



Study of serum levels of calcium, phosphorus and alkaline phosphatase in chronic kidney disease

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ABSTRACT

Chronic kidney disease (CKD) is a worldwide public health problem, with increasing prevalence and lethal adverse outcomes like progressive loss of kidney function, cardiovascular disease and premature death. Disturbances in mineral metabolism and bone disease are common complications of CKD and an important cause of morbidity and decreased quality of life in patients with CKD. Patients with renal failure have an increased risk of cardiovascular mortality that may be due in part to vascular calcification. To measure serum levels of calcium, phosphorus and alkaline phosphatase in patients in various stages of CKD and to correlate the same with creatinine and estimated Glomerular Filtration Rate (eGFR) values. This is a cross sectional study done at Thanjavur Medical College Hospital. 60 CKD patients and 50 healthy controls were enrolled in this study. Serum levels of creatinine, calcium, phosphorus and alkaline phosphatase were measured and eGFR values correlated with the serum creatinine. The mean values of creatinine (4.9 ± 2.23 mg/dl), calcium (9.8 ± 0.456 mg/dl), phosphorus (4.19 ± 0.404 mg/dl) and alkaline phosphatase (94.01 ± 15.10 U/L) in the study group are significantly higher than the control group in which the mean levels are 0.89 ± 0.102 mg/dl, 10.17 ± 0.37 mg/dl, 4.02 ± 0.16 mg/dl and 25.16 ± 4.65 U/L respectively. We have found that there is a significant difference in the above said parameters among patients in different stages of CKD (stage 3-5) indicating the progression of mineral bone disease with advancing stage of CKD.

Key words: chronic kidney disease, alkaline phosphatase, calcium, phosphorus.

INTRODUCTION

Chronic kidney disease (CKD) is a worldwide public health problem, with increasing prevalence and potentially lethal adverse outcomes like progressive loss of kidney function, cardiovascular disease and premature death. Disturbances in mineral metabolism and bone disease are common complications of CKD and an important cause of morbidity and decreased quality of life. Importantly, there is increasing evidence suggesting that these disorders in mineral and bone metabolism are associated with increased risk for cardiovascular and soft tissue calcification, renal osteodystrophy etc.¹

CHRONIC KIDNEY DISEASE:

Chronic Kidney Disease (CKD) affects about 5- 10% of the world population and the expected incidence is approximately 5-8% every year.² In South India, the main causes of CKD in decreasing order of prevalence are diabetic nephropathy (29.6%), chronic interstitial nephritis (20.4%), chronic glomerulonephritis (17.4%), and hypertensive nephropathy(11%).³ The Kidney Disease Outcomes Quality Initiative (KDOQI) of the National Kidney Foundation (NKF) defines “ chronic kidney disease as either kidney damage or a glomerular filtration rate (GFR) of less than 60mL/min/1.73 m² for 3 or more months”.⁴

In 2002, KDOQI published its classification of the stages of chronic kidney disease as follows:

- Stage 1 : Kidney damage with normal or increased GFR (>90 mL/min/1.73m²)
- Stage 2 : Mild reduction in GFR ($60 - 89$ mL/min/1.73m²)

- Stage 3 : Moderate reduction in GFR (30-59mL/min/1.73m²)
- Stage 4: Severe reduction in GFR (15-29 mL/min/1.73m²)
- Stage 5: Kidney failure (GFR <15mL/min/1.73m²)

Patients with chronic kidney disease stages 1-2 are generally asymptomatic, clinical manifestations typically appear in stages 3-5.

Risk factors for CKD:

According to KDOQI guidelines, the risk factors for the development of CKD can be divided into following;

Susceptibility factors:

There is increased susceptibility to kidney damage seen in older age, family history of chronic kidney disease, reduction in kidney mass and low birth weight.

Initiation factors:

They directly initiate kidney damage like Diabetes, high blood pressure, autoimmune diseases, systemic infections, urinary tract infections, urinary stones, lower urinary tract obstruction and drug toxicity.

Progression factors:

They cause faster decline in kidney function after initiation of kidney damage they include higher level of proteinuria, higher blood pressure, poor glycaemic control in diabetes, smoking.

