Association Between C-Reactive Protein and Biomarkers of Bone and Mineral Metabolism in Chronic Hemodialysis Patients: A Cross-Sectional Study

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**Objective:** Both chronic inflammation and dysregulation of bone and mineral metabolism are closely related with long-term outcomes of dialysis patients. Our objective was to investigate the relationship between these two abnormalities.

**Design:** This was a cross-sectional study.

**Setting:** This study was performed at a hospital-based hemodialysis center.

**Patients:** We enrolled 448 (male, 198; female, 250) clinically stable hemodialysis patients. Patients with chronic inflammatory disease, malignancy, or viral hepatitis were excluded. Their age (mean ± SD) was 57.4 ± 12.5 years.

**Main Outcome Measures:** Biomarkers, including high-sensitivity C-reactive protein (hsCRP), total calcium, phosphate, and intact parathyroid hormone levels, were measured and compared with the recommended range in the K/DOQI guidelines. Correlations between these parameters were analyzed, and factors independently associated with hsCRP and the calcium phosphate product (Ca × P) were identified by regression analysis.

**Results:** Most patients did not achieve the K/DOQI recommended therapeutic range in the four parameters, and only 50 patients (11%) met their treatment goals. The hsCRP level was directly related to calcium, phosphate, and Ca3P. Patients who achieved the guidelines’ range had lower hsCRP levels (1.97 mg/L vs. 2.71 mg/L, \( P < .05 \)). A high hsCRP level (≥ 10 mg/L) was associated with higher calcium, phosphate, and Ca × P levels, and lower albumin levels. Serum albumin, Ca × P, alkaline phosphatase, and diabetes independently predicted hsCRP levels.

**Conclusion:** There is a strong association between chronic inflammation and the disturbance of bone mineral metabolism in chronic hemodialysis patients.

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Patients with end-stage renal failure have increased cardiovascular mortality and premature death.1 In addition to conventional risk factors, new emerging contributors such as chronic inflammation, oxidative stress, and abnormal mineral metabolism were identified as factors in initiating and causing cardiovascular disease in dialysis patients.2 Enhanced chronic inflammation was reported in dialysis patients, and this finding was linked to cardiovascular and all-cause mortality.3 Markers of chronic inflammation such as C-reactive protein (CRP) and interleukin-6 were both good at predicting patient survival.4,5 Calcium and phosphate dysregulation in renal failure is very common, and contributes significantly to uremic hyperparathyroidism. Together with bone pathology, determinations of serum calcium, phosphate, parathyroid hormone, and other molecules are considered specific biomarkers in the evaluation of chronic kidney disease-related mineral and bone metabolism.6 In recent years, abnormal levels of calcium and phosphate were found to be related to long-term outcomes in dialysis patients.7–9 This abnormality,
in association with other factors such as aging, underlying diabetes, dyslipidemia, and vitamin D therapy, can lead to generalized cardiovascular calcification.\textsuperscript{10,11} The extent and severity of this extraosseous calcification can affect patient survival.\textsuperscript{12}

It remains unclear whether the systemic nature of chronic inflammation can enhance calcification or any interplay between these two risk factors. We conducted a cross-sectional analysis in hemodialysis patients to investigate treatment results regarding bone and mineral metabolism. This study also analyzed the relationship between biomarkers of chronic inflammation and calcium, phosphate, and bone biomarkers.

**Methods**

In total, 448 endstage renal failure patients who had been on dialysis for more than 6 months were enrolled. All these patients received maintenance hemodialysis, three sessions per week, and 4 hours for each session. All hemodialyzers contained semisynthetic materials, and hemodialysis therapy was performed with a bicarbonate-based dialysate. Patients without any active infection, malignancy, viral hepatitis, or chronic inflammatory diseases were eligible. Patients with any hospitalizations in the past month or with fever of unknown cause were excluded. In this study population, 23.4\% of patients (n = 105) were diabetic. Other underlying renal diseases included chronic glomerulonephritis (n = 162, 36.1\%), hypertension (n = 31, 6.9\%), polycystic kidney disease (n = 21, 4.6\%), analgesic nephropathy (n = 10, 2.2\%), and unknown (n = 119, 26.5\%). For phosphate control, aluminum hydroxide, calcium bicarbonate, calcium acetate, and sevelamer hydrochloride were prescribed to achieve the therapeutic ranges recommended by K/DOQI. Hyperparathyroidism was treated by either oral or intravenous calcitriol. Blood samples were collected upon the initiation of midweek hemodialysis. Biochemical data, including serum albumin, total calcium, phosphate, total alkaline phosphatase, and intact parathyroid hormone (iPTH), were measured. The iPTH level was determined by chemiluminescent technology (ADVIA Centaur Intact PTH assay, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA), and the normal range is 10 to 57 pg/mL. Total serum alkaline phosphatase was measured using a colorimetric assay (Alkaline Phosphatase (ALP) IFCC Liquid, Roche Diagnostics Corporation, Indianapolis, IN, USA); the normal range is 28 to 94 U/L. High-sensitivity CRP (hsCRP) was determined by a nephelometry technique (Behring Diagnostics, Marburg, Germany). Treatment results were assessed according to K/DOQI recommended guidelines.\textsuperscript{13} Demographic data, such as age, gender, duration on dialysis, and underlying renal disease, were reviewed and recorded. The adequacy of hemodialysis is presented in terms of Kt/V.

