

Regulation of Calcium Homeostasis in Acute Kidney Injury: A Prospective Observational Study

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ABSTRACT

Background: Maintaining homeostasis is an integral part of all physiological processes both in health and disease including critically ill patients and may impact clinical outcomes. The present study was designed to assess prevalence of serum calcium, phosphate, vitamin-D3, FGF-23, and PTH levels abnormalities in AKI.

Patients and methods: Single-center, prospective, observational study in a tertiary care hospital. Patients meeting KDIGO criteria for AKI were included. Paired blood samples were drawn from eligible patients—first sample within 24 hours of AKI diagnosis and second after 5 days or at time of hospital discharge, whichever was earlier for measuring serum calcium (albumin corrected), phosphate, PTH, 25(OH)Vit-D, and FGF-23 levels. Clinical outcomes analyzed included survival status, utilization of RRT, and hospital stay.

Results: Of the 50 patients with AKI, about three-fourths were males. Mean age of the participants was 57.32 ± 11.47 years. Around half of patients had hypocalcemia and four-fifths had low serum phosphate. Nearly 82% had low 25(OH)Vit-D and 52% cases had high PTH level. Patients who underwent RRT had numerically higher but not significant serum calcium and PTH levels. FGF-23 levels (pg/mL) were significantly higher in patients on RRT (81.70 ± 17.30 vs non-RRT, 72.43 ± 20.27 , $p = 0.049$), nonsurvivors (87.96 ± 18.82 vs survivors 57.11 ± 15.19 , $p = 0.045$), and those hospitalized for time of stay above median (109.67 ± 26.97 vs below median 70.27 ± 20.43 , $p = 0.046$). Among all the bone and mineral parameters analyzed high FGF23 levels were consistently linked with poor clinical outcomes in AKI.

Conclusion: The present study found high prevalence of calcium and phosphate disorders in AKI with dysregulated phosphate homeostasis as evidenced from elevated FGF-23 levels linked with morbidity and mortality in AKI.

Keywords: Acute kidney injury, Calcium homeostasis, Fibroblast growth factor-23, Parathyroid hormone, Vitamin D.

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HIGHLIGHTS

Calcium and phosphate play an essential physiological role in homeostasis. Despite extensive epidemiological data on dysregulated bone and mineral metabolism in CKD, not much is known about its prevalence and outcome association in AKI. The present study found high prevalence of calcium and phosphate disorders in AKI with dysregulated phosphate homeostasis as evidenced from elevated FGF-23 levels which showed strong association with morbidity and mortality. Given the context of poor clinical outcomes in AKI, the present study leads us to hypothesize that FGF-23, phosphate axis could, potentially, play a role in this conundrum.

INTRODUCTION

Acute kidney injury (AKI) indicates abrupt deterioration of renal function. While AKI often implies anuria and urgent need of emergency renal replacement therapy (RRT), it is a broader term which also encompasses an initial phase of reduced glomerular filtration rate (GFR) where potential interventions can prevent progression to advanced disease.¹ AKI occurs in as many as 60% of the intensive care unit (ICU) patients with and its presence magnifies the morbidity and mortality of these critically ill patients.¹ Sepsis-associated AKI is a part of sepsis-associated multiple organ dysfunction syndromes that has shown to be independently associated with adverse clinical outcomes.²

Electrolyte or mineral imbalances are common findings in AKI patients in the ICU reported in almost two-third of the patients.³ Calcium and phosphate play an essential physiological role in homeostasis and they are regulated by three key hormones

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involve parathyroid hormone (PTH), 25(OH)Vit-D, and fibroblast growth factor-23 (FGF-23). PTH and activated vitamin D enhance calcium reabsorption in the renal tubules. FGF-23 is a novel key regulator of phosphorus excretion and contributes to abnormal bone metabolism in CKD.⁴ A few studies done previously analyzing levels of FGF-23 in AKI found this hormone level increased in AKI as compared to healthy controls and that it was associated with

adverse outcomes.^{4,5} However, there is a paucity of data on the incidence of calcium, phosphate related mineral imbalances, and their association with outcomes among patients with AKI. In order to address this lacuna in medical literature, we designed the present study to assess serum calcium, phosphate, 25(OH)Vit-D, FGF-23, and parathyroid hormone (PTH) levels in AKI patients and to test their association with relevant clinical outcomes.

