Prevalence of Vitamin D Deficiency in Patients with Stage 3/4 Chronic Kidney Disease and Its Relation to Secondary Hyperparathyroidism

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Abstract

Objective: The extent of bone mineral disorders in predialysis patients is not well defined. This study aimed to detect the prevalence of both 25-hydroxyvitamin D (25(OH)D) and 1,25-dihydroxyvitamin D (1,25(OH)2D) deficiency in patients with stage 3–4 chronic kidney disease (CKD) and its relation to secondary hyperparathyroidism.

Materials and Methods: In this study, 113 patients with stage 3–4 CKD admitted to our outpatient clinic were included. Clinical, demographic, and lifestyle characteristics of patients were recorded. Patients’ serum creatinine, calcium, phosphorus, 25(OH)D, 1,25(OH)2D, and parathormone (PTH) levels were measured.

Results: We found that 85 patients (75.2%) had stage 3 CKD, and 28 patients (24.8%) had stage 4 CKD. A low 25(OH)D level was detected in 89.4% of the patients (stage 3 CKD 89.4%, stage 4 CKD 89.3%). The PTH level was higher in patients with low 25(OH)D level with a borderline statistical significance (p=0.057). The number of patients with 1,25(OH)2D level lower than 54.53 pg/ml was 28 (24.77%), which was accepted as a cut-off value. The mean PTH level was significantly higher in the group with lower 1,25(OH)2D (P=0.048). The prevalence of secondary hyperparathyroidism was found as 57.5%.

Conclusion: We found a high prevalence of bone mineral disorders among patients with stage 3–4 CKD. These results point out the importance of timely diagnosis and appropriate treatment.

Keywords: Vitamin D deficiency, secondary hyperparathyroidism, chronic kidney disease, late-stage kidney disease

INTRODUCTION

Bone mineral disorders are observed from the early stages of chronic kidney disease (CKD). According to the Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guideline on the management of chronic kidney disease-mineral and bone disorders published in 2017, a regular follow-up of serum calcium, phosphorus, parathormone (PTH), alkaline phosphatase, and vitamin D levels is suggested starting from CKD stage 3 (1).

The patients with CKD commonly suffer from 1,25-dihydroxyvitamin D (1,25(OH)2D) deficiency because of lack of 25-hydroxyvitamin D (25(OH)D), which is the precursor form of vitamin D, and decreased activity of 1α-hydroxylase, which converts 25(OH)D into its active form (2). It can be even observed in more than 80% of patients with CKD (3). Low vitamin D levels are associated with increased bone turnover, secondary hyperparathyroidism, and decreased bone mineral density in patients with CKD (4). Moreover, it has been shown that
low vitamin D levels increase risk of progression to dialysis and mortality in patients with predialysis CKD (5).

Despite the high burden of bone mineral disorders, there is lack of systemic studies to reveal the prevalence of vitamin D deficiency and its relation to secondary hyperparathyroidism in predialysis patients.

In this study, we aimed to detect the prevalence of both 25(OH)D and 1,25(OH)2D deficiency in patients with stage 3-4 CKD and its relation to secondary hyperparathyroidism.

MATERIALS AND METHODS

Study Subject and Design

We performed a cross-sectional study in a tertiary-care outpatient clinic center. Between November 2012 and February 2014, we examined medical records of 772 patients. Adult patients (>18 years of age) with an estimated glomerular filtration rate (eGFR) value between 15 and 59 mL/min/1.73 m² were eligible for the study. The following exclusion criteria were used: being on a renal replacement treatment; having a malignancy; the presence of primary hyperparathyroidism; severe liver failure; and severe malabsorption. Furthermore, being on treatment with medications containing vitamin D, calcium, and phosphate binding agents, calcimimetic agents, denosumab, and biphosphonates in the last six months was also considered as an exclusion criterion. According to these criteria, 159 patients were eligible. From these, 113 patients gave an informed consent to be a participant in this study.

eGFR was calculated using Modification of Diet in Renal Disease formula with the latest creatinine value of the patients measured in the last one year.

