Deoxycholic Acid, a Metabolite of Circulating Bile Acids, and Coronary Artery Vascular Calcification in CKD

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Background: Vascular calcification is common among patients with chronic kidney disease (CKD), and it is associated with all-cause and cardiovascular disease mortality. Deoxycholic acid, a metabolite of circulating bile acids, is elevated in CKD and induces vascular mineralization and osteogenic differentiation in animal models.

Study Design: Cohort analysis of clinical trial participants.

Setting & Participants: 112 patients with moderate to severe CKD (estimated glomerular filtration rate, 20-45 mL/min/1.73 m^2) who participated in a randomized controlled study to examine the effects of phosphate binders on vascular calcification.

Predictor: Serum deoxycholic acid concentration.

Outcomes: Baseline coronary artery calcification (CAC) volume score and bone mineral density (BMD) and change in CAC volume score and BMD after 9 months.

Measurements: Deoxycholic acid was assayed in stored baseline serum samples using liquid chromatography–tandem mass spectrometry, CAC was measured using a GE-Imitron C150 scanner, and BMD was determined using computed tomographic scans of the abdomen with calibrated phantom of known density.

Results: Higher serum deoxycholic acid concentrations were significantly correlated with greater baseline CAC volume and lower baseline BMD. After adjusting for demographics, coexisting illness, body mass index, estimated glomerular filtration rate, and concentrations of circulating markers of mineral metabolism, including serum calcium, phosphorus, vitamin D, parathyroid hormone, and fibroblast growth factor 23, a serum deoxycholic acid concentration > 58 ng/mL (the median) was positively associated with baseline CAC volume (β = 0.71; 95% CI, 0.26-1.16; P = 0.003) and negatively associated with baseline BMD (β = −20.3; 95% CI, −1.5 to −39.1; P = 0.04). Serum deoxycholic acid concentration > 58 ng/mL was not significantly associated with change in CAC volume score after 9 months (β = 0.06; 95% CI, −0.09 to 0.21; P = 0.4). The analysis for the relationship between baseline deoxycholic acid concentrations and change in BMD after 9 months was not statistically significant, but was underpowered.

Limitations: The use of nonfasting serum samples is a limitation because deoxycholic acid concentrations may vary based on time of day and dietary intake. Few trial participants with complete data to evaluate the change in CAC volume score (n = 75) and BMD (n = 59). No data for changes in deoxycholic acid concentrations over time.

Conclusions: Among patients with moderate to severe CKD, higher serum deoxycholic acid concentrations were independently associated with greater baseline CAC volume score and lower baseline BMD.

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Am J Kidney Dis. 71(1): 27-34. Published online August 9, 2017.

doi: 10.1053/j.ajkd.2017.06.017

Many people live with chronic kidney disease (CKD); in the United States, the overall prevalence of CKD is ~14%.[1] CKD carries significant morbidity and mortality.[2] Among Medicare patients, the adjusted mortality rate among those with CKD is 118 deaths/1,000 person-years compared to 48 deaths/1,000 person-years among those without CKD.[1] Many patients with CKD are more likely to suffer a cardiovascular disease event or die than to progress to end-stage renal disease.[2] For those with CKD, cardiovascular disease represents an outsized factor associated with poor outcomes. Not only is there a greater prevalence of traditional cardiovascular risk factors compared with those with normal kidney function,[3] CKD also confers nontraditional cardiovascular risk factors such as abnormal mineral metabolism, anemia, malnutrition, increased oxidative stress, inflammation, and volume overload.[4] A well-established cardiovascular risk factor in CKD is systemic and coronary artery calcifications (CACs), which are highly prevalent among both non–dialysis-dependent[5] and dialysis-dependent[6] patients with CKD. Among those with non–dialysis-dependent CKD, CAC has been shown to increase the risk for all-cause mortality, cardiovascular disease events and mortality, and hospital admission.[7-9] Likewise, among patients with dialysis-dependent CKD, CAC is associated with vascular dysfunction,[10] a risk factor for cardiovascular disease, and is an independent and incremental predictor of all-cause mortality.[11]

Vascular calcification occurs when hydroxyapatite crystals deposit in the intimal or medial layer of arteries. It is an actively regulated process[12] involving various signaling pathways. Many clinical factors are associated with vascular calcification: advancing age, diabetes, kidney function decline, inflammatory states, and rare genetic conditions. In CKD, inflammatory cytokines and abnormal mineral metabolism, especially hyperphosphatemia, induce vascular calcification.[13] However, other mechanisms are also implicated.[14]

Circulating bile acid concentrations are elevated in CKD.[15,16] Furthermore, the composition of bile acids is

perturbed, characterized by a decrease in the proportion of the primary bile acid, cholic acid, and an increase in the proportion of the secondary bile acid, deoxycholic acid.[15] Deoxycholic acid is directly toxic when applied to vascular smooth muscle cells.[17] Bile acids and their nuclear receptor, farnesoid X receptor (FXR), regulate key processes such as lipid and glucose metabolism,[18] and FXR is found in numerous tissues, including liver, kidney, intestine, macrophages, and vasculature.[18] FXR activation attenuates vascular calcification in a CKD model,[19] reduces atherosclerotic plaque formation in animal models,[20,21] and reduces circulating deoxycholic acid concentrations.[21] Thus, a plausible hypothesis is that elevated circulating deoxycholic acid concentrations in CKD promote vascular calcification through reduced FXR activation and direct vasculature toxicity.

