

# Measurement of Serum Levels of Calcium, Phosphorus and Parathormone to Study the Prevalence and Pattern of Mineral Bone Disorder in Chronic Kidney Disease Patients

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**Abstract- Background:** Chronic kidney disease(CKD) is an international health problem affecting 5-10% of the world population. There occur changes in serum and tissue concentrations of phosphorus, calcium and parathormone (PTH) in CKD, leading to pathological changes in bones.

**Objectives:** To study the prevalence of mineral bone disorder(MBD) in CKD stage 3 to stage 5D patients using calcium, phosphorus & parathormone(PTH) as parameters; & to correlate the biochemical abnormalities with clinical disease.

**Methods:** Study was conducted between May 2011 to Dec 2012 at IPGMER & SSKM hospital, Kolkata in 190 patients with CKD stages3-5D. In all patients, serum levels of calcium, phosphorus & parathormone were estimated and results analysed.

**Results:** 60% patients had normal calcium levels. 38.9% patients had hypocalcemia. 45.3% patients had hyperphosphataemia. 69.5% patients had raised serum parathormone levels.

**Index Terms-** Chronic kidney disease, mineral bone disorder,calcium, phosphorus, parathormone.

## I. INTRODUCTION

Mineral bone disorder(MBD) is one of the most important complication of chronic kidney disease(CKD) [1]. As renal function declines, biochemical abnormalities involving calcium & phosphorus metabolism lead to pathological changes in bones which can predispose to bone pain & fractures, thus increasing the morbidity and mortality in CKD patients.

In CKD patients, the ability of the kidneys to appropriately excrete phosphate load is diminished, leading to hyperphosphatemia, elevated parathormone(PTH), & decreased 1,25-dihydroxyvitamin D [1,25(OH)<sub>2</sub>D]. The conversion of 25-hydroxyvitaminD [25(OH)D] to 1,25,(OH)<sub>2</sub>D is impaired, thus reducing intestinal calcium absorption & increasing parathormone(PTH). The kidney fails to respond adequately to PTH, which normally promotes phosphaturia and calcium reabsorption.

In recent years, it has been found that these biochemical abnormalities not only lead to bone disease, but also predispose to vascular calcification & increased risk of cardiovascular morbidity & mortality.

Therapy is focused on correcting these biochemical & hormonal abnormalities in an effort to limit the consequences.

So, this study was undertaken to know the prevalence of mineral bone disorder(MBD) in CKD stages 3 to 5D, & to correlate biochemical abnormalities of calcium, phosphorus & parathormone with clinical disease.

## II. MATERIALS AND METHODS

This is a prospective single centre study conducted at Department of Nephrology at IPGMER & SSKM Hospital, Kolkata. Study period-May 2011 to Dec 2012.

All Patients from age 12 years to 65 years of both male and female sex with CKD stages 3-5D [2,3,4] attending opd / admitted in nephrology ward are included in study.

**Inclusion criteria** - All patients of 12-65 years of either sex with proven CKD stage 3 to 5D .

**Exclusion criteria-**

- 1) Patients suffering from systemic diseases like SLE(systemic lupus erythematosus) / RA(rheumatoid arthritis),
- 2) Patients on steroids and other drugs which have effect on bone,
- 3) Patients with primary bone diseases.

Detailed history and physical examination was done with reference to bone pain, fractures, and patients were subjected to following investigations:

- 1) Serum calcium(corrected for albumin)
- 2) Serum phosphorus
- 3) Intact Parathyroid hormone assay(Ipth), and Other routine investigations for kidney disease.

## III. INVESTIGATIONS

- 1) Serum Calcium was measured by colorimetric assay by OCP method.

**OCP METHOD** : Principle: Calcium is an alkaline medium combines with O-cresolphthalein to form a purple coloured complex. Intensity of the colour formed is directly proportional to the amount of calcium present in the sample.

Calcium + ocp -> purple coloured complex.

Normal reference range value :  
Serum/plasma - 8.5 to 10.5mg/dl

2) S.phosphorus measured by colorimetric assay by Modified Gommorri's method.