We used a standardized form to collect information about demographic data, physical activity level, way of dressing, nutritional habits, medications, weight, and height. Adequate sun exposure was defined as exposure of face and arms to sun at least 30 minutes in sunny days according to the patients’ declaration (6). Physical activity was classified as sedentary, moderate, and heavy as per the self-assessment of the patients. The patients who did not do moderate exercises, such as running, walking, cycling were recorded as sedentary; the patients who exercised for 30 minutes were recorded as moderate; and those who exercised more than 30 minutes were recorded as heavy. Data on cardiovascular disorders, diabetes, and hypertension were recorded from the patient’s files.

To measure serum creatinine, calcium, phosphorus, 25(OH)D, 1,25(OH)2D, and PTH levels, a sample of blood was drawn from the patients.

The study protocol was approved by the local medical ethics committee.

Laboratory Assessment

Serum creatinine, calcium, and phosphorus were analyzed using photometric methods with Abbott Architect c8000 autoanalyzer (Abbott Diagnostics, Abbott Park, IL, USA). The PTH levels were determined by means of the chemiluminescent immunoassay (Liaison N-tact; DiaSorin Inc, Stillwater, MN, USA). Serum calcium levels were corrected according to albumin level. The reference ranges for laboratory parameters were as following: serum creatinine, 0.8-1.3 mg/dL; calcium, 8.9-10.1 mg/dL; and phosphorus, 2.5-4.5 mg/dL.

Measurements and Definition of Laboratory Range for Vitamin D Levels

The 25(OH)D and 1,25(OH)2D levels were measured with ELISA (Eastbiopharm, Hangzhou, China) Catalog no: CK-E10878. A level of 25(OH)D lower than 15 ng/dl was considered as vitamin D deficiency. The threshold for low 1,25(OH)2D was defined by the 25th percentile of patients included in our study, which was the lower percentile (n=28). In accordance with this threshold, low 1,25(OH)2D was determined as 54.53 pg/mL.

Statistical Analysis

Statistical analysis was performed with the SPSS (Statistical Package for Social Sciences for Windows) 17.0 software (SPSS Inc., Chicago, IL, USA). Data were expressed as the median±standard deviation (SD). Normality was determined using histograms, probability graphics, and Kolmogorov-Smirnov/Shapiro-Wilk tests.

The t-test was used for normally distributed variables, and the Mann-Whitney U test was used for non-normally distributed variables. For categorical variables (gender, stage etc.), the chi-square test (Fisher exact test) was used. Correlation was analyzed using Pearson or Spearman correlation tests. p<0.05 was considered as statistically significant.

RESULTS

Demographic and Clinical Data

In our study, 113 patients were evaluated. Among the patients, 85 (75.2%) had stage 3 CKD, and 28 (24.8%) had stage 4 CKD. Demographic, clinical data, and lifestyle-related characteristics of the patients stratified by CKD stage are shown in Table 1. The mean age was 59.94±11.6 years. Male sex was more prominent (n=67, 59.3%). The average body mass index (BMI) of the patients was 28.75±5.2 kg/m². The most commonly seen comorbid diseases were hypertension (85%), diabetes mellitus (45.1%), and cardiovascular diseases (34.5%).

Only 37.2% of the patients stated an adequate sun exposure, and 33.6% of them had a sedentary lifestyle. Female patients generally preferred to wear hijab (69.6%) that reduces sun exposure. In female patients with stage 3 CKD, 66.7% preferred to wear hijab; and in female patients with stage 4 CKD, 76.4% preferred to wear hijab (p=0.724). Between the two groups,
there was no statistically significant difference regarding those parameters.

Considering nutrition habits, patients consumed on average 4 meals of fish, 13 meals of egg, 14 meals of meat, and 24 meals of milk and milk products in a month. Those amounts might roughly reflect daily intake of 250 IU (7).

