Profile of mineral bone disease in Pre-dialytic stage 3-5 Chronic Kidney Disease Patients: A Cross-Sectional Study

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ABSTRACT

Background: Kidneys and bones are the major metabolic buffer systems in our body that help us to maintain the internal milieu. The importance being given to the mineral abnormalities in CKD is due to its high association with the cardiovascular disease and death due to CVD. So an ideal approach is to help to reduce the renal mineral load rather than suppressing the compensation. This study aims to contribute some data about the prevalence of mineral abnormalities and bone diseases in stage 3-5 pre-dialytic CKD patients in the south Indian population where the data about the disease is insufficient. **Methods:** It is a cross-sectional study conducted from January 2016 to June 2016 in government kilpauk medical college, Chennai. 83 Pre- dialytic stage 3 to 5 CKD patients were analyzed for various biochemical parameters. The data were analysed using SPSS (Statistical Package for Social Science) Ver 16.01. Appropriate statistical tools are used wherever required. **Results:** Of the 83, the prevalence of hypoparathyroidism is 6.02%, Hyperparathyroidism is 65.06%, Hyperparathyroidism more than 2 times upper limit is 47%, hypocalcemia is 12%, Hyperphosphatemia is 22.89%. Vitamin D deficiency in stages 3, 4 & 5 are 96%, 76% & 100% respectively. Prevalence of high bone turnover, mild turnover, osteomalacia & adynamic bone disease based on Bone-specific Alkaline phosphatase are 24%, 22%, 19% & 6% respectively. **Conclusion:** We concluded that symptoms of MBD alone are not enough to predict the bone health state. Bone turnover markers should be measured to assess it.

KEYWORDS: Adynamic bone disease, Bone-specific Alkaline Phosphatase, bone turnover, CKD-MBD, Pre-dialytic CKD

INTRODUCTION

Kidneys and bones are the major metabolic buffer systems in our body that help us to maintain the internal milieu. Disease in one naturally is going to affect the other in long term. The association between the two has long been known but not brought into the limelight till the recent decades. This increase in the importance being given to the mineral abnormalities is due to its high association with the cardiovascular disease and death due to CVD. Hence the parathyroids overwork to reduce some of the mineral load to the kidneys by depositing them on the tissues. They achieve it by mobilizing the calcium from the bones, form adducts with the excess phosphates and the products get deposited in the tissues. This compensatory mechanism becomes a disease when the deposition starts to affect the functionality of the tissues, just like what obesity does to the body. So an ideal approach is to help reducing the renal mineral load rather than suppressing the compensation. This study aims to contribute some data about the mineral abnormalities of CKD in the south Indian population where the data about the disease is insufficient.

Aims & Objectives:

1. To study the patterns of various mineral abnormalities in stage 3-5 Chronic Kidney Disease patients in south Indian population.
2. To find out the correlation between calcium, phosphorus, magnesium, iPTH, Vit-D and bone-specific alkaline phosphatase.
3. To elucidate the relationship between symptomatic MBD with the biochemical marker.

MATERIALS AND METHODS

This is a cross-sectional study, conducted from January 2016 to June 2016 in Govt. Kilpauk Medical College, Chennai, Tamil nadu. A total of 83 CKD patients attending the Nephrology department, Govt kilpauk Medical College, Chennai were included in the study.
We included Stage 3-5 predialytic CKD patients and for those whom dialysis had been done for less than a month. Patients already on dialysis, who underwent renal transplantation, those with known parathyroid abnormalities, liver disease & rickets and those on anti-epileptics were excluded. None of the patients were on Vitamin D supplementation. But all of them were on calcium supplementation.

Ethics committee approval was obtained prior to the study. Questionnaire regarding the symptoms of CKD-MBD is given to all the patients in our study. Those who are having one or more of the symptoms such as, non-

How to cite this article:
Velayudham B, Balaguru P, Vijayakumar N, Shankar P.. Profile of mineral bone disease in Pre-dialytic stage 3-5 Chronic Kidney Disease Patients: A Cross-Sectional Study. Int J Oral Health Med Res 2017;4(3):39-43.

pains that are deep dull aching pains that cannot be often localized by the patient, fractures occurring to trivial injuries, are considered as symptomatic patients. Ophthalmic fundus examination is done.

