STUDY OF ASSOCIATION BETWEEN BIOCHEMICAL PARAMETERS OF MINERAL BONE DISEASE AND BONE HISTOMORPHOMETRY IN CHRONIC KIDNEY DISEASE PATIENTS

THESIS

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DOCTORATE OF MEDICINE (DM)

(NEPHROLOGY)

JUNE 2022. **DR. SANTOSH KUMAR MAURYA**

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DECLARATION

I hereby declare that the thesis titled "**Study of association between biochemical parameters of mineral bone disease and bone histomorphometry in chronic kidney disease patients"** embodies the original work carried out by the undersigned in All India Institute of Medical Sciences, Jodhpur.

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CERTIFICATE

This is to certify that the thesis titled "**Study of association between biochemical parameters of mineral bone disease and bone histomorphometry in chronic kidney disease patients"** is the bonafide work of Dr Santosh Kumar Maurya carried out under our guidance and supervision, in the department of Nephrology, All India Institute of Medical Sciences, Jodhpur.

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INDEX

LIST OF ABBREVIATIONS

ABBREVIATIONS

CKD	:	CHRONIC KIDNEY DISEASE
ESRD	:	END STAGE RENAL DISEASE
MUO	:	MIXED UREMIC OSTEODYSTROPHY
HPT	:	HYPERPARATHYROIDISM
iPTH	:	INTACT PARATHYROID HARMONE
ROD	:	RENAL OSTEODYSTROPHY
KDOQI	:	KIDNEY DISEASE OUTCOMES QUALITY INITIATIVE
KDIGO	:	KIDNEY DISEASE: IMPROVING GLOBAL OUTCOMES
Hb	:	HEMOGLOBIN
HD	:	HEMODIALYSYIS
NIH	:	NATIONAL INSTITUTE OF HEALTH
NKF	:	NATIONAL KIDNEY FOUNDATION
DEXA	:	DUEL ENERGY X RAY ABSORBTIOMETRY
TRPV	:	TRANSIENT RECEPTOR POTENTIAL VANILLOID

CaSR. : CALCIUM SENSING RECEPTOR

ADH : ANTIDIURETIC HARMONE

IRMA : IMMUNOREDIOMETRIC ASSAY

RIA : RADIOIMMUNOASSAY

MBD : MINERAL BONE DISEASE

ABD : ADYNAMIC BONE DISEAS

BFR : BONE FORMATION RATE

BMU : BASIC MULTICELLULAR UNIT

BV/TV : BONE VOLUME/TISSUE VOLUME

Tb Wi : TRABECULAR WIDTH

Tb N : TRABECULAR NUMBER

OV/BV : OSTEOID VOLUME/BONE VOLUME

OS/BS. : OSTEOID SURFACE/BONE SURFACE

O.Th. : OSTEOID THICKNESS

ES/BS : ERODED SURFACE/BONE SURFACE

Ob.S/BS : OSTEOBLAST SURFACE/BONE SURFACE

Oc.S/BS : OSTEOCLAST SURFACE/BONE SURFA

LIST OF TABLES

LIST OF FIGURES

40	ABD WITHOUGHT OSTEOID AND WITHOUGHT CELLULAR ACTIVITY	79

SUMMARY OF THE PROJECT

Background: The estimated prevalence of CKD as per the data from International Society of Nephrology data center study is 17%[1.]. The Kidney Disease Outcome Quality Initiative (KDOQI) of the National Kidney Foundation (NFK) has classified categories of CKD according to the glomerular filtration rate (GFR)[2] as follows: Stage 1 - GFR 90 mL/minute/1.73 m² or higher, Stage 2 - GFR 60 to 89 mL/minute/1.73 m^2,Stage 3a - GFR 45 to 59 mL/minute/1.73 m^2,Stage 3b – 30 to 44 mL/minute/1.73 m^2,Stage 4 - GFR 15 to 29 mL/minute/1.73 $m^2$and Stage 5 - GFR less than 15 mL/minute/1.73 m^2. The presence of chronic kidney disease has multiple effects on mineral metabolism. The nomenclature mineral bone disorder of CKD (CKD-MBD) includes bone disease which includes abnormal bone turnover, mineralization and volume, soft tissue calcification and biochemical abnormalities. Renal osteodystrophy (ROD) is bone part of CKD MBD.In ROD, multiple type of bone disease present like high or low turnover, normal and abnormal mineralization, normal, low or high volume.

Aims and Objectives: AIMS

- By bone biopsy histopathology, we identify type of bone turn over disorders like high, low or mixed type.

Primary objective

- To identify correlation between biochemical parameter of mineral bone disease and bone histomorphometry.

Secondary objective

- To identify the prevalence of type of mineral bone disorder among CKD patients stage 3-5 including on maintenance hemodialysis.

Methods: A cross sectional study has been conducted after seeking written informed consent from the study participants, who attend the OPD or IPD services of Department of Nephrology. After taking consent, bone biopsy from iliac crest was done, fixed in alcohol and processed in Pathology department and staining was done by Goldner stain. Histomorphometric analysis was done by using NIH software Image J Adobe Photoshop software for image masking into black and white for bone volume calculation.

Results: Total of twenty-eight patients with CKD were enrolled in the age group of 18- 55 years. The median age was 33 years. The mean age was 33.07 ± 10.42 years. Out of the total twenty-eight patients, 20 (71%) were males and 8 (29%) were females. All 28 patients were taken from indoor who were admitted in AIIMS. All patients were in stage 5 CKD. Most of the patients were with uremic symptoms with first time diagnosis as CKD (78%).18 % patients were on MHD and 1 patient(4%) was not on dialysis. After bone histomorphometry analysis and interpretation ROD was described mainly in 4 parts; High turnover, MUO, Normal and Low turnover or adynamic bone disease. Most common ROD was MUO(46%) followed by normal histology(29%),adynamic or low turnover disease in 14% and pure high turnover disease in 11% of patients. Serum intact PTH was significantly correlated with high turnover disease.

Conclusion: Intact PTH is the main determinant of the type of bone histomorphometry. The level of intact PTH depend not only on one biochemical parameter but multiple factors which influence it's levels. KDIGO guidelines suggested that the intact PTH levels should be 2 to 9 times of the upper limit of normal along with other biochemical parameters monitored. In most of the studies there were no significant correlation between biochemical parameters and bone histomorphometry. It may be due to different population groups like dialysis and non dialysis patients, stage of CKD, calcium supplements prescribed and type of phosphate binders. In our study also, the most common ROD is Mixed Uremic Osteodystrophy. Our study suggests that bone biopsy is desired to ascertain CKD-MBD renal osteodystrophy profile, with existent indications of bone biopsy, as a related diagnostic tool in providing guide for diagnosis and treatment of mixed pattern of renal osteodystrophy not delineated by biochemical parameters alone and otherwise missed in CKD patients who are on chronic HD/PD.

INTRODUCTION

Chronic Kidney disease (CKD) is an important public health related problem on account of its high prevalence and disease related morbidity and mortality. The growing burden of Diabetes mellitus and hypertension, infections, exposure to nephrotoxins and obstructive uropathy due to nephrolithiasis account for major causes of CKD in Indian population. The estimated prevalence of CKD as per the data from International Society of Nephrology data centre study is 17%[1.]. The Kidney Disease Outcome Quality Initiative (KDOQI) of the National Kidney Foundation (NFK) has classified categories of CKD according to the glomerular filtration rate (GFR)[2] as follows:

• Stage 1 - GFR 90 mL/minute/1.73 m² or higher

• Stage 2 - GFR 60 to 89 mL/minute/1.73 m^2

• Stage 3a - GFR 45 to 59 mL/minute/1.73 m^2

• Stage 3b – 30 to 44 mL/minute/1.73 m^2

• Stage 4 - GFR 15 to 29 mL/minute/1.73 m^2

• Stage 5 - GFR less than 15 mL/minute/1.73 m^2.

The presence of chronic kidney disease has multiple effects on mineral metabolism. The nomenclature mineral bone disorder of CKD (CKD-MBD) includes bone disease which includes abnormal bone turnover, mineralization and volume; vascular and soft tissue calcifications as well as the myriad spectrum of abnormal biochemical profile of calcium, phosphorous, parathyroid hormone and vitamin D metabolism in chronic kidney disease patients. With deterioration of kidney function and decline in GFR, there is a pertinent need to excrete phosphorous. This is mediated by increased release of Fibroblast Growth Factor (FGF-23) that regulates phosphorous excretion. With gradual decrease in renal mass and increased FGF-23, the active form of vitamin D- calcitriol levels are reduced with concomitant low serum levels of calcium leading to elevated serum parathyroid hormone levels. The increased levels of parathyroid hormone levels lead to altered bone metabolism causing increased bone turn over and vascular calcifications.

Bone diseases in CKD

Bone morphology is assessed with respect to

1. Turnover: it could be low, normal or high
2. Mineralization: which could be normal or abnormal
3. Volume: it reflects net bone formation and resorption rates and related to bone porosity, strength and fragility- it could be low, normal or high.

Renal osteodystrophy refers to various bone histomorphologic abnormalities due to disturbances in bone turn over, mineralization and volume as a result of CKD-MBD

A. High turn over bone disease This is a classical form of bone disease seen in CKD patients similar to osteitis fibrosa cystic seen in primary hyperparathyroidism. There is sustained elevated levels of serum parathyroid hormone (secondary hyperparathyroidism) which leads to increased bone formation and bone resorption due to increased osteoblast and osteoclast activity with high fraction of trabecular surface covered by osteoid seams with abnormal collagen deposition and bone marrow fibrosis. Cystic areas arise due to increased bone resorption activity. Thus bone turnover is high as evidenced by increased tetracycline uptake; mineralization may be normal or defective and the volume is variable. Clinically patient complains of aches all over the body/bone pains with increased susceptibility to fractures.

B. Adynamic bone disease/low turnover bone disease- there is reduced bone formation and resorption evidenced by low tetracycline uptake. This bone disease is associated with low parathyroid levels and observed in elderly and diabetic individuals with CKD, aluminium toxicity and with continuous ambulatory peritoneal dialysis. There is over suppression of secondary hyperparathyroidism due to overzealous supplementation of active vitamin D and calcium. Bone turn over is low with absence of active bone resorption and bone formation; the mineralization is abnormal with no osteoid or mineralization and volume is normal. Bones tend to be brittle with increased tendency to fracture.

C. Mixed

D. Osteomalacia- this affliction of bone is also low turn over type but characterized by increase in unmineralised bone and observed in severe vitamin D deficiency. The bone turnover is normal, mineralization is abnormal and volume is increased.

E. Osteoporosis is associated with increased bone breakdown relative to bone production. The bone density(mass) is at least 2.5 standard deviation less than peak bone mass.

Bone biopsy is the gold standard method of determining the type of bone disease present in CKD. Bone samples are taken from the iliac crest after a time spaced administration of two doses of tetracycline spaced by 14 days interval. It provides information regarding the bone micro architecture and kinetic properties by the amount of bone formation and mineralization between two layers of bone labeled with tetracycline. Tetracycline binds to newly formed bone at the unmineralized bone interface. Measuring the distance between the two lines allows for calculation of bone formation rate during that interval.

There is evidence that CKD is associated with low bone mass and accelerated bone loss with decreased bone quality. Several studies have reported that kidney function is significantly associated with declines in BMD measured by DEXA[3].

CKD 2-5D patients have significant cortical bone loss, with decreased BMD in hip, distal radius measured by DEXA and significant declines in cortical area, density, and thickness and increased porosity evaluated by high-resolution peripheral quantitative computed tomography[4].

There are also studies showing a gradual increase in bone resorption associated with decreased bone formation and impairment in bone mineralization on histomorphometry, as CKD progresses.[5]

In 2006, the Kidney Disease Improving Global Outcomes (KDIGO) Working Group classified these disorders as a single clinical designated CKD–mineral and bone Disorder (CKD–MBD) manifested by either one or the combination of: laboratory abnormalities of bone and mineral metabolism, including altered phosphorus, calcium, parathyroid hormone (PTH), or vitamin D metabolism; abnormalities in bone turnover, mineralization, volume, linear growth, or strength; and/or bone disease and vascular and other soft-tissue calcifications[6].

According to the KDIGO Working Group, the WHO definition of osteoporosis can be

applied for the diagnosis and management of stages 1 to 3a CKD, as long as there are no biochemical abnormalities suggesting CKD-MBD[7].

However, diagnosis of osteoporosis in stages 3b-5 CKD (or in earlier stages if CKD-MBD is suspected) is more complex and an exclusionary one. In these stages, patients have significant alterations in mineral metabolism and the probability of having features of ROD is high[7]. These are associated with bone strength impairment and may lead to low BMD and/or fragility fractures. Thus, the WHO criteria are not valid in these situations[8].

The 2017 KDIGO clinical practice guidelines state that, in patients with CKD stage 3-5D, it is reasonable to perform a bone biopsy if knowledge of type of ROD will impact treatment decisions. Currently, biochemical markers and imaging tests are not accurate predictors of bone histology[9].

Thus, the gold standard for the diagnosis and specific classification of renal osteodystrophy (ROD) remains the histomorphometric analysis of the bone biopsy.

Table No:1. Normal range of biochemical parameter in CKD patient

CKD STAGE	GFR RANGE (mL/min/1.73 m2)	PHOSPHORUS (mg/dL)	CALCIUM (CORRECTED)(mg/dL)	CA X P	INTACT PTH (pg/mL)
3	30-59	2.7-4.6	8.4-10.2		35-70
K/DOQI KDIGO					
4	15-29	2.7-4.6	8.4-10.2		70-110
K/DOQI KDIGO					
5	<15,dialysis	3.5-5.5	8.4-9.5	<55	150-300
K/DOQI KDIGO	Normal range	Norml range Avoid hyperphosphatemia	Normal range Avoid hypercalcemia	Use not Endorsed	Avoid PTH <2 or >9 times the upper normal limit

REVIEW OF LITERATURE

Historical Perspectives

In 1883 Lucas[10] suggested the term “renal rickets”. the correlation between kidney disease and bone abnormality is seen by him in patients with albuminuria. on further study of paper published by Lucas, shows that the case described were not example of renal infantilism.in 1911 Morley Fletcher[11]showed a typical example of renal infantilism occurring in a child who had developed genu valgum when five years of age.

Bauer et al[12] in 1930 established Correlation between bone lesions (osteitis fibrosa cystica) and the parathyroid gland abnormality following a review of 88 patients with endocrine bone disorders.

The term "renal osteodystrophy" was coined in 1943,[13] 60 years after an association was identified between bone disease and kidney failure. The traditional types of renal osteodystrophy[14] have been defined on the basis of turnover and mineralization as follows:

1) mild, slight increase in turnover and normal mineralization;

2) osteitis fibrosa, increased turnover and abnormal mineralization

3) osteomalacia, decreased turnover and abnormal mineralization

4) adynamic, decreased turnover and acellularity

5) mixed, increased turnover with abnormal mineralization.

Bricker and Slatopolsky gives the “trade-off hypothesis” in which they describe the pathogenesis of renal osteodystrophy. The theory states that progressive nephron loss in

CKD patients leads to several compensatory mechanisms such as elevated FGF 23 and PTH in response to retained phosphate.

In 1960s and 1970s, renal osteodystrophy in patients with end-stage kidney disease (ESKD) were osteitis fibrosa and mixed uraemic osteodystrophy predominantly with a minority of patients presenting with osteomalacia prior to dialysis.[15]

In some dialysis centre (Ottawa and Newcastle) due to high concentration of Aluminium and fluoride in their tap water, osteomalacia became a major problem following initiation of dialysis. this type of renal osteodystrophy(osteomalacia) was characterized by microcytic anemia and encephalopathy[16]

Adynamic bone disease was not only peculiar to aluminum contamination of tap water used for dialysis but also associated with the use of large amounts of aluminum containing phosphate binders and active vitamin D analogue therapy and calcium supplement.[17]

Subsequently, there was a rapid decline in the occurrence of this disease entity with improvement in water purification systems and reduced prescription of aluminum-containing phosphate binders.