METHODS

Study Population

This was a prospective single-center study conducted among consecutive 50 hospitalized patients with AKI in general medical floor and intensive care unit (ICU) of a tertiary care academic hospital. The inclusion criterion for the study was diagnosis of AKI as per KDIGO-2012 criteria, i.e., an abrupt increase in SCr ≥ 0.3 mg/dL within 48 hours or $\geq 50\%$ from baseline which is known or presumed to have occurred within the prior 7 days and/or urine volume < 0.5 mL/kg/hour for 6 hours⁶ and age more than 18 years but less than 70 years. Exclusion criteria for the study were patients with current or recent therapy with elemental vitamin D at doses of 800 IU/day, patient with a history of parathyroid disease, metabolic bone disease, fat malabsorption, or duodenal resection, pregnancy, patients who were intubated, sedated or suffered from altered mental status, patients with CKD having baseline eGFR < 60 mL/minute/1.73 m², and those undergoing dialysis. The study protocol was approved by the Institutional Ethics Committee.

Study Procedure

Patients found eligible for study as per inclusion criteria and exclusion criteria were enrolled in study after obtaining informed consent. Initial blood sample was drawn within 24 hours of establishing a clinical diagnosis of AKI. Test measured included albumin corrected serum calcium, serum phosphate, intact PTH levels, 25(OH)Vit-D, and FGF-23 levels. A second blood sample was withdrawn 5 days after the clinical diagnosis of AKI or at the time of discharge whichever was earlier. Vitamin 25(OH)Vit-D and PTH levels were assessed by a paramagnetic particle two-step competitive binding immunoassay in Beckman Coulter "UniCelDxl 600 Immunoassay Systems" in serum samples. FGF-23 was measured in stored samples since this is not tested regularly at hospital pathology laboratory. A 96-well ELISA kit from "Bioassay Technology Laboratory" was used for FGF-23 assay using blood samples that were allowed to clot for 10–20 minutes in room temperature, centrifuged for 20 minutes at 2000–3000 RPM and stored at -80°C .

Data Collection

The data were arranged in two groups based on various outcomes and these parameters were compared between the groups:

- Duration of hospital stay below the median (considered as short duration) and above the median (considered as long duration).
- Survivors and nonsurvivors of present hospitalization.
- Receive any form of renal replacement therapy (RRT) during current hospitalization (dialyzed) vs nondialyzed AKI patients.

Outcome Variables

Abnormal calcium/phosphate homeostasis was inferred based on abnormal levels of serum calcium, phosphate, 25(OH)Vit-D, PTH, and FGF-23. Outcomes analyzed were a composite of (1) need

for RRT; (2) hospital mortality; and (3) length of hospital stay (as a percentage variable based on median duration of hospital stay).

Statistical Analysis

All the statistical analyses were performed using SPSS (version 20.0. Armonk, New York, USA: IBM Corp.). Categorical variables were presented as percentages and continuous variables as mean, SD, and median. Normality of data was tested by the Kolmogorov-Smirnov test. The clinical profile of patients was analyzed by Chi-square test for qualitative variables and student "t" test for quantitative variables. Alpha error of 5% was taken for test hypothesis.

Sample Size

Based on the prevalence rates of abnormal calcium homeostasis in patients with AKI and in those without AKI, taking the values of these metabolites effect size was 0.774 and the minimum required sample size with 80% power of study and two-sided alpha of 5% (using formula $n > 2(Z\alpha + Z\beta)^2 (ES)^2$) was 27 patients, where $Z\alpha$ is Z at two-sided alpha error of 5% and $Z\beta$ is the value of Z at the power of 80% and ES is effect size. Hence based on the statistical calculation, a total number of minimum 27 cases were required but a total of 50 patients attending the Inpatient Department and ICU of Department of Medicine and Nephrology with clinical and laboratory evidence of AKI were recruited from a tertiary care hospital over 2 years from 2017 to 2018.

RESULTS

Descriptive Statistics

Of the 50 patients included, majority were males (70%) and mean age was 57.32 ± 11.47 years. Notable comorbid conditions and acute events included anemia (62%), sepsis (62%), circulatory shock (56%), hypertension (52%), diabetes mellitus (48%), and coronary heart disease (36%) (Figs 1 and 2). Distribution of study variables is illustrated in Table 1 and Figure 3.

Comparison of Laboratory Parameters According to Outcome

Table 2 compares the laboratory parameters based on various outcomes, i.e., survival status, utilization of RRT, and duration of hospital stay. Patients who had undergone RRT had higher calcium