After obtaining consent from the patient, about 8 ml of venous blood is obtained from the median cubital vein without applying tourniquet. All the samples are collected at 10 am to 12 pm. Blood collection is done like this because factors like central vein blood or peripheral blood, winter season or summer season, nocturnal or daylight, will affect the PTH concentration. 3 ml of blood is collected in K2-EDTA tube for separating plasma. 5 ml of blood is collected in Red topped Standard serum separating tube. After allowing the sample to clot at room temperature for about one hour, samples are centrifuged, and serum & plasma are separately aspirated using Pasteur pipettes. Samples are separately stored in 1 ml eppendorf tubes. Thus 4 aliquots are allotted for every patient. Serum and plasma samples are stored at -20o C ice lined refrigerator. All samples are analyzed within 3 months of collection. Plasma samples are analyzed for 25-OH Vitamin D and iPTH. Serum samples are analyzed for all other biochemical parameters.

Vitamin D: The kit used in this study is ms-05894913 190 by Roche diagnostics and was carried out on Cobas e411 ECLIA analyzer measures 25-OH vitamin D. The stability of vitamin d in K2 EDTA plasma at -20^{o}C is for 24 weeks. The measuring range is 3.00-70.0 ng/ml. The shelf life of unopened kit is upto expiry date; opened and at 2-8^{o}C is 8 weeks; On cobas e 411 analyzer is 3 weeks.

Parathormone: The iPTH assay is carried out using the 2 site Electro Chemi Luminescence Immuno Assay. The kit used is ms-11972103122 on Cobas e-411 analyzer. The antibodies used in this test reacts at N-terminal 1-37 amino acids at 26-32 amino acid epitope and C-terminal 38-84 amino acids at 37-42 amino acid epitope. Measuring range is 1.20-5000pg/ml. The stability of PTH at -20^{o}C in plasma is for 6 months. This method has no cross-reactivity with PTH 1-37, PTHrP 1-86. The shelf life of unopened kit at 2-8^{o}C is up to expiry date; opened and at 2-8^{o}C is 12 weeks; on the analyzer is 8 weeks.

Bone Specific Alkaline Phosphatase: This assay was carried out by ELISA in microtitre strip method, with Microvue BAP ELISA kit-046001 from Quidel Corporation, San Diego, USA in Robonik readwell Touch ELISA plate analyzer and Robonik washwell plate ELISA washer. The stability of the enzyme in serum is 5 days at 2-8^{o}C; twelve months at -20^{o}C; 36 months at -80^{o}C.

Calcium: This assay is done using the Calcium Gen 2 kit- 05061482 190 from Roche and analyzed using Cobas c-311 analyzer. The assay is based on the change in absorbance of 376nm wavelength light that is measured photometrically. Stability of the serum calcium at -20^{o}C is 8 months. The range of measurement of this method is 0.8-20.1 mg/dl. The measured parameter is the serum total calcium. It is corrected according to the serum albumin to calculate corrected calcium.

Phosphorus: This assay is done using Inorgnic Phosphate ver.2 kit- 03183793 122 from Roche, on Cobas c-311 analyzer. The assay is based on Molybdate UV principle, which measures the change in absorbance of the Ammonium phosphomolybdate which subsequently reduces to molybdenum. The stability of the phosphate ions in serum stored at -20^{o}C is 1 year.

Urea: This assay is done using the UreaL kit- 04460715 190 from Roche, on Cobas c-311 analyzer. The assay is based on the Kinetic test principle with Urease and Glutamate dehydrogenase enzymes. The resulting NADH concentration is measured photometrically and is directly proportionate to the concentration of urea. The stability of urea in serum stored at -20^{o}C is 1 year.

Creatinine: This assay is done using the CREJ2 kit- 04810716 190 from Roche, on Cobas c-311 analyzer. The assay is based on kinetic colorimetric principle using Jaffe's method that forms a Yellow-orange complex that is measured photometrically. The serum stability for creatinine at -20^{o}C is 3 months, the shortest of all parameters in our study. Hence all analyses are completed within three months of sample collection. Patients are staged according to eGFR calculation using CKD-EPI equation.

Magnesium: This assay is done using Robonik Prietest Touch photocolorimeter machine based on Xylidyl blue method. The normal range by this method is 1.6 to 2.8 mg/dl.

eGFR – calculation is done by CKD-EPI equation.

Statistical Analysis: The data were analysis using SPSS (Statistical Package for Social Science) Ver 16.01. Student t-test was used for testing the significance of all the variables mean and standard deviation in groups. Chi-square test was used to compare proportions. Pearson Correlation test was used to find out the relationship between the variables. All the statistical results were considered significant at P value ≤ 0.05.

