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Dysregulated Mineral Metabolism in Patients with Acute Kidney Injury and Risk of Adverse Outcomes

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Abstract

Objective—Numerous studies have evaluated the prevalence and importance of vitamin D deficiency among patients with chronic kidney disease and end-stage renal disease, however, little is known about vitamin D levels in acute kidney injury (AKI). We evaluated the association between vitamin D metabolites and clinical outcomes among patients with AKI.

Design—Prospective cohort study.

Patients—30 participants with AKI and 30 controls from general hospital wards and intensive care units at a tertiary care hospital.

Measurements—Plasma levels of 25-hydroxyvitamin D [25(OH)D], 1,25-dihydroxyvitamin D [1,25(OH)₂D], 24R,25-dihydroxyvitamin D₃, vitamin D binding protein (VDBP), and fibroblast growth factor 23 (FGF23) were measured within 24 hours of AKI onset and 5 days later. Bioavailable 25(OH)D and 1,25(OH)₂D levels, defined as the sum of free- and albumin-bound 25(OH)D and 1,25(OH)₂D, were estimated using equations.

Results—Compared to controls, participants with AKI had lower levels of 1,25(OH)₂D [17 (10–22) versus 25 (15–35) pg/ml, $p=0.01$], lower levels of VDBP [23 (15–31) versus 29 (25–36) mg/dl, $p=0.003$], and similar levels of bioavailable 25(OH)D and 1,25(OH)₂D at enrollment. Levels of bioavailable 25(OH)D were inversely associated with severity of sepsis in the overall sample ($p<0.001$). Among participants with AKI, bioavailable 25(OH)D, but not other vitamin D metabolites, was significantly associated with mortality after adjusting for age and serum creatinine (adjusted odds ratio per 1 SD ln [bioavailable 25(OH)D]=0.16, 95% confidence interval=0.03 to 0.85).

Conclusions—Bioavailable 25(OH)D could have a role as a biomarker or mediator of adverse outcomes among patients with established AKI.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest. The results presented in this paper have not been published previously in whole or part.

Keywords

acute kidney injury; 1-alpha-hydroxylase; 25(OH)D

INTRODUCTION

Vitamin D deficiency is common among patients with chronic kidney disease (CKD) and end-stage renal disease (ESRD), and is associated with secondary hyperparathyroidism,¹ anemia,² erythropoietin resistance,³ impaired immune response,^{4,5} and increased morbidity and mortality.⁶ In contrast, little is known about vitamin D deficiency or its association with outcomes among patients with acute kidney injury (AKI).

Deficiencies of both 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25(OH)₂D] have been reported in small studies in rats,⁷ dogs,⁸ and humans with AKI.^{9, 10} Larger studies among critically-ill patients have shown strong associations between 25(OH)D deficiency and adverse outcomes, including increased length of stay,¹¹ infection,¹² and mortality.¹¹⁻¹⁴ However, these studies were focused on critical illness and did not specifically evaluate the association between vitamin D deficiency and AKI. Patients with AKI may be particularly at risk of having low levels of 1,25(OH)₂D due to diminished renal synthesis. Most studies of vitamin D among hospitalized patients, however, only measured 25(OH)D and did not simultaneously measure levels of other vitamin D metabolites, vitamin D binding protein, and other hormones that regulate mineral metabolism, such as parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23). Accordingly, the metabolism, pathophysiology, and clinical relevance of dysregulated mineral metabolism among patients with AKI remain largely unknown.

We tested the hypotheses that AKI is associated with decreased levels of 25(OH)D, 1,25(OH)₂D, and bioavailable 25(OH)D (the sum of free- and albumin-bound 25(OH)D), and that decreased levels of these vitamin D metabolites are associated with greater severity of sepsis and greater risk of death among patients with AKI.

SUBJECTS AND METHODS

Study Design

We conducted a single-center, prospective cohort study among inpatients at New York Presbyterian Hospital-Columbia University Medical Center (CUMC). All protocols were approved by the CUMC Institutional Review Board and were in accordance with the Helsinki Declaration.

