Brief Report

Vitamin C deficiency and secondary hyperparathyroidism in chronic haemodialysis patients

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Abstract

Background. Maintenance haemodialysis patients often suffer from secondary hyperparathyroidism and serum parathyroid hormone levels may be influenced by nutritional variables.

Methods. We examined serum bio-intact parathyroid hormone (BiPTH) and plasma vitamin C in 117 chronic haemodialysis patients. Plasma vitamin C was measured by high-performance liquid chromatography with electrochemical detection, on samples collected before start of the dialysis treatment.

Results. Plasma vitamin C showed a significant positively skewed distribution, ranging from <2 µM to >300 µM. We found 15% (n = 17) of the patients with severe vitamin C deficiency (<10 µM), 66% (n = 77) in the range 10–80 µM, and 19% (n = 23) with plasma vitamin C >80 µM, the upper limit of normal for non-renal disease population. High plasma vitamin C was associated with lower plasma BiPTH (P = 0.005, one-way analysis of variance), and this association persisted after stepwise multiple regression for other factors known to influence PTH. Low vitamin C levels were also associated with increased serum alkaline phosphatase, a further indicator of the impact of vitamin C status on bone metabolism. Patients who reported dietary vitamin C intake of ≥100 mg/day had lower BiPTH (P = 0.015), consistent with findings from plasma measurements of vitamin C. This novel observation of the interaction between vitamin C and the parathyroid hormone linked signalling pathways, and may uncover mechanisms of therapeutic importance.

Keywords: haemodialysis; metabolic bone disease; secondary hyperparathyroidism; vitamin C

Introduction

Secondary hyperparathyroidism (SHPT) affects up to 50% of chronic haemodialysis (HD) patients [1]. This disorder is diagnosed by elevated parathyroid hormone (PTH) in the presence of low or normal serum calcium levels. SHPT is primarily due to increased PTH synthesis and secretion, although impaired renal clearance of PTH may also contribute [2]. PTH synthesis and secretion is stimulated by low serum levels of ionized calcium and high levels of serum phosphate [3]. Additional factors such as vitamin D [3,4] race, gender [5], age [6] and diabetic status [1] may also influence generation of PTH. The levels of PTH in dialysis patients can be very high, and in these patients PTH is considered a uraemic toxin [7]. Resultant fibrotic changes in the bone marrow from elevated PTH lead to impaired erythropoiesis and an increased erythropoietin (EPO) requirement [8–10]. High PTH and phosphate levels are associated with increased cardiovascular mortality in HD patients [11].

Dietary vitamin C is indispensable in humans, who lack the enzyme gulonolactone synthetase needed for its biosynthesis [12]. Maintenance haemodialysis patients (MHD) are especially prone to vitamin C deficiency because of dietary restrictions, malnutrition and clearance of vitamin C during dialysis treatment, and this deficiency may have significant impact on patient outcomes [13]. A single haemodialysis treatment may result in a 50 to 75% drop in plasma vitamin C level [14–16] which usually rebounds to baseline levels before the next dialysis treatment if adequate intake is provided, but many patients show very low levels even before dialysis [14–16]. A daily intake of 60 to 100 mg of vitamin C is sufficient to maintain health in a person with...
normal renal function [12] but may not be adequate for a patient on MHD. We present evidence for severe vitamin C deficiency in a substantial fraction of MHD patients. Our data show a potential link between low vitamin C levels and the occurrence of SHPT.

Materials and methods

This cross-sectional study was conducted in two Beth Israel Medical Center/Renal Research Institute dialysis units in New York city during April 2005. All patients were dialisized with high-flux polysulfone dialyzers (Fresenius Optiflux). Patients were excluded because of age <18 years, positive serostatus for HIV, hepatitis B or hepatitis C, chronic infections, enrollment in another research protocol, or decision not to provide informed consent. None of the patients showed signs or symptoms of overt nephrotic syndrome. Dietary vitamin C intake was assessed by a nutritionist via chart review. Nutritional questionnaire and subsequent interview with the patient was performed to calculate prescribed vitamin C dosage. Weekly dose of vitamin D and vitamin D analogues were calculated from the dosage of doxercalciferol, calcitriol or paricalcitol given at haemodialysis. Dietary protein intake was estimated from the equilibrated normalized protein catabolic rate (enPCR) derived from formal urea kinetic models [17]. No formal assessment of dietary calcium and phosphorus intake was undertaken.