End – stage factors:

These factors increase morbidity and mortality in patients which include lower dialysis dose (Kt/V), temporary vascular access, anaemia, low serum albumin level and late referral.⁵

Approximately 1 million nephrons are present in each kidney, contributing to the total GFR. In the case of renal injury, the kidney has an ability to maintain GFR by hyperfiltration and compensatory hypertrophy of the remaining healthy nephrons. Thus, nephron adaptability allows for continued normal clearance of plasma solutes. Plasma levels of substances such as urea and creatinine start to show significant increases only after total GFR has decreased to 50%. A rise in plasma creatinine from baseline value of 0.6mg/dl to 1.2mg/dl in a patient even though still within the reference range actually represents a loss of 50% of functioning nephron mass.

Decreased renal function interferes with the kidney's ability to maintain fluid and electrolyte homeostasis. The ability to concentrate urine declines early and is followed by decrease in ability to excrete phosphate, acid and potassium. Many studies have demonstrated that as renal function declines, there is a progressive deterioration in mineral homeostasis, with a disruption of normal serum and tissue concentrations of phosphorus, calcium and alkaline phosphatase.

In patients with chronic kidney disease, including those undergoing maintenance hemodialysis (CKD 5D) therapy and those with predialysis CKD stages various abnormalities related to mineral and bone disorders have been implicated as novel risk factors of mortality.⁹⁻¹⁵ This study proposes to study the serum levels of calcium, phosphorus and alkaline phosphatase in various stages of CKD.

AIMS AND OBJECTIVES:

1. To measure serum creatinine, calcium, phosphorus and alkaline phosphatase in patients with various stages of CKD. To study the relationship between these levels and the progression of the disease.
2. To demonstrate the disturbance in mineral metabolism secondary to CKD using calcium, phosphorus levels and alkaline phosphatase levels, thereby ascertaining the presence of mineral bone disease in patients with CKD.

MATERIALS AND METHODS

This is a cross sectional study done at Department of Biochemistry, Thanjavur Medical College. 60 patients (aged 20 – 60 yrs, 41 males) clinically diagnosed to have chronic kidney disease were enrolled from the Nephrology Outpatient Department and Ward. This included patients with CKD of diverse etiologies in various stages of the disease. Fifty (age group 20 – 60 yrs, 33 males) apparently healthy individuals were taken as controls. Blood samples were collected after overnight fasting and were analysed for serum creatinine, urea, calcium, phosphorus and alkaline phosphatase.

ANALYTES	METHOD
UREA	UREASE – GLDH METHOD
CREATININE	MODIFIED JAFFE'S METHOD
CALCIUM	ELECTROLYTE REAGENT KIT METHOD
PHOSPHORUS	PHOSPHO MOLYBDATE KIT METHOD
ALKALINE PHOSPHATASE	ALP REAGENT BY KINETIC METHOD (AMP BUFFER)
eGFR	CALCULATED USING COCKCROFT & GAULT FORMULA: 140-AGE*BODY WEIGHT * 0.85(FOR FEMALES) 72*SERUM CREATININE

RESULTS

Descriptive and inferential statistical analysis has been carried out in the present study using SPSS version 20. Results on continuous measurements are presented as mean \pm S.D. Significance is assessed at 5% level of significance. Analysis of variance (ANOVA) has been used to find the significance of study parameters between patients in three stages of CKD (3-5), student t – test (two tailed independent) has been used to find the significance of study parameters on continuous scale between study and control groups. P value < 0.05 considered as significant. Statistical analysis are described in the following tables and bar diagrams.