RESULTS

Using CKD-epi equation, the eGFR of the patients were calculated. They were staged according to the calculated eGFR into stages 3, 4 & 5. The demographic pattern of the 83 pre-dialytic stage 3-5 CKD patients is as follows (Table 1):

Parameter	For n=83
age	54.3+/-12.6
M:F	0.9:1
eGFR	23.6+/-13
Urea	67(50,104)
Creatinine	2.5(1.9,4.2)
calcium	9.2(8.7,9.4)
Phosphorus	3.8(3.1,4.5)
iPTH	93.9(49.3,172.7)
Vitamin D	15.8(9.9,21.1)
BAP	28(19.6,41)
Symptom occurrence	40.96%

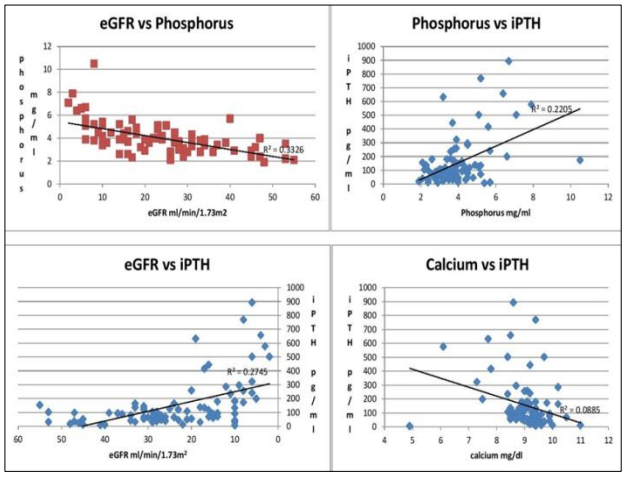
Table-1: Demographic profile of CKD 3-5 patients. Age & eGFR are expressed as mean+/-SD. Urea, creatinine, calcium, phosphorus, iPTH, vitamin D, Bone specific alkaline phosphatase are expressed as median with first and third quartile values in parantheses.

The biochemical parameters of the patients are as follows (Table 2):

Parameter	CKD stage 3(n=28)		CKD stage 4 (n=34)		CKD stage 5 (n=21)		Total (n=83)		p val
	no	%	no	%	no	%	no	%	
Hypercalcemia >10mg/dl	3	12	3	8.82	1	4.17	7	8.43	0.11
Hypocalcemia <8.6 mg/dl	3	12	4	11.76	9	37.5	16	19.28	
Hyperphosphatemia>4.5 mg/dl	1	4	6	17.65	12	50	19	22.89	0.001
Hypophosphatemia <2.5mg/dl	5	20	3	8.82	0	0	8	9.64	
Hypomagnesemia <1.5 mg/dl	4	16	6	17.65	5	20.83	15	18.07	0.53
iPTH <15pg/ml	3	12	1	2.94	1	4.17	5	6.02	0.04
iPTH >65pg/ml	13	52	20	58.82	21	87.5	59	65.06	
Vit D <30ng/ml	26	96	26	76.4	21	100	8	9.64	0.02
BAP normal	21	84	24	70.59	17	70.83	62	74.70	0.18
BAP low	2	8	0	0	1	4.17	3	3.61	
BAP high	2	8	10	29.41	6	25	18	21.69	

Table-2: Biochemical parameters

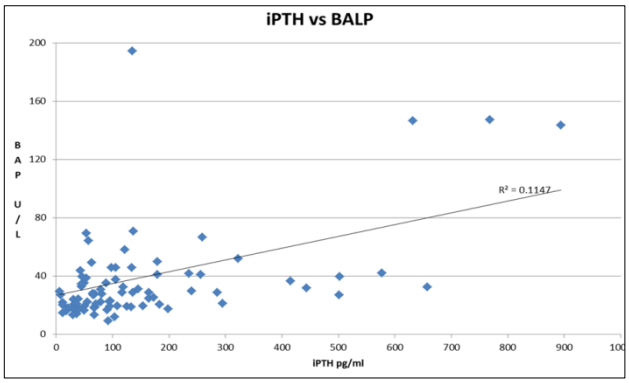
In our study, as the eGFR falls down, the phosphate retention increases along with a rise in the parathormone levels. This rise in the parathormone levels also is in parallel with the fall in the calcium levels. As hyperparathyroidism occurs, bone turnover increases as measured by the bone turnover marker, Bone-specific Alkaline Phosphatase. A statistically significant correlation, as analyzed by Pearson coefficient, is seen between iPTH and calcium (r = -0.30; p = 0.01); iPTH and BAP (r = 0.34; p = 0.001); BAP and Vitamin D (r = 0.28; p = 0.01). These are clearly presented in the following Graphs 1and 2.