Definition of chronic kidney disease–mineral bone disease (CKD-MBD)[18] :

A systemic disorder of mineral and bone metabolism due to CKD manifested by one or a combination of the following:

1.Abnormalities of calcium, phosphorus, parathyroid hormone, or vitamin D metabolism
2.Abnormalities in bone turnover, mineralization, volume, linear growth, or strength
3.Vascular or other soft tissue calcification

Biochemical Abnormalities

KDOQI guidelines recommend that laboratory monitoring should begin in CKD 3 in adult and earlier in children at CKD stage 2.

Calcium:• Serum calcium levels are controlled tightly in the range of 8.5-10.5 mg/dL . Extracellular calcium is measured as total calcium: 50% is free (the measured part), 10% is bound to anions, and 40% is bound to albumin. Calcium absorption occurs across intestinal epithelium through vitamin D–dependent TRPV5 and TRPV6 transporters, as well as paracellular pathways. Absorbed calcium enters 3 compartments: blood, soft tissue, and bone

60%-70% is reabsorbed passively in proximal tubules with sodium and water reabsorption 10% is reabsorbed in the thick ascending limb by the paracellular route . The rest is reabsorbed through transcellular pathways in the distal convoluted tubule, connecting tubule, and cortical collecting duct through TRPV5 and TRPV6 calcium channels .TRPV6 predominates in the intestine, whereas TRPV5 predominates in the kidney.

Calcium-sensing receptor (CaSR)

- G-protein–coupled protein that binds calcium to sense small changes in ionized calcium levels; decreased ionized calcium stimulates PTH secretion . CaSR is expressed in parathyroid cells, thyroid C cells, intestine, kidney, and likely bone . In the kidney, CaSR is in mesangial cells and throughout tubules

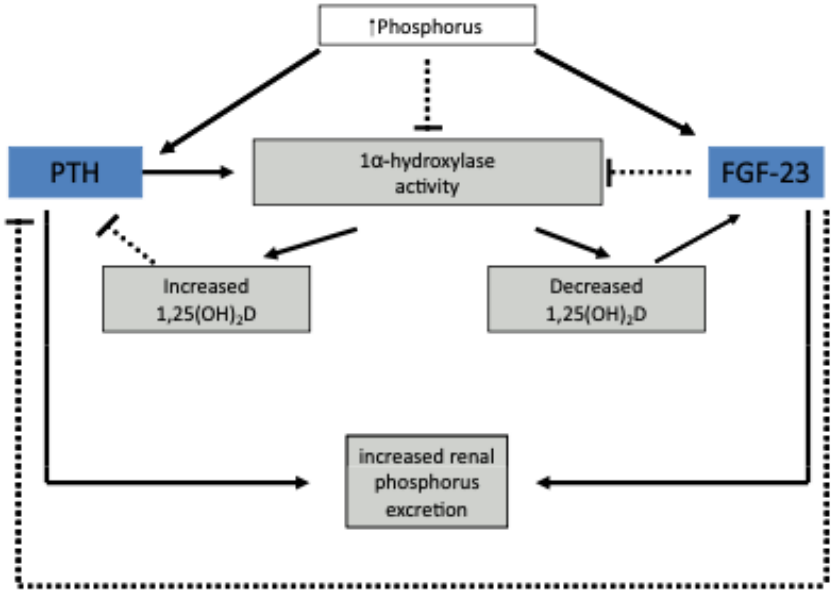
- ➢ Activation of CaSR on the thick ascending limb decreases paracellular calcium reabsorption Upregulation of CaSR in hypercalcemia inhibits antidiuretic hormone (ADH)-induced free water reabsorption, leading to urinary dilution.
- ➢ Renal effects of CaSR are both dependent and independent of PTH

Phosphorus: Serum phosphorus concentration reference range is 2.5-4.5 mg/dL; total-body stores of phosphorus equal 700 g. Of total-body stores, 85% is in bone as hydroxyapatite; 14%, intracellular; and 1%, extracellular.Of extracellular phosphorus, 70% is within phospholipids (organic), 30% is inorganic. 15% of inorganic fraction is protein bound. 85% of inorganic fraction is complexed with cations or circulating in free mono hydrogen or dihydrogen forms. This 85% is the fraction measured in phosphorus assays and therefore not a reliable estimate of total-body phosphorus, especially in CKD . 60%-70% of dietary phosphorus is absorbed in all intestinal segments

- ➢ Dependent on luminal concentration
- ➢ Absorbed through sodium/phosphate cotransporter 2b (Npt2b)
- ➢ Stimulated by calcitriol

Inorganic phosphorus is filtered by glomeruli, then 70%-80% is reabsorbed in proximal tubule through the Npt2a cotransporter. Npt2a is moved to or removed from the brush border to facilitate phosphorus reabsorption or excretion, respectively. 20%-30% of filtered phosphorus is reabsorbed in distal tubule. Renal phosphorus excretion is sensitive to serum phosphorus levels; PTH and FGF-23 increase phosphorus excretion. Phosphorus depletion decreases its own excretion.

Fig No. : 1.Regulation of serum phosphorus.



(A solid line indicates stimulation a dashed line indicates inhibition)

circulating levels of calcium and phosphorus is to maintain normal laboratory range at all stage of CKD ,according to KDIGO guidelines.in CKD stage 5 Phosphorus levels should not be simply equal to the upper phosphorus level of 5.5 mg/dl as suggested by KDOQI guidelines but should be lowered as much as possible toward the laboratory limit, which is lower than 5.5mg/dl.

Calcium levels should also be maintained with in the normal Laboratory range. calcium levels >9.5 mg/dL or even higher are associated with increased mortality in

CKD patients[19-23]. there is not much evidence of an association between low levels of calcium and mortality, and data of literature are controversial[20]. considering ionized calcium as gold standard, the "corrected calcium" formula does not offer any superiority over total calcium for diagnosis[24].

Parathyroid Hormone

The intact PTH is a single chain 84 amino acid peptide hormone. K/DOQI guidelines recommend that serum PTH levels of patients with CKD should be measured regularly and maintained within target ranges. For this reason, the methodology for PTH should be accurate because treatment decisions are made on the levels of PTH.

The first generation RIA used polyclonal antibodies directed toward C-terminal or mid region PTH molecule. In addition, PTH fragments were also measured. Therefore, the PTH levels were greatly elevated. Thus, the first generation assays became obsolete.

The second generation PTH assays were introduced in mid-1980s as first and second generation assays. These second generation assays were called "INTACT PTH" assays as they were thought to measure only the full length 1–84 PTH . 2^{nd} generation IRMA used two different antibodies; one directed toward the 39–84 portion and the second toward the 15–20 portion of the PTH molecule.

these assays had certain limitations; in particular, their values were high and overestimated (in the range of 400–500 pg/mL) because of recognition of another fragment with amino acids from 7 to 84 of the PTH molecule. The high intact PTH values in dialysis patients made the physician to take steps for suppression by either medical or surgical interventions, leading to low-turnover bone disease.

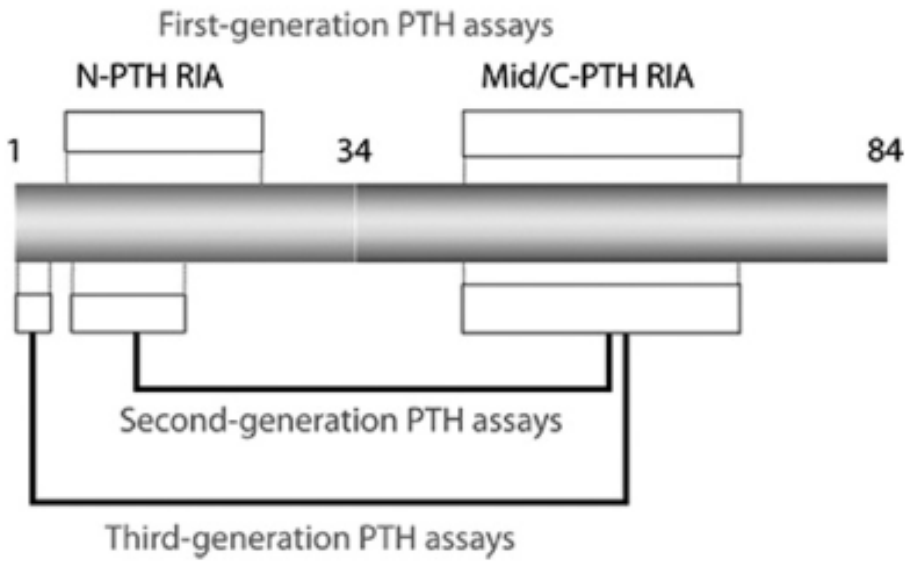
Further studies[25] have shown that the newly measured 7–84 fragment has effects that are opposite to the intact 1–84 PTH, such as a decrease in serum Ca^{2+} and urine PO_4

excretion and inhibition of bone resorption. These inhibitory effects of the 7–84 fragment seem to be mediated through a receptor called PTHR1, which is different from PTH/PTHrp receptor.

The third generation PTH assay was first developed in 1999. It also used two antibodies; one directed toward C-terminal amino acids, and the second toward the first amino acids (1–4). Thus, the third generation IRMA does not recognize the 7–84 fragment, and therefore, measures the bio-intact PTH (1–84).

The CHOICE study[26] showed that elevated levels of PTH as measured by the third generation assay were strongly associated with increased risk of mortality in incident HD patients than those values measured by the second generation assays.

Fig No.2. Assays for parathyroid hormone (PTH).



{The intact PTH molecule is composed of 84 amino acids; different regions of the protein are targeted by first- through third-generation assays. Abbreviations: Mid/C-PTH, mid/carboxyl terminus of PTH; N-PTH, amino terminus of PTH; RIA, radioimmunoassay}

In another study, Martin et al[27]. observed a decrease in both bio-intact and intact PTH levels in HD patients treated with cinacalcet by 38 %, but the ratio of bio-intact to intact PTH was not affected. Thus, the study shows that PTH as measured by both assays can be used in HD patients to follow the response to cinacalcet.

In the past, a desired PTH level between 150 and 300 pg/mL was suggested for dialysis patients. However, bone biopsy studies showed that a low bone turnover can be seen within this range and sometimes even with higher PTH levels. In a recent study, according to the K/DOQI PTH ranges, 15 patients out of 40 (40%) with iPTH > 300 pg/mL showed an histomorphometric pattern of low turnover[28-29].

Table No 2. Markers of Bone Turnover

1. bone formation marker

Specific bone alkaline.	Glycoprotein derives from osteoblasts. Good surrogate marker of phosphatase (b-ALP) bone formation
Osteocalcin (OC)	Originates from osteoblasts, odontoblasts and hypertrophic chondrocytes. Plays a vital role in osteoid mineralization.

C-terminal propeptide of type I procollagen (PICP)	Arises from proliferating osteoblasts and fibroblasts. Not recommended for routine use as a marker of mineral bone disorders.
N-terminal propeptide of type I procollagen (PINP)	Arises from proliferating osteoblast and fibroblasts.

2.

Bone Resorption Marker

Hydroxyproline	Present in both the newly synthesized and the mature collagen.
Hydroxylysine glycosides	Hydroxylysine in collagen is glycosylated to varying degrees,depending on tissue type
Carboxyterminal crosslinked telopeptide of type I collagen (CTX-I)	Collagen type I, predominantly from the bone. Isomerisation of aspartyl to s-aspartyl occurs with ageing of collagen molecule.

Aminoterminal crosslinked telopeptide of type I collagen (NTX-I)	Collagen type I, predominantly from the bone.
Bone Sialoprotein (BSP)	Synthesized by osteoblasts and osteoclastic cells.
Tartrate-resistant acid phosphatase (TRAP)	Six isoenzymes are present in the human tissues. Expressed by Osteoclasts, dendritic cells and macrophages. TRAP 5b is considered as a marker of bone resorption, while TRAP 5a is a known marker of inflammatory conditions.

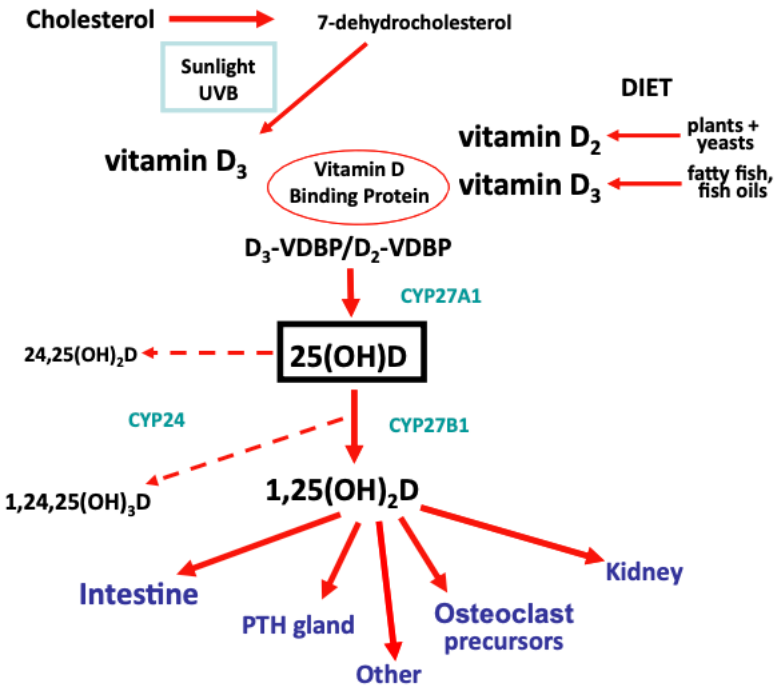
Vitamin D

Cholesterol is converted to 7-dehydrocholesterol, which in the presence of sunlight is converted to vitamin D_3 . Vitamin D_2 (ergocalciferol) is obtained from dietary sources. D_2 and D_3 are hydroxylated by CYP27A1 in the liver to 25(OH)D_2 (ercalcidiol) and 25(OH)D_3 (calcidiol), together termed 25(OH)D. Ercalcidiol and calcidiol have a half life of 3 weeks and are the best assessment of vitamin D intake from sun and food. 25(OH)D is converted by 1alfa-hydroxylase in the kidney to calcitriol

$1,25(OH)_2 D_3$ actions:

- Increase TRPV5 and TRPV6, the calcium adenosine triphosphatase and sodium/calcium transporters in the intestine and kidney
- This increases oral calcium absorption and calcium reabsorption in renal tubules
- Decreases PTH synthesis by binding to the vitamin D receptor in the parathyroid gland, inhibiting PTH gene expression, and decreasing PTH cell proliferation

Fig No. 3 Vitamin D Metabolism



CKD patients are characterized not only by a deficit in renal 1 alpha hydroxylase, which causes low levels of 1,25 dihydroxyvitamin D (1.25VD), but also by a high-frequency deficit in circulating 25-hydroxyvitamin D[30-32].

However, it has also been emphasized that testing methods for 25 VD are not yet standardized, and definitions of “insufficiency” and “deficiency” need to be further validated in CKD patients.

Alkaline Phosphatases

ALP provide additional information on bone turnover. some comparative histological studies have demonstrated, PTH alone is not a good predictor of bone mineral disease with high and low turnover, except for respectively very high or very low values[33].