Study Participants

Participants from general medical wards and intensive care units (ICUs) were recruited into two groups: AKI and control. AKI was defined in accordance with criteria established by the Acute Kidney Injury Network: an abrupt increase in serum creatinine 0.3 mg/dl or 50% within 48 hours.¹⁵ Urine output was not included in the definition of AKI because of variable availability and accuracy of urine flow data. An additional criterion for AKI was the exclusion of prerenal azotemia, defined as resolution of increased serum creatinine within 24-48 hours of the administration of intravenous volume repletion or discontinuation of diuretics. Control participants were not formally 1:1 matched but were selected to be closely representative of participants with AKI with regard to age, gender, race, and hospital location (i.e. general medical ward vs. ICU), and were required to have a stable serum creatinine < 1.0 mg/dl.

Additional inclusion criteria for all participants included age 18 and 70 years, capacity to give informed consent, and baseline eGFR > 60mL/min/1.73m², based on the MDRD equation, assessed within the preceding 3 months by electronic medical records. Patients greater than 70 years old were excluded to circumvent the issue of age-related decline in GFR.

Additional exclusion criteria for all participants included current or recent therapy with elemental vitamin D at doses 800 IU/day or a history of parathyroid disease, metabolic bone disease, fat malabsorption, or duodenal resection, as assessed by electronic medical records.

Patients who lacked capacity to provide written informed consent were deemed by the institutional review board not to be eligible for enrollment by surrogate consent. Accordingly, patients who were intubated, sedated or suffered from altered mental status were excluded.

Study Procedures

Participants were identified as having AKI using a daily automated query of the hospital's central data warehouse to rapidly identify inpatients with elevations in serum creatinine. After initial identification, further review of chart records was performed to determine eligibility. Patients who met all criteria for the study, and who were agreeable to participate, provided written informed consent.

Upon enrollment in the study, 5 cc of blood was collected in EDTA-containing vacutainers. Among those with AKI, enrollment collections were drawn 24-48 hours after the clinical diagnosis was established. Samples were centrifuged, aliquoted, and stored at -80°C. A second blood collection was obtained 5 days after the first from participants who remained hospitalized.

Clinical Outcome Measures

The primary clinical outcome was in-hospital mortality. The secondary clinical outcome was severity of sepsis. For the latter, participants were categorized into one of four categories on enrollment: no sepsis, sepsis, severe sepsis, or septic shock, in accordance with criteria established by the International Sepsis Definitions Conference.¹⁶

Laboratory Analyses

Mineral Metabolites—Assays were performed by Columbia University's Clinical and Translational Science Award Core Laboratory. 25(OH)D (both D₂ and D₃) and its major metabolite 24R,25-dihydroxyvitamin D₃ [24R,25(OH)₂D₃] were measured using ultra performance liquid chromatography with tandem mass spectrometry (UPLC/MS/MS). The ratio of 24R,25(OH)₂D₃ to 25(OH)D levels was used to standardize the results of the primary vitamin D metabolite to the total vitamin D stores. 1,25(OH)₂D (sum of D₂ and D₃) was measured using a standard radioimmunoassay kit (DiaSorin, Stillwater, MN). The 25(OH)D calibrators of the assays were standardized against reference material of the National Institute for Standards and Technology. For the 25(OH)D and the 1,25(OH)₂D assays, the lab participates successfully in the international Vitamin D External Quality Assessment Scheme (London, UK). Intra- and inter-assay coefficients of variation (CVs) were <10% and 3.4-4.8% for 25(OH)D; 7.7% and 12.3% for 1,25(OH)₂D; and <10% for 24R,25(OH)₂D₃.

C-terminal FGF23 and vitamin D binding protein (VDBP) were measured in duplicate by commercial ELISA assays (Immutopics, San Clemente, CA, and Alpco Diagnostics, Salem,

NH, respectively). Inter- and intra-assay CVs were 4.7% and 2.4% for FGF23 and 12.7% and 5.0% for VDBP, respectively.

Bioavailable Vitamin D—Because 25(OH)D circulates bound to VDBP (85-90%) and albumin (10-15%), less than 1% of circulating hormone exists in its free form.¹⁷ The sum of free- and albumin-bound hormone is referred to as “bioavailable”, because 25(OH)D and 1,25(OH)₂D bound to VDBP is thought to have limited biological activity. Formulas have been developed and validated in both normal and cirrhotic subjects for the calculation of free and bioavailable 25(OH)D and 1,25(OH)₂D levels based on serum concentrations of total 25(OH)D, total 1,25(OH)₂D, VDBP, and albumin.^{17, 18} Using these formulas, bioavailable compared to total 25(OH)D levels correlate more strongly with bone mineral density among young healthy adults¹⁸ and correlate more strongly with serum calcium and PTH levels among incident hemodialysis patients.¹⁹ We therefore used these equations to estimate levels of bioavailable 25(OH)D and bioavailable 1,25(OH)₂D. The equations used to calculate these levels are as follows:

$[D_{\text{total}}]$ = total measured vitamin D concentration (either 25(OH)D or 1,25(OH)₂D)

$[Alb]$ = measured albumin concentration

$[DBP]$ = measured vitamin D binding protein concentration

$[D_{Alb}]$ = concentration of albumin-bound vitamin D

$[D_{DBP}]$ = concentration of DBP-bound vitamin D

$[D_{\text{free}}]$ = concentration of free (unbound) D

$[D_{\text{bioavailable}}]$ = concentration of Bioavailable D = $[D_{\text{free}}] + [D_{Alb}]$

K_{alb} = affinity constant between vitamin D and albumin = $6 \times 10^5 \text{ M}^{-1}$ (for 25-OH D) or $5.4 \times 10^4 \text{ M}^{-1}$ (for 1,25-OH₂ D)

K_{DBP} = affinity constant between vitamin D and DBP = $7 \times 10^8 \text{ M}^{-1}$ (for 25-OH D) or $3.7 \times 10^7 \text{ M}^{-1}$ (for 1,25-OH₂ D)

$a = K_{DBP} \cdot K_{alb} \cdot [Alb] + K_{DBP}$

$b = K_{DBP} \cdot [DBP] - K_{DBP} \cdot [D_{\text{total}}] + K_{alb} \cdot [Alb] + 1$

$c = -[D_{\text{total}}]$

$[D_{\text{free}}] = \frac{-b + \sqrt{b^2 - 4ac}}{2a}$

$[D_{\text{bioavailable}}] = [D_{\text{free}}] + [D_{Alb}] = (K_{alb} \cdot [Alb] + 1) \cdot [D_{\text{free}}]$

Statistical Analyses

Statistical analysis was performed with SAS Version 9.2 (SAS Institute Inc., Cary, NC). Data are reported as median and interquartile range (IQR, 25th-75th percentiles). Differences between groups were assessed by the Mann-Whitney U test. Differences within groups comparing enrollment and follow-up parameters were assessed by the Wilcoxon signed-rank test. Correlations between mineral metabolites and severity of sepsis were analyzed using Spearman's rank correlation coefficient. Multivariable logistic regression models were used to compute adjusted odds ratios between enrollment bioavailable 25(OH)D (natural log-transformed, given its skewed distribution, and standardized to mean 0 and SD 1) and mortality. The models were adjusted for age and serum creatinine at the time of enrollment. Additional exploratory analyses were also performed to adjust for VDBP and albumin (in addition to age and creatinine) at the time of enrollment. All comparisons are two-tailed, with $p < 0.05$ considered significant.

RESULTS

Baseline characteristics

Thirty participants were enrolled into the AKI and control groups (N=60 total). Baseline characteristics are shown in Table 1. Participants in the two groups were similar with regard to age, gender, race, hospital location, baseline renal function, and comorbidities, with the exception of a greater proportion of diabetics and septic patients in the AKI group. Among those with AKI, the etiologies included ischemic acute tubular necrosis (47%), nephrotoxins (20%), sepsis (17%), hepatorenal syndrome (7%), rhabdomyolysis (6%), and acute interstitial nephritis (3%).

Enrollment serum parameters

At enrollment, virtually all participants (30/30 with AKI and 28/30 controls) had serum 25(OH)D levels less than 30 ng/ml, and the majority (25/30 with AKI and 21/30 controls) had levels less than 20 ng/ml (Table 2). There were no differences in the prevalence of vitamin D deficiency (plasma level <20 ng/ml) or insufficiency (plasma level 20-29 ng/ml) between the AKI and control groups. The majority of participants (22/30 with AKI and 17/30 controls) had undetectable levels of 25(OH)D₂ and only two participants (both in the AKI group) had levels greater than 5 ng/ml, indicating minimal prior use of supplemental vitamin D₂ in both groups.