**Statistical Analysis**

All data were analyzed for normality of distribution, using the Kolmogorov-Smirnov test. Results are expressed as mean $\pm$ SD for normally distributed data and as median (interquartile range) for nonparametric data. Student’s t-test was used for comparisons of means between two groups, and the Mann-Whitney U-test was used for nonparametric data. A $\chi^2$ test was used for category group comparisons. We further determined correlations between all variables with Pearson’s correlation test. The hsCRP was Ln-transformed in all statistical analyses and was presented as LnhsCRP. Multivariate regression was used to find factors independently associated with calcium phosphate product (Ca $\times$ P) and hsCRP.

**Results**

**Treatment Results and Data Distribution**

Figure 1 demonstrates the treatment results of bone and mineral metabolism, based on K/DOQI guidelines. The mean levels of total calcium, phosphate, and Ca $\times$ P were within therapeutic range. Mild elevations of alkaline phosphatase and iPTH were evident (Table 1). Half of the patients satisfied their therapeutic calcium goal, but 40.6\% had a lower level (<8.5 mg/dL). In patients with an acceptable calcium level (8.5 to 9.5 mg/dL), 16.1\% (36 patients) achieved all K/DOQI guidelines. Hyperphosphatemia (>5.5 mg/dL) was observed in 33\% of the study population, and 54.5\% of patients met their treatment goal. Among these patients, 17.6\% (43 patients) achieved all K/DOQI guideline. Almost a quarter of the enrolled patients (23.7\%) had a Ca $\times$ P greater than 55 mg$^2$/dL$^2$, with an average of 65.5 $\pm$ 9.7 mg$^2$/dL$^2$, whereas patients with a
Ca \times P of less than 55 \text{mg}^2/\text{dL}^2 \text{ had a significantly lower average value (40.9 \pm 8.4 \text{mg}^2/\text{dL}^2, P < .05). The hsCRP level was also significantly higher in patients with a Ca \times P greater than 55 \text{mg}^2/\text{dL}^2 \text{ (median, 3.86 mg/L vs. 2.4 mg/L, P = .014). In patients whose Ca \times P met the guideline, 15.1\% (50 patients) achieved the other three guideline-recommended targets. The distribution of iPTH levels was uneven, and one third of patients had a lower iPTH (<150 pg/mL), whereas another 42.8\% had higher iPTH levels (>300 pg/mL). About one fifth (20.8\%) of our patients were within the recommended level, and 53.8\% (50 patients) achieved the four treatment targets. Overall, only 50 patients achieved the guideline range of the four parameters; they constituted 11.2\% of the total study population.

Comparison Studies and Correlations Between Parameters

A comparison between patients who satisfied all parameters and other patients showed significant differences in calcium, phosphate, Ca \times P, and iPTH levels (Table 1). The hsCRP level was also significantly lower in those patients who achieved all four measurements. There was no difference in albumin, hemoglobin, and alkaline phosphatase levels. Table 2 includes the four parameters and the demographic data of study patients according to their hsCRP levels. Patients with more inflammation (hsCRP $\geq$10 mg/dL) had significantly higher levels of calcium, phosphate, and Ca \times P. Furthermore, these patients were older and had lower albumin levels. In the patient group of hsCRP less than 10 mg/L, 11.9\% (48/402) achieved the four K/DOQI recommended targets, whereas only 4.3\% (2/50) of the high hsCRP group achieved four acceptable parameters.