Low bone mineral density (BMD) and fracture are strongly related to vascular calcification and poor cardiovascular outcomes. Among postmenopausal women and older men, vascular calcification is linked with low BMD[22,23] and increased fracture rate.[24-26] Likewise osteoporosis severity is proportionally associated with cardiovascular disease event risk.[27] Similar associations among vascular calcification, low BMD, and osteoporosis have been observed in dialysis patient[28-32] and nondialysis CKD[33,34] cohorts. Precise mechanisms that underlie these observations are still unclear. However, vascular calcification and bone remodeling share many of the same signaling pathways and genes and may be mediated by age, inflammation, and in the case of CKD, mineral metabolism abnormalities.[35]

Understanding the mechanisms associated with the development of vascular calcification and decreased BMD will identify potential treatment targets and strategies. Because FXR activation is associated with reduced deoxycholic acid concentrations and attenuated vascular calcification, we hypothesized that elevated circulating deoxycholic acid concentrations among patients with CKD would be associated with more severe vascular calcification and decreased BMD. This is a post hoc analysis of the randomized controlled trial Effects of Phosphate Binders in Moderate CKD.[36] Here, we report results from a cross-sectional and longitudinal analysis of patients with CKD stages 3b to 4 (estimated glomerular filtration rate [eGFR], 20-45 mL/min/1.73 m^2) examining the association of baseline deoxycholic acid concentrations with baseline CAC volume scores, a measure of vascular calcification, and baseline BMD, as well as the association of baseline deoxycholic acid concentrations with change in CAC scores and BMD after 9 months.

Methods

Participants

Details of the study that assessed the effects of phosphate binding on serum markers of mineral metabolism, vascular calcification, and lumbar BMD were described previously.[36] Briefly, 148 participants with CKD stages 3b to 4 (eGFR, 20-45 mL/min/1.73 m^2 calculated with the MDRD [Modification of Diet in Renal Disease] Study equation) and phosphorus concentrations of 3.5 to 6.0 mg/dL were randomly assigned to calcium acetate, lanthanum carbonate, sevelamer, or matching placebo and followed up for 9 months. The primary end point was change in serum phosphorus concentrations, and secondary end points included changes in concentrations of serum parathyroid hormone (PTH), fibroblast growth factor 23 (FGF-23), active vitamin D (1,25-dihydroxyvitamin D [1,25 $[OH]_2D$]), and urine phosphorus and fractional excretion of phosphorus, as well as changes in vascular calcification scores (coronary arteries, thoracic, and abdominal aorta), and change in lumbar BMD. The study was approved by the Schulman Institutional Review Board (Cincinnati, OH) and registered at ClinicalTrials.gov (study number: NCT00785629). The Colorado Multiple Institution Review Board protocol number is 09-0870. All study participants provided written documentation of informed consent. Procedures were in accordance with the ethics standards of the institutional review board and the Declaration of Helsinki.

A subset of participants with available serum and complete baseline information for CAC and lumbar BMD were included in the present cross-sectional analysis, resulting in a final cohort of 112 participants. Additionally, there were available data for 75 of the 112 participants for a longitudinal analysis examining the relationship between baseline deoxycholic acid concentrations and change in CAC volume score after 9 months; only 59 participants had complete data to examine the relationship between baseline deoxycholic acid concentrations and change in BMD after 9 months.

Variables

Nonfasting baseline stored serum samples were assayed for the predictor variable, circulating deoxycholic acid concentration, using liquid chromatography–tandem mass spectrometry as previously described.[21] In brief, human serum (100 μL) was diluted in 300 μL of cold acetonitrile containing 3 ng of D6-deoxycholic acid (Cambridge Isotope Laboratory) as internal standard. The mixture was passed through a Phree phospholipid removal plate (Phenomenex). The eluate was evaporated with nitrogen gas stream, then redissolved in 100 μL of 10 mM of ammonium acetate buffer (pH 8.0)/methanol (1:1, v/v). A 10-μL aliquot of each sample solution was then injected into a liquid chromatography–electrospray ionization–tandem mass spectrometry system (QTRAP 3200; SCIEX) for analysis. Outcome variables were baseline total CAC volume score and lumbar BMD. CAC volume score was obtained using a GE-Imitron C150 scanner and a standard protocol as previously described.[37] Atherosclerotic calcium was defined as a plaque area ≥ 1 mm^2 with density ≥ 130 Hounsfield units. Total calcium volume score was derived by the sum of all lesion volumes in cubic