TABLE:1 Descriptive statistics of control and study group

PARAMETERS	CONTROL		CASES	
	MEAN	S.D	MEAN	S.D
AGE (yrs)	41.8	9.65	44.45	8.66
WEIGHT (kg)	66.32	8.74	66.13	7.99
CREATININE (mg/dl)	0.89	.102	4.9	2.23
eGFR	96.63	17.01	21.66	11.44
CALCIUM (mg/dl)	10.17	.37	9.08	.456
PHOSPHORUS (mg/dl)	4.02	.16	4.19	.404
ALKALINE PHOSPHATASE (U/L)	25.16	4.65	94.01	15.10

TABLE: 2 Student's t – test: comparison of means between control and study group

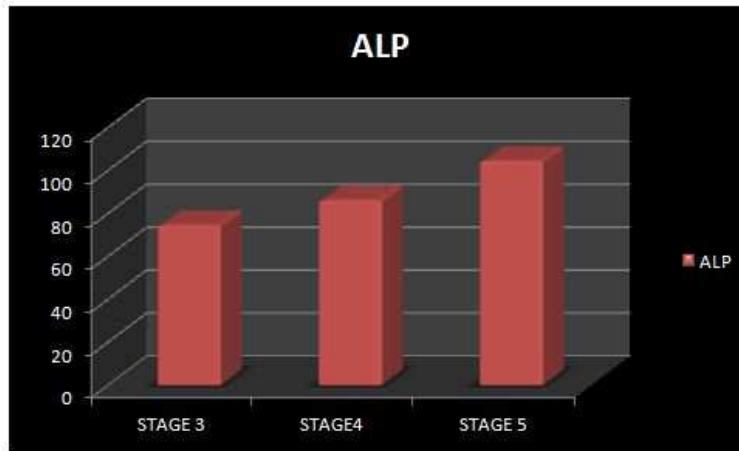
ANALYTES	CONTROL		CASES		t values	STATISTICAL SIGNIFICANCE
	MEAN	S.D	MEAN	S.D		
CREATININE (mg/dl)	0.89	.102	4.9	2.23	t = -13.786	p = .000(<0.05) significant
eGFR	96.63	17.01	21.66	11.44	t = 26.54	p = .000(<0.05) significant
CALCIUM (mg/dl)	10.17	.37	9.08	.456	t = 13.86	p = .000(<0.05) significant
PHOSPHORUS (mg/dl)	4.02	.16	4.19	.404	t = -2.98	p = 0.004(<0.05) significant
ALKALINE PHOSPHATASE (U/L)	25.16	4.65	94.01	15.10	t = -33.45	p = .000(<0.05) significant

TABLE:3 ANOVA Comparison of creatinine, calcium, phosphorus and alkaline phosphatase between different stages of chronic kidney disease

ANALYTES	STAGE 3 (n=16)	STAGE 4 (n=17)	STAGE 5 (n=27)	F value	P- VALUE
CREATININE (mg/dl)	2.18 \pm 0.68	4.2 \pm 1.23	7.04 \pm 1.05	116.700	p = 0.000 (<0.05) significant
eGFR	42.68 \pm 6.09	22.61 \pm 3.72	11.15 \pm 2.03	346.215	p = 0.00 (<0.05) significant
CALCIUM (mg/dl)	9.22 \pm 0.45	9.19 \pm 0.59	8.93 \pm 0.29	2.945	p = 0.06 (>0.05) not significant
PHOSPHORUS (mg/dl)	3.9 \pm 0.288	4.44 \pm 0.46	5.2 \pm 0.36	7.270	p = 0.002 (<0.05) significant
ALKALINE PHOSPHATASE (U/L)	84.37 \pm 16.27	87 \pm 10.54	104.0 \pm 10.28	16.626	p = 0.00 (<0.05) significant