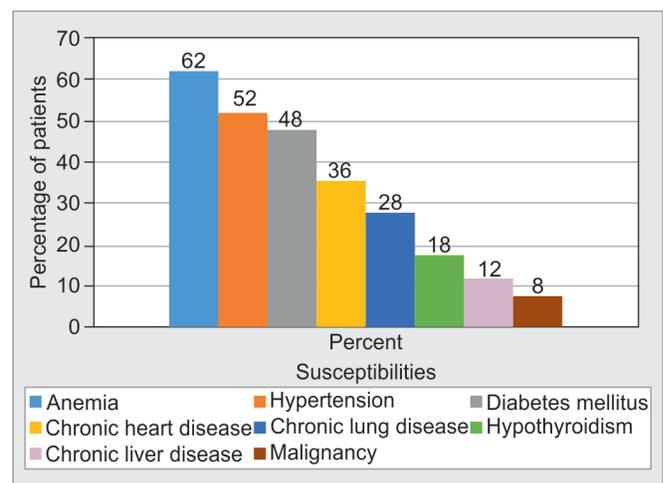


Fig. 1: Prevalence of comorbidities in study population

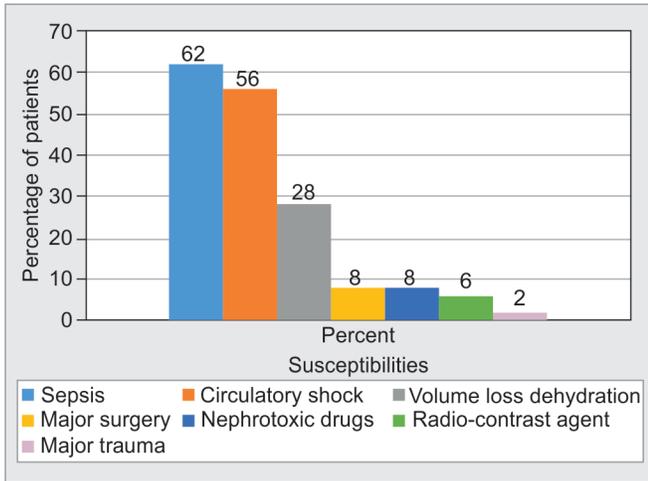


Fig. 2: AKI risk factors in study population

Table 1: Descriptive statistics of different parameters among study population

Parameters	Mean ± SD
Creatinine (mg/dL)	4.12 ± 2.23
Urea (mg/dL)	92.89 ± 31.23
Calcium (mg/dL)	8.54 ± 0.82
Phosphate (mg/dL)	2.28 ± 0.66
25(OH)Vit-D (ng/mL)	21.02 ± 10.43
PTH (pg/mL)	101.66 ± 50.02
FGF-23 (pg/mL)	81.18 ± 168.63
Hospital stay (days)	9.98 ± 4.39

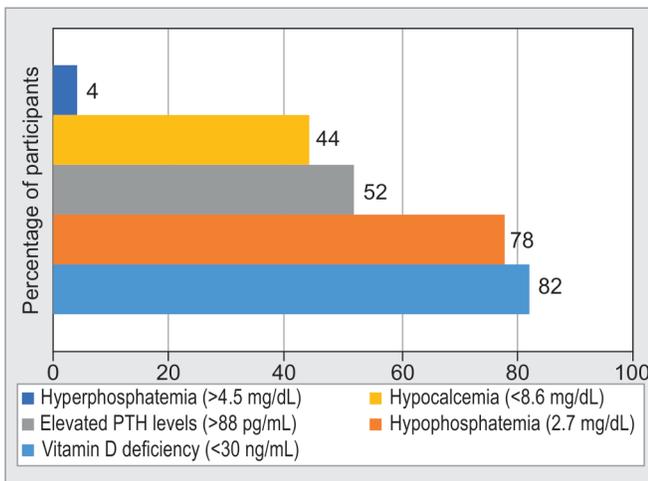


Fig. 3: Mineral and bone disease abnormalities in study population

(8.9 ± 0.53 mg/dL, vs 8.5 ± 0.84 mg/dL, $p = 0.218$) and higher PTH (103.48 ± 52.13 pg/mL vs 85.3 ± 19.6 pg/mL, $p = 0.446$) as compared to the non-RRT group but was not statistically significant. However, FGF-23 levels (81.70 ± 17.30 pg/mL vs 72.43 ± 20.27 pg/mL, $p = 0.049$) were significantly higher in patients who were dialyzed as compared to the nondialyzed group. Patients who stayed in hospital for longer duration had significantly lower serum calcium levels and significantly higher FGF-23 as compared to the patients admitted for short duration. Serum FGF-23 was found to be significantly

higher in the patients who did not survive (87.96 ± 18.82 pg/mL vs 57.11 ± 15.19 pg/mL, $p = 0.045$). No significant change was observed in other laboratory parameters.

DISCUSSION

Electrolyte, mineral, and acid-base disturbances commonly occur in AKI patients. Maintaining homeostasis is an integral part of the management of critically ill patients and it directly affects the outcome. Interestingly, although hypocalcemia is commonly observed in patients with AKI, the literature on dysregulated mineral metabolism in this patient population is relatively limited. Thus, the present study was designed to determine if an association existed between the levels of calcium, phosphate, 25(OH)Vit-D, PTH, and FGF-23 with the adverse outcomes, viz., RRT, death in the hospital, and length of hospital stay in patients with AKI. In the groups based on the survival status of patients and the need for RRT, there was no significant difference between values of serum calcium, phosphate, 25(OH)Vit-D, and PTH levels. On the other hand, FGF-23 was found to be significantly higher in nonsurvivors and dialyzed patients.

