



Original Research Article

Mineral disturbances in patients with CKD: A case control study

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ABSTRACT

Introduction: Mineral bone Disease in CKD manifest as a combination of abnormalities of PTH, calcium, phosphorus and vitamin D metabolism. Abnormalities of bone turnover, mineralization, Vascular or other soft tissue calcification. Mineral disturbances are common complications of CKD they begin early in the course of disease. Derangements in mineral metabolism is also associated with cardiovascular disease and all-cause mortality. Cardiovascular disease accounts for 70% of all deaths in patients with CKD, with an overall mortality of 20% per year in patients on dialysis.

Material & Methods: 50 patients diagnosed with CKD and 50 healthy controls were included in the study. Serum calcium, Serum Phosphorus, Serum PTH, Serum urea and Serum creatinine were estimated in both cases and controls.

Results: Statistically significant increase in calcium and phosphorus levels were seen in cases as compared to controls. The mean level of calcium in cases is 8.35 ± 1.07 and control is 8.98 ± 0.98 and the mean level of phosphorus in cases is 4.40 ± 1.70 and control is 3.47 ± 0.62 ($p < 0.001$).

Conclusion: Alteration in minerals like calcium and Phosphorus occurs early in the course of disease and are responsible for various cardiovascular manifestations and bone osteodystrophy. Early medical management like calcium supplementation and phosphate binders help in better management of Mineral bone disease in CKD.

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1. Introduction

Mineral bone metabolism in CKD manifest as a combination of abnormalities of PTH, calcium, phosphorus and vitamin D metabolism. Abnormalities of bone turnover, mineralization, Vascular or other soft tissue calcification.¹

Mineral disturbances are common complications of CKD they begin early in the course of disease. In stage 3 CKD, the kidneys are unable to fully excrete phosphorus load nor can convert vitamin D into its active metabolite calcitriol. This leads to a compensatory secondary hyperparathyroidism.²

Elevated PTH and decreased calcitriol levels are found in 40% of patients with stage 3 and 80% of patients with stage 4.³ The minerals and endocrine functions disrupted in

CKD are c important in the regulation of bone remodelling. Bone abnormalities like altered remodelling and loss of bone volume are found in most of the patients with CKD.⁴ The skeletal changes result in an increased prevalence of hip fracture compared to the general population across the entire range of CKD stages 3-5 patients.⁵

Derangements in mineral metabolism is also associated with cardiovascular disease and all-cause mortality.^{6,7} Cardiovascular disease accounts for 70% of all deaths in patients with CKD, with an overall mortality of 20% per year in patients on dialysis.⁸ In patients with kidney failure the cardiovascular mortality rates are 10 to 500 times higher than in the general population.⁹ Individuals at earlier stages of CKD not yet on dialysis (stages 3-4) have 17-times more chances to die of cardiovascular disease rather than progressing to dialysis.¹⁰

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The mechanism by which abnormal mineral metabolism may increase cardiovascular risk is by inducing or accelerating arterial and valvular calcification. Patients on dialysis have 2 to 5-times more coronary artery calcification than age-matched controls.¹¹

Calcification is also very common in the peripheral arteries where both intimal and medial calcification are seen.¹² The coronary artery and peripheral artery calcification are associated with increased mortality in patients on dialysis. The risk factors associated with arterial calcification include advanced age, diabetes, obesity, hypertension, dyslipidaemia, inflammatory markers, hypoalbuminemia, use of calcium-containing phosphate binders and disordered mineral metabolism.³ Vascular calcification was previously thought to be a passive process, due to the elevations in calcium and phosphorus observed in patients with advanced CKD.

Thus, this study was undertaken to see changes in calcium and phosphorus levels in patients with CKD.