Total alkaline phosphatases can contribute to correctly evaluating the status of skeletal metabolism, even in the presence of treatments interfering with bone turnover. In case of Presence of high levels of total ALP it should be looked for conditions such as hepatopathy and cholestasis. Bone alkaline phosphatases can help to resolve interpretative doubts about the extra bone origin of alkaline phosphatases.

FGF23

Belongs to a group of molecules called phosphatonins . Phosphatonins are hormones that regulate phosphorus excretion. Three phosphatonins have been identified: sFRP-4, MEPE, and FGF-23. Produced almost exclusively in osteocytes and bone-lining cells, but also found in heart, liver, thyroid/parathyroid, intestine, and skeletal muscle. FGF-23 receptor on the proximal tubule requires a coreceptor (klotho) for signal transduction. Klotho is found in the distal renal tubule and parathyroid gland Klotho is downregulated in aging and CKD. FGF-23 has the following actions

- ➢ Downregulates luminal sodium/phosphate cotransporters in the proximal tubule, decreasing phosphorus reabsorption and therefore increasing its excretion
- ➢ Inhibits 1alfa-hydroxylase (*CYP27B1*), decreasing the conversion of 25-hydroxyvitamin D (25[OH]D) to 1,25-dihydoxyvitamin D ($1,25[OH]_2D_3$; calcitriol)
- ➢ Stimulates 24-hydroxylase (*CYP24*), leading to vitamin D degradation
- ➢ Inhibits PTH secretion

There is a strong correlation between serum FGF23 levels and declining eGFR. As renal function declines FGF23 levels increase. In severe renal failure, FGF23 levels can be

raised up to 1000-fold above the normal range, likely due to retained phosphate or decreased renal clearance.[34]

Non Invasive Diagnostic Approach of MBD

Dual X-rays absorptiometry (DXA) is considered the gold standard for the diagnosis of osteoporosis in general population .it is one of the main predictors of fragility fractures[35].

The Word Health Organization Osteoporosis defines osteoporosis as a reduction in bone density expressed as a T-score lower than −2.5 Standard Deviation (SD) compared to normal population. bone strength is the result of a combination of bone density and bone quality, consisting of bone microarchitecture, turnover and mineralization. The disorders in bone quality can explain the finding that about half of all osteoporotic fractures occur in patients with T scores > −2.5 SD.

It is well established that patients with CKD G3a–G5D have increased fracture rates compared with the general population[36-38]

There are lack of evidence that DXA BMD predicted fractures in CKD patients as it does in the general population, and the inability of DXA to indicate the histological type of bone disease, the 2009 Guideline recommended that BMD testing not be performed routinely in patients with CKD G3a to G5D with CKD-MBD.

Bone Histomorphometry in Renal Osteodystrophy

Healthy bone is a dynamic tissue, continually resorbing bone and replacing it with new bone in discrete areas know as basic multicellular units, also called bone metabolic units (BMUs).[39]

A BMU is a temporary structure. It forms in response to signal or stimulus, performs its function, and disbands, leaving a few residual lining cells and osteocytes. Every BMU

function in a sequential manner: activation of osteoclasts, resorption of old bone, recruitment of osteoblasts, formation of new bone matrix, and mineralization. lifespan of a BMU is not well defined. Cortical BMUs can wander for months. Parfitt[40] estimates the duration is 2 to 8 months. This is harder to measure in cancellous bone because the 2-dimensional sections do not capture the entire serpentine course of the BMU.

Thus, the turn- over of bone is different from the turnover on the skin, where the entire surface continuously is forming skin and shedding it. BMU origination have two type of stimulus, one when the bone has been damaged; others may originate at random surfaces on the bone, under the influence of local or systemic hormones. Marrow stromal cells near the bone are under tonic inhibition by sclerostin from the interior osteocytes, which detect the need for new bone formation.[41]

The osteocytes then stop secreting the sclerostin and start secreting other factors such as prostaglandins, nitric oxide, and growth factors. The stromal cells respond by secreting macrophage colony- stimulating factor, which helps promote pre- osteoclasts. The pre-osteoclasts have RANK receptors, and when these are activated they fuse and form mature osteoclasts that will resorb bone. These eroded surfaces are the first events detected by light microscopy.

Under the appropriate conditions, the stromal cells generate pre-osteoblasts, which express receptor activator of nuclear factor (NF)- kappaB (RANK) ligand on the cell surface. At a given spot on the bone surface, resorption is rapid for the first 10 days, and continues for about a month. The osteoclasts undergo apoptosis; the lifespan and activity of the osteoclasts determine the depth of the resorption cavity.

the pre-osteoblasts that were generated by the marrow stem cells have proliferated, and when the osteoclasts retire the osteoblasts line the cavity. The team of osteoblasts forms a

matrix, and after about 14 days directs mineralization of the matrix. The time to fill in the cavity at any given place on the surface is 124 to 168 days in normal individuals.[42]

After formation is completed, that place on the surface is quiescent. The duration of time for the cycle on one point of the surface is called the total period (resorbing plus forming plus quiescent periods), which varies from 2 to 5 years. The inverse of the total period is the activation frequency.

After restoration of the bone volume, the newly formed bone undergoes further mineralization for about 3 years. Older bone has more densely packed crystals, and micro radiographic studies have shown that newly formed bone may be 25% less dense than older bone.[43]

Definition of Turnover

If the bone resorption rate and bone formation rate were equal, then the turnover could be defined as either rate. The bone volume would not be changing, and the bone balance would therefore be zero. This situation is generally seen in healthy young adults.

If the bone resorption rate is greater than the formation rate, because the resorbed cavities are not completely filled with new bone, then the turnover would be reflected by the bone formation rate, and the bone balance would be negative. This is seen frequently with aging and postmenopausal osteoporosis.

On the other hand, if the bone formation rate is greater than the bone resorption rate in an adult skeleton, the turnover would be determined by the bone resorption rate, and the bone balance would be positive. This un- usual condition could be seen, for example, during recovery from lactation or treatment with an anabolic medication.

During growth, the bone is modelling as well as remodelling, and the concept of turnover would apply only to that bone that is remodelling. Therefore, bone formation rate (BFR) and bone turnover are not the same, and either low turnover or high turnover can be associated with loss of bone volume and reduction in bone strength.

In renal osteodystrophy, those with high bone formation rates also have high resorption rates, and those with adynamic bone disease have low rates of both formation and resorption. Thus, the bone formation indices are used to describe turnover because they can be measured with much more accuracy than the bone resorption rate.

Measurement of Bone Formation Rate

1.Tetracycline label(Dynamic)

Bone areas where new bone is formed calcium will deposit in that area. tetracycline will deposit in that bone area where new calcium deposited and can be seen under a fluorescent microscope. The length of the tetracycline labels multiplied by the distance between the labels is the area of new bone formed during the label interval. The BFR can be expressed in reference to the bone surface, bone volume, or tissue volume. The most easily understood concept of turnover is the BFR as a percentage per year of the bone volume.

Tetracycline labels has some limitation in measurement of bone formation rate. patients have poor compliant due to complicated dose schedule, gastrointestinal side effect. when there is very rapid bone formation, the labels become blurry and diffuse, making them difficult to measure. Conversely, when the bone is forming very slowly the labels do not show separation and it is difficult to tell if a label is a double label or a single label.

Fig No. 4 Tetracycline double labeling

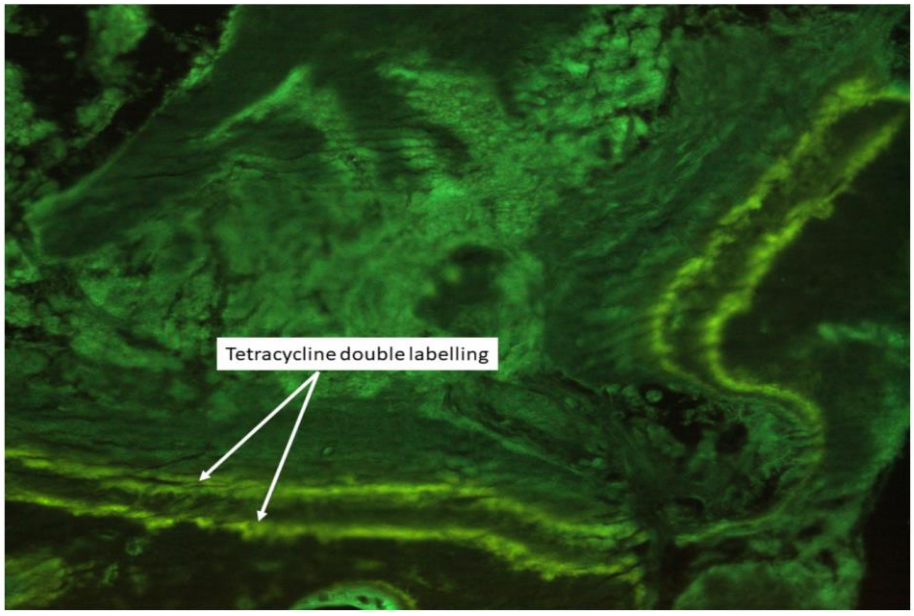
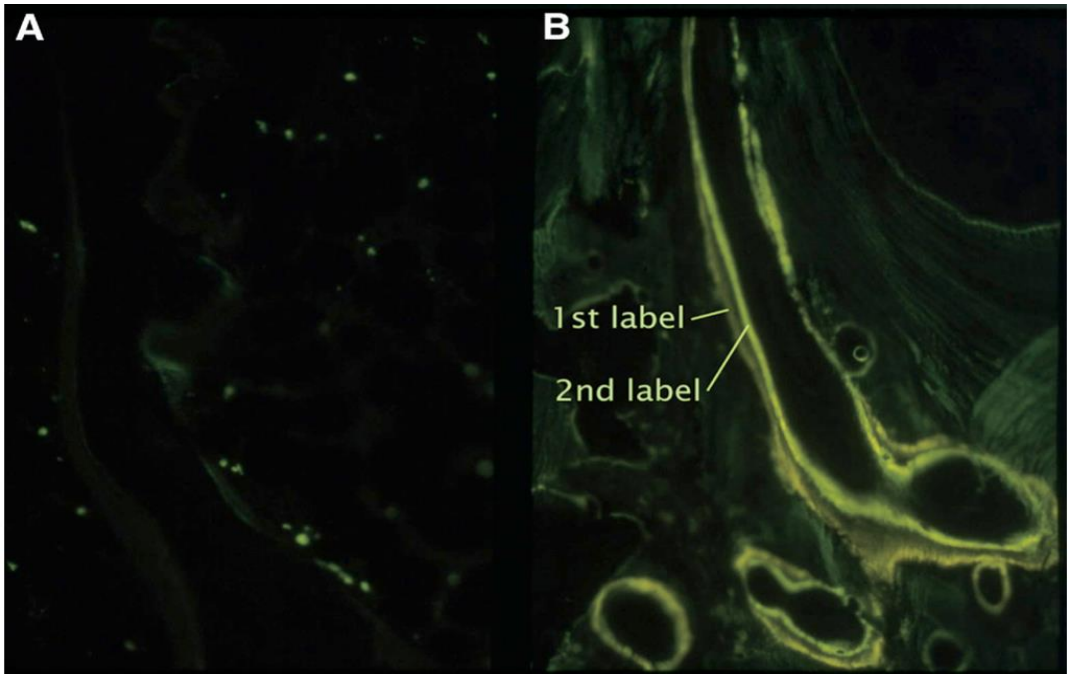


Fig No. 5: Tetracycline labeling.

(A) Adynamic bone without label, (B) normal labels}



Other Measurements Related to Bone Turnover (Static)

Other measurements that relate to the formation aspects of bone turnover include the osteoblastic surface, the number of active osteoblasts, and the osteoid surface. Bone resorption is related to the number of osteoclasts and the depth of resorption cavities.

MINERALIZATION IN RENAL OSTEODYSTROPHY

The second axis of TMV classification is mineralization.it reflects the amount of unmineralized osteoid. The classic disease with an abnormality of mineralization is osteomalacia, in which the BFR is low and osteoid volume is high. Some patients who have high bone formation rate also have high osteoid volume but do not actually have a problem with the mineralization process. opposite to this some patient who have low bone formation rate and normal osteoid have adynamic disease. they do not manifest a problem with mineralization because they do not even form the osteoid matrix.

Mineralization is measured by the osteoid maturation time or the mineralization lag time, both of which depend heavily on the osteoid width as well as the distance between tetracycline labels. The osteoid maturation time is the osteoid width divided by the distance between labels per day. The mineralization lag time is the osteoid maturation time adjusted for the percentage of osteoid surface that has a tetracycline label.

Osteomalacia has been defined by different investigators as increased osteoid volume, increased osteoid maturation time, or increased mineralization lag time.

Parfitt[44] defined osteomalacia in patients with malabsorption if they had a combination of osteoid volume/bone volume greater than 10%, osteoid thickness greater than 12.5 micro m, and mineralization lag time greater than 100 days.

BONE VOLUME IN RENAL OSTEODYSTROPHY

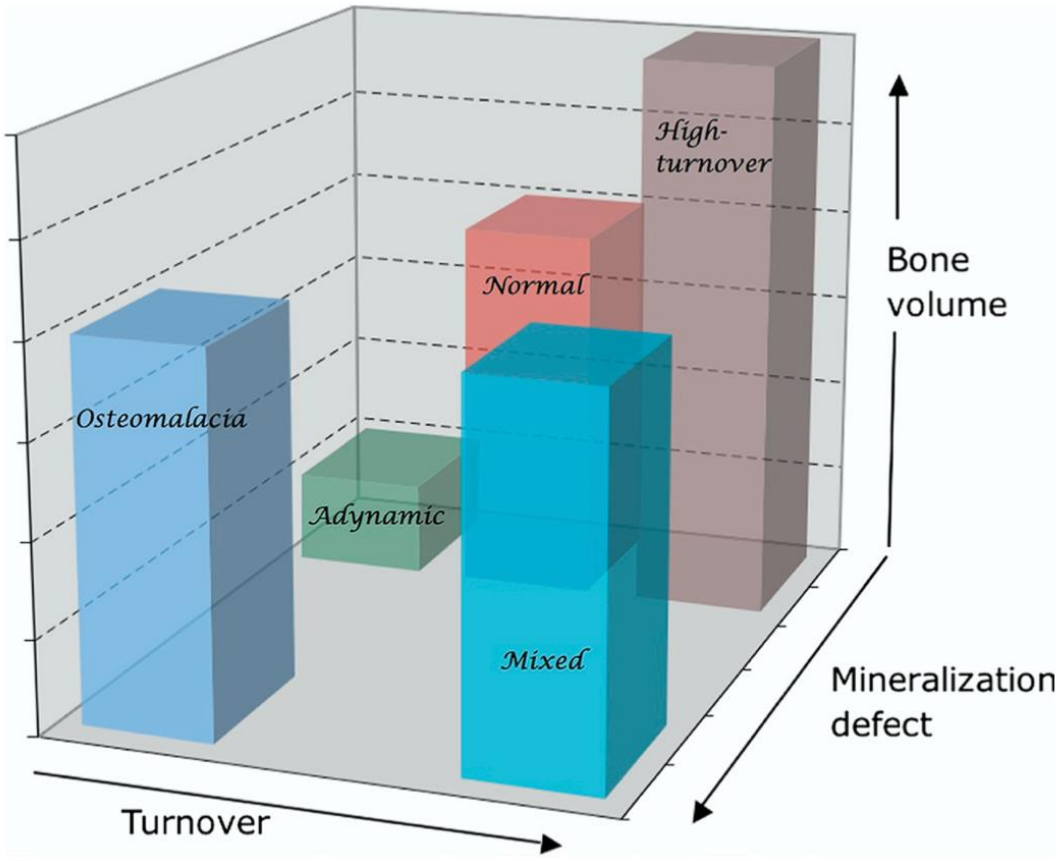
The final axis is bone volume. The bone volume is the end result of changes in bone formation and resorption rates. If overall BFR is greater than overall bone resorption rate, the bone is in positive balance and the bone volume will increase. If mineralization remains constant, an increase in bone volume also would result in an increase in bone mineral density and should be detectable by radiographic densitometry.

bone volume also would result in an increase in bone mineral density and should be detectable by radiographic densitometry. both cortical and cancellous bone volume decrease in typical idiopathic osteoporosis, these compartments frequently are different in patients with CKD. For example, with high PTH levels, cortical bone volume is decreased but the cancellous volume is increased.[45]

There is a large error, however, from one biopsy to the next in the same person. From contiguous iliac crest biopsies there is an average of 29% difference.[46]

Bone volume is related to bone strength, but the same volume can have different microarchitecture. The trabecular thickness can be calculated from measurements of the bone surface per volume relationship, and this is an index related to bone strength. There is consensus that bone volume is expressed as bone volume per tissue volume, which is the same as bone area per tissue area when expressed in 2 dimensions. This can be measured in cortical or in cancellous bone.