Principle:

Phosphate ions in an acidic medium reacts with ammonium molybdate to form a phosphomolybdate complex. This complex reacts with metal and is reduced to a molybdenum blue complex . Intensity of the molybdenum blue complex formed is directly proportional to the amount of inorganic phosphorus present in the sample.

Phosphorous + Ammonium molybdate -> Phosphorous molybdate complex.

Phosphomolybdate complex+ Metal -> Molybdenum Blue complex.

Normal reference range:

Serum : 2.5 to 4.5 mg/dl

3)iPTH(intact parathormone) - measured by 2 site immunoradiometric assay(2<sup>nd</sup> generation assay)

Principle: This elisa kit applies the competitive enzyme immunoassay technique utilizing a monoclonal antibody for the target antigen and a target antigen HRP conjugate. The assay sample and buffer are incubated together with target antigen HRP conjugate precoated plate for one hour. After the incubation period the wells are decanted and washed five times . The wells are then incubated with a substrate for HRP enzyme. The product of the enzyme – substrate reaction form blue coloured complex. Finally a stop solution is added to stop the reaction, which will then turn the solution yellow. The intensity of colour is inversely proportional to the target antigen concentration since the target antigen from samples and target antigen HRP conjugate compete for the antibody binding site. Since the number of sites is limited ,as more sites are occupied by the target antigen from the sample ,fewer sites are left to bind the conjugate . A standard curve is plotted relating the intensity of the colour to the concentration of standard . The target antigen concentration in each sample is interpolated from this standard curve[5].

CKD3-5D normal range= reference limits of particular assay.

CKD 5D:-normal range=- 2 to 9 times upper reference limit for assay.

**Statistical methods:** variables are presented as distributions (i.e., frequencies and percentages) using microsoft excel.

#### IV. RESULTS

190 patients with chronic kidney disease stage 3-5D were tested for evidence of mineral bone disorder. Out of 190 patients, two thirds were males and one third were females. Majority of patients were middle aged. 47% patients were diabetic and 84% patients had hypertension.

##### **CKD Stage 3:**

Out of 190 patients, 30 patients were in CKD stage 3. Majority(80%) had calcium levels in normal range. 20% had

calcium below normal. 86.7% patients had phosphorus levels within normal range. In 13.3% patients phosphorus was elevated. 53.3% patients had normal Ipth levels, whereas in significant number(46.7%) of patients ipth was elevated above normal range.

##### **CKD Stage 4 :**

Out of 190 patients, 58 patients were in CKD stage 4. In 38(65.5%) patients calcium was within normal range. 31% patients had low calcium levels and 3% patients had hypercalcemia. In 72.4% patients, phosphorus was within normal range. While 27.6% patients had hyperphosphataemia. 69% patients had ipth above normal range and in only 31% patients ipth was normal.

**CKD Stage 5 :** Out of 190 patients, 70 patients were in CKD stage 5. In 54% patients, calcium was within normal range. While a significant number of patients (46%) had hypocalcemia. Phosphorus was above normal in 60% patients and in 40% it was within normal range.

##### **CKD Stage 5D:**

Out of 190 patients, 32 patients were in CKD stage 5D. Hypocalcemia was noted in 56% patients. Phosphorus was elevated in 75% patients and normal in rest. iPTH was elevated in 44% patients and below normal in 37% patients. Only 19% patients had ipth in normal range.

#### V. DISCUSSION

Abnormalities of mineral metabolism occur early in chronic kidney disease[6]. Recently, increased attention has been focused on endocrine abnormalities in patients with CKD as a way to explain some of these associations[7]. Mineral bone disorder(MBD) was common in our patients with CKD. Calcitriol deficiency plays a major role in the development of secondary hyperparathyroidism(HPTH), as 1,25(OH)<sub>2</sub>D deficiency promotes parathyroid gland hyperplasia & increased PTH synthesis through loss of the ability to upregulate vitamin D receptor expression within parathyroid cells[8].The end result is elevated serum PTH & abnormal calcium and phosphorus balance. Beginning in CKD stage 3, secondary hyperparathyroidism was the earliest change noted which was present in nearly half of patients. As CKD progressed prevalence of hyperparathyroidism increased to involve more than 90% patients in CKD stage 5. Also, the severity of hyperparathyroidism was more as CKD stage progressed.