2. Materials and Methods

The study is conducted at Vydehi Institute of Medical Sciences and Research Centre, Bangalore, Karnataka. After a written consent, 50 patients diagnosed with CKD were included in the study. The exclusion criteria were autoimmune disorders, paediatric patients and patients with congenital renal disorders. The base line demographic data & clinical history was taken from each patient. 50 healthy individuals attending routine health check-up and healthy staff members were included in the study as controls. Venous blood samples were collected into vacutainers and transported to the laboratory. They were centrifuged at 3000 rpm for 10 minutes. Serum was separated and analysed.¹³ The base line investigations like Serum urea, creatinine, were done and patients were categorised into various stages of CKD by calculating eGFR. Other parameters like Serum calcium, Phosphorus, PTH and Alkaline phosphatase were analysed. Beckman coulter Unicel DXC 600 was used to analyse all chemistry analytes and PTH was analysed in Beckman coulter Access 2 immunoassay system. Bio Rad Controls were used for all parameters.

Estimation of calcium: total calcium concentration was determined by indirect potentiometry utilizing a calcium ion selective electrode in conjunction with a sodium reference electrode.¹⁴

Estimation of Phosphorus: The phosphorus concentration by a timed endpoint method. In the reaction, inorganic phosphorus reacts with ammonium molybdate in an acidic solution to form a coloured phosphomolybdate complex.¹⁵

Measurement of Urea: Urea reagent by enzymatic Conductivity rate method.¹⁶

Measurement of Creatinine: Creatinine reagent is used to measure the analyte concentration by a modified rate Jaffé

method.

Estimation of Alkaline phosphorus: ALP reagent is used to measure alkaline phosphatase activity by a kinetic rate method.¹⁷

2.1. Statistical analysis

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean \pm SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5% level of significance. Student t test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups Inter group analysis) on metric parameters. Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups. The Statistical software SPSS 15.0 was used for the analysis of the data..(20-21)

2.2. Significant figures

+Suggestive significance (P value: $0.05 < P < 0.10$), * Moderately significant (P value: $0.01 < P \leq 0.05$), ** Strongly significant (P value: $P \leq 0.01$).

3. Results

The mean age of patients with CKD is 49.26 ± 12.73 years. Maximum patients were in the age group of 51-60 years. In healthy controls the mean age was 43.83 ± 15.12 years. Among the cases 70% were males and 30% were females. In healthy controls 60% were males and 40% were females. Samples are age and gender matched.

There was a statistically significant increase in urea levels was seen in cases as compared to controls ($p < 0.001$). The mean level in cases is 76.60 ± 69.77 and control is 223.50 ± 7.46 ($p < 0.001$). (Figure 1)

Statistically significant increase in creatinine levels was seen in cases as compared to controls ($p < 0.001$). The mean level in cases is 4.11 ± 4.25 and control is 0.56 ± 0.10 ($p < 0.001$). (Figure 1)

The mean level of calcium in cases is 8.35 ± 1.07 and control is 8.98 ± 0.98 . A Statistically significant decrease in the levels of calcium is seen in cases as compared to controls. ($p = 0.001$). (Figure 2)

The normal reference range of calcium is 8.5-10.2 mg/dl. 51.9% of cases had calcium levels between 8.5-10.2 mg/dl and 76% of controls had values within the normal reference range.

48.1% of cases had calcium low levels and only 24% of controls had levels < 8.5 mg/dl.

None of the study groups had high calcium levels above 10.2mg/dl.