Fig No:6. Relationship between TMV and classic types hypothetical; actual studies show variability in the bone of renal osteodystrophy. The bone volume in this diagram is volume for each type of disease.



Correlations between Fractures and Bone Histomorphometry

One study of 31 dialysis patients found that those with low turnover osteodystrophy had a fracture rate of 0.2 per year compared with 0.1 per year in those with osteitis fibrosis; this was owing to a high number of rib fractures in the low turnover patients.[47]

A review of 2,340 biopsies performed in Brazilian patients for clinical indications found that the frequency of fractures was significantly higher in those with osteomalacia compared with other forms. There were no differences in fracture history between those

with adynamic bone disease, high bone turn- over, or mixed bone disease.[48] A study that followed up 62 patients for 5 years after bone biopsy found a higher rate of fractures in those with adynamic bone disease.[49]

Correlations between Bone density and Bone Histomorphometry

In patients with CKD, Lindergard[50] measured bone volume/tis- sue volume on 71 biopsies from dialysis patients, and did not see a correlation with bone mineral density (BMD) at the radius.

The relationship between BMD and type of renal osteodystrophy varies among reports. Four studies found similar BMD in all types.[51-54] Lower BMD has been reported in both high and low bone turnover, with wide ranges.[55,56]

The main literature on bone biopsy

Previous studies showed a relatively good correlation between intact PTH level and bone histomorphometric parameters[57].therefore, the 2003 national kidney foundation kidney disease outcomes quality initiative (NKF/KDOQI) guidelines[58], recommended the target iPTH levels of 150-300 pg/ml in dialysis patient which was correlated with normal bone turnover. however certain patients who had PTH within the target range still have abnormal bone turnover. after the guidelines was published ,the prevalence of low bone turnover was markedly elevated while the prevalence of high bone turnover was decreased.

Suthanit laowalert et.al[59] studied on 22 chronic HD patient. bone turnover marker was measured. double tetracycline labelled iliac crest bone specimen were evaluated. bone

histomorphometry revealed osteitis fibrosa -50%,ABD -50%,mixed uremic osteodystrophy -5% were found. Serum i PTH level predict high bone turnover with area under the ROC of 0.833(p=0.008).

Barbara M. Misof et al[60] done a retrospective study and measured bone mineralization density distribution. bone biopsy was done from 58 patients with CKD-MBD. Outcomes were studied in relation to serum parathyroid hormone (PTH), alkaline phosphates (APH), histomorphometric bone turnover and treatment with clinacalet or phosphate binders. Results showed, lower calcium concentrations in bone from high turnover versus low turnover patients were observed. OLS(osteocyte lacunae sectioned)-characteristics were distinctly different in patients with highest compared to those with lowest turnover. Patients with cinacalcet had different OLS-characteristics compared to those without cinacalcet. Furthermore, patients with phosphate binders had difference in BMDD(bone mineral density distribution) and OLS-characteristics compared to patients without phosphate binders.

Lobão R .et al[61] studied Pre-dialysis CKD patients (n = 103, 46 females/57 males), median creatinine clearance of 29 (10 - 78) ml/min/ 1.73 m2, were evaluated using biochemical analysis and DEXA. Bone biopsies were obtained from those with low BMD.

Fifty (48.5%) out of the 103 patients had low BMD (LBD group) and 53 (51.5%) had normal BMD (NBD group). The risk for low BMD was increased in those patients with alkaline phosphatase levels above 190 U/l and intact-PTH (iPTH) below 70 pg/ml ($p < 0.05$). Demographic and biochemical parameters from both groups were comparable,

except for lower body mass index (BMI) in LBD subjects (p = 0.04). Women who had been post-menopausal for at least 1 year comprised 65% (13/20) and 50% (13/26) of the LBD and NBD groups, respectively (p = NS). In 40 LBD patients, bone histomorphometry revealed adynamic bone disease (ABD, 52.5%), osteomalacia (OM, 42.5%) and mixed bone disease (MBD, 5%). Trabecular bone volume (BV/TV) was lower in ABD and OM patients. A nearly significant association was found between ABD and iPTH < or = 150 pg/ml (p = 0.056), whereas higher values of iPTH were associated with OM. Total alkaline phosphatase < or = 190 U/l was significantly associated with ABD, whereas higher values were associated with OM. No correlation was observed between BV/TV and BMD.

Ruth G. G. Hiller et al[62] studied In Germany ; Bone biopsies were taken from 12 embalmed body donors at the iliac crest, the proximal tibia, and the lumbar vertebral body, respectively. Masson-Goldner stained sections of methyl methacrylate embedded biopsies were used for trabecular bone volume calculation.

Median values of trabecular bone volume were comparable between all body donors with median 18.3% (10.9–22.9%) at the iliac crest, 21.5% (9.5–40.1%) at the proximal tibia, and 16.3% (11.4–25.0%) at the lumbar spine.

Khuraijam Bembem et al[63] studied 32 cases of diagnosed CKD-MBD formed the study group. Detailed clinical history and biochemical analysis was done for them. Bone marrow trephine biopsies were conducted and the histology was studied. The clinicobiochemical and the histomorphological findings were correlated. Based on the bone biopsy findings, Hyperparathyroid bone disease consisted of-14 cases (44%), Mixed uremic osteodystrophy of-16 cases (50%) and one case (3%) each of Low turnover disease (Adynamic bone disease) and Normal histology.

The mean blood urea, S. Creatinine, S Phosphate and the S. Vit D3 were found to be statistically significant between the two major subgroups. The area of the bone trabeculae and the osteoid percentage was found to be more in the MUO group and was found to be statistically significant.

According an Indian study Vikrant S et al[64], they studies on 462 patients of ckd stage 3-5D. and found the frequency of various biochemical abnormalities. These was hypocalcemia-23.8%,hypercalcemia-5.4%,hypophosphatemia-2.8%,hyperphosphatemia 55.4%,secondary hyperparathyroidism-82.7%,raised ALP -56.9%.

Table No:3. Different studies for Histomorphometry

Suthanit laowalert et.al	Observational	22 chronic HD patient	osteitis fibrosa -50%,ABD -50%,mixed uremic osteodystrophy -5%	APSN 2019
M. . Barbara Misof et al	Observational	58 patients with CKD-MBD		JMNI 2019
Lobão R et al.	Observational	n = 103, 46 females/57 males	ABD 52.5%,OSTEOMALACIA 42.5%,MIXED 5%	Clin Nephrol. **2004**
Ruth G. G. Hiller et al.	Observational		ABD 40%,MUO 30%,OSTEOMALACEA 30%	BMC Nephrology 2017

. Khuraijam Bembem et at.			HIGH TURN OVER 44% MIXED 50% ABD 3% NORMAL 3%	Indian J Hematol Blood Transfus. 2017:
Coen et al[65].	Observational	79, CKD 107, on hemodialysIS	69% mixed osteodystrophy 11% adynamic bone disease 2.5% hyperparathyroidism 1% osteomalacia 57% hyperparathyroidism 28% mixed osteodystrophy 11% adynamic bone disease 2.8% osteomalacia	Nephron, 2002
Miller et al[66].	Observational	6, CKD stages IV-V	33% low, 33% high bone turnover, 33% osteomalacia	CJASN 2008
Lehmann et al[67].	Observational	36, CKD stages III-IV 92, CKD stage V	47.2% Osteitis fibrosa 61.4% Osteitis fibrosa	Clin Nephrol, 2008
Sprague et al[68].	Cross sectional	492, on hemodialysis	59% low, 24% normal, 17% high bone turnover	Am J Kidney Dis., 2016
Evenepoel et al[69].	Observational	36, kidney transplant	44.4% low, 52.8% normal, 2.8% high bone turnover	Kidney Int., 2017
Sharma et al[70].	Observational	14,CKD stage 5	50% high,29% normal,21% low bone turnover	Am J Nephrol, 2018

Liangos et al[71].	Observational	33,CKD stage 3-5 53, on hemodialysis	High-turnover mixed uremic osteodystrophy	Kidney blood Press Res. 2018
Novel-Catin et al[72].	Observational	*n* = 68, ESRD	45% Osteitis fibrosa ,21%MUO,12%ABD,10 % OM	Bone 2020
Jørgensen et al[73].	Observational	205,kidney transplant	16% high 60% normal 24% low turnover	Bone ,2021
Salam et al[74].	Cross-sectional	N 43,CKD stage 4-5	40% high 34% normal 26% low bone turnover	Bone ,2021
Aaltonen et al[75].	Observational	N 26,ESRD	12% High 27% normal 61% Low bone turnover	Calcific Tissue Int. 2021
Gerakis et al[76].	Observational	62, on hemodialysis	64.5% hyperparathyroidism 22.6% adynamic bone disease 9.7% mixed bone disease 3.2% osteomalacia	J Nephrol., 2000
Lavigne et al[77].	Observational	N 11,CKD	18.1 High 18.1 MUO 45.4% ABD 9.1% Osteomalacia 9.1% Not defined	J Nephrol 2021

AIMS AND OBJECTIVES

AIMS

- By bone biopsy histopathology we identify type of turn over disorder like high, low or mixed type.

Primary objective

- To identify correlation between biochemical parameter of mineral bone disease and bone histomorphometry.

Secondary objective

- To identify the prevalence of type of mineral bone disorder among CKD patient stage 3-5 including on maintenance hemodialysis.

MATERIALS AND METHODS

PLACE OF STUDY : The study will be conducted from January 2020 to October 2021 at Dept. of Nephrology AIIMS, Jodhpur.

STUDY DESIGN: Single centre cross sectional study.

PATIENT SELECTION:

Inclusion Criteria:

-newly diagnosed CKD stage 3 to stage 5ND and 5D

- age >18 years and less than 60 years

Exclusion

1. CKD Stage 3-5 patients on calcium supplements, calcium containing phosphate binders, vitamin D or its active metabolites and analogues and calcimimetics.

2. patients on medications which affect bone mineral metabolism; HRT, corticosteroid, cinacalcet, warfarin, phenytoin and bisphosphonate within one year before enrolment.

3. Age < 18 years and > 60 years.

4. patient having bone disease/history of fractures in preceding 6 months.

5. Patient having skin infection at biopsy site, deranged bleeding and cogulation profile.

6. Patient not giving consent.

SAMPLE SIZE:28

ESTIMATED EXPENDITURE: Rs. 40000/.

METHODS

The study was conducted after getting clearance from the institute ethics committee. Each case was assessed with relevant clinical history including bone pains and fractures. The biochemical profile included blood urea, serum creatinine, uric acid, calcium, phosphorous, alkaline phosphatase (ALP), PTH and 25-hydroxycholecalciferol.

Cohort

All bone biopsies were performed at AIIMS Jodhpur between Feb 2020 and Oct 2021. Biopsy was done in admitted patients only. After taking consent biopsy was done under local anaesthesia (2% lignocaine) at biopsy. Total 28 biopsies were done through iliac crest 2 cm posterior and inferior to anterior superior iliac Spine using an 8G trephine with an external/internal diameter of 4.50/3.55 mm. Bone histomorphometric analyses were performed at the Laboratory of Pathology AIIMS Jodhpur.. Tetracycline Labelling (Dynamic study) was not done in this study. BM trephine biopsy was carried out in all cases. All biopsy samples was taken and fixed in 100 percent ethanol and submitted for pathology assessment.

Bone histomorphometry

Un-decalcified 5- μm thick sections were stained by the Goldner trichrome method to determine static bone parameters. Image J and Adobe Photoshop® were used to measure static and bone histomorphometric parameters.

Before evaluation of bone sections in Image J, black and white image masks were created using Adobe Photoshop® Version CS 3. Images were visualized under light microscopy and regions of interest were captured for analysis. Adobe Photoshop® was used to prepare the images for analysis. Color images were converted to black and white masks using the wand tool in Adobe Photoshop® to highlight areas of bone. All bone tissues

were designated in black color. The remaining tissue was designated in white color, creating a black and white mask for analysis by Image J software (Figure 1). Similarly, a mask was created for analysis of osteoid, with osteoid selected using the wand tool and colored black; the remaining tissue being colored white.

The tissue volume, bone volume, and osteoid volume were quantified using freely-available Image J software which is a NIH validated tool for assessment of bone histomorphometry[98].

Histomorphometric analyses are based on the identification of cells and extracellular matrix by chemical staining within a region of interest and defined by primary parameters of areas they occupy, their boundary perimeters, or their distances from other points of reference.

Image J is available as a no-cost download from NIH website and is frequently updated and validated. It allows freedom to work on any computer, adding flexibility to when and where the analysis of sections is performed. All measurements in Image J are made on archived images and so determination of histomorphometric parameters are semi-automated rather than live (i.e., in real time from tissue sections).

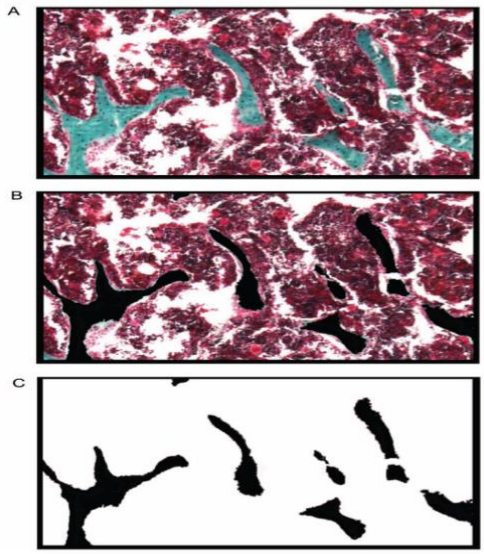
All bone histomorphometric parameters are reported in two dimensions, using standardized nomenclature[78].

Parameters assessed included active osteoblasts per bone and osteoid perimeters (ObPm/BPm, ObPm/OPm, %), active osteoclasts per bone and eroded perimeters (OcPm/BPm, OcPm/EPm, %), eroded per bone perimeter (EPm/BPm), osteoid per bone area (OAr/BAr) and the presence or absence of fibrosis. Patients were diagnosed as having low, normal, or high bone turnover in a semi-quantitative assessment by an experienced bone pathologist, based primarily on the key dynamic parameter BFR on

total tissue area, using a previously published normative reference range of 97–613 $\mu m^2/mm^2/day$[79].

Patients were categorized as high turn over if BFR > 613, or signs of excessive bone resorption (EPm/BPm above normal range of 0.5–3.4%), or evidence of disordered bone formation (marrow fibrosis > 5%). Patients were categorized as low turnover if BFR < 97, and with limited amounts of osteoid (OAr/BAr, normal range 0.23–5.83%) and without the presence of fibrosis.