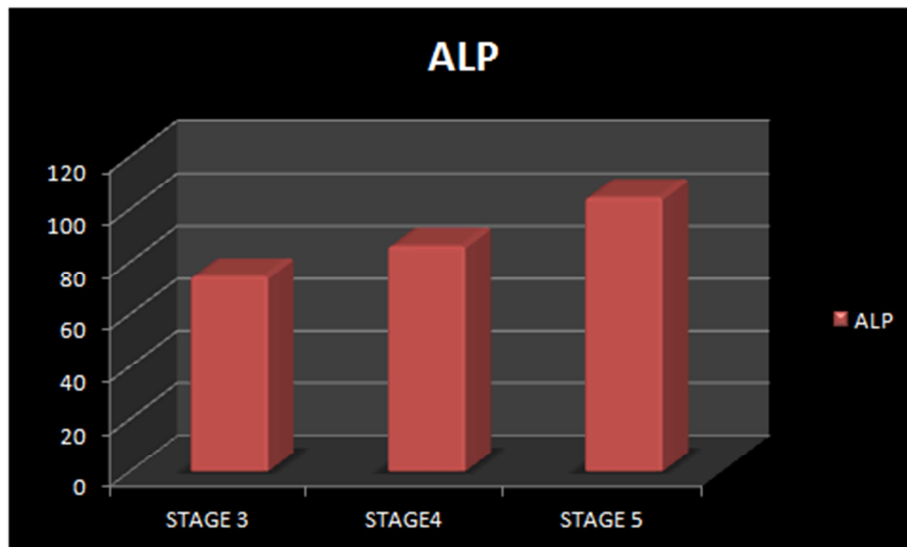
BAR DIAGRAM: 3

COMPARISON OF ALKALINE PHOSPHATASE LEVELS IN VARIOUS STAGES OF CKD.



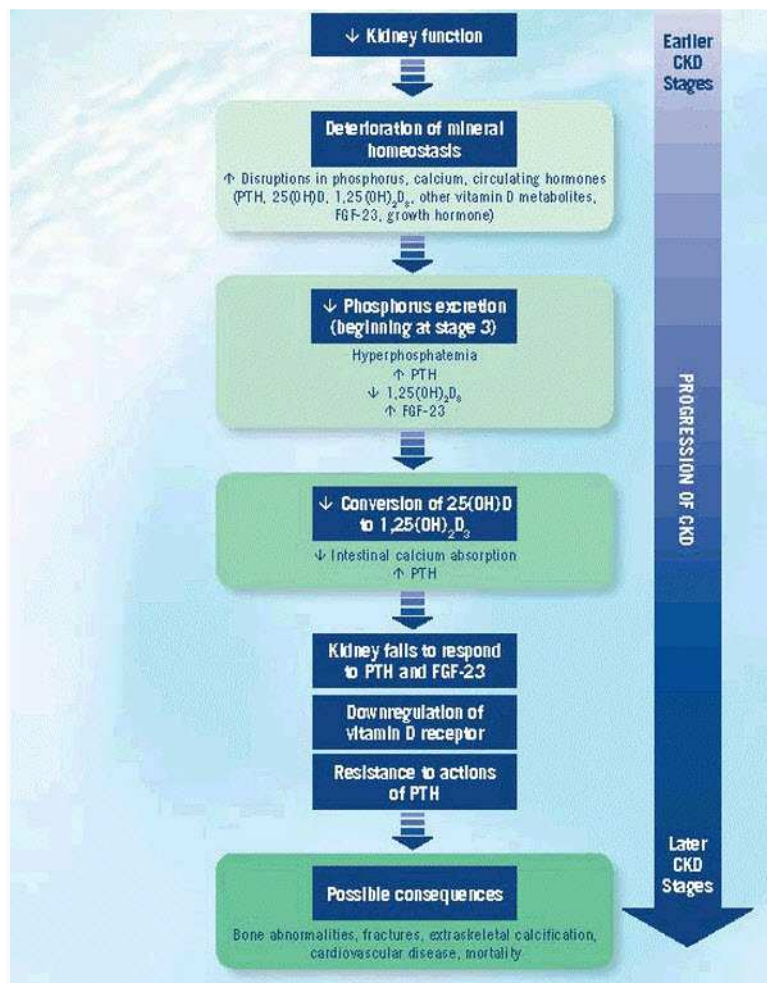
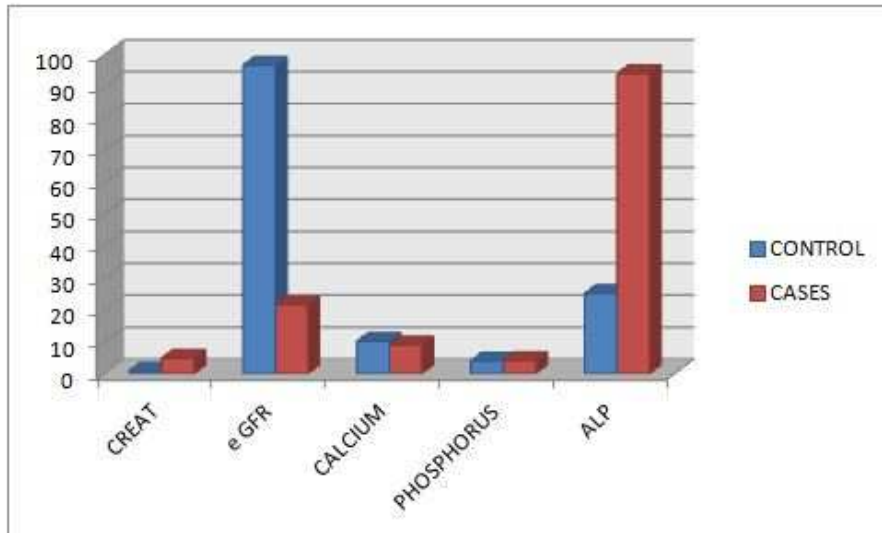
BAR DIAGRAM: 3