The study protocol was approved by the Institutional Review Board of the Beth Israel Medical Center and an informed consent was obtained from all enrolled subjects.

Sample collection and laboratory procedures

Blood samples for vitamin C determination were obtained in non-fasting subjects from the arterial line at the beginning of the mid-week HD session, using heparin anticoagulant. Plasma was isolated by centrifugation immediately after blood collection, stabilized with 10% metaphosphoric acid [18] and stored at −80°C until analysis, which was conducted within 60 days of sample collection. Vitamin C was determined by HPLC on a Beckmann C18 column (250 mm × 4.6 mm) with electrochemical detection. Spike-recovery experiments confirmed the accuracy, reliability and sensitivity of the assay over the very broad range needed for this investigation, from 1 µM to 400 µM (data not shown). Measurement of vitamin C via HPLC with electrochemical detection has become widely accepted in a variety of clinical and epidemiological studies [13, 19–21].

Additional samples were obtained for the measurement of high-sensitivity C-reactive protein (hs-CRP). The hs-CRP (reference range 0.5–3.0 mg/L) was measured by nephelometry on a Dade-Behring SN-100 [22].

Other laboratory indicators including serum levels of total calcium, phosphate, alkaline phosphatase (AP; reference range 35–104 U/L), albumin and haematological parameters were measured by standard techniques (Spectra East Laboratory, Rockleigh, NJ, USA). Measurement of PTH was done with the immunometric bio-intact PTH (BiPTH) assay (Nichols Institute Diagnostics; reference range 12.6–53.5 pg/mL). This method assesses 1–84 PTH, the bioactive portion of PTH, without cross-reaction to PTH 7–84. All bio-intact PTH measurements were performed at Spectra East Laboratory with a high quality control.

Data analysis

Patients were ranked according to plasma vitamin C levels: <10 µM (indicating severe deficiency), 10–80 µM and >80 µM (above the range generally attained in the normal population). Levels of BiPTH in each group were assessed by one-way analysis variance (1-way-ANOVA). A multiple linear regression model with backward elimination ($P < 0.1$ for variable retention) was developed to explore the relationship between log$_{10}$ BiPTH (as the dependent variable) and gender, race, age, body mass index, dialysis vintage, diabetic status, equilibrated normalized protein catabolic rate (enPCR), serum levels of calcium, phosphate, albumin, cumulative monthly intravenous vitamin D dose and vitamin C as independent variables. BiPTH was analyzed in relation to dietary vitamin C supplement use reported on questionnaire, and levels of BiPTH were compared with the Students $t$-test after subjects were stratified by reported vitamin C supplement use. The Spearman rank-test was used for correlation analysis on non-normally distributed variables. A Bonferroni correction was applied for multiple comparisons. The Kolmogorov–Smirnov test was used to test for normal distribution. Statistical analyses were performed using SPSS version 11.0 (SPSS, Inc., Chicago, IL, USA).

Results

A total of 117 patients (67 males, 50 females) undergoing three times a week in-center haemodialysis treatment from two different dialysis facilities were studied. The baseline characteristics are reported in Table 1.

Table 1. Baseline characteristics of the study population ($n = 117$)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63 ± 13</td>
<td>64 (26–88)</td>
</tr>
<tr>
<td>Dialysis vintage (years)</td>
<td>3.2 ± 2.6</td>
<td>2.5 (0.06–14.4)</td>
</tr>
<tr>
<td>Vitamin C (µM)</td>
<td>59 ± 65</td>
<td>41 (1.6–330)</td>
</tr>
<tr>
<td>Vitamin C supplement (mg/day)</td>
<td>90 ± 155</td>
<td>60 (0–1000)</td>
</tr>
<tr>
<td>BiPTH (pg/mL)</td>
<td>283 ± 258</td>
<td>200 (10–1518)</td>
</tr>
<tr>
<td>AP (U/L)</td>
<td>113 ± 74</td>
<td>94 (35–512)</td>
</tr>
<tr>
<td>Ca (mg/dL)</td>
<td>9.2 ± 0.8</td>
<td>9.2 (6.8–11.4)</td>
</tr>
<tr>
<td>P (mg/dL)</td>
<td>5.3 ± 1.5</td>
<td>5.0 (2.2–9.2)</td>
</tr>
<tr>
<td>Ca × P (mg²/dL²)</td>
<td>48.8 ± 14.8</td>
<td>46.1 (20.2–83.7)</td>
</tr>
<tr>
<td>hsCRP predialysis (mg/L)</td>
<td>12.0 ± 15.0</td>
<td>6.8 (0.4–104.2)</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.0 ± 0.3</td>
<td>4.0 (3.4–6.6)</td>
</tr>
<tr>
<td>Vitamin D therapy (µg/month)</td>
<td>36.4 ± 31.1</td>
<td>30 (0–127.0)</td>
</tr>
</tbody>
</table>
Plasma vitamin C levels

