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Osteopontin Levels in Patients With Chronic Kidney Disease Stage 5 on Hemodialysis Directly Correlate With Intact Parathyroid Hormone and Alkaline Phosphatase

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Abstract

Chronic kidney disease stage 5 (CKD5) marks the fifth stage of renal failure, frequently causing dysregulation of bone and mineral metabolism. Challenges exist in evaluating and managing chronic kidney disease–mineral bone disorder (CKD-MBD) with the standard panel of biomarkers. Our objective was to profile osteopontin (OPN) in patients with CKD5 on maintenance hemodialysis (CKD5-HD) and elucidate its relationship to phosphorus (P), calcium (Ca^{2+}), alkaline phosphatase (AP), and intact parathyroid hormone (iPTH) to improve understanding of the present model of CKD-MBD. Elevation of plasma OPN was seen in the CKD5-HD cohort (n = 92; median: 240.25 ng/mL, interquartile range [IQR]: 169.85 ng/mL) compared to a normal group (n = 49; median: 63.30 ng/mL, IQR: 19.20 ng/mL; $p < .0001$). Spearman correlation tests revealed significant positive correlations of OPN with iPTH ($p < .0001$; $r = 0.561$, 95% confidence interval = 0.397-0.690) and OPN with AP ($p < .0001$; $r = 0.444$, 95% confidence interval = 0.245-0.590) in CKD5-HD patients. Ultimately, OPN may play an integral role in the MBD axis, suggesting that it may be important to actively monitor OPN when managing CKD5-HD.

Keywords

kidney disease, hemodialysis, biomarkers, mineral and bone disorder, osteopontin

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Introduction

Chronic kidney disease stage 5 (CKD5) is the fifth stage of renal failure, with treatment necessitating dialysis or kidney transplant. The number of CKD5 prevalent cases rises by approximately 20,000 per year, and in 2016, the number of prevalent cases reached 726,331.[1] Chronic kidney disease leads to dysregulation of calcium (Ca^{2+}), phosphorus (P), parathyroid hormone (PTH), and vitamin D metabolism, resulting in biochemical laboratory abnormalities, significant bone disease, and/or vascular calcification that define chronic kidney disease–mineral and bone disorder (CKD-MBD).[2-4] Kidney Disease Improving Global Outcomes (KDIGO) recommends evaluation of CKD-MBD by monitoring serum Ca^{2+}, phosphate, PTH, and alkaline phosphatase (AP) starting in stage G3a of CKD.[2-4] However, even with careful monitoring of these markers, patients with CKD5 on maintenance hemodialysis (CKD5-HD) have poorer health outcomes related to MBD, such as increased risk of developing cardiovascular disease and increased fracture risk.[5-7] While obtaining abdominal radiographs, computed tomography–based imaging, or dual-energy X-ray absorptiometry scans or performing a bone biopsy to help evaluate calcification and bone mineral density (BMD) status, the strength of

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recommendation of these practices is low.[3,4] Therefore, the utility in identifying additional biomarkers to better evaluate, diagnose, and treat patients with CKD5-HD with MBD is appealing.[8-11]

Osteopontin (OPN) is a glycol-phosphoprotein found in bone, acute and chronic inflammatory cells, smooth muscle, epithelial, and endothelial cells, neurons, and fetal renal tissue and is expressed in the thick ascending limb of the loop of Henle.[12,13] Some of its functions include increasing macrophage and T-cell counts, perpetuation of inflammation, wound healing, tumor development and progression, roles in diabetes, and possible roles in the regulation of nephrolithiasis and nephrogenesis.[12,14] OPN was also found to promote angiogenesis, encourage growth and invasion of renal cancer, impact the development of lupus nephritis in patients with systemic lupus erythematosus, and potentially be useful as a marker of acute allograft rejection in kidney transplants.[15-20] Additionally, OPN is important in regulation of vascular calcification and bone mineralization. Local increases in OPN in vessel walls have been linked to atherosclerotic plaque formation, inflammation within arteries, and smooth muscle mineralization.[12,21,22] The function of OPN in bone is defined by its ability to anchor osteoclasts via the $\alpha_v\beta_3$ integrin.[23] By anchoring osteoclasts, OPN plays a significant role in bone resorption and turnover.

A number of biomarkers have been evaluated in CKD-MBD, but there is a clear need for better characterization of novel markers, especially within the context of CKD5-HD.[9,10,11] Serum monitoring of OPN may satisfy this need for improved diagnosis and management of CKD-MBD as it (1) has clear physiologic functions associated with bone and mineral regulation and (2) has been shown to have high sensitivity and specificity in diagnosing bone turnover disease and coronary calcification in certain populations.[24,25] Our study sought to evaluate the plasma levels of OPN in a CKD5-HD population and describe its relationship to the current markers used for evaluating MBD established by the KDIGO group.

Table 1. Demographics of Patient With CKD5-HD, Loyola University Hospital System Outpatient Dialysis Center.