Fig No. :7. Construction of the black and white image mask.



(A) A region of interest was selected at 40X magnification from a Goldner's Trichrome stained section. This captured image was then opened in Adobe Photoshop® to prepare the black and white mask. (B) Within the selected region of interest, bone was identified and represented in black. (C) The remaining, non-bone tissue, was selected to be white.

Assays for parathyroid hormone (PTH): The intact PTH molecule is composed of 84 amino acids; different regions of the protein are targeted by first- through third-generation assays. The LIAISON® N-TACT® PTH Gen II were used. is an *in vitro* chemiluminescent immunoassay (CLIA) intended for the quantitative determination of intact human parathyroid hormone in serum. Sample was sent in EDTA Vial. The test was performed on the LIAISON® Analyzer family.

VITAMIN -D: The ADVIA Centaur Vitamin D assay is an 18-minute antibody competitive immunoassay that uses an anti-fluorescein monoclonal mouse antibody covalently bound to paramagnetic particles (PMP), an anti-25(OH)vitamin D monoclonal mouse antibody labeled with acridinium ester (AE), and a vitamin D analog labeled with fluorescein.

An inverse relationship exists between the amount of vitamin D present in the patient sample and the amount of relative light units (RLU) detected by the system.

CREATININE: Enzymatic assay for the quantitative determination of creatinine in human serum, plasma and urine on Beckman Coulter AU analysers. For *in vitro* diagnostic use only. Creatinine is hydrolysed by creatininase to creatine. The creatine formed is hydrolysed by creatinase to sarcosine and urea. Sarcosine oxidase catalyzes the oxidative demethylation of the sarcosine to yield glycine, formaldehyde and hydrogen peroxide. In the presence of peroxidase (POD), the hydrogen peroxide formed reacts by quantitative oxidation condensation with N-(3-sulfopropyl)-3-methoxy-5-methylaniline (HMMPS) and 4-aminoantipyrine to yield a blue pigment. The creatinine concentration is proportional to the change in absorbance at 600/700 nm.

UREA: Kinetic UV test for the quantitative determination of urea in human serum, plasma and urine on Beckman Coulter analysers. For *in vitro* diagnostic use only. Urea is hydrolysed in the presence of water and urease to produce ammonia and carbon dioxide. The ammonia produced in the first reaction combines with 2-oxoglutarate and NADH in the presence of glutamate-dehydrogenase (GLDH) to yield glutamate and NAD+. The decrease in NADH absorbance per unit time is proportional to the urea concentration.

CALCIUM: Calcium ions react with o-Cresolphthalein-complexone in an alkaline medium to form a purple coloured complex. In this method the absorbance of the Ca-

oCPC complex is measured bichromatically at 570/660 nm. The resulting increase in absorbance of the reaction mixture is directly proportional to the calcium concentration in the sample.

PHOSPHOROUS: Inorganic phosphorous reacts with molybdate to form a heteropolyacid complex. The use of a surfactant eliminates the need to prepare a protein free filtrate. The absorbance at 340/380 nm is directly proportional to the inorganic phosphorous concentration in the sample.

STATISTICAL ANALYSIS: Quantitative data was expressed as mean and Standard deviation. Statistical analysis were done using Chi square to correlate the bone histology with the clinical biochemical parameters.

RESULTS

Patient characteristics:

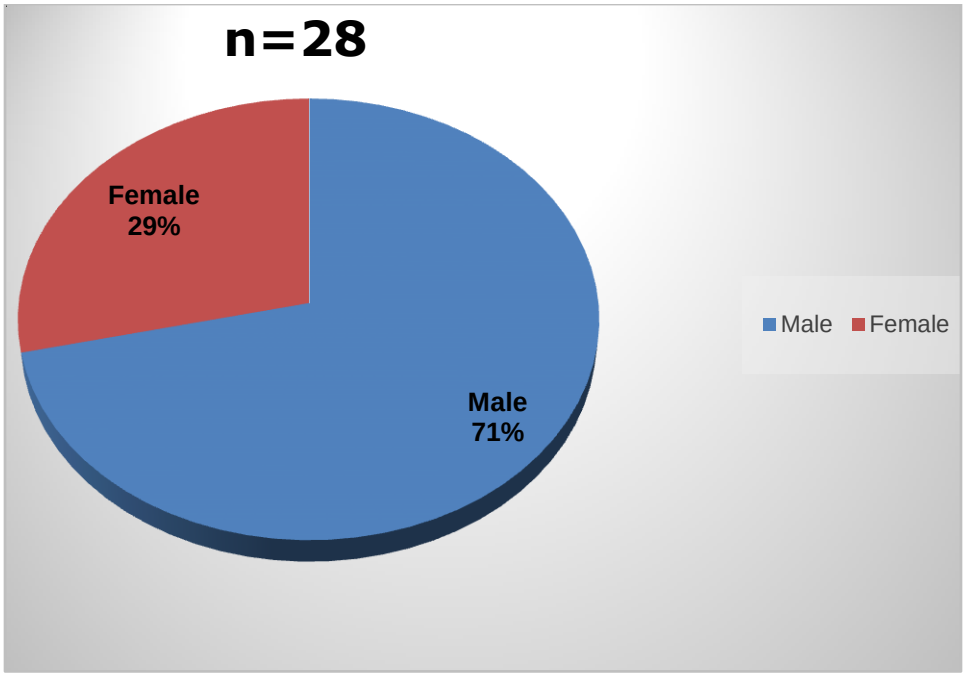
Table No. 4:Age and gender characteristics of the group

Sex(n)	Median	Mean	SD	Percentage	IQR
Male(20)	36	35.10	10.12	71	13
Female(8)	29	29.3	9.41	29	16
Total(28)	33	33.07	10.42	100	16.5

Total of twenty-eight patients with CKD were enrolled in the age group of 18- 55 years.

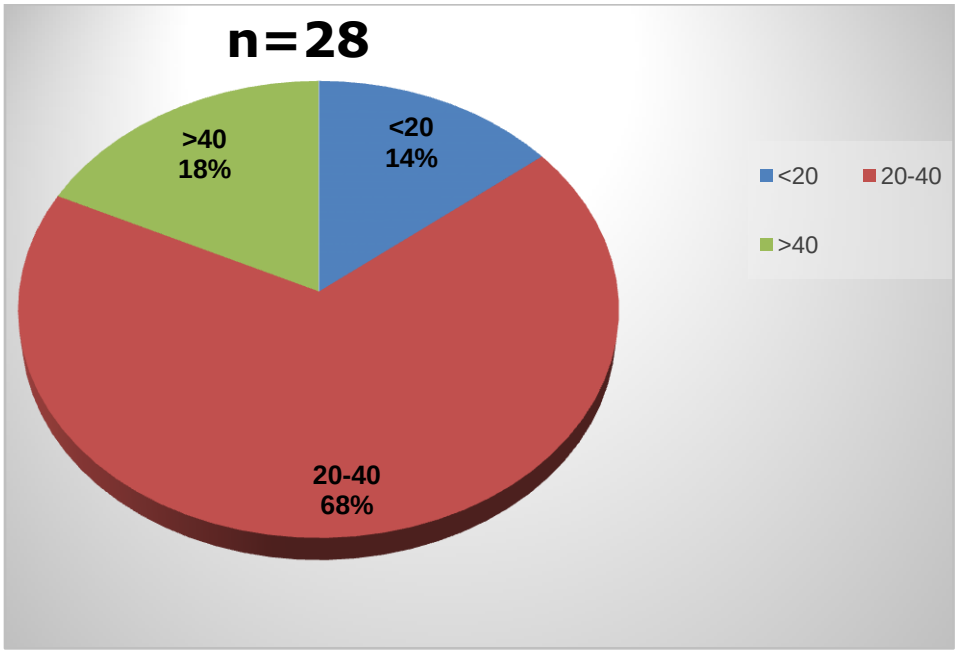
The test of normality was applied on the values of age and found to be nonparametric. The median age was 33 years and interquartile range (IQR) of 16.5. The mean age was 33.07 ± 10.42 years. Out of the total twenty-eight patients, 20 (71%) were males and 8 (29%) were females. Median age for male patients was 36 years with IQR 16 years and mean age was 35.10 ± 10.12 years. While the median age for female patients was 29 years with IQR 16 years and mean 26.57 ± 6.35 years.

Fig No. 8: Distibution of patients according to sex



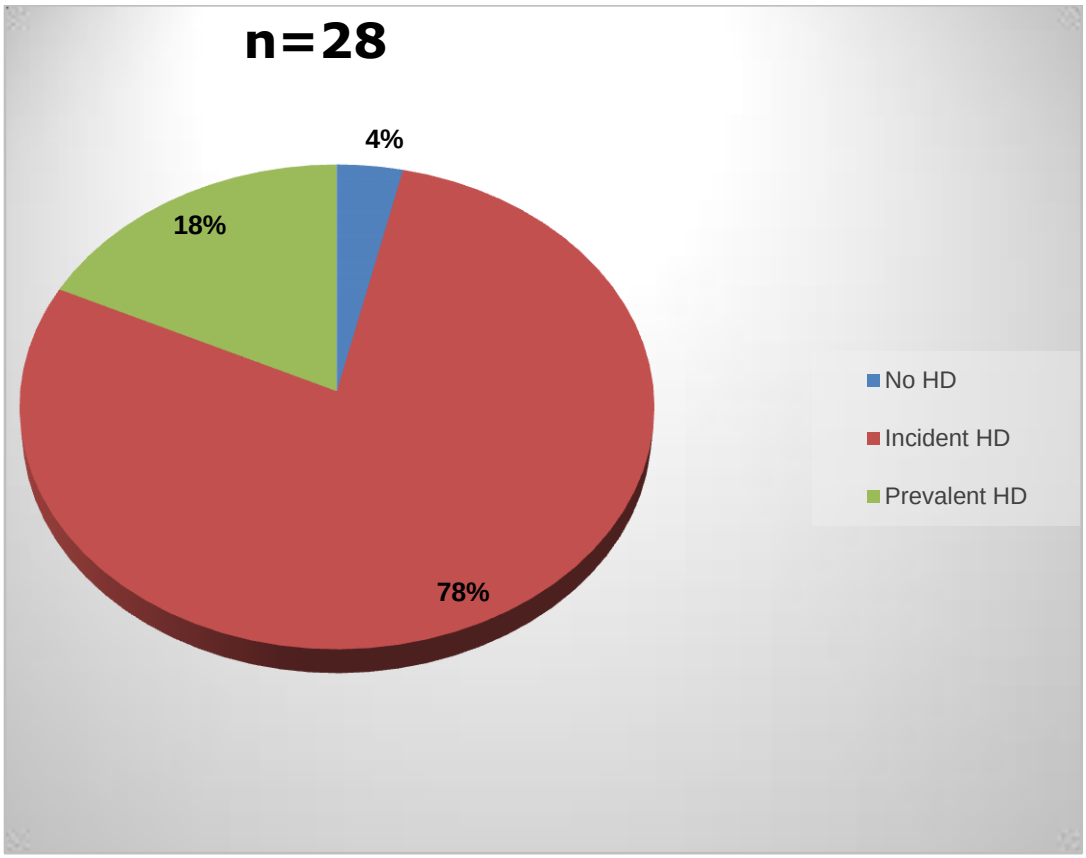
Total 28 patients taken in the study in which 8 patients were female(29%).

Fig No.9.Age distribution of patients



Age of patients taken in this study were between 18 years to 55 years. most of the patients is between 20-40 years of age(68%).14% patients were < 20 years of age and 18% were >40 years of age.

Fig No.10:RRT (HD) prevalence of patients



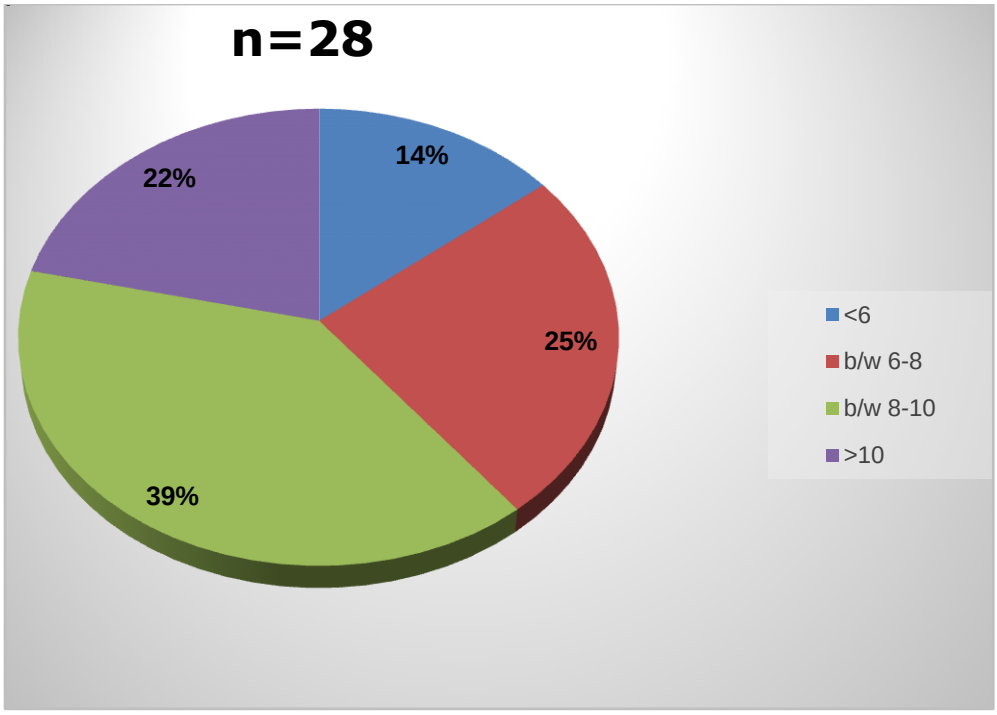
All 28 patients were taken who were admitted in AIIMS for initial management of disease. All patients were in stage 5 CKD. Most of the patients were in uremic symptoms with diagnosis as CKD(78%).18 % patients were on MHD and 1 patient(4%) was not on dialysis.

Table No:5. Descriptive table of lab variables

	N	Minimum	Maximum	Mean	Standard deviation
Hb	28	3.3	15	8.05	2.30
Blood urea	28	87	454	197.96	85.3
Uric acid	28	1.7	13.5	8.45	2.60
Calcium	28	3.02	9.54	7.89	1.32
Phosphorus	28	2.52	12.61	6.86	2.71
Vitamin D	28	5.19	40.70	18.18	9.56
I PTH	28	62.3	1900	650.7	466.0
ALP	28	52	1379	219.1	311.3

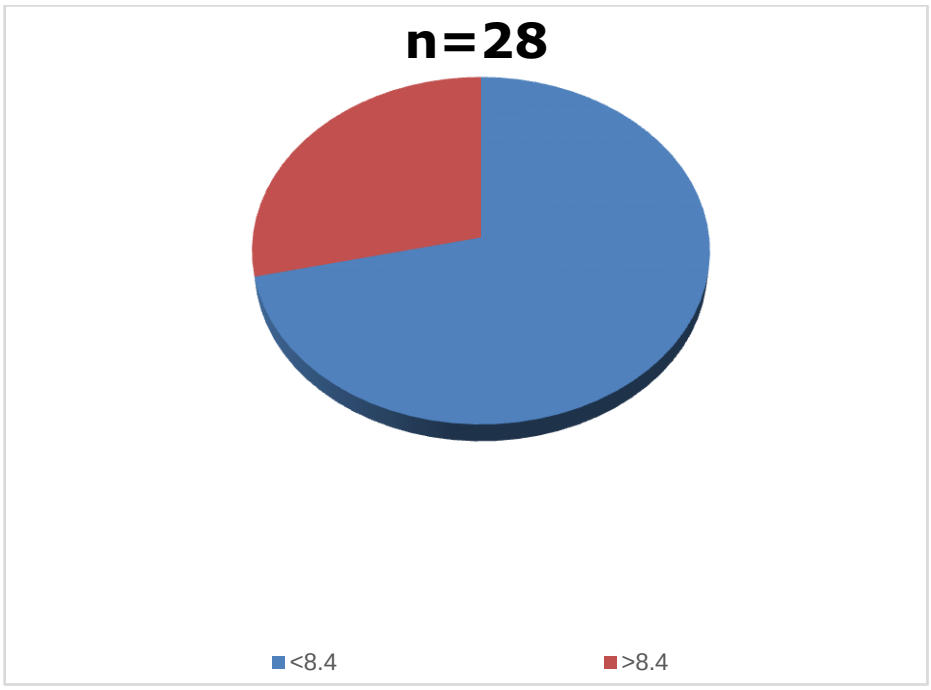
Of the 28 patients most patients have anemia. mean Hb was 8.05 with standard deviation of 2.3.the Mean and Standard deviation of Blood urea, Uric acid, Calcium, Phosphorus, Vitamin D was 197.96±85.3;8.45 ± 2,60;7.89±1.32;6.89±2.71;18.18±9.56 respectively. mean intact PTH was 650.7 and standard deviation was 466.Standard deviation is very high due to two patients having very high value of intact PTH. Mean of ALP was 219.1 and standard deviation was greater than mean-311.1.It is because two patients had very high value of ALP.