Recent studies have reported that AKI was associated with an increased length of stay ranging from 2 to 18 extra days spent in the hospital.⁷⁻¹¹ This finding persisted even after adjustment for age, NIHSS score, previous stroke, and insurance status.⁸ However, the association of calcium homeostasis with the length of hospital stay in AKI patients has not been studied in any study. Multiple studies have demonstrated that elevated FGF-23 levels are associated with major cardiovascular events and mortality in CKD and end-stage kidney disease.¹²⁻¹⁶ However, there is limited data regarding status of FGF-23 levels in AKI.^{17,18} In a pilot study, Leaf et al. found elevations in cFGF23 levels in patients both with and without AKI, though the magnitude of the rise was far greater in patients with AKI and even greater in severe AKI.⁴ On the contrary, we did not have a comparator arm to examine FGF-23 levels in other patients admitted to the hospital.

Implications of elevated cFGF-23 levels in critical illness are still not known. Mechanistically, the association of elevated FGF-23 levels with lower 1,25(OH)Vit-D levels can be explained by known inhibitory effects of FGF-23 on 1-alpha-hydroxylase activity both in kidney and in extra-renal tissues.¹⁹ The implications of the expected low level of 1,25(OH)Vit-D in AKI due to FGF-23 suppression are unclear since it was associated with mortality in one study⁴ but not in others.²⁰ In our study too, no association was seen in 25(OH)Vit-D levels among RRT vs non-RRT group and between survivor and nonsurvivor groups. It is possible that FGF-23 may have an independent role to play in mortality since one study found that 70% of AKI patients in the highest tertile of FGF-23 values either died or required RRT.⁴ This is further supported by the finding that FGF-23 levels correlate with the severity of sepsis syndrome regardless of renal involvement.²¹ Similarly, Zeid et al. demonstrated a significant positive correlation between FGF23 with both APACHE and SOFA scores.²²

Limitations of the present study include small sample size, single-center data, and observational nature; hence it is difficult to exclude unknown confounders affecting outcomes. Furthermore, associations of clinical covariates with adverse outcomes were not compared with known factors affecting clinical outcomes in AKI. Additionally, we did not have a control group to compare these parameters in AKI vs a non-AKI group to say if noted changes in calcium-phosphate homeostasis are unique in AKI population. Future studies should be conducted

Table 2: Comparison of laboratory parameters according to outcome

Parameters	Outcomes			RRT			Hospital stay [#]		
	Non-survivors (n = 11)	Survivors (n = 39)	p value	Yes (n = 5)	No (n = 45)	p value	Below median (n = 18)	Above median (n = 19)	p value
Calcium (mg/dL)	8.77 ± 0.68	8.48 ± 0.85	0.311	8.98 ± 0.52	8.49 ± 0.84	0.218	8.78 ± 0.83	8.11 ± 0.76	0.015*
Phosphate (mg/dL)	2.27 ± 0.49	2.28 ± 0.70	0.963	2.35 ± 0.21	2.27 ± 0.69	0.795	2.29 ± 0.74	2.25 ± 0.72	0.872
25(OH)VitD (ng/mL)	20.46 ± 10.39	23.03 ± 10.83	0.475	18.90 ± 3.17	21.26 ± 10.94	0.636	23.64 ± 6.62	17.09 ± 13.04	0.060
PTH (pg/mL)	110.34 ± 44.04	99.21 ± 51.85	0.520	103.48 ± 52.13	85.30 ± 19.68	0.446	94.06 ± 41.39	105.91 ± 63.84	0.505
FGF-23 (pg/mL)	87.96 ± 18.82	57.11 ± 15.19	0.045*	81.70 ± 17.30	72.43 ± 20.27	0.049*	70.27 ± 20.43	109.67 ± 26.97	0.046*

*Statistical significant value at 95% confidence interval (CI); #Status, survival with no RRT

to identify mechanisms responsible for dysregulated calcium/phosphate homeostasis and their role in adverse outcomes in AKI. Moving beyond associations, causality of notable associations in the current study would need multicentric interventional studies targeting FGF-23 molecule and its prominent role in cardiovascular events as seen in CKD.

To summarize, our study found high prevalence of calcium and phosphate disorders in AKI with dysregulated phosphate homeostasis as evidenced from elevated FGF-23 levels which showed strong association with morbidity and mortality. Given the context of poor clinical outcomes in AKI, our study leads us to hypothesize that FGF-23, phosphate axis could, potentially, play a role in this conundrum.

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