Demographic	No. of Patients[a] (% of Total)
Age	60.5 (20.2-94.8)[b]
Sex	
Male	44 (47.8%)
Female	48 (52.2%)
Race/ethnicity	
White	24 (26.1%)
Black	49 (53.3%)
Asian/Pacific Islander	0 (0.0%)
Native American/Alaskan Native	0 (0.0%)
Hispanic/Latino/other	19 (20.7%)
BMI	
<20 kg/m^2	4 (4.3%)
20-24.9 kg/m^2	25 (27.2%)
25-30 kg/m^2	26 (28.3%)
>30 kg/m^2	37 (40.2%)
Comorbidities	
Diabetes	60 (65.2%)
Coronary artery disease	44 (47.9%)
Osteoporosis/osteopenia	23 (25.0%)
Comorbidities	
Atrial fibrillation	14 (15.2%)
Stroke history	17 (18.5%)
Medications	
Calcium acetate	29 (31.5%)
Lanthanum	5 (5.4%)
Cinacalcet	16 (17.4%)
Sevelamer carbonate	42 (45.7%)
ACE inhibitor	15 (16.3%)
ARB	5 (5.4%)
Supplementation	
Vitamin D	82 (91.3%)
Calcium carbonate	10 (10.9%)

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; BMI, body mass index; CKD5-HD, chronic kidney disease on maintenance hemodialysis.
[a] n = 92.
[b] Age reported as mean age (minimum age-maximum age).

Methods

Whole blood samples were collected in sodium citrate tubes from stable patients with CKD5-HD treated at Loyola University Hospital System's Outpatient Dialysis Center (n = 92). Normal plasma was obtained from 49 nonsmoking, drug-free healthy volunteers (25 males and 24 females) from George King Bio-Medical, Inc (Overland Park, Kansas). Both CKD5-HD and normal plasma samples were frozen at −80°C for storage and later analysis. Plasma levels of OPN in both populations were measured using a commercially available OPN sandwich enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, Minnesota). Epic electronic medical record charts of the 92 CKD5-HD patients were reviewed for levels of iPTH, AP, P, and Ca^{2+} at the time blood samples were collected. Demographics such as age, sex, race/ethnicity, body mass index, comorbidities, medications, and supplementation were obtained through the chart review and are represented in Table 1.

Statistical Analyses

Comparison of OPN levels between the CKD5-HD and normal population was performed utilizing a Mann-Whitney t test, with a $p < .05$ demonstrating statistical significance. Results were expressed as median and interquartile range (IQR: IQR presented as one value, Q3 - Q1). Relationships of OPN levels with iPTH, AP, P, and Ca^{2+} levels were identified via Spearman correlation tests. Results were deemed statistically significant with $p < .05$. The r values were produced to determine the strength of correlation between 2 markers. The data was collected in Microsoft Excel and analyzed using Windows GraphPad Prism v7 software (GraphPad Software, La Jolla, California). Normality was determined via the IBM SPSS Statistics software (SPSS Inc., Chicago, IL).

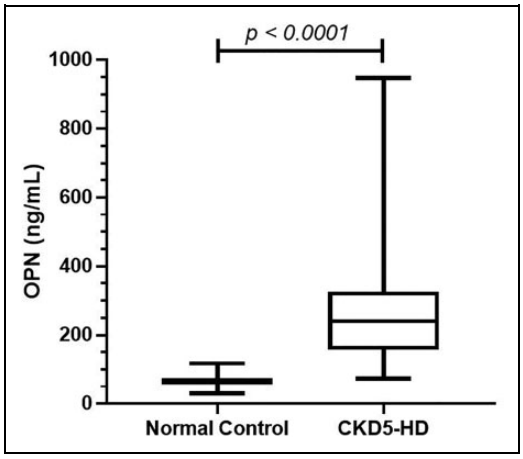

Figure 1. Plasma level differences of osteopontin (OPN) in a normal control group and patients with chronic kidney disease stage 5 on hemodialysis (CKD5-HD). Normal control: n = 49; median = 63.3 ng/mL; interquartile range (IQR) = 19.20 ng/mL; CKD5-HD: n = 92; median = 240.25 ng/mL; IQR = 169.85 ng/mL. Plasma levels of OPN are elevated in CKD5-HD compared to normal control group ($p < .0001$ via Mann-Whitney t test).

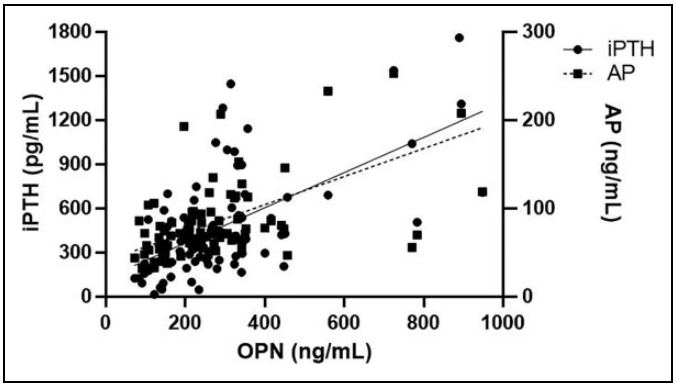

Figure 2. Spearman correlational analysis comparing osteopontin (OPN) to intact parathyroid hormone (iPTH), and OPN to alkaline phosphatase (AP) in patients with chronic kidney disease stage 5 on hemodialysis (CKD5-HD). Results show a significantly positive correlation between OPN and iPTH ($p < .0001$; $r = 0.561$, 95% confidence interval = 0.397-0.690) and OPN and AP ($p < .0001$; $r = 0.444$, 95% confidence interval = 0.245-0.590).