COMPARISON OF ALKALINE PHOSPHATASE LEVELS IN VARIOUS STAGES OF CKD.



BAR DIAGRAM: 1

COMPARISON OF CREATININE, GFR, CALCIUM, PHOSPHOROUS AND ALKALINE PHOSPHATASE BETWEEN CASES AND CONTROLS



DISCUSSION

Chronic Kidney Disease- Mineral Bone Disorder is a systemic disorder of mineral and bone metabolism due to CKD manifested by either one or a combination of the following:

1. Abnormalities of calcium, phosphorus and vitamin D metabolism.
2. Abnormalities of bone turnover, mineralization, volume, linear growth or strength.
3. Vascular or other soft tissue calcification.[6]

Kidney disease wasting (KDW) (also known as the malnutrition–inflammation complex), renal anemia, and kidney bone disease (KBD) appear to be the 3 most important nontraditional risk factors associated with cardiovascular disease in CKD[23]. The progression of mineral bone disease is depicted in fig.1

Elevated serum phosphorus has been related to vascular and coronary artery calcification and resulting cardiovascular morbidity. Among mineral abnormalities, the most prevalent is hyperphosphatemia which is a common problem among patients with ESRD. It is speculated that elevated phosphorus may aggravate the effects of coronary atherosclerosis through increased vascular calcification and smooth muscle proliferation. Elevated phosphorus may alter microcirculatory hemodynamics through increased extra vascular resistance and further compromise myocardial perfusion.

Shanthi K et al studies have found a significant increase in serum phosphorus levels in patients in CKD.⁷ These findings are similar to our study. We observed a statistically significant increase in serum phosphorus levels in cases as compared to controls.

J. Floege et al reported a significant increase in phosphorus levels and concluded that high levels of phosphorus as a significant risk factor for mortality in CKD.[8]

Hyperphosphatemia and hypercalcemia have been shown to promote calcification of the vasculature, myocardium and cardiac valves. Vascular calcification, manifested in reduced vessel wall elasticity, increased intima-media layer thickness and enhanced pulse-wave velocity, has been linked to left ventricular hypertrophy.

Calcium exerts negative feedback on PTH secretion through the calcium-sensing receptors on the parathyroid. Decrease in serum calcium during the course of CKD caused by phosphate retention and decreased 1,25 dihydroxycholecalciferol attenuates this feedback and leads to increased PTH mRNA levels and proliferation of parathyroid cells. The number of calcium-sensing receptors also may decrease in hypertrophied parathyroid tissue and lead to inadequate suppression of PTH secretion even in the setting of normal or high calcium levels.

In the present study increase in phosphorus level and corresponding decrease in the calcium levels with increase in stage of the disease has been depicted in Bar diagram 2.