Compared to controls, participants with AKI had a trend toward lower levels of 25(OH)D; significantly lower levels of 1,25(OH)₂D, 24R,25(OH)₂D₃, and VDBP; and no difference in levels of bioavailable 25(OH)D or bioavailable 1,25(OH)₂D (Table 2). Despite lower absolute levels of 24R,25(OH)₂D₃ among participants with AKI, there was no difference when standardized to 25(OH)D levels. Participants with AKI had higher levels of parathyroid hormone, FGF23, phosphate, and creatinine, lower levels of calcium, and similar levels of albumin.

Correlations between enrollment 25(OH)D, bioavailable 25(OH)D, and other mineral metabolites in the overall sample are presented in Figure 1. Enrollment 25(OH)D correlated positively with calcium, and negatively with PTH and FGF23. Bioavailable 25(OH)D showed similar trends, though the associations were less robust and did not reach statistical significance with PTH. Neither 25(OH)D nor bioavailable 25(OH)D correlated with phosphate (p=0.35 and p=0.48, respectively).

In the overall sample, non-whites compared to whites had lower levels of enrollment 25(OH)D [median (IQR) 8 (4-14) versus 16 (8-23) ng/ml, p=0.04] but differences in bioavailable 25(OH)D did not reach significance [0.8 (0.5-1.2) versus 1.4 (0.7-1.7) ng/ml, p=0.11]. There were no differences in enrollment 25(OH)D or bioavailable 25(OH)D according to hospital location (general ward versus intensive care unit). Among participants with AKI, there were no differences in 25(OH)D, bioavailable 25(OH)D, or FGF23 according to the etiology of AKI.

In the overall sample, participants with liver disease had lower VDBP levels compared to those without liver disease [22 (13-26) versus 28 (24-33) mg/dl, p=0.02]. VDBP levels were not associated with the presence or absence of other comorbidities. Similarly, FGF23 levels were not associated with any of the comorbidities (with the exception of diabetes mellitus, which was more prevalent in the AKI group).

Day 5 serum parameters

Due to interim death or hospital discharge, fewer participants (16 controls and 19 AKI participants) were available for blood collections on day 5 after enrollment. By day 5, levels of 25(OH)D, 1,25(OH)₂D, 24R,25(OH)₂D₃, and VDBP remained significantly lower among participants with AKI compared to controls (Table 3). No significant within-group differences were detected among the vitamin D metabolites between enrollment and day 5.

Mineral metabolites and severity of sepsis

The relation between enrollment mineral metabolites and severity of sepsis is shown in Figure 2. Severity of sepsis was negatively associated with 25(OH)D and bioavailable 25(OH)D, positively associated with FGF23, and not associated with PTH, VDBP, 1,25(OH)₂D, or bioavailable 1,25(OH)₂D. Among all seven mineral metabolites, bioavailable 25(OH)D had the strongest statistical association with severity of sepsis ($r = -0.45$, $p < 0.001$).

Mineral metabolites and mortality in AKI

Among the 30 participants with AKI, 10 died during their hospitalization [median (IQR) time to death was 24 (12-40) days after enrollment]. The cause of death was septic shock and multi-organ failure in all except a single participant who expired from cardiogenic shock. None of the control participants died, therefore analyses between mineral metabolites and mortality were restricted to the AKI group.

The association between tertiles of enrollment vitamin D metabolites and mortality is shown in Figure 3. Levels of bioavailable 25(OH)D, but not other vitamin D metabolites, were lower among participants who died compared to those who survived [0.5 (0.4-0.8) versus 1.2 (0.6-2.0) ng/ml, $p = 0.05$]. After adjustment for age and enrollment serum creatinine, levels of bioavailable 25(OH)D were inversely associated with mortality (odds ratio 0.16 per SD of natural log-transformed bioavailable 25(OH)D, 95% C.I. 0.03 to 0.85). After additional adjustment for enrollment albumin and VDBP, bioavailable 25(OH)D remained significantly inversely associated with mortality (odds ratio 0.05, 95% C.I. 0.004 to 0.64).

In contrast to bioavailable 25(OH)D, total 25(OH)D, 1,25(OH)₂D, bioavailable 1,25(OH)₂D, VDBP, PTH, calcium, phosphate, albumin, and creatinine were not associated with mortality. Higher enrollment FGF23 levels were significantly associated with mortality, as reported previously.²⁰

DISCUSSION

The principal findings are that levels of bioavailable 25(OH)D were strongly and inversely associated with severity of sepsis in the overall sample, and, unlike other vitamin D metabolites, inversely associated with hospital mortality among participants with AKI. In addition, we found that participants with AKI had lower levels of 1,25(OH)₂D and VDBP, a trend toward lower levels of 25(OH)D, and similar levels of bioavailable 25(OH)D and bioavailable 1,25(OH)₂D compared to controls. Cumulatively, these findings suggest a complex interplay between AKI, vitamin D, and adverse outcomes.