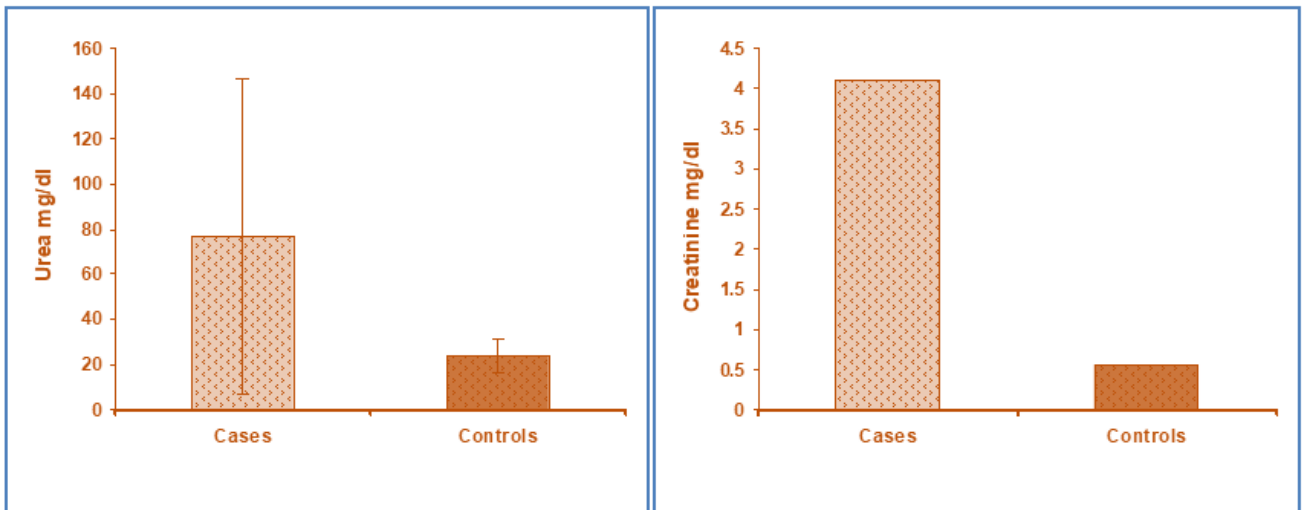


Fig. 1: Mean urea and creatinine levels in cases and controls

Table 1: Distribution of Calcium in two groups studied

Bio chemical parameter	Cases (n=50)		Controls (n=50)	
	No	%	No	%
Calcium mg/dl				
<8.5	24	48.1	12	24.0
8.5-10.2	26	51.9	38	76.0
>10.2	-	-	-	-

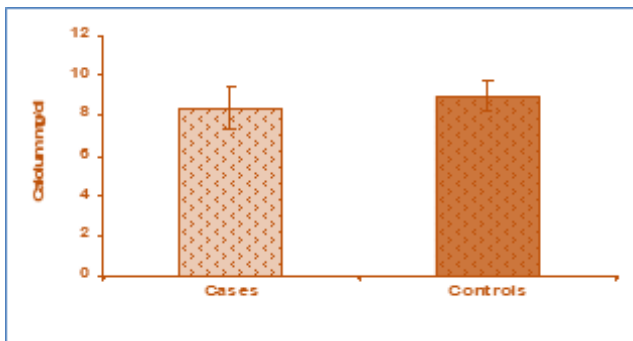


Fig. 2: Mean calcium levels in cases and controls

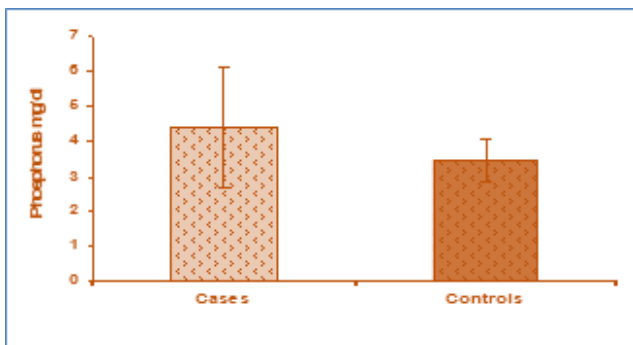


Fig. 3: Mean phosphorus levels in cases and controls

Statistically significant increase in levels of phosphorus was observed in cases as compared to controls. The mean level of phosphorus in cases is 4.40 ± 1.70 and control is 3.47 ± 0.62 ($p < 0.001$).

The normal levels of Phosphorus are 2.5-4.5 mg/dl.

62% of cases had Phosphorus levels between 2.5-4.5mg/dl and 98% of controls were in this range.

20% of cases had levels between 4.6-5.5 mg/dl whereas only 2% of controls were in the same range.