Figure 2 demonstrates the correlations between LnhsCRP and the four K/DOQI parameters. We found that LnhsCRP was positively correlated with calcium ($r = 0.116$, $P < .05$), phosphate ($r = 0.108$, $P < .05$), Ca \times P ($r = 0.130$, $P < .01$), and alkaline phosphatase ($r = 0.124$, $P < .01$). There was no association between LnhsCRP and iPTH ($r = -0.033$, $P > .05$) and dialysis duration ($r = 0.17$, $P > .05$). The analysis of other relationships revealed that serum albumin levels were positively correlated with iPTH levels ($r = 0.136$, $P < .01$) and hemoglobin ($r = 0.101$, $P < .05$), but were negatively correlated with age ($r = -0.427$, $P < .001$), calcium ($r = -0.129$, $P < .01$), and LnhsCRP ($r = -0.295$, $P < .001$). The iPTH level was directly related to dialysis duration ($r = 0.235$, $P < .001$), calcium ($r = 0.161$, $P < .01$), phosphate ($r = 0.351$, $P < .001$), Ca \times P ($r = 0.389$, $P < .0001$), and alkaline phosphatase ($r = 0.602$, $P < .0001$). The reverse association was found between iPTH and age ($r = -0.203$, $P < .0001$). In addition to albumin and iPTH, patient age was negatively correlated with phosphate ($r = -0.146$, $P < .001$) and Ca \times P ($r = -0.108$, $P < .05$), but was positively correlated with LnhsCRP and calcium ($r = 0.127$, $P < .01$).
Multivariate regression analysis revealed that the independent associations of hsCRP in the present study were with underlying diabetes, albumin, Ca × P, and alkaline phosphatase levels (Table 3). Calcium, phosphate, and alkaline phosphatase levels were independently related to Ca × P.

**Discussion**

Our results clearly demonstrate that few dialysis patients achieved the therapeutic goals recommended by K/DOQI guidelines in terms of four targets: calcium, phosphate, Ca × P, and parathyroid hormone levels. The average levels of calcium, phosphate, and Ca × P were within recommended ranges, and only iPTH was mildly elevated. About half of our patients met the goals of calcium and phosphate ranges, but less than one third of patients achieved the recommended Ca × P and iPTH levels. The most difficult target involved maintaining parathyroid hormone levels within the recommended range. Overall, only 11% of patients under our care fulfilled the four-parameter criteria. This treatment result is very similar to that reported in the Dialysis Outcomes and Practice Patterns Study.\(^{14}\) Undoubtedly it remains challenging for nephrologists to manage bone mineral metabolism appropriately.\(^{15}\) We also note that patients who achieved four parameters of the K/DOQI guidelines had less inflammation, because their hsCRP levels were significantly lower. Because hsCRP is a well-known representative of microinflammation and also an outcome predictor among dialysis populations, the measurement of this biomarker should be recommended in the routine monitoring of chronic dialysis patients. Further research is necessary to establish how to develop a practical strategy for detection, follow-up, and especially treatment.

Abnormal calcium and phosphate homeostasis is one of the early complications in renal failure. As renal failure progresses, signs of abnormal hormonal regulation develop, such as vitamin D insufficiency and hyperparathyroidism. Adequate control of calcium, phosphate, and parathyroid hormone levels constitutes a fundamental element for managing renal osteodystrophy.\(^{16}\) Many agents are available to correct these abnormalities of renal failure and dialysis patients. Most of the time, the treatment endpoint is confined to uremic hyperparathyroidism and skeletal involvement. Recent studies to assess the effect of these abnormalities
on long-term outcomes revealed that both baseline levels and time-varying measurements of calcium, phosphate, parathyroid hormone, and Ca × P strongly predicted mortality and morbidity. How these biomarkers correlate with long-term outcomes remains unclear. Several studies indicated that cardiovascular calcification predicts long-term outcomes in dialysis patients. The uremia-associated vascular calcification in media layers can evolve into cardiovascular complications and significantly affect patient outcome. The pathomechanism of vascular calcification involves a variety of contributing factors. In addition to calcium phosphate deposits, several novel and active processes, such as hyperphosphatemia-induced phenotypic changes of vascular smooth muscle cells, play important roles. Chronic inflammation is currently considered an integral component of atherosclerosis. It is therefore reasonable to hypothesize that inflammatory reactions contribute to vascular calcification, although this pathological process was originally thought to be confined to the intima. Systemic inflammation can also inhibit fetuin-A synthesis, which is an inhibitor of vascular calcification. Because chronic inflammation is prevalent in the dialysis population, this generalized reaction may be associated with vasculopathy in uremia. On the other hand, whether active calcification within the vascular wall can initiate or enhance the inflammatory reaction remains unclear. Clinical studies found that the presence and the extent of vascular calcification were associated with higher CRP levels as well as mortality. Our results showed that dialysis patients with more inflammation had higher levels of calcium, phosphate, and Ca × P than those with less inflammation. In multivariate regression analysis, after adjusting for calcium and phosphate, Ca × P independently correlated with hsCRP. These findings indicate a close relationship between chronic inflammation and disturbance in calcium and phosphate.