These findings are consistent with and extend those from prior reports in both animals^{7, 8} and humans.^{9, 10, 20} In one study,⁹ patients with AKI requiring hemodialysis were found to have low levels of both 25(OH)D and 1,25(OH)₂D. However, the sample size was limited to eight patients, the control group consisted of healthy volunteers rather than more comparable hospitalized patients without AKI, and other mineral metabolites such as VDBP were not measured. In contrast, strengths of the current study included a larger sample size, an

inpatient control group, a comprehensive evaluation of mineral metabolites, and their association with clinical outcomes.

We found lower levels of $1,25(\text{OH})_2\text{D}$ among participants with AKI, however, the precise mechanisms could not be discerned. Reduced levels of circulating $25(\text{OH})\text{D}$, severely reduced GFR, or global nephron dysfunction may have contributed to diminished delivery of $25(\text{OH})\text{D}$ substrate available for activation by renal 1α -hydroxylase. Alternatively, elevated levels of FGF23, through inhibition of 1α -hydroxylase and/or stimulation of the catabolic 24 -hydroxylase, may have resulted in diminished conversion of $25(\text{OH})\text{D}$ to $1,25(\text{OH})_2\text{D}$.^{21, 22} However, we measured $24\text{R},25(\text{OH})_2\text{D}_3$, the major metabolite of $25(\text{OH})\text{D}$, and found that levels were not elevated in AKI. Accordingly, we did not find evidence that enhanced catabolism of $25(\text{OH})\text{D}$ is the primary mechanism of reduced $1,25(\text{OH})_2\text{D}$ in AKI. These findings are consistent with a study of rats with acute tubular necrosis, which found that lower levels of $1,25(\text{OH})_2\text{D}$ resulted from decreased production and not enhanced catabolism of the hormone.⁷

We also found that levels of bioavailable $25(\text{OH})\text{D}$ were inversely associated with severity of sepsis in the overall sample and with hospital mortality among participants with AKI. These outcomes were more tightly associated with bioavailable $25(\text{OH})\text{D}$ than with other mineral metabolites, findings which are consistent with previous studies in both normal healthy individuals¹⁸ and in hemodialysis patients¹⁹ demonstrating bioavailable $25(\text{OH})\text{D}$ to be more closely linked to mineral metabolism than total $25(\text{OH})\text{D}$. However, unlike these previous studies, we did not observe bioavailable $25(\text{OH})\text{D}$ to be more tightly associated with other mineral metabolites than total $25(\text{OH})\text{D}$, perhaps reflecting the role of bioavailable $25(\text{OH})\text{D}$ as a global severity of illness marker rather than a marker of mineral metabolism. Alternatively, our findings of a stronger association between bioavailable vs. total $25(\text{OH})\text{D}$ levels and severity of sepsis may be related to selective uptake of bioavailable $25(\text{OH})\text{D}$ by macrophages and other nontraditional target organs. These sites may be more dependent on adequate bioavailable $25(\text{OH})\text{D}$ than the renal proximal tubules, which take up VDBP- $25(\text{OH})\text{D}$ via megalin-dependent endocytosis.²³

The greater prognostic value of bioavailable $25(\text{OH})\text{D}$ compared to other mineral metabolites may be partially attributable to a confounding effect from albumin or VDBP, both of which are variables in the equations used to estimate bioavailable $25(\text{OH})\text{D}$. In univariate analyses, neither albumin nor VDBP showed a significant association with mortality, unlike bioavailable $25(\text{OH})\text{D}$. Nonetheless, potential residual confounding cannot be excluded, particularly since multiple studies have shown albumin²⁴ and VDBP levels^{25, 26} to be negative predictors of adverse outcomes among hospitalized patients.