Fig No.11.Serum uric acid distribution



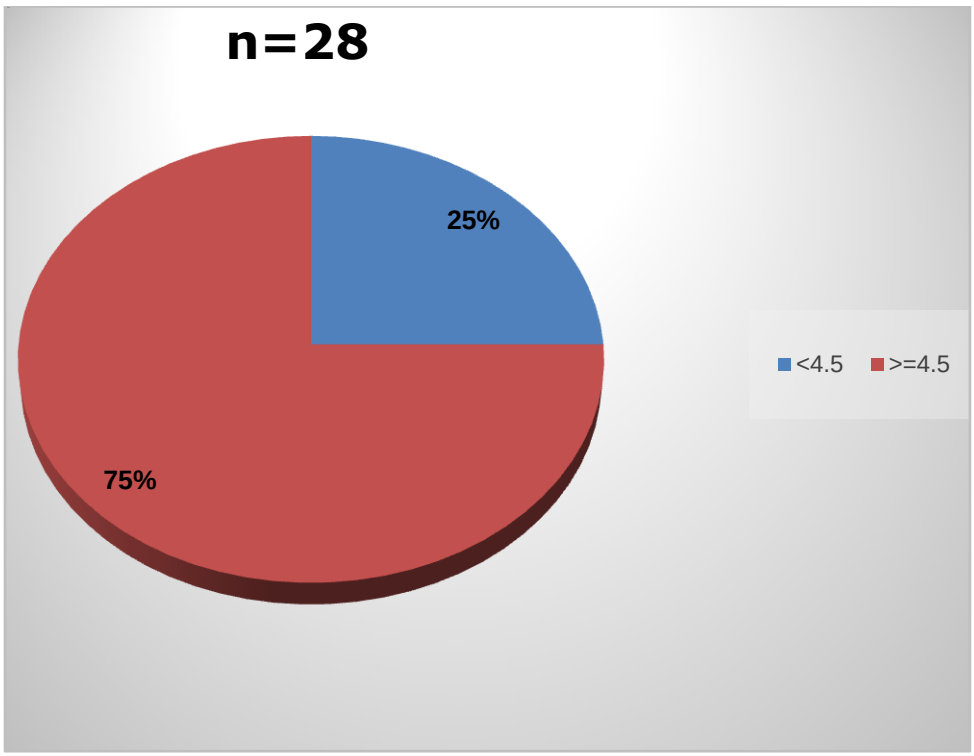
Most of the patients had increased serum uric acid(86%).14 % patients had normal serum uric acid.In increased serum uric acid group, 25% were between 6-8 mg/dl,39% were between 8-10 mg/dl and 22% had serum uric acid levels >10 mg/dl.

Fig No.12 Corrected calcium prevalence



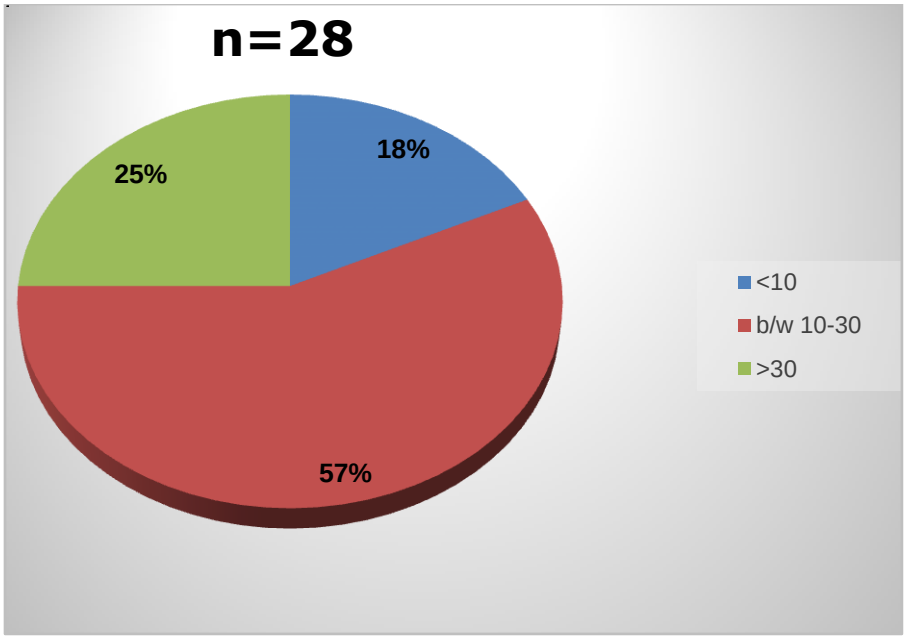
Most of the patients had decreased serum calcium level (71%).Twenty percent patients had serum calcium level >8.4mg/dl. Most of patients had low levels of albumin so corrected calcium was used in this study.

Fig No.13.Phosphorus prevalence



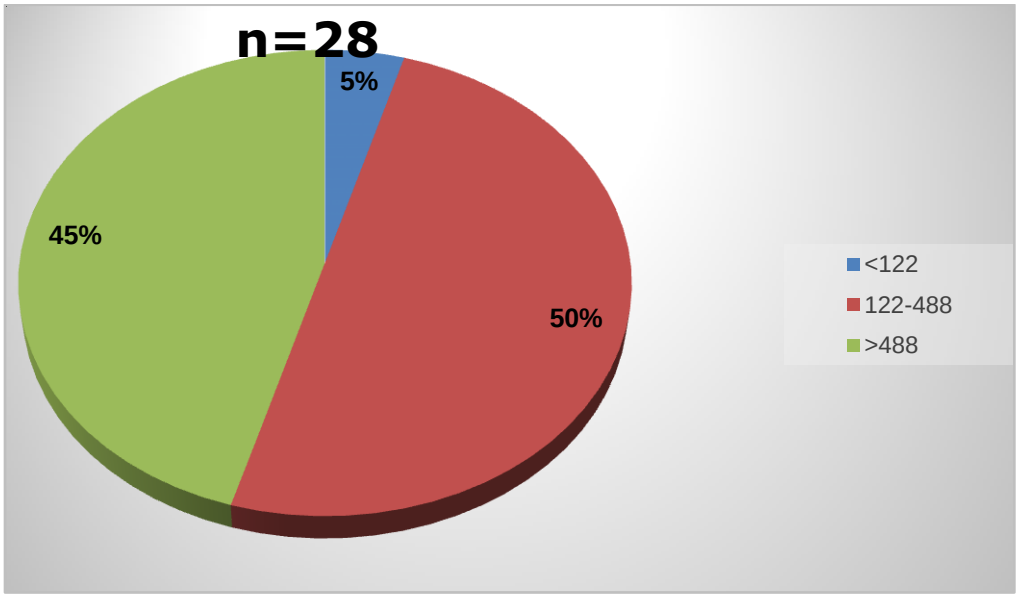
75% patients had increased phosphorus level at the time of presentation. Twenty five patients have normal phosphorus level. most of the patients who have low phosphorus level are on MHD.

Fig No.14: 25(OH) Vitamin d prevalence



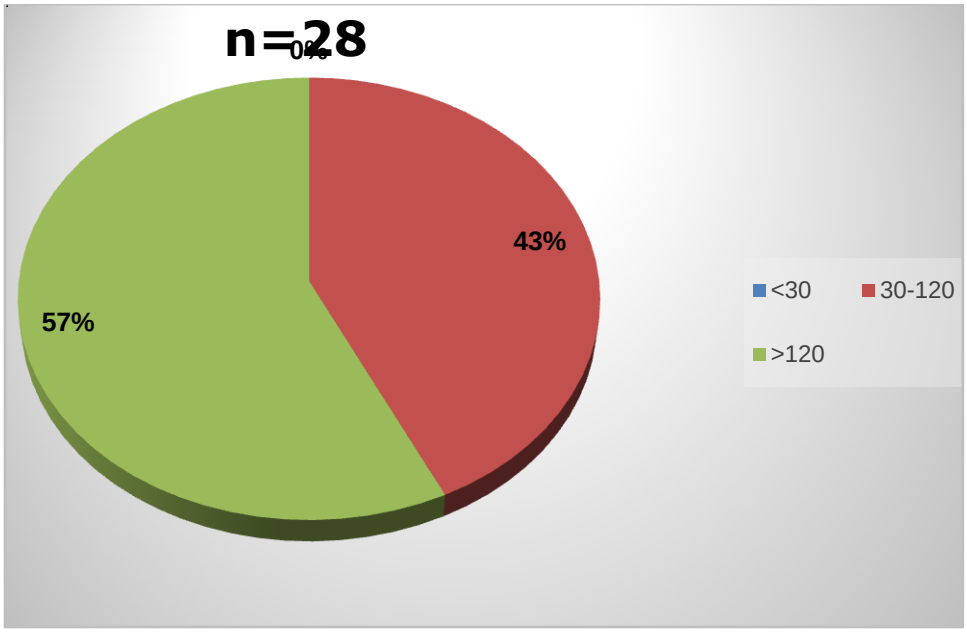
Most of the patients had vitamin D insufficiency. Vitamin D insufficiency is defined as Vitamin D level between 10-30 ng/ml. 57% patients were vitamin D insufficiency.25% patients had normal Vitamin D level and 18 % patients had vitamin D deficiency.

Fig No.15. Serum Intact PTH prevalence



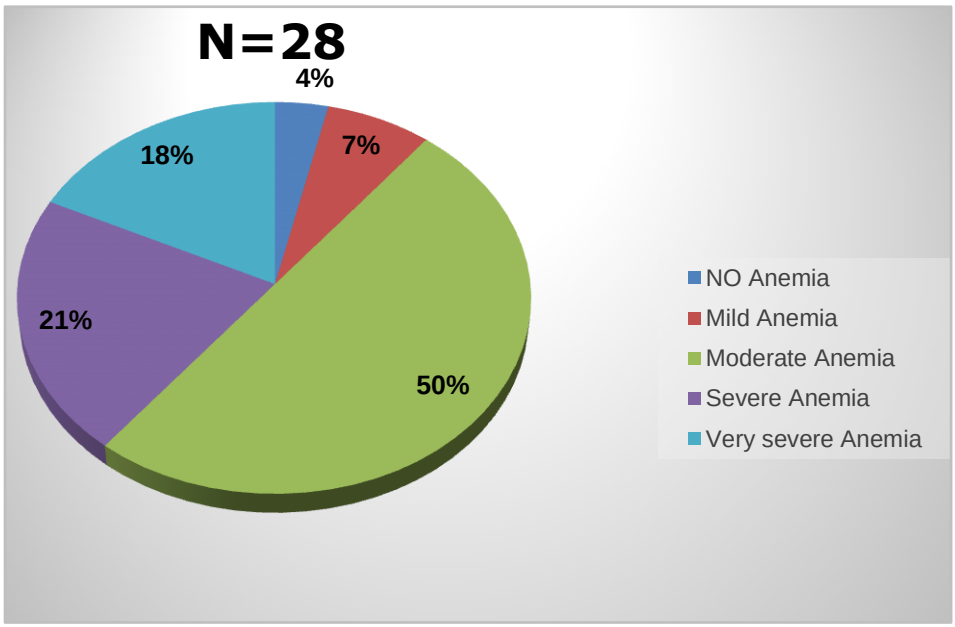
All patients have elevated serum intact PTH level. only 5% patients have appropriately elevated intact PTH according to GFR. 50 % Patients have moderately increased serum intact PTH and 45% patients have severely increased serum intact PTH level.

Fig No.16: Alkaline phosphatase prevalance



All patients have normal or increased level of serum alkaline phosphatase levels. fourty percent patients had normal ALP level and 57% patients had increased ALP level.

Fig No:17.Anemia prevalence in population



Most of the patients (96%) were anemic. Of these, 7% were mildly anemic,50% had moderate anemia, 21% were severely anemic and 18% had very severe anemia.four percent of patients had no evidence of anemia.

Table No:6.a.Correlations of biochemical parameters of ROD with each other

		I PTH	Phosphorus	Vitamin D	ALP	Corrected Calcium
I PTH	Pearson Correlation	1	.201	-.303	.274	-.448*
	Sig. (2-tailed)		.304	.117	.158	.017
	N	28	28	28	28	28
Phosphorus	Pearson Correlation	.201	1	-.229	-.348	-.366
	Sig. (2-tailed)	.304		.241	.069	.055
	N	28	28	28	28	28
Vitamin D	Pearson Correlation	-.303	-.229	1	.167	.403*
	Sig. (2-tailed)	.117	.241		.395	.034
	N	28	28	28	28	28
ALP	Pearson Correlation	.274	-.348	.167	1	-.183
	Sig. (2-tailed)	.158	.069	.395		.352
	N	28	28	28	28	28
Corrected Calcium	Pearson Correlation	-.448*	-.366	.403*	-.183	1
	Sig. (2-tailed)	.017	.055	.034	.352	8
	N	28	28	28	28	28

*. Correlation is significant at the 0.05 level (2-tailed).

Table No:6.b
Spearmans rho

			I PTH	Phosphorus	Vitamin D	ALP	Corrected Calcium
Spearman's rho	I PTH	Correlation Coefficient	1.000	.077	-.264	.327	-.338
		Sig. (2-tailed)	.	.695	.174	.089	.079
		N	28	28	28	28	28
	Phosphorus	Correlation Coefficient	.077	1.000	-.196	-.447*	-.240
		Sig. (2-tailed)	.695	.	.318	.017	.219
		N	28	28	28	28	28
	Vitamin D	Correlation Coefficient	-.264	-.196	1.000	-.066	.370
		Sig. (2-tailed)	.174	.318	.	.738	.053
		N	28	28	28	28	28
	ALP	Correlation Coefficient	.327	-.447*	-.066	1.000	-.113
		Sig. (2-tailed)	.089	.017	.738	.	.568
		N	28	28	28	28	28
	Corrected Calcium	Correlation Coefficient	-.338	-.240	.370	-.113	1.000
		Sig. (2-tailed)	.079	.219	.053	.568	.
		N	28	28	28	28	28

*. Correlation is significant at the 0.05 level (2-tailed).