**Laboratory Findings**

We analyzed and presented our laboratory results using two different classifications; first, by grouping the patients according to CKD stage, and second by grouping the patients according to 25(OH)D level.

In the whole study population, hyperparathyroidism was detected as 57.5%. The frequency of hyperparathyroidism in patients with stage 4 CKD was significantly higher than that of the patients with stage 3 CKD (stage 3 CKD 47.1%, stage 4 CKD 57.5%, p<0.05). There was no significant difference between the stages both for 1,25(OH)

### Table 1. Demographic, clinical data, and lifestyle characteristics

<table>
<thead>
<tr>
<th></th>
<th>All patients (n=113)</th>
<th>Stage 3 CKD (n=85)</th>
<th>Stage 4 CKD (n=28)</th>
<th>p **</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male %)</td>
<td>59.3</td>
<td>61.2</td>
<td>53.6</td>
<td>0.477</td>
</tr>
<tr>
<td>Age (year)</td>
<td>59.94±11.6</td>
<td>60.56±12.2</td>
<td>58.05±9.7</td>
<td>0.321</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28.75±5.1</td>
<td>28.74±4.8</td>
<td>28.76±5.8</td>
<td>0.161</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>45.1</td>
<td>49.4</td>
<td>32.1</td>
<td>0.111</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>85</td>
<td>88.2</td>
<td>75</td>
<td>0.125</td>
</tr>
<tr>
<td>Cardiovascular diseases (%)</td>
<td>34.5</td>
<td>35.3</td>
<td>32.1</td>
<td>0.761</td>
</tr>
<tr>
<td>Adequate sun exposure (%)</td>
<td>37.2</td>
<td>38.8</td>
<td>32.1</td>
<td>0.526</td>
</tr>
<tr>
<td>25-hydroxyvitamin D 15mcg/L (%)</td>
<td>89.4</td>
<td>89.4</td>
<td>89.3</td>
<td>1</td>
</tr>
<tr>
<td>25-hydroxyvitamin D&gt;15 mcg/L (%)</td>
<td>10.6</td>
<td>10.6</td>
<td>10.7</td>
<td>1</td>
</tr>
<tr>
<td>Physical activity (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedentary</td>
<td>33.6</td>
<td>35.3</td>
<td>28.6</td>
<td></td>
</tr>
<tr>
<td>Adequate</td>
<td>38.9</td>
<td>38.8</td>
<td>39.3</td>
<td>0.746</td>
</tr>
<tr>
<td>Active</td>
<td>27.4</td>
<td>25.9</td>
<td>32.1</td>
<td></td>
</tr>
</tbody>
</table>

CKD: Chronic Kidney Disease

**Comparison of stage 3 and 4 CKD

<table>
<thead>
<tr>
<th></th>
<th>CKD (n=113)</th>
<th>Stage 3 CKD (n=85)</th>
<th>Stage 4 CKD (n=28)</th>
<th>p**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.51±0.6</td>
<td>1.26±0.3</td>
<td>2.29±0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Estimated Glomerular Filtration Rate (mL/min 1.73m²)</td>
<td>40.12±12.1</td>
<td>45.7±7.9</td>
<td>23.14±4.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>8.15±0.6</td>
<td>8.14±0.56</td>
<td>8.18±0.62</td>
<td>0.915</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>3.12±0.5</td>
<td>3.09±0.5</td>
<td>3.23±0.48</td>
<td>0.213</td>
</tr>
<tr>
<td>PTH (pg/mL)***</td>
<td>89.54±68</td>
<td>68.77±33.5</td>
<td>152.6±102</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>25-hydroxyvitamin D (mcg/L)</td>
<td>10.55±7.6</td>
<td>10.99±8.06</td>
<td>9.21±5.8</td>
<td>0.28</td>
</tr>
<tr>
<td>1,25-dihydroxyvitamin D (pg/mL)</td>
<td>83.8±92.7</td>
<td>87.2±95.8</td>
<td>73.5±83.3</td>
<td>0.221</td>
</tr>
</tbody>
</table>

CKD: Chronic Kidney Disease

**Comparison of stage 3 and 4 CKD

*** (Parathormone) PTH>65 pg/ml: upper limit of the normal range for the laboratory kit.
89.3%, p<0.001). Compared with patients with stage 3 CKD, the PTH levels were also significantly higher in patients with stage 4 CKD (p<0.001) (Table 2).