Our observations of bioavailable $25(\text{OH})\text{D}$ and mortality are consistent with previous reports of total $25(\text{OH})\text{D}$ levels and risk of mortality among critically ill patients.¹¹⁻¹⁴ While the exact mechanism of this association is unknown, vitamin D plays a critical role in host defense, both by stimulating innate immunity and inhibiting adaptive immunity.²⁷ Specifically, the antimicrobial peptide cathelicidin (LL-37) is regulated by the vitamin D receptor and can be increased *in vitro* by administration of $1,25(\text{OH})_2\text{D}$.²⁸ LL-37 levels correlate positively with $25(\text{OH})\text{D}$ levels in both healthy adults²⁹ and in critically-ill patients³⁰ and are inversely associated with risk of infectious disease mortality among patients undergoing hemodialysis.³¹ Markers of systemic inflammation, on the other hand, such as interleukin-6, are elevated among vitamin D deficient patients and can be significantly decreased by vitamin D supplementation,³² suggesting that vitamin D also plays a role in limiting an exaggerated inflammatory response. Randomized controlled studies will be needed to determine whether vitamin D supplementation can beneficially

influence host defense parameters and ultimately improve outcomes among patients with AKI, critical illness, or both. One such study is currently underway (NCT01130181).

These findings must be interpreted in the context of the study design, including modest sample size, single-center, a maximum of only two data points per participant, relatively short duration of follow-up (until hospital discharge), and observational design. We did not have vitamin D levels prior to the onset of acute illness and therefore cannot exclude the possibility of reverse causality. Levels of bioavailable 25(OH)D and bioavailable 1,25(OH)₂D were estimated using equations which were not developed and validated in AKI. Consequently, estimated values for these vitamin D metabolites should be viewed as preliminary and in need of validation. The AKI group had a disproportionate number of diabetics compared to the control group, which may have resulted in bias. However, the groups were similar with regard to other baseline demographics and comorbidities, and analyses between mineral metabolites and mortality were restricted to the AKI group. We did not have data available on body mass index, a potential source of residual confounding since 25(OH)D levels have been shown to be inversely associated with obesity.³³

Additionally, surrogate informed consent was not approved for this study, which precluded enrollment of critically-ill patients lacking decisional capacity due to sedation, intubation, or other acute processes affecting consciousness or cognition. However, given our observations that the lowest levels of vitamin D metabolites tended to occur among participants with the most severe critical illness, it is likely that the above limitation would have, if anything, biased our results toward the null. Future studies should aim to include the full spectrum of patients with and without AKI, including those who are most critically ill.

Additional studies will be needed to further define the pathophysiology and prognostic importance of vitamin D deficiency among patients with AKI and critical illness. Our data suggest that bioavailable 25(OH)D could have a role as a biomarker of adverse outcomes among those with established AKI and perhaps hospitalized patients in general. Whether low levels of bioavailable 25(OH)D may be directly linked to adverse outcomes through an effect on nontraditional targets, such as the innate immune response, is an intriguing possibility that will require interventional studies of vitamin D supplementation. Future studies should aim to validate the equations used to estimate levels of bioavailable 25(OH)D in AKI patients through actual measurement of the free- and albumin-bound vitamin D concentrations and should continue to assess whether bioavailable levels, compared with total levels, are a more meaningful marker of vitamin D status.

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Figure 1. Correlations between enrollment 25(OH)D and bioavailable 25(OH)D and other mineral metabolites

PTH=Parathyroid Hormone; FGF23=Fibroblast Growth Factor 23.

Figure 2. Enrollment mineral metabolites and severity of sepsis

PTH=Parathyroid Hormone; FGF23=Fibroblast Growth Factor-23; VDBP=Vitamin D

Binding Protein. Bioavailable 1,25(OH)₂D levels (data not shown) were not associated with severity of sepsis (r= -0.02; p=0.90).

Figure 3. Tertiles of enrollment vitamin D metabolites and mortality among participants with AKI

*p=0.03 for multivariable logistic regression model, after adjustment for age and enrollment creatinine.

Table 1
Baseline characteristics of the participants

ATN=Acute Tubular Necrosis. HRS=Hepatorenal Syndrome. AIN=Acute Interstitial Nephritis. *Represents renal function prior to study enrollment.