Vitamin C values (Figure 1) departed significantly from the normal distribution (\( P < 0.001 \) by the Kolmogorov–Smirnov test), ranging from 1.6 \( \mu \)M to 329.7 \( \mu \)M (mean 58.9 \( \mu \)M \( \pm \) 65.3; median 41.1 \( \mu \)M). Fifteen percent of the patients (\( n = 17 \)) had vitamin C levels <10 \( \mu \)M (a range seen in very deficient subjects), 66% (\( n = 77 \)) had levels between 10 and 80 \( \mu \)M, and 23 patients (19%) had vitamin C levels >80 \( \mu \)M, which is considered the upper limit of normal for the healthy population.

Thirty percent (\( n = 35 \)) reported no vitamin C supplement usage. Forty-nine percent (\( n = 57 \)) of our patients declared an intake of 60 mg of supplemental vitamin C per day, and 21% (\( n = 25 \)) reported an intake of 100 mg or greater of supplemental vitamin C per day (Table 2). Among the group that reported daily supplement of 100 mg or more vitamin C, only 8% showed a severe vitamin C deficiency (plasma vitamin C <10 \( \mu \)M), compared to 20% among those who reported no supplement use, indicating a trend toward increased levels of plasma vitamin C with supplement use in this patient sample (\( \chi^2 = 8.261; P = 0.0825; 4 \text{ d.f.} \)).

Interaction of vitamin C with plasma PTH

The distribution of plasma BiPTH is shown in Figure 2. BiPTH was analyzed in relation to categories of plasma vitamin C (<10 \( \mu \)M, 10–80 \( \mu \)M and >80 \( \mu \)M); the results are shown in Figure 3. Higher levels of plasma vitamin C were associated with lower levels of BiPTH (\( P = 0.005, 1\text{-way ANOVA} \)).

In a further analysis of BiPTH stratified by reported patients’ vitamin C intake, the levels of BiPTH were lower in patients who reported consuming \( \geq 100 \text{ mg/day} \) of vitamin C (Figure 4) (\( P = 0.015, \text{ Student’s } t\text{-test} \)).

Multivariate analysis for effects on PTH levels

In a multiple linear regression model with backward elimination with \( \log_{10} \) BiPTH as the dependent variable and gender, race, age, dialysis vintage, diabetic status, enPCR, vitamin D therapy, body mass index (BMI), serum levels of calcium, phosphate, albumin, and \( \log_{10} \) vitamin C as independent variables the inverse relationship of vitamin C and BiPTH was confirmed (\( R^2 = 0.396, \text{ adjusted } R^2 = 0.356, P < 0.0001 \)) (Table 3), along with other parameters known to influence PTH levels, including serum phosphate, serum calcium and vitamin D therapy.
Table 2. Plasma vitamin C levels in patients stratified by vitamin C supplement use (χ² = 8.26; P = 0.083; 4 d.f.)