Further, 18% of cases had levels >5.5 mg/dl and none of controls had values in this range.

The cases were categorised into various stages of CDK using the MDRD formula and the levels of Calcium, Phosphorus, Ca^*Po_4 and PTH.

Distribution of Alkaline Phosphatase in two groups studied: Alkaline Phosphatase levels were measured in cases and controls.

The normal range is 56-153IU/L. 94% of cases had alkaline phosphatase levels between 56-153 IU/L where as 98% of controls had in the same range.

6% of cases had levels >153 IU/L and only 2% of controls had in the same range.

4. Discussion

Phosphorus homeostasis is impaired when the GFR falls. As GFR decreases to 60 mL/min, there is a gradual increase

Table 2: Distribution of phosphorus in two groups studied

Bio chemical parameters Phosphorus mg/dl	Cases (n=50)		Controls (n=50)	
	No	%	No	%
<2.5	4	8	5	10
2.6-3.5	14	28	21	42
3.6-4.5	13	26	23	46
4.6-5.55	10	20	1	2
>5.5	9	18	0	0.0

Table 3: Distribution of Ca*PO4 product in two groups of patients studied

Ca*PO4 Product	Cases		Controls	
	No	%	No	%
<70	49	98.1	50	100.0
>70	1	1.8	0	0.0
Total	50	100.0	50	100.0
Mean ± SD	36.35±13.39		31.00±5.00	

Ca*PO4 is significantly raised in cases when compared to control with P= 0.012*

Table 4: Comparison of Ca, P and Ca*P, PTH according to CKD stage

Variables	Stage of CKD					P value
	Stage I	Stage II	Stage III	Stage IV	Stage V	
Calcium mg/dl	8.3±1.01	8.48±1.12	7.43±1.46	8.54±0.91	8.38±1.01	0.500
Phosphorus mg/dl	3.25±0.62	3.99±0.76	5.55±1.72	5±2.8	4.66±2.07	0.209
Ca*PO4 product	26.66±3.86	33.41±5.7	40.09±9.05	42.28±24.42	38.9±16.28	0.281
PTH pg/dl	67.72±29.92	75.09±33.38	96.47±33.88	158.98±115.1	211.13±88.0	<0.001**

Table 5: Comparison of mean levels of various bio chemical parameters in cases and controls

Biochemical parameters	Cases	Controls	P value
Urea mg/dl	76.60±69.77	23.54±7.46	<0.001**
Creatinine mg/dl	4.11±4.25	0.56±0.10	<0.001**
Calcium mg/dl	8.35±1.07	8.98±0.76	0.001**
Phosphorus mg/dl	4.40±1.70	3.47±0.62	0.001**
Alkaline Phosphatase IU/L	90.92±46.37	82.91±21.78	0.285
PTH pg/dl	136.80±92.70	52.47±16.34	<0.001**

in serum phosphorus levels. During this period, normal phosphorus levels are maintained by continuous increases in FGF-23 and PTH levels. As the GFR further falls these compensatory mechanisms fail leading to Secondary hyperparathyroidism.¹⁸

Craver et al. studies observed a significant increase in phosphorus levels in patients who were in various stages of CKD (p<0.001). They observed that elevated PTH levels were associated with increased cardiovascular risk, loss of arterial elasticity and left ventricular hypertrophy. Relevant mechanism could be direct action on vascular and cardiac cells, which express PTH receptors.¹⁹

G. Floege et al. reported a significant increase in phosphorus levels and concluded that high level of phosphorus is a significant risk factor for mortality in CKD.²⁰

We observed a statistically significant increase in serum phosphorus levels in cases as compared to controls (p<0.001).