This is commensurate with the studies of Scialla JJ [21] et al., who have reported that fibroblast growth factor (FGF23), Parathormone, and phosphate levels rose over time in patients with renal disease and that participants with faster rates of decline in measured GFR had the greatest increases in these parameters. Higher baseline levels of FGF23, PTH, and phosphate each associated with increased risk for End Stage Renal Disease or death independent of GFR. Kim et al.²² have described decrease in levels of 25(OH)D/1,25(OH)2D with progression of CKD.

ALP is a hydrolyze enzyme that dephosphorylates various molecules, most effectively operating in an alkaline environment. ALP is fairly ubiquitous in the human body, but it is especially concentrated in the bone, liver, placenta, leukocytes and kidneys. ALP is produced by osteoblasts in bone tissue in response to decreased calcium levels and plays an important role in bone mineralization by hydrolyzing pyrophosphate in the extracellular milieu. ALP is a biochemical marker of bone turnover and is used to monitor the metabolic bone disease associated with renal insufficiency. Elevated ALP levels can be seen with worsening magnitude of bone turnover with the rate of elevation a reliable marker of severity of the high turnover osteodystrophy. The processes of bone absorption and resorption are closely regulated in healthy individuals. Renal osteodystrophy arises as a consequence of bone remodelling dysregulation. While the pathogenesis of End Stage Renal Disease varies from high to low bone turnover. Elevated PTH stimulate bone demineralization and lead to high- turn over, a condition characterised by accelerated rates of bone absorption and resorption with concurrent production of alkaline phosphatase from osteoblast cells contributing to its high levels in plasma as the renal function or GFR declines.

Furthermore, pathologic conditions most commonly associated with elevations in ALP include diseases of the bones (such as high-turnover bone disease in CKD) and the liver.[17-19] The origin of circulating ALP can be determined

by measuring tissue-specific ALP such as bone-specific ALP. Elevation in total ALP is a known feature of CKD-MBD.

High serum Alkaline Phosphatase is associated with increased mortality. An analysis of Dialysis Outcomes and Practice Patterns Study (DOPPS) database found that elevated serum alkaline phosphatase in hemodialysis patients were associated with higher risk of hospitalization and death. Lee et al studies concluded that alkaline phosphatase can promote vascular calcification by hydrolyzing pyrophosphate in the arterial wall.

ALP has been shown in histological sections of vessels obtained from dialysis associated calcific uremic arteriolopathy. Indeed genetic ablation of tissue-nonspecific ALP leads to amelioration of soft tissue calcification in animal studies. ALP has been found to be up-regulated in calcified diabetic arteries, and the rate of pyrophosphate hydrolysis in the aorta was shown to be higher in uremic rats than in controls and the increase was inhibited by levamisole, a non-specific inhibitor of ALP, suggesting a role for ALP in vascular calcification. In another study Lee et al²³. have found alkaline phosphatase to have an incremental association with mortality in patients with CKD.

In this study, there was a significant increase in alkaline phosphatase levels in cases as compared to control. There was also a significant increase in the ALP levels with increase in the stage of CKD (Bar diagram 3).

To summarise, this study brings to light the fact that there is derangement of mineral metabolism secondary to mineral bone disease in patients with CKD evident from hyperphosphatemia and hypocalcemia in patients. There is also a significant increase in alkaline phosphatase levels in patients with CKD. All these parameters were found to worsen with increase in the stage of CKD highlighting the higher risk of lethal complications secondary to impaired metabolism in patients with poorer renal function.

A small cohort size and lack of correlation with imaging of calcific changes in both arteries and bone are potential drawbacks of this study .

CONCLUSION

Abnormalities of mineral bone metabolism are common in CKD patients. These abnormalities start in early stages of CKD and worsen with disease progression. In the present study the changes in the serum levels of calcium ,phosphorus and alkaline phosphatase were significant in relation to the progression of the disease. This underscores the importance of early recognition of Mineral Bone Disorder, understanding of the pathophysiological consequences of the same and planning management strategies to prevent its progression. Given the prognostic importance of ALP, it may serve as a target for the treatment of hemodialysis patients and further research may be directed to study the role of novel ALP inhibitors that suppress vascular smoother muscle cell calcification in reducing the cardiovascular morbidity and mortality in patients with CKD .

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