Hypoparathyroidism (PTH<15 pg/mL; lower limit of the normal range for the laboratory kit) was detected in 0.9% of the patients. There were no significant differences between the two stages (stage 3 CKD 1.2%, stage 4 CKD 0%, p=1).

Hypocalcemia was present in 69% of the patients (stage 3 CKD 69.4%, stage 4 CKD 67.9%), and hypophosphatemia was present in 14.2% of the patients (stage 3 CKD 16.5%, stage 4 CKD 7.1%). There was no patient with hypercalcemia or hyperphosphatemia. The calcium and phosphorus levels were similar between the two stages (p=0.915, p=0.213).

Patient subgroups classified according to 25(OH)D levels are shown in Table 3. The male gender was more common among patients with low 25(OH)D; however, there was no significant difference between the two groups regarding age, CKD stage, BMI, diabetes mellitus, and hypertension. History of cardiovascular disease was more common among patients with low 25(OH)D.
levels ($p=0.008$). In women wearing hijab, a significantly lower level of $25(OH)D$ was detected (76.9%); on the other hand, 28.6% of women wearing hijab had a $25(OH)D$ value higher than 15 mcg/L ($p=0.02$). The creatinine, eGFR, and calcium levels were similar between the two groups. The PTH level was higher in patients with low $25(OH)D$ level with a borderline statistical significance ($p=0.057$). The phosphorus and $1,25(OH)_2D$ levels were found significantly lower in patients with low vitamin D level (Table 3).

Finally, we examined the associations among PTH, $25(OH)D$ and $1,25(OH)_2D$. Compared with patients with a normal $1,25(OH)_2D$ level (78.43±43.15 pg/mL), the PTH level of patients with a low $1,25(OH)_2D$ level was significantly higher (124.38±109.8 pg/mL) ($p=0.048$) (Figure 1). On the other hand, in patients with low $25(OH)D$ level ($n=101$), $1,25(OH)_2D$ levels were not significantly different between patients with stage 3 and stage 4 CKD (62.8±20.4 pg/mL vs. 52.74±23.67 pg/mL; $p=0.129$) (Figure 2).

**DISCUSSION**

We detected that 57.3% ($n=65$) of the patients with stage 3 or stage 4 CKD had hyperparathyroidism. The prevalence of hyperparathyroidism in predialysis patients is between 20% and 60% in various studies (5, 8, 9, 10). Probably this difference in prevalence between different studies is because of a variation of $25(OH)D$ levels among the study participants. This study revealed a significantly higher frequency of hyperparathyroidism and higher PTH level in patients with stage 4 CKD compared with those in stage 3 CKD. This finding is similar to the previous studies (5, 9, 11).

In 89.4% of the patients, $25(OH)D$ levels were low. There was no significant difference between stage 3 and 4 CKD considering this finding ($p=1$). A study with 273 peritoneal dialysis patients from Turkey and Greece showed vitamin D insufficiency in 92% of the patients (12). Another study from Germany found that 74% of 444 patients with an eGFR < 60 mL/min had a $25(OH)D$ level lower than 20 ng/mL (13). Furthermore, in a study from Canada 34.5% of 168 patients with stage 2-5 CKD (8), in USA 12% of 1814 patients with CKD (5), in another study again in USA 15% of 12763 patients with stage 3-4 CKD (14) had a $25(OH)D$ level lower than 15 ng/mL. In the UK in a study, 39% of 112 patients with stage 3-4 CKD had vitamin D insufficiency (11). However, in Australia, which is a sunny region, 9.8% of 593 patients with stage 1-5 CKD revealed vitamin D insufficiency (15). In our study, in line with previous data, we also did not detect significantly high phosphorus levels in early stages of CKD (5, 9, 10); on the other hand, hypocalcemia was frequent (69%) in our patients. The high frequency of hypocalcemia is possibly related to low $25(OH)D$ levels.