Goodman et al. in their observational study highlighted the increased prevalence and extent of coronary artery calcification in young dialysis patients compared with normal controls.²¹

Hyperphosphatemia is a common problem in patients with CKD. Elevated serum phosphorus has been associated with the progression of secondary hyperparathyroidism, deposition of calcium in soft tissues, and vascular calcification.²²

Higher serum PO4, Ca×PO4 product is associated with increased coronary artery calcification. In addition to this, it also contributes to vascular smooth muscle cell proliferation and compromise flow in the coronary microcirculation.²³

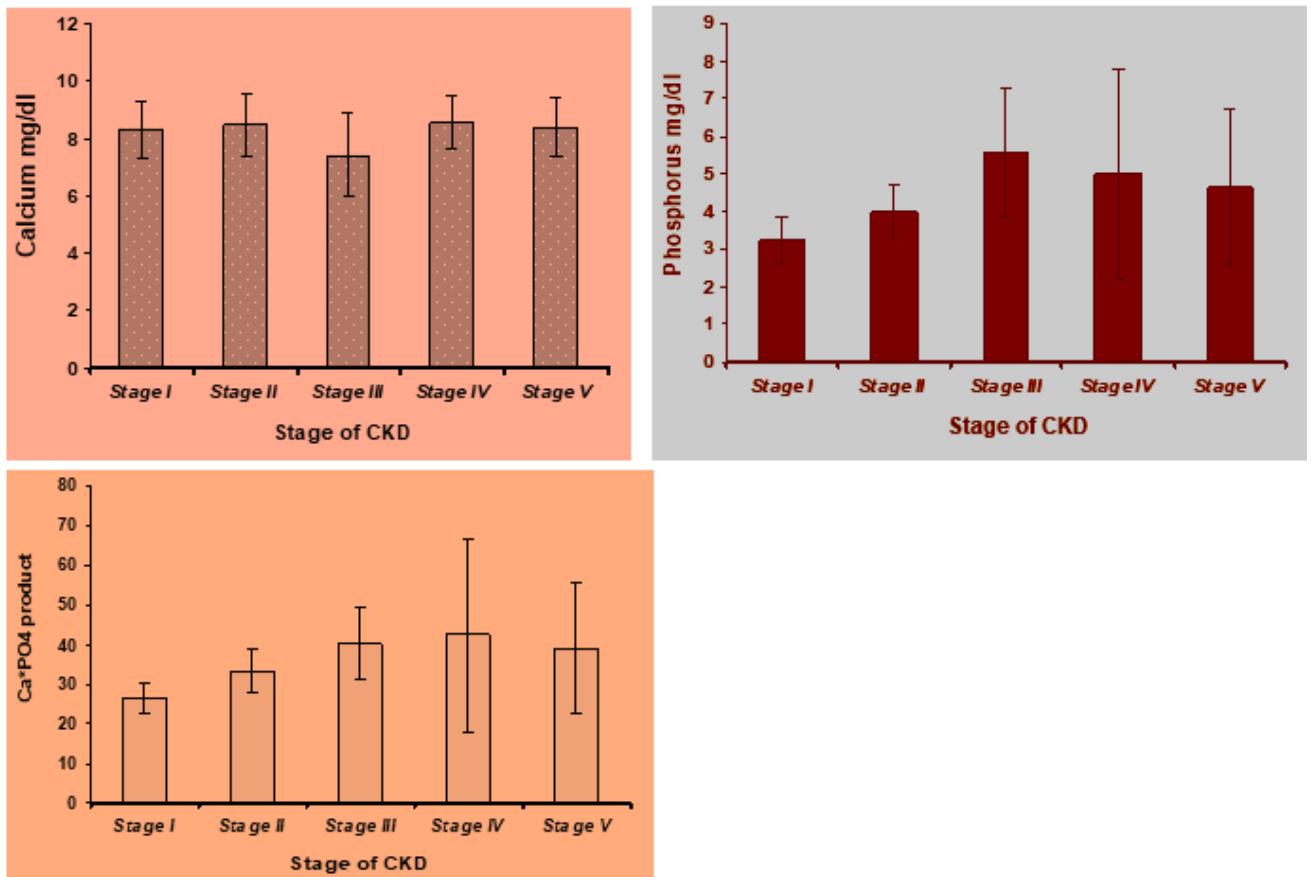


Fig. 4: Distribution of Calcium, Phosphorus, PTH in two groups studied

The mean values of Phosphorus seen in cases in our study are 4.40 ± 1.70 whereas in controls the values are 3.47 ± 0.62 . There was a statistically significant increase in phosphorus levels in cases as compared with controls with $p=0.001$ and the mean Calcium levels in cases are 8.35 ± 1.07 and controls have a mean of 8.98 ± 0.76 . The values were statistically significant with $p=0.001$. In our study we observed a rise in serum phosphorus levels in cases and fall in serum calcium levels. There was a statistically significant increase in serum PTH levels in cases as compared to controls. The mean value of PTH was 136.80 ± 92.70 in cases and 52.47 ± 16.34 in controls.

In our study we found a statistically significant increase in PTH level in cases as compared to controls ($p < 0.001$). The findings are similar to Block et al. study, a significant increase in PTH levels was observed in CKD. They also identified high PTH levels as a significant correlate of all-cause mortality. They concluded that elevations in serum PTH might be associated with increased risk of death from cardiac causes.²⁴

A multicentre study from Italy reported that serum P was >5.5 mg/dL in 51.6% and the Ca \times P product was >55 mg²/dL² in 35.5% of the patients.²⁵

In a cohort study in Japan (J-DOPPS), the mean values of serum Ca, P, Ca \times P product, and iPTH were 9.4 ± 1.0 mg/dL, 5.7 ± 1.6 mg/dL, 52.8 ± 15.9 mg²/dL², and 194 ± 263 pg/mL, respectively.²⁶

Schwartz et al. studies showed an association between higher levels of serum phosphorus and calcium-phosphorus product with an unfavourable renal outcome.²⁷ In their study higher serum phosphorus was associated with significantly higher risk for progression of CKD, even after adjustment for multiple potential confounders. The association of higher serum phosphorus with progressive CKD was more accentuated in patients with higher serum calcium.