Graph 1: Scatter plots showing relationship among eGFR, phosphorus, calcium and Parathormone. eGFR- estimated Glomerular filtration rate; iPTH- intact parathormone.

The correlations among the biochemical parameters are mentioned in Table 3.

This indicates that there is a significant positive correlation between BAP and iPTH as well as Vit D. This shows that a high bone turnover parallels a high parathormone level and good vitamin D level. The



Graph 2: Scatter plot showing relationship between parathormone and the bone turnover marker. iPTH- Intact parathormone; BAP- Bone specific Alkaline Phosphatase.

		BAP	iPTH	Vit D
BAP	Pearson correlation	1	0.34	0.28
	Sig. (2-tailed)	-	0.001	0.01
	N	83	83	83
iPTH	Pearson correlation	0.34	1	-0.10
	Sig. (2-tailed)	0.001	-	0.36
	N	83	83	83
Vit D	Pearson correlation	0.28	-0.10	1
	Sig. (2-tailed)	0.01	0.36	-
	N	83	83	83

Graph 2: Scatter plot showing relationship between parathormone and the bone turnover marker. iPTH- Intact parathormone; BAP- Bone specific Alkaline Phosphatase.

opposite is also statistically significant i.e., a low bone turnover state parallels vitamin D deficiency and hypoparathyroidism.

As per our study, the prevalence of various bone abnormalities, suspected with biochemical parameters alone, have been described in Diagram 1.

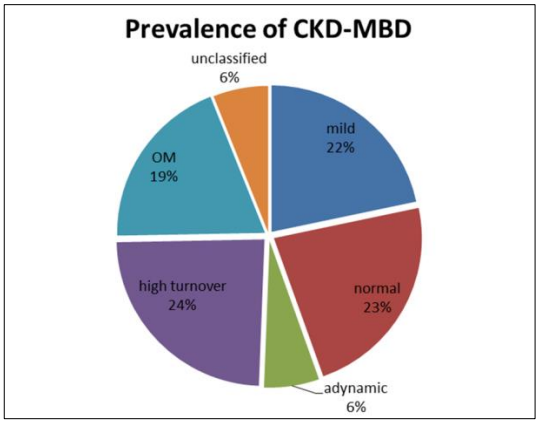


Diagram 3: Prevalence of mineral bone diseases in CKD 3-5 pre-dialytic patients. OM- osteomalacia; adynamic- adynamic bone disease

A Bone biopsy is the gold standard in diagnosing the bone disease.[1] Without a bone biopsy, it is difficult to classify bone disease. The Adynamic bone disease occurs because of a very low iPTH which is less than 100pg/ml that decreases both osteoblast and osteoclast activities. Bone-specific Alkaline Phosphatase levels fall in Adynamic bone disease.[2,3] In our study, with biochemical parameters alone, we considered MBD as follows (Table 4) .

MBD	BAP (Female age 25-44= 11.6-29.6U/L, Female age >44=14.2-42.7U/L, Male age>25 = 15-41.3U/L)	iPTH (15-65pg/ml)	Vit D (deficiency= <30ng/ml)
Normal	Normal	Normal or elevated but less than twice the upper limit	Deficient
Adynamic bone disease	Low	Low	Deficient
High turnover bone disease	High	Elevated or normal	
Mild turnover disease	Normal	Elevated more than twice the upper limit	Deficient
Osteomalacia	Normal	Normal	Deficient
Unclassified	Normal	Low	Deficient

Table-4: MBD classes according to the biochemical parameters alone. MBD-Mineral bone disease; BAP-Bone specific Alkaline Phosphatase; iPTH-intact parathormone; Vit D- Vitamin D.

The prevalence of MBD symptoms in patients with abnormal bone turnover is (n=9 out of 25) 36% (p=0.816) which is not statistically significant. This indicates that symptoms can't predict the nature of the MBD.