The Ca × P product has long been recognized as a risk factor for extrasosseous calcification. One study showed that mortality was increased significantly when the product was greater than 72 mg²/dL. However, other studies indicate that lower Ca × P promised a better outcome. The strong association between hsCRP and Ca × P found in this study may confirm its therapeutic potential. By reducing Ca × P, it was found that

<table>
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<tr>
<th>Table 2. Demographic and Biochemical Data of Study Patients, Based on hsCRP (mg/L) Levels</th>
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<td>Groups (No., %)</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>hsCRP &lt; 10</td>
</tr>
<tr>
<td>hsCRP ≥ 10</td>
</tr>
<tr>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Ca × P, calcium phosphate product; iPTH, intact parathyroid hormone; Alk-p, alkaline phosphatase; hsCRP, high-sensitivity C-reactive protein.</td>
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*P < 0.05, hsCRP < 10 mg/L versus hsCRP ≥ 10 mg/L.
inflammation diminished. In the present study, a significant difference was noted between low-grade versus high-grade inflammation (46.7 ± 13.7 mg/dL vs. 52.0 ± 14.2 mg/dL, respectively). Although the value of the more inflamed patient group was lower than 55 mg/dL, an increased mortality was evident at greater than 50 mg/dL. Further study is required to confirm whether a lower Ca × P is beneficial. Measurement of parathyroid hormone levels is an important indicator for diagnosing chronic kidney disease-associated mineral and bone disorders. Most studies found that the level of parathyroid hormone can predict patient outcome, in association with the other three parameters: calcium, phosphate, and Ca × P. In this study, both univariate and multivariate analyses did not establish any correlation between iPTH and hsCRP levels. Similarly, the role of parathyroid hormone in vascular calcification is also controversial.

Figure 2. Correlations of LnhsCRP with serum calcium, phosphate, calcium phosphate product (Ca × P), intact parathyroid hormone (iPTH), and alkaline phosphatase (alk-P).
lack of association between parathyroid hormone and inflammatory markers in cross-sectional studies suggests that uremic hyperparathyroidism may not contribute to chronic inflammation in dialysis patients. However, longitudinal studies may help determine the interactions between these two common complications, along with their impacts on patient outcome. Interestingly, serum total alkaline phosphatase level was shown to be a good indicator of survival in chronic hemodialysis patients. We also found that alkaline phosphatase level independently correlated with hsCRP level. Along with a direct relationship with hyperparathyroidism and bone activity, levels of parathyroid hormone and alkaline phosphatase may represent different aspects of interpreting mineral and bone metabolism in renal failure.

We conclude that the success rate of achieving treatment goals, as recommended in K/DOQI guidelines, is not high. There was an association between chronic inflammation and markers of phosphocalcic metabolism as well as serum alkaline phosphate levels. With all parameters within therapeutic ranges, the severity of inflammation declined in chronic hemodialysis patients. It remains unclear whether a better control of chronic kidney disease-associated mineral and bone disorders leads to less inflammation, or on the contrary, if a high degree of inflammation makes effective control of these dysregulations more difficult or impossible. Further study is needed to clarify these complex relationships.

References


Table 3. Multivariate Stepwise Linear Regression Analysis of Variables Significantly Related to Levels of LnCRP in Chronic Hemodialysis Patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standardized β-Coefficient</th>
<th>95% Confidence Interval</th>
<th>P Value</th>
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</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>0.113</td>
<td>0.067 to 0.542</td>
<td>.012</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.286</td>
<td>−1.423 to −0.753</td>
<td>.000</td>
</tr>
<tr>
<td>Ca × P</td>
<td>0.131</td>
<td>0.004 to 0.018</td>
<td>.004</td>
</tr>
<tr>
<td>Alk-p</td>
<td>0.116</td>
<td>0.000 to 0.003</td>
<td>.01</td>
</tr>
</tbody>
</table>

Ca × P, calcium phosphate product; Alk-p, alkaline phosphatase.