Fig. No: 18 distribution of Intact PTH in population

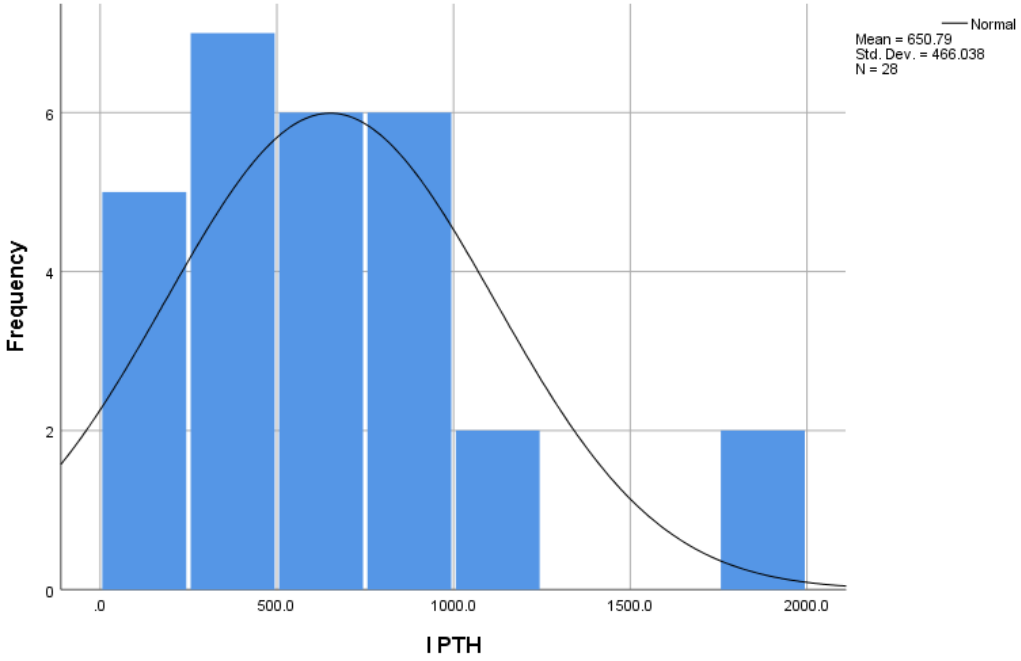


Fig. No: 19 distribution of Vitamin D in population

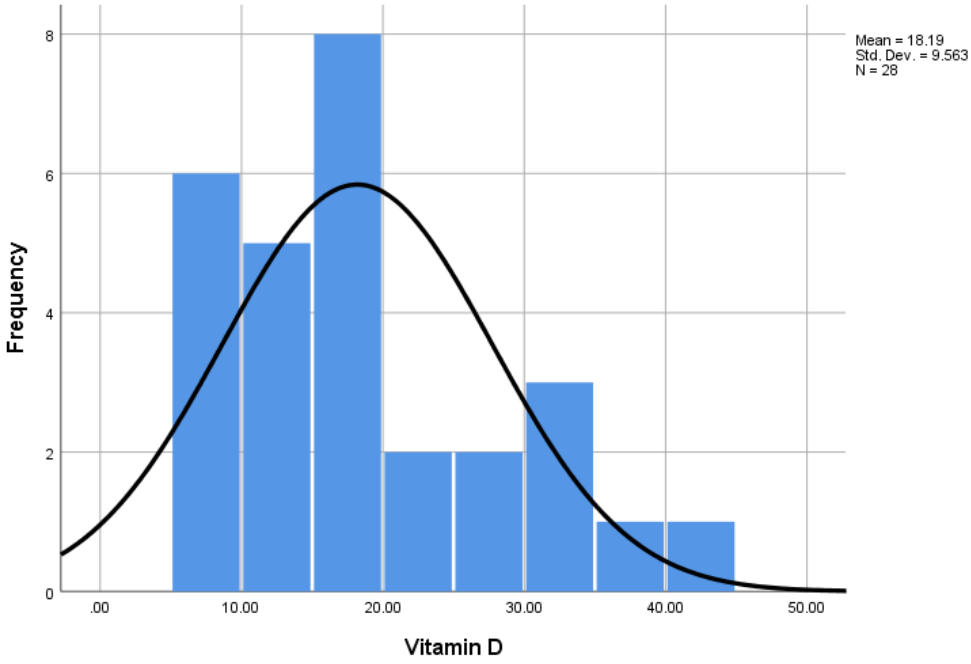


Fig. No: 20 distribution of ALP in population

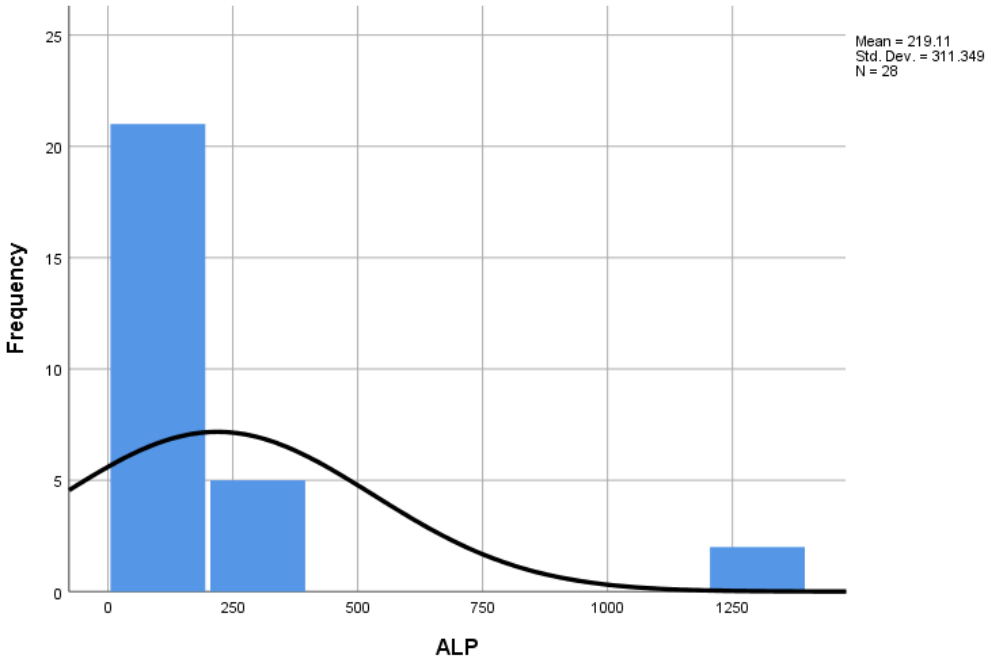


Fig. No. 21: Distribution of phosphorus in population

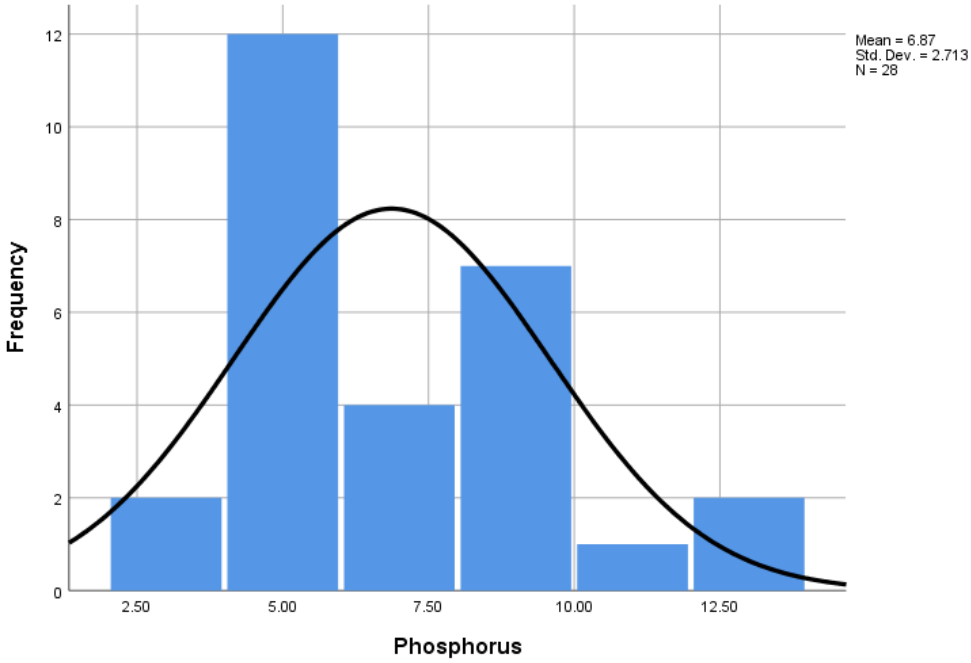
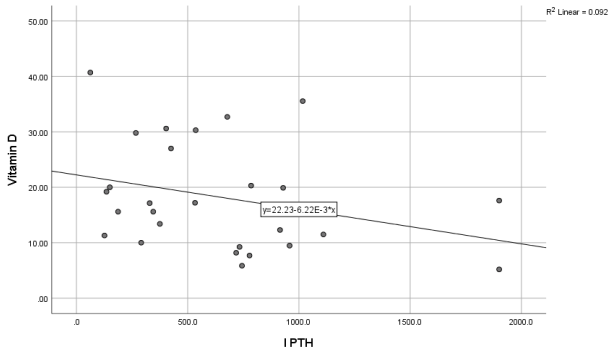
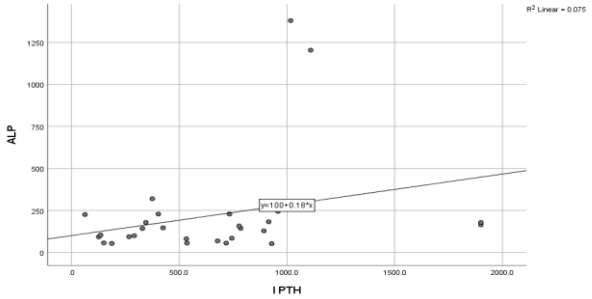

Mean = 6.87
Std. Dev. = 2.713
N = 28
Frequency
0
2
4
6
8
10
12
2.50
5.00
7.50
10.00
12.50
Phosphorus

Fig No:22. Scatter plot showing the correlation between Vitamin D and S Intact PTH levels



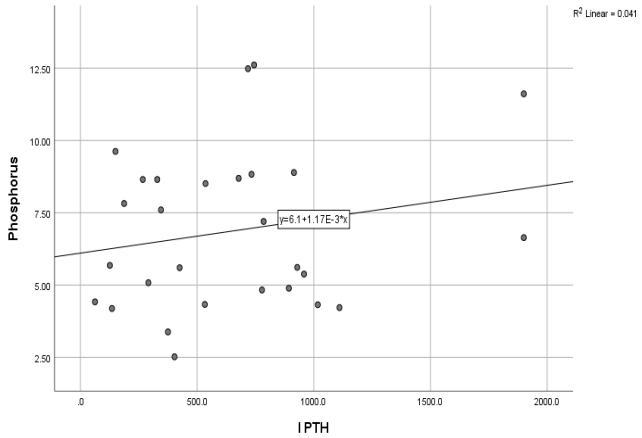
The value of R is -0.303.The value of R^2 ,the coefficient of determination, is 0.092.The p value is 0.3593.the result is not significant at p <0.05.although technically a negative correlation, the relationship between variables is only weak.

Fig No:23.Scatter plot showing the correlation between ALP and S Intact PTH levels



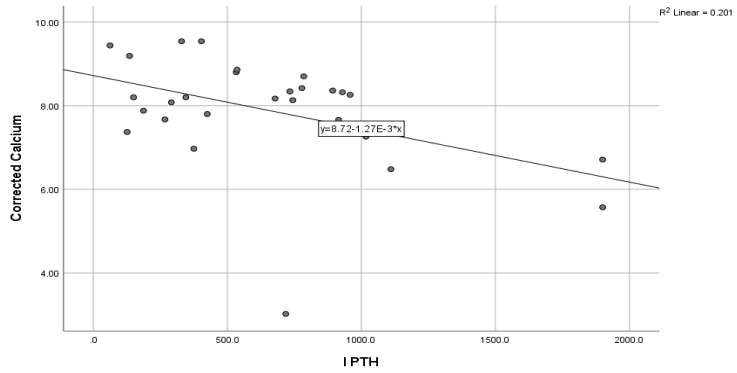
the value of R is 0.273.the value of R^2,the coefficient of determination, is 0.075.the p value is 0.1259.the result is not significant at p<0.05.although technically a positive correlation, the relationship between variables is weak.

Fig No:24. Scatter plot showing the correlation between Phosphorus and S Intact PTH levels



The value of R is 0.202.the value of R^2, the coefficient of determination, is 0.041.the p value is 0.14.The result is not significant at p<0.05.although technically a positive correlation, the relationship between variables is weak.

Fig No:25.Scatter plot showing the correlation between corrected calcium and s Intact PTH levels



The value of R is -0.0967.the value of R^2, the coefficient of determination, is 0.201.The p value is 0.064.The result is not significant at p<0.05.

Fig No.26.Prevalence of aortic valve calcification

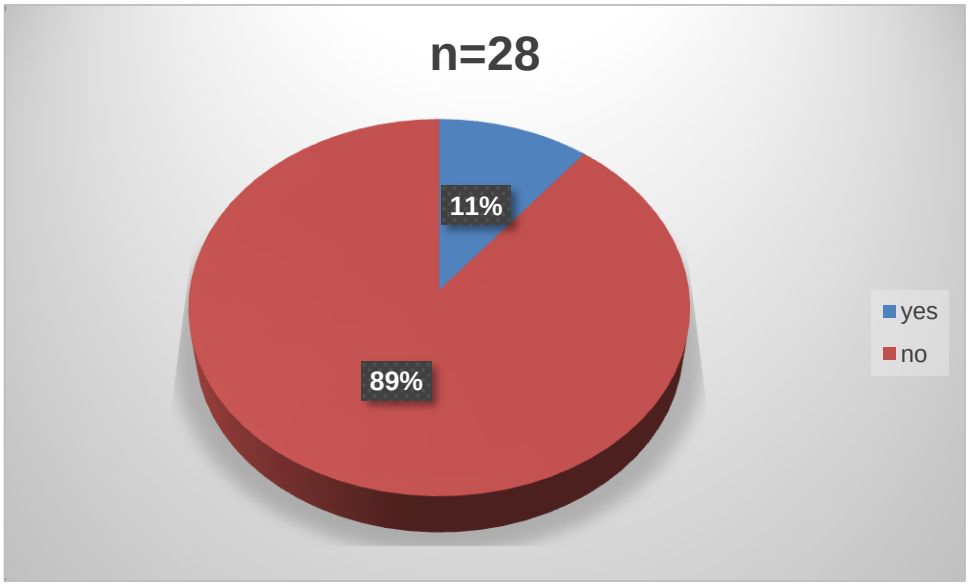
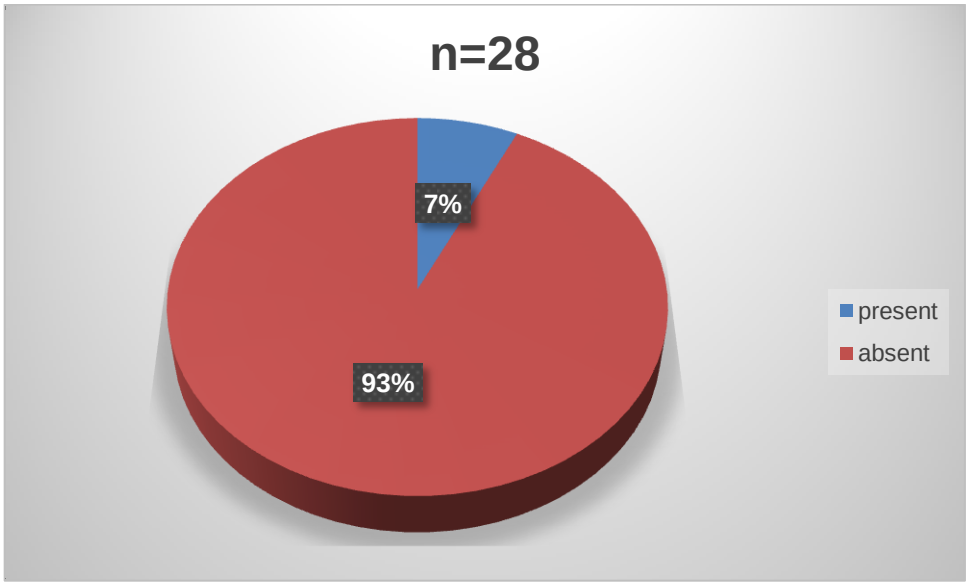