<table>
<thead>
<tr>
<th>Plasma vitamin C levels</th>
<th>None (n = 35)</th>
<th>60 (n = 57)</th>
<th>100 or greater (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10 µM</td>
<td>20% (n = 7)</td>
<td>14% (n = 8)</td>
<td>8% (n = 2)</td>
</tr>
<tr>
<td>10–80 µM</td>
<td>60% (n = 21)</td>
<td>70% (n = 40)</td>
<td>64% (n = 16)</td>
</tr>
<tr>
<td>&gt;80 µM</td>
<td>20% (n = 7)</td>
<td>16% (n = 9)</td>
<td>28% (n = 7)</td>
</tr>
</tbody>
</table>

Table 3. Results of a multiple regression analysis with backward elimination with log₁₀ PTH as the dependent variable

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>SE (B)</th>
<th>β</th>
<th>t</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>3.110</td>
<td>0.414</td>
<td>−0.01</td>
<td>7.512</td>
<td>&lt; 0.000</td>
</tr>
<tr>
<td>Serum phosphate (mg/dL)</td>
<td>0.080</td>
<td>0.021</td>
<td>0.315</td>
<td></td>
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<tr>
<td>Vitamin D therapy (µg/month)</td>
<td>0.003</td>
<td>0.001</td>
<td>0.271</td>
<td>3.409</td>
<td>0.001</td>
</tr>
<tr>
<td>Log₁₀ serum vitamin C(µmol/L)</td>
<td>−0.214</td>
<td>0.062</td>
<td>−0.271</td>
<td>−3.435</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum calcium (mg/dL)</td>
<td>−0.131</td>
<td>0.040</td>
<td>−0.258</td>
<td>−3.276</td>
<td>0.001</td>
</tr>
<tr>
<td>Dialysis vintage (months)</td>
<td>0.003</td>
<td>0.001</td>
<td>0.244</td>
<td>3.108</td>
<td>0.002</td>
</tr>
<tr>
<td>enPCR (g/kg/day)</td>
<td>−0.279</td>
<td>0.125</td>
<td>−0.172</td>
<td>−2.240</td>
<td>0.027</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.005</td>
<td>0.002</td>
<td>0.171</td>
<td>2.053</td>
<td>0.043</td>
</tr>
</tbody>
</table>

R² = 0.396, adjusted R² = 0.356; P (final model) < 0.0001; B = regression coefficient; SE(B) = standard error of B; β = standardized regression coefficient.

Variables removed from the model (P-value > 0.1) by backward regression were diabetes mellitus (yes/no), gender (female/male), body mass index (kg/m²), serum albumin concentration (mg/dL).

Vitamin C and serum alkaline phosphatase (AP) levels were inversely correlated (Spearman rank coefficient of correlation rₛ = −0.192, P = 0.045). AP and BiPTH were positively correlated (rₛ = 0.441, P < 0.001). Vitamin C and hs-CRP levels were not related (R² = 0.01, P = 0.361).

Discussion

The key and novel finding in the present study is a negative association between indicators of vitamin C status and BiPTH. Moreover, the present study reports that a significant proportion of chronic dialysis patients are very deficient in vitamin C. In this sample 15% of MHD patients showed severe vitamin C deficiency, with plasma vitamin C concentrations <10 µM. Vitamin C was detected via HPLC with electrochemical detection. HPLC assays offer many advantages over other methods in providing specificity, sensitivity and reproducibility [23,24]. Vitamin C levels were confirmed with spike-recovery measurements on plasma vitamin C levels. In population studies of subjects without renal disease, such levels are often associated with scurvy and are considered a marker of severe vitamin C deficiency [12]. In HD patients these low vitamin C levels can be explained in part by the removal of vitamin C during haemodialysis. Even severe vitamin C deficiency can be overlooked in MHD patients because they overlap with common signs and symptoms found in this population. These signs include ecchymoses, bleeding gums, arthralgias and unspecific systemic symptoms like weakness, oedema, depression, neuropathy and vasomotor instability. To reach normal vitamin C levels it is necessary to prescribe vitamin C supplements to all MHD patients, to assure compliance and to check vitamin C levels if deficiency is suspected. Plasma vitamin C levels <10 µM were observed even in patients who were prescribed adequate vitamin C supplements. Intestinal mucosal iron deposition has been documented in the majority of MHD patients [25], which may lead to oxidation of dietary vitamin C in the gut and decrease the bioavailability of dietary supplements.

In our study BiPTH and plasma vitamin C levels were inversely related both in univariate and multivariate analyses. This finding was corroborated by the observation that patients who reported ≥100 mg/day of vitamin C intake had lower BiPTH levels. To the best of our knowledge, this association has not been previously reported.