Results

Primary analysis compared plasma OPN levels in the CKD5-HD cohort to the normal population. A statistically significant elevation of OPN plasma levels in the CKD5-HD cohort (median: 240.25 ng/mL, IQR: 169.85 ng/mL) was observed compared to the normal group (median: 63.30 ng/mL, IQR: 19.20 ng/mL) with a p value of <.0001 via the Mann-Whitney t test (Figure 1).

Correlation analyses were conducted between OPN and iPTH, OPN, and AP, OPN and Ca^{2+}, and OPN and P. Spearman correlation tests revealed a significant positive correlation of OPN with iPTH ($p < .0001$; $r = 0.561$, 95% confidence interval = 0.397-0.690) and OPN with AP ($p < .0001$; $r = 0.444$, 95% confidence interval = 0.245-0.590) in the CKD5-HD cohort (Figure 2). No other correlations were found between OPN and total Ca^{2+} ($p = .36$, $r = 0.096$; data not shown) or OPN and P ($p = .269$, $r = 0.116$; data not shown).

Discussion

Our results show a significant increase in the plasma level of OPN in patients with CKD5-HD. These findings are consistent with previous studies in CKD showing elevated OPN.[26] Elevation of OPN in our patient samples may be due to its upregulation in chronic inflammatory states and/or due to MBD.[9,27] High levels of OPN are important to note as they are associated with all-cause mortality in patients with CKD5-HD.[28]

Chronic kidney disease leads to Ca^{2+}-P-vitamin D-PTH dysregulation.[26] Progression of CKD causes more severe MBD, which can lead to severe bone disease such as osteitis, osteomalacia, and possibly osteoporosis.[29,30]

The relationship of OPN to the Ca^{2+}-P-vitamin D-PTH axis is more complex. A study from Shen and Christakos demonstrated marked increase in OPN transcription with stimulation of the vitamin D receptor.[31] Animal studies of vascular calcification have demonstrated an increase in OPN expression after treatment with vitamin D receptor agonists.[32] OPN knockout mice have shown to have higher bone formation secondary to PTH-induced increase in osteoblast activity, and PTH infusion has been shown to increase OPN levels.[33,34] These studies exemplify how OPN expression can be significantly altered by changes in a number of variables in the Ca^{2+}-P-vitamin D-PTH axis.

AP is a common marker of bone turnover and has shown to be elevated in patients with CKD with low BMD and is also reported to be associated with higher mortality in patients with or without MBD.[4,35-37] Our results show a positive correlation between OPN and AP. OPN has been shown to play a role in the inhibition of vessel calcification.[21] Lomashvili and colleagues demonstrated in rat models how AP can both dephosphorylate OPN and increase aortic calcification.[21,38] Both OPN and AP have increased expression in high bone turnover states.[23,36] However, variability of duration on dialysis, degree of inflammation, and status of calcification and bone turnover states in our CKD5-HD cohort make it difficult to predict the cause of the direct relationship between OPN and AP.[35,36,38]

Limitations

Our study has several limitations. The sample size is relatively small, and samples were collected at varying time points during the day. Circadian rhythm and diet may have affected the levels of proteins and minerals that were measured.[39] The amount of time since starting dialysis varied for each patient, which could have affected levels of certain biomarkers.[35] The patients with CKD5-HD had a significant number of comorbidities, such as diabetes, hypertension, and coronary/peripheral artery disease which could have affected the regulation of Ca^{2+}, P, OPN, and

iPTH. However, our cohort represents the typical patients with CKD5-HD as most are found to have upward of 3 comorbidities at any given time.[40] Serum bicarbonate and bone-specific AP have been studied in CKD-MBD; however, we did not include these markers in our study due to lower strength of recommendation outlined by KDIGO as well as cost of add-on laboratory testing.[4] Future studies may warrant multivariate modeling of OPN with these markers in addition to the ones studied.

Conclusion

This study demonstrates a significant elevation in OPN in patients with CKD5-HD compared to normal blood samples and identified a direct relationship between OPN and iPTH and OPN and AP in the same CKD5-HD cohort. These findings suggest OPN may play an integral role in ion homeostasis, vascular calcification, and bone turnover axes in CKD5-HD. This underscores that it may be important to include OPN in the group of markers used to evaluate patients with CKD5-HD for mineral and bone dysregulation.

Authors' Note

Ethical approval to report this case was obtained from Loyola University Chicago Health Sciences Institutional Review Board (#LU107346). Verbal informed consent was obtained from the patients for their anonymized information to be published in this article.

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Declaration of Conflicting Interests

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