Variable	AKI (N=30)	Control (N=30)
Age (yr) – median (IQR)	57 (50-64)	56 (45-61)
Female sex – no. (%)	10 (36)	12 (40)
Race – no. (%)		
White	13 (43)	13 (43)
Non-white	17 (57)	17 (57)
Comorbidities – no. (%)		
Congestive Heart Failure	6 (20)	9 (30)
Hypertension	14 (47)	14 (47)
Liver disease	8 (27)	9 (30)
Diabetes mellitus	17 (57)	8 (27)
Sepsis – no. (%)	15 (50)	10 (33)
Hospital Location – no. (%)		
General Medical Ward	16 (53)	17 (57)
Intensive Care Unit	14 (47)	13 (43)
Baseline Renal Function*		
Serum Creatinine (mg/dl) – median (IQR)	0.9 (0.7-1.0)	0.8 (0.7-0.9)
eGFR (mL/min/1.73m ²) – median (IQR)	103 (82-131)	111 (92-126)

Table 2**Enrollment serum parameters**

Values represent median (interquartile ranges). VDBP=Vitamin D Binding Protein. FGF23=Fibroblast Growth Factor 23.

	Reference Values	Control (N=30)	AKI (N=30)	p-value
25(OH)D (ng/ml)	30-80	14 (8-21)	8 (4-15)	0.06
1,25(OH) ₂ D (pg/ml)	18-72	25 (15-35)	17 (10-22)	0.01
24R,25(OH) ₂ D ₃ (ng/ml)	N/A	1.5 (0.6-2.6)	0.9 (0.3-1.5)	0.01
24R,25(OH) ₂ D ₃ / 25(OH)D	N/A	0.09 (0.07-0.19)	0.07 (0.04-0.11)	0.10
VDBP (mg/dl)	20-55	29 (25-36)	23 (15-31)	0.003
Bioavailable 25(OH)D (ng/ml)	N/A	1.0 (0.7-1.5)	0.8 (0.4-1.8)	0.54
Bioavailable 1,25(OH) ₂ D (pg/ml)	N/A	3.0 (1.8-5.3)	2.5 (1.8-3.3)	0.64
FGF23 (RU/ml)	7-71	263 (96-574)	1471 (224-2534)	0.003
PTH, intact (pg/ml)	8-51	40 (31-78)	76 (51-182)	0.004
Albumin (g/dl)	4.1-5.3	3.3 (2.6-3.6)	3.0 (2.4-3.4)	0.18
Calcium (mg/dl)	8.7-10.0	8.7 (8.1-9.0)	8.1 (7.5-8.6)	0.004
Phosphate (mg/dl)	2.5-4.3	3.4 (2.9-4.0)	4.7 (3.8-5.3)	<0.001
Creatinine (mg/dl)	0.5-1.0	0.7 (0.6-0.9)	2.2 (1.8-2.7)	<0.001

Table 3**Day 5 serum parameters**

Values represent median (interquartile ranges). VDBP=Vitamin D Binding Protein. FGF23=Fibroblast Growth Factor 23.

	Reference Values	Control (N=16)	AKI (N=19)	p-value
25(OH)D (ng/ml)	30-80	15 (8-18)	6 (5-12)	0.03
1,25(OH)₂D (pg/ml)	18-72	20 (16-28)	13 (12-20)	0.05
24R,25(OH)₂D₃ (ng/ml)	N/A	1.7 (0.8-2.5)	0.6 (0.3-1.1)	0.04
24R,25(OH)₂D₃ / 25(OH)D	N/A	0.11 (0.08-0.15)	0.08 (0.04-0.11)	0.22
VDBP (mg/dl)	20-55	29 (24-38)	19 (15-25)	0.05
Bioavailable 25(OH)D (ng/ml)	N/A	1.1 (0.9-1.6)	0.9 (0.4-1.1)	0.12
Bioavailable 1,25(OH)₂D (pg/ml)	N/A	2.5 (2.0-4.9)	3.1 (1.8-3.4)	0.63
FGF23 (RU/ml)	7-71	286 (127-454)	459 (215-1478)	0.17
PTH, intact (pg/ml)	8-51	48 (39-95)	71 (46-108)	0.56
Albumin (g/dl)	4.1-5.3	3.3 (2.7-3.7)	3.2 (2.3-3.5)	0.25
Calcium (mg/dl)	8.7-10.0	8.9 (8.1-9.3)	8.1 (7.5-8.7)	0.07
Phosphate (mg/dl)	2.5-4.3	3.7 (3.3-4.1)	3.4 (2.8-3.7)	0.07
Creatinine (mg/dl)	0.5-1.0	0.8 (0.7-0.9)	1.3 (1.1-2.4)	<0.001