Previous studies revealed a decrease in renal functions induces a decreased 1-alpha-hydroxylase activity (5, 8, 9). Although patients with stage 3 CKD revealed a lower $1,25(OH)_2D$ and a higher phosphorus level, there was no significant decrease in $1,25(OH)_2D$ levels in patients with stage 3 CKD compared with those in patients with stage 4 CKD. An increase in PTH levels prevents hyperphosphatemia, and hence because of a disinhibition in 1-alpha-hydroxylase activity, $1,25(OH)_2D$ levels persist as relatively high. This phenomenon can be the cause of statistical insignificance in $1,25(OH)_2D$ decrease.

In patients with low $25(OH)D$ levels, an expected low $1,25(OH)_2D$ level was detected. Also, a significant lower phosphorus level was found in these patients because of high PTH levels.

According to World Health Organization, daily vitamin D requirement is 400 IU for patients younger than 65 years and 600 IU for patients older than 65 years (16). According to the results of our dietary questionnaire, low vitamin D intake could be considered as an additional causative cause of vitamin D deficiency besides inadequate sun exposure. Considering data of United States Department of Agriculture (USDA) Food Composition Databases, fish, egg, meat, milk, and milk products are basic vitamin D resources. The amount of vitamin D is approximately 40 IU in one egg, 42 IU in 100 g red meat, 120 IU in one cup of milk, and 440 IU in 100 g salmon (7). However, we could not calculate the exact amount of vitamin D intake using our questionnaire, because exact amount of the meals was unknown.

In our study population, the most frequently seen comorbid diseases were hypertension (85%), diabetes mellitus (45.1%), and cardiovascular diseases (34.5%). Other studies support this finding (2, 13). Despite the similar demographic data of the patients with low or normal $25(OH)D$ levels, we detected a higher frequency of low $25(OH)D$ level among women who preferred to wear hijab. In the patients with low $25(OH)D$ level, the frequency of cardiovascular diseases was significantly higher compared with that of the normal $25(OH)D$ group ($p=0.008$). This finding is supported by many other studies (5, 8, 13, 14, 17).

Our study has several limitations. Firstly, we were not able to calculate an accurate threshold for low $1,25(OH)_2D$. A study with a larger group can provide a more accurate threshold for $1,25(OH)_2D$. Secondly, we did not compare the patients in this study to a healthy control group. Thirdly, we excluded patients who received any treatment with vitamin D, calcium, phosphate binding agents, calcimimetic agents, denosumab, and bisphosphonates because those medications might interfere with the laboratory parameters that we examined. Additionally, by excluding patients on treatment, we excluded patients with CKD with severe bone mineral metabolism disorders. Our results were possibly affected by this exclusion criterion. Furthermore, we did not collect data on physical activity and nutritional habits using a more detailed and validated questionnaire.

**CONCLUSION**

A follow-up for bone mineral metabolism disorders is recommended to patients with CKD in predialysis period. An early treatment including the substitution of vitamin D must be considered. For prevention, adequate sun exposure and appropriate nutrition must be advised.
**Ethics Committee Approval:** Ethics Committee approval was received for this study from the Ethics Committee of Istanbul University-Cerrahpaşa School of Medicine (12.11.2013, 31879).

**Informed Consent:** Written informed consent was obtained from all patients who participated in this study.

**Peer-review:** Externally peer-reviewed.


**Conflict of Interest:** The authors have no conflicts of interest to declare.

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**REFERENCES**


