium were significantly higher in patients with serum 25(OH)D less than 15 ng/ml. Ozbey et al. supported this finding [28]. In contrast, serum 25(OH)D was not significantly related to parathyroid gland size in other reports [20, 17, 19]. Low preoperative 25(OH)D levels were associated with postoperative symptoms of hypocalcemia and secondary hyperparathyroidism in 190 patients with pHPT [29]. Thus, controversy exists about the relationship between serum 25(OH)D levels and the severity of pHPT. In the present study, serum levels of calcium and PTH seemed to be related to the serum 25(OH)D level. These findings were compatible with previous evidence that the severity of pHPT is related to the status of vitamin D [19]. The lack of significance might be due to the lack of adequate patient number. When pHPT patients with serum PTH levels of more than 1000 ng/l were omitted, the relationship between 25(OH)D and serum PTH levels was significant. The levels of 1,25(OH)2D3 were negatively correlated with serum 25(OH)D levels in female patients with pHPT in the present study. PTH activates vitamin D by inducing the expression of vitamin D 1α-hydroxylase in the proximal renal tubule. Excess serum PTH leads to the increased production of activated vitamin D, which might result in the consumption of vitamin D. This might explain the frequent vitamin D insufficiency in patients with pHPT. Since serum 1,25(OH)2D3 levels were significantly correlated to BMD at the lumbar spine, as serum 25(OH)D levels were correlated to BMD in the present study, enhanced activation of vitamin D by an increase in the severity of pHPT might lead to worsening vitamin D insufficiency, resulting in decreased BMD at the lumbar spine and increased fracture risk. Further study is necessary to clarify whether vitamin D insufficiency causes the severity of pHPT or an elevation of PTH causes vitamin D insufficiency. The correlation between 1,25(OH)2D3 but not 25(OH)D3 and BMD at the radius, was significant. This might be due to the relatively small number of subjects, because 25(OH)D seemed to be related to the BMD score at the radius. Alternatively, PTH may directly affect 1,25(OH)2D3 and radial BMD, compared to 25(OH)D, which might explain the present results.

Vitamin D deficiency causes bone loss and increased fracture risk [1, 4, 5]. Vitamin D supplementation is important for bone health, and several studies suggested that activated vitamin D decreases vertebral fracture risk in patients with postmenopausal osteoporosis [30]. Thus, vitamin D state might influence bone fragility in pHPT. Moosgaard et al. reported that low plasma 25(OH)D was associated with femoral and radial BMD in 289 patients with pHPT [19]. Moreover, there was a trend toward increased risk of osteoporotic fractures with low plasma 25(OH)D in that study. In a cross-sectional study of 62 female patients with pHPT, the influence of 25(OH)D levels on BMD was overwhelmed by the effects of PTH excess, age and body mass index [31]. In the present study, serum levels of 25(OH)D were significantly related to age-matched BMD (Z-score) at the lumbar spine, but not at the radius and femoral neck in postmenopausal female patients with pHPT. In pHPT, cortical bone is easily affected, and lumbar BMD is relatively preserved [32]; however, the present data suggested that vitamin D status affects BMD at the lumbar spine more potently. Therefore, vitamin D insufficiency may increase the risk of vertebral fractures in female patients with pHPT, since decreased BMD of the lumbar spine greatly elevates the risk of vertebral fractures. In our previous study [13], only lumbar spine BMD was lower in the fracture group, but radial and femoral BMD were not lower in female patients with pHPT. These data suggest that BMD measurement, which is specific to fracture sites, is important for the prediction of fracture risk in pHPT. Although reduced BMD in the forearm is common in pHPT patients, reduced radial BMD is not considered to be appropriate for the prediction of vertebral fracture risk. The present data indicated that the relationship between serum 25(OH)D levels and BMD seemed potent compared with their relationship with serum calcium or PTH levels. These findings suggest that the vitamin D state affects bone more potently than the severity of pHPT. Moreover, it is possible that vitamin D insufficiency increases the fracture risk in pHPT patients. Further study is necessary to clarify these issues.

Some discrepancies exist between the present study and studies by Moosgaard et al. or Carnevale et al. [19, 31], which might be due to differences in the proportion of postmenopausal patients, because the estrogen state greatly affects BMD at the lumbar spine. More-
over, BMI was relatively smaller in the present study, compared with previous reports [19, 31]. Higher BMI also might prevent the effects of vitamin D insufficiency on BMD at the lumbar spine by means of increased mechanical loading and the promotion of intrinsic estrogen production in adipose tissue; however, we cannot completely rule out the possibility that 25(OH)D was related to BMD through BMI. In multiple regression analysis, the relationships between serum levels of 25(OH)D levels and BMD (Z-score) were still significant at the lumbar spine, but not at the radius and femoral neck (p = 0.0206, 0.1017 and 0.4714, respectively), when serum 25(OH)D and BMI were employed as independent variables, suggesting that the vitamin D state affected BMD at the lumbar spine independently of BMI.

Mezquita-Raya et al. reported no differences in the biochemical markers of bone turnover, including BAP and OCN, between 25(OH)D \( \leq 15 \) ng/ml and 25(OH)D >15 ng/ml groups in healthy or osteoporotic women except urine carboxy-terminal telopeptide of type I collagen [1]. In the present study, serum 25(OH)D levels were not correlated with the levels of any bone metabolic indices, compatible with previous evidence [17]. These findings suggest that bone metabolic indices are not markers with high sensitivity for vitamin D insufficiency. Serum levels of non-specific alkaline phosphatase were significantly related to serum 25(OH) D levels in pHPT patients, although there was no relationship between serum BAP and 25(OH)D levels in the present study. Our previous study suggested that serum ALP measurement is useful in predicting BMD change after parathyroidectomy [7]. On the other hand, serum BAP was not related to lumbar spine BMD change after parathyroidectomy in our later study [24]. Non-specific alkaline phosphatase is derived from tissues such as bone and liver, and the measurement of serum BAP is more specific for the assessment of bone metabolism. Although the reason for these discrepancies is unknown, assessment by serum ALP to evaluate bone metabolism in pHPT patients may lead to misunderstanding regarding bone metabolism.

Renal manifestations, such as renal stone or renal dysfunction, are also important phenotypes of pHPT. In several studies, serum 25(OH)D levels were related with urinary calcium excretion in patients with pHPT [19, 28]; however, the present study indicated that urinary calcium excretion and the prevalence of renal stones were not associated with serum levels of 25(OH)D in female patients with pHPT, compatible with a previous report [27]. Taken together, the vitamin D state does not seem to influence the severity of renal manifestation, compared with its effect on bone in female patients with pHPT.

There are several limitations in the present study. First, the sample size was not large enough to make definite conclusions. Second, the definite diagnosis of pHPT comes from the histological finding and the normalization of hypercalcemia after parathyroidectomy, limiting this present study. The subjects included patients with an abnormal parathyroid gland identified by at least two imaging techniques. Therefore, subjects with a smaller adenoma which was not identified by two imaging techniques might have been excluded from the study, which also might limit definite conclusions. Third, we cannot rule out the possibility that timing variations affected serum levels of 25(OH)D level in pHPT patients, because we cannot adjust for when 25(OH)D was measured, since parathyroidectomy is usually performed soon after diagnosis and the time of 25(OH)D measurement is difficult to adjust in not so many subjects.

In conclusion, vitamin D status seemed to be related to the severity of disease in postmenopausal female patients with pHPT. In particular, the relationship between serum 25(OH)D level and BMD at the lumbar spine was predominant.

References