We propose that the inverse interaction between BiPTH and vitamin C observed by us is explained by the action of vitamin C on post-receptor events in the super-family of seven membrane-spanning receptors [26], which includes the PTH receptor in the bone [27] and calcium-sensing receptor in the parathyroid gland (CaSR) [28]. Binding of PTH to its receptor initiates signalling through the alpha-subunit of the stimulatory G-protein resulting in elevated cAMP and activation of cAMP-dependent protein kinases [29,30]. Vitamin C enhances the cAMP response to PTH in preosteoblast-like cells [26] and in other organ systems [31]. Vitamin C deficiency may result in end-organ resistance to PTH. Ligand binding to the CaSR encompasses cAMP-dependent post-receptor events [32]. It is conceivable that blunted CaSR signalling as a result of low vitamin C levels may result in inappropriately increased PTH release.

We also demonstrated an inverse correlation between vitamin C and AP, a protein produced primarily in bone and liver. Bone AP is generated by osteoblasts and participates in the mineralization process [33]. Elevated AP in CKD patients frequently reflects increased bone turnover associated with increased PTH levels [34,35].
Furthermore, vitamin C may play a critical role in vitamin D metabolism and vitamin D binding in target tissues [36]. It has been shown that vitamin C deficiency is accompanied with an inhibition of 25-hydroxycholecalciferol-1-hydroxylase (vitamin D 1-OHase) activity and an augmentation of 25-hydroxycholecalciferol-24-hydroxylase (vitamin D 24-OHase) activity, which catalyzes both 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D into biologically inactive, water-soluble calcitroic acid. This may lead to decreased serum calcium levels and via feedback mechanism to elevated PTH serum levels. Vitamin C-dependent alteration of vitamin D 1-OHase activity may reflect a change in cAMP-dependent phosphorylation/dephosphorylation reactions in the kidney. Vitamin C also plays a crucial role in vitamin D binding. Decreased 1,25-dihydroxycholecalciferol (1,25-(OH)2D3) receptor concentration in the intestinal mucosa and reduced 1,25-(OH)2D3 receptor binding performance in vitamin C deprivation may contribute to lower calcium serum levels [36].

Moreover, oxidative stress leads to a decreased activity of vitamin D 1-OHase and vitamin D 24-OHase. Vitamin D 1-OHase seems to be more sensitive to oxidative stress compared to vitamin D 24-OHase [37]. A stabilizing and protective effect of vitamin C on renal cholecalciferol hydroxylases may play an important role in vitamin D metabolism. Unfortunately plasma vitamin D levels were not assessed in this study.

Our observations generate testable hypotheses on interactions between vitamin C and PTH. We propose that increasing vitamin C levels by dietary supplementation results in a fall of PTH in vitamin C-deficient MHD patients with SPTH. In addition to clinical trials with vitamin C intervention, further research on the cellular basis of our findings is warranted, as could be undertaken with established cell lines from bone and parathyroid gland. As a caveat, one should not conclude that intentionally increasing vitamin C supplementation for treatment of SHPT is warranted from our observation.

Vitamin C deficiency is prevalent in a significant proportion of chronic HD patients. Low vitamin C levels may be linked to secondary hyperparathyroidism. Studies including a more detailed analysis of bone biochemistry and possibly intervention with vitamin C in patients with SHPT are needed to further elucidate the impact of vitamin C on bone metabolism in CKD patients.

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References

22. Albert MA, Danielson E, Rifiab N et al. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. JAMA 2001; 286: 64–70
27. Friedman PA, Coutermash BA, Kennedy SM et al. Parathyroid hormone stimulation of calcium transport is mediated by dual signaling mechanisms involving protein kinase A and protein kinase C. *Endocrinology* 1996; 137: 13–20
34. Pierides AM, Skillen AW, Ellis HA. Serum alkaline phosphatase in azotemic and hemodialysis osteodystrophy: a study of isoenzyme patterns, their correlation with bone histology, and their changes in response to treatment with 1alphaOHD3 and 1,25(OH)2D3. *J Lab Clin Med* 1979; 93: 899–909
36. Sergeev IN, Arkhapchev YP, Spirichev VB. Ascorbic acid effects on vitamin D hormone metabolism and binding in guinea pigs. *J Nutr* 1990; 120: 1185–1190

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