Adynamic bone disease as evident by low ipth levels was uncommon in nondialytic population but affected more than one third of patients on dialysis..

Elevated PTH & hyperphosphatemia were recently identified as risk factors for mortality in dialysis patients[9]. Serum calcium and phosphorus abnormalities were uncommon in CKD stages 3& 4(<1/3rd), but were seen in more than half to two thirds of patients as they entered stage 5 and dialysis. Hypercalcemia was rarely seen. Levels of calcium correlated inversely with iPTH levels. Phosphorus levels correlated positively with iPTH levels. Median calcium & phosphorus values were within normal ranges, and increases in iPTH began to occur from CKD stage4 [6].

Hyperparathyroidism presents early in CKD & worsens with progression of CKD stages. There is an increase in the prevalence of hyperparathyroidism from CKD stage 4. Hyperparathyroidism was present in 69% patients in CKD stage 4 & 91.4% patients in CKD stage 5 which was similar to Levin A et al. study [6] in which 56% patients in CKD stage 4 had hyperparathyroidism.

Literature on the prevalence of these abnormalities with current assays is limited. A study by Levin et al., one of the largest multi center study found that calcium & phosphate levels do not change till advanced stages of CKD. Hyperparathyroidism presents early in CKD & worsens with progression of CKD stages.

## VI. CONCLUSION

Abnormalities of mineral bone metabolism are common in CKD patients. Hypocalcemia & hyperphosphataemia are noted in later CKD stages & worsen with disease progression. Hence, this shows the importance of early recognition of abnormalities, understanding of their patho-physiological consequences, & planning management strategies to prevent their progression. Thus, reducing the cardiovascular morbidity & mortality.

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**Table/fig- 1: Baseline characteristics :**

Parameters		Number(n=190)	Percent(%)
Age(years)	20-40years	42	22.1
	41-60years	95	50.5
	61-80years	53	27.9
	Sex		
	Male	128	67.4
	Female	62	32.6

**Table/fig- 2 : Distribution of cases according to serum calcium levels in CKD stages 3 to 5D :**

CKD stages	Calcium (mg/dl)			Total
	<8.5	8.5-10.5	>10.5	
CKD stage3	6(20.0%)	24(80.0%)	0	30
CKD stage4	18(31.0%)	38(65.5%)	2(3.4%)	58
CKD stage5	32(45.7%)	38(54.3%)	0	70
CKD stage5D	18(56.3%)	14(43.8%)	0	32
Total	74(38.9%)	114(60.0%)	2(1.1%)	190

Data presented as No. of patients (%)

**Table/fig-3: Distribution of cases according to serum phosphorus levels in CKD stages 3 to 5D :**

CKD stages	phosphorus (mg/dl)		Total
	2.5-4.5	>4.5	
CKD stage 3	26(86.7%)	4(13.3%)	30
CKD stage 4	42(72.4%)	16(27.6%)	58
CKD stage 5	20(40.0%)	42(60.0%)	70
CKDstage 5D	8(25.0%)	24(75.0%)	32
Total	104(54.7%)	86(45.3%)	190

Data presented as No. of patients (%)

**Table/fig- 4 : Distribution of cases according to serum ipth levels in CKD stages 3 to 5D :**

CKD stages	Intact PTH levels			Total
	Below normal	Normal	Above normal	
CKD stage 3	0	16(53.3%)	14(46.7%)	30
CKD stage 4	0	18(31.0%)	40(69.0%)	58
CKD stage 5	3(4.3%)	3(4.3%)	64(91.4%)	70
CKDstage 5D	12(37.5%)	6(18.8%)	14(43.8%)	32
Total	15(7.9%)	43(22.6%)	132(69.5%)	190

Data presented as No. of patients (%)