DISCUSSION

Prevalence of CKD-MBD is available in western countries.[4] The prevalence of CKD in our Indian population is estimated to be between 0.78% and 0.87%[5]. The main purpose of our study is to describe the profile of CKD-MBD patients in our hospital. We studied 83 CKD stage 3-5 patients. The average age of the CKD patients in our study is 54.3 +/- 12.67 years. Of them 42 were females and 41 males leading to an M:F ratio of 0.9:1. The symptomatic CKD-MBD individuals according to our study comprised 40.96%. Remaining 59.03% didn't have any symptoms related to CKD-MBD. This is in concurrence with other Indian studies.[5] This further reveals how disguising the clinical picture of MBD is in our setup.

The prevalence of hypoparathyroidism is 6.02% indicating the possible load of low turnover bone disease which when assessed by BAP is 6%. In our population, Vitamin D supplementation is low. This may explain the low prevalence of adynamic bone disease in our setup.[2]

Hyperparathyroidism, with a cutoff of iPTH >65 pg/ml, is noted in 58.82% in stage 4 and 87.5% in stage 5, with overall 65.06% indicating the possibility high turnover bone disease. Other Indian studies show hyperparathyroidism to be 73%[6] , 57.3% in stage 4 CKD and 89.5% in stage 5 CKD[5] and 84.62% in stage 4 and 88.29% in stage 5.[7]

High turnover state assessed by Z. Jabbar et al is 60%[6] with a cutoff of iPTH as >300pg/ml. In our study 47% (n=30) of patients have an iPTH value of two to nine times the normal upper limit, out of which 40% (n=12) have high BAP indicating that truly high turnover state exists in less than what is actually estimated by iPTH.

Those with iPTH elevations more than twice the upper limit but BAP in normal level (n=18; 22%) might still be in a mild turnover bone state. This group of patients need to be followed up to find whether they are going for a high turnover or a low turnover state. At present they are considered as mild turnover state.

The prevalence of hypocalcemia in our study is noted to be 12%, 11.76% and 37.5% in stages 3, 4 & 5 respectively. Study by Agarwal et al showed that 29.9% and 49.6% of hypocalcemia is prevalent in the stages 4 and 5.[8]

Hyperphosphatemia is found in 4%, 17.65% & 50% in stages 3, 4 & 5 of CKD with an overall of 22.89% in our study. It is in concordance with other Indian studies- Valson et al 59%[5], Ghosh et al 64.10% in stage 4 and 70.27% in stage 5.[7] It can be seen that phosphate retention increases with fall in GFR. These are the potential candidates for phosphate lowering therapies if clinically feasible.

Prevalence of hypomagnesemia in stage 3-5 CKD, in our study shows that magnesium is not associated with bone turnover abnormalities. Hypomagnesemia occurrence in symptomatic individuals is also not statistically significant (p=0.561).

Vitamin D deficiency is found only in stages 3 and 4 of CKD, 96% and 76.4% namely, in our study. In stage 5 CKD, all of them are deficient (p=0.02). Previous studies showed a prevalence of up to 80% of vitamin D deficiency.[6,7]

Prevalence of an abnormal BAP, in our study, is not found in a statistically significant (p=0.816) percentage with any of the following ie., symptomatic individuals / diabetic individuals / patients with abnormal fundus examination.

Thus none of the above features in a CKD-MBD patient can predict an abnormal bone turnover. Only bone turnover markers next to bone biopsy can predict bone turnover diseases in CKD.

Limitations of the Study are as follows:

- Extra skeletal calcification was not studied due to lack of Electron Beam Computerised Tomography (EBCT) which is the gold standard for this parameter.
- Bone biopsy was not done to confirm the data analysed.
- The Bio-intact PTH is not compared with BAP in assessing serum bone turnover marker[(9)].
- This is a cross-sectional study. True burden of CKD-MBD in the population can be assessed in longitudinal studies.
- National Data about CKD-MBD is not sufficient to correlate at this point of time.
- Trend of biochemical parameters over time can help diagnosing the bone turnover state rather than one time value. Thus a follow up study is needed for the same set of patients.

CONCLUSION

- Symptoms of CKD-MBD need not predict the bone turnover rate

- Diabetic Retinopathy need not indicate bone turnover rate.
- Bone turnover rate is best assessed by turnover markers next only to bone biopsy.
- Clinically quiet nature of this disease tells that early screening is the best way to alter the course of the disease.

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Source of Support: Nil
Conflict of Interest: Nil