Reynolds et al. showed that higher ambient serum calcium level led to more significant phosphorus-driven calcification of vascular smooth muscle in vitro.²⁸

In our study we found that the Ca \times P was increased in cases but was <70 mg²/dL². It was observed that the cases had an elevated phosphorus levels and low calcium levels.

The controls also had the same Ca \times P as they had normal calcium levels and low phosphorus levels. As a result of this the Ca \times P remains the same in both the groups studied = 0.012.

In our study increase in alkaline phosphatase levels were observed in cases as compared to controls and the rise was of significant only in stage 5. High serum alkaline phosphatase is associated with increased mortality. An analysis of the Dialysis Outcomes and Practice Patterns Study (DOPPS) database found that elevated serum alkaline phosphatase levels in hemodialysis patients were associated with higher risk of hospitalization and death. Lee et al studies concluded saying, that alkaline phosphatase can promote vascular calcification by hydrolysing pyrophosphate in the arterial wall.²⁹

Sigrist et al conducted a longitudinal study and found elevated levels of alkaline phosphatase in stage IV and V of CKD, they found that higher levels of serum alkaline phosphatase were associated with progressive arterial calcification.³⁰

When the levels of minerals were compared with various stages of CKD, we found that the levels of phosphorus increased as the disease progressed and the levels of phosphorus were less in stage V. A probable reason for this may be the initiation of dialysis in such patients. The calcium levels were maintained in the lower range in all stages of disease. There was an increase in ALP in cases and it remained high in all the stages of CKD. PTH levels also increased as the disease progressed.

5. Conclusion

Alteration in minerals like calcium and Phosphorus occurs early in the course of disease and are responsible for various cardiovascular manifestations and bone osteodystrophy. Early medical management like calcium supplementation and phosphate binders help in better management of Mineral bone disease in CKD.

6. Limitations

Vit D could not be assessed in the given population which is also an important marker in CKD-MBD. The patients were assessed only at the time of presentation and were not followed up.

7. Source of Funding

None.

8. Conflict of Interest

The authors declare no conflict of interest.

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