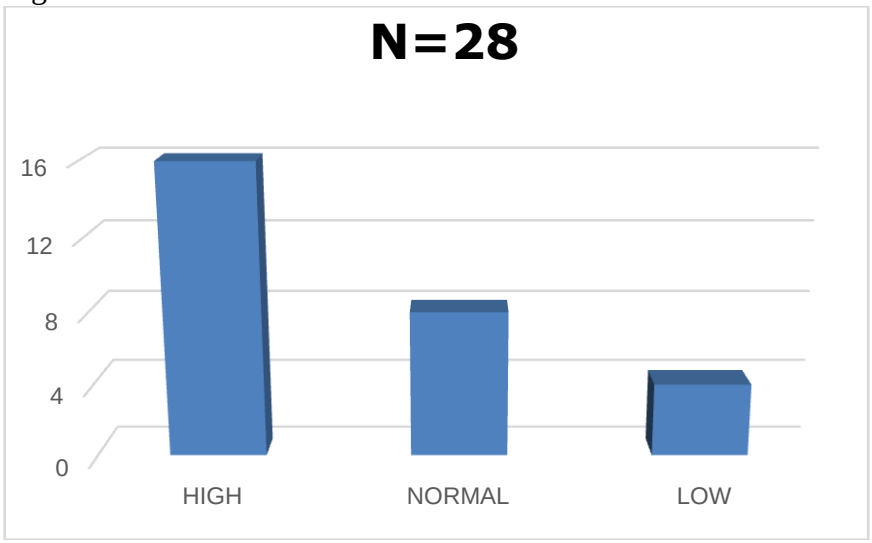
Fig No:27. Vessel Calcification Prevalence

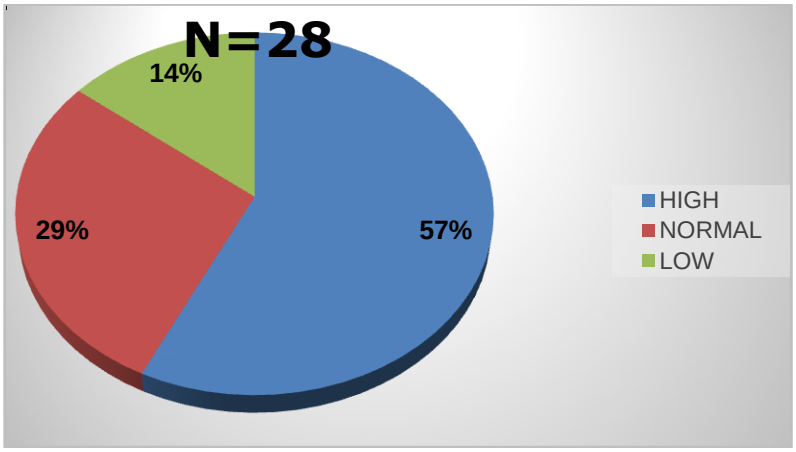


X ray abdomen lateral and AP view was done. Seven percent patients had calcification in Aorta. Ninty three percent of patients had no aortic calcification.

TURNOVER

Figure No:28 a and b Bone turnover

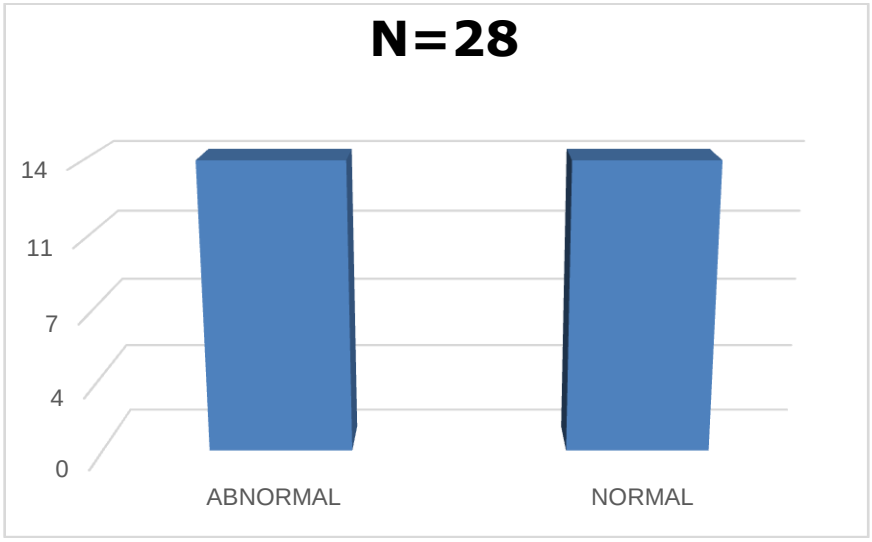


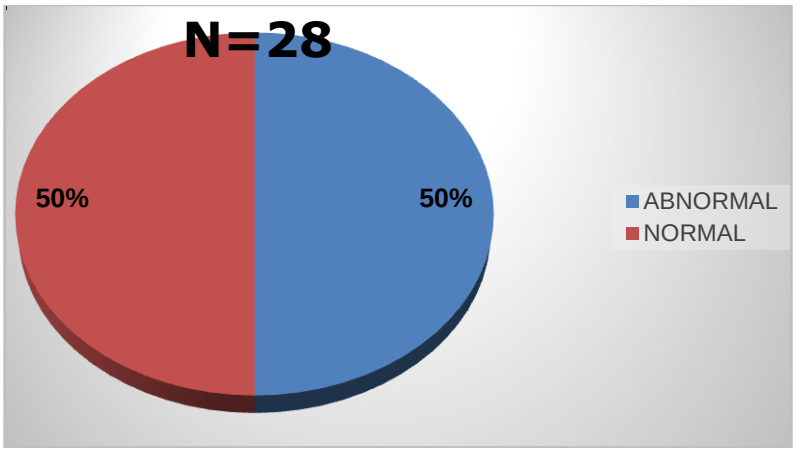


Most common outcome was high turnover disease in 16 (57%) of patients.Eight (29%) patients had normal turnover and 4(14%) patients had low turnover bone disease.

Mineralization

Fig No:29 a and b Mineralization

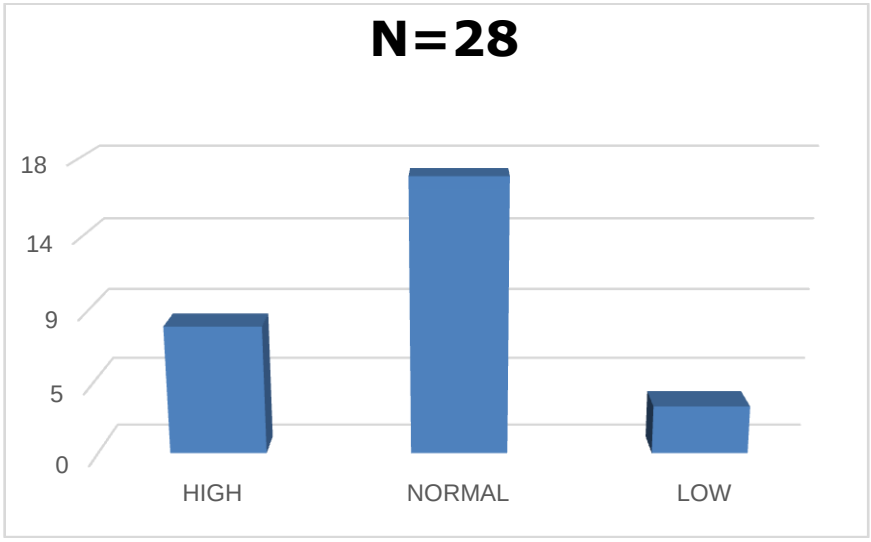


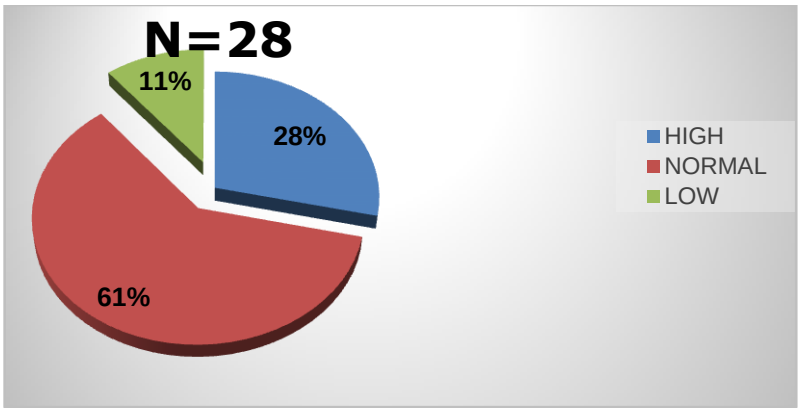


Of total 28 patients 14 (50%) patients had abnormal mineralization and 50% patients have normal mineralization.

VOLUME

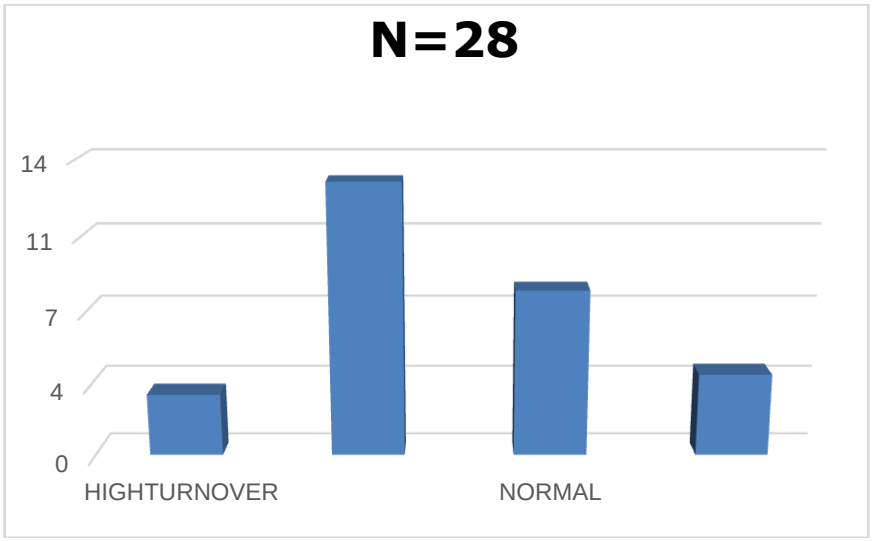
Fig No: 30 a and b Volume of bone

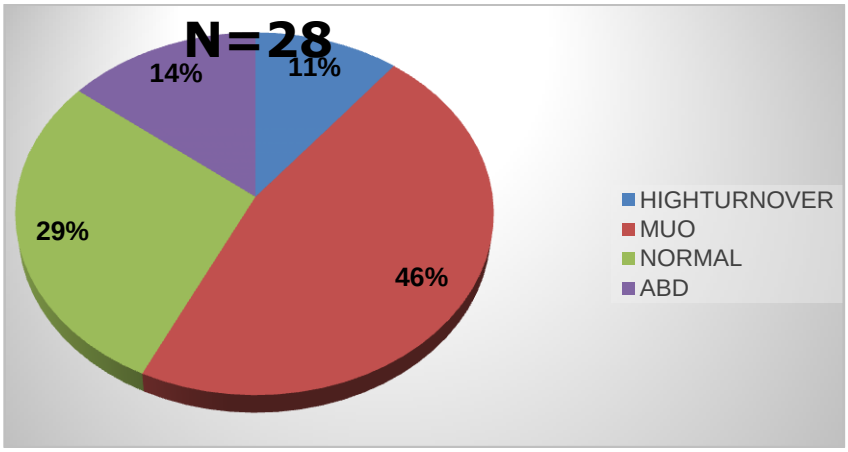




Most of the patients were young so 61% patients had normal bone volume.Twenty eight % had high bone volume which had mainly high bone turnover disease. Eleven percent patients had low bone volume.

Fig No. 31 a and b Renal osteodystrophy prevalence





After bone histomorphometry analysis and interpretation ROD was described in mainly four parts: High turnover, MUO, Normal and Low turnover (adynamic bone disease). Most common ROD was MUO(46%) followed by normal histology(29%),adynamic or low turnover disease in 14% and pure high turnover disease in 11% of patients.

Table No:7. Biochemical profile of the types of ROD compared to the total population as mean + SD

	S Urea	S Ca	S Po4	S ALP	S Vit D3	S PTH
Total case	197.96+85.36	7.89+1.32	6.86+2.71	219.10+311.34	18.18+9.56	650.78+466.03
HPT(N=3)	237.43+84.2	7.95+0.51	7.72+4.2	101.33+24.43	11.01+5.03	388.33+437.16
MUO(N=13)	186.86+69.42	7.36+1.62	7.47+2.6	316+438.9	16.91+10.24	996.67+429.31
ABD(N=4)	247.25+156.95	9.01+0.76	6.27+2.29	130.7+72.6	23.16+11.78	178.32+112.71
NORMAL(N=8)	166.75+71.44	8.15+0.76	5.84+2.54	150+88.5	20.45+7.80	348.5+115.18

Mean serum urea was highest in ABD patients, not being a determinant of bone histomorphometry. Mean serum calcium was highest in ABD/low turnover disease. Mean serum Phosphate was highest in HPT group. Mean ALP was highest in MUO group. Mean Vitamin D was highest in ABD group. Mean Intact PTH was highest in MUO group.

Table No:8.Correlations between Biochemical and Bone Histomorphometry Parameters

		I PTH	Ob.S/BS(0-9.5)%	OcS/BS(0-2.0)%	ES/BS(1.75-7.0)%	Fibrosis area/Tissue area(<5)%
I PTH	Pearson Correlation	1	.341	.578**	.538**	.396*
	Sig. (2-tailed)		.076	.001	.003	.037
	N	28	28	28	28	28
Ob.S/BS(0-9.5)%	Pearson Correlation	.341	1	.490**	.577**	.635**
	Sig. (2-tailed)	.076		.008	.001	.000
	N	28	28	28	28	28
OcS/BS(0-2.0)%	Pearson Correlation	.578**	.490**	1	.679**	.680**
	Sig. (2-tailed)	.001	.008		.000	.000
	N	28	28	28	28	28
ES/BS(1.75-7.0)%	Pearson Correlation	.538**	.577**	.679**	1	.736**
	Sig. (2-tailed)	.003	.001	.000		.000
	N	28	28	28	28	28
Fibrosis area/Tissue area(<5)%	Pearson Correlation	.396*	.635**	.680**	.736**	1
	Sig. (2-tailed)	.037	.000	.000	.000	
	N	28	28	28	28	28

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

FIG NO: 32 I PTH and ObS/BS correlation

The value of R is 0.340. Although technically a positive correlation, the relationship between variables is weak.The value of R^2, the coefficient of determination, is 0.116.The P-Value is .051.the result is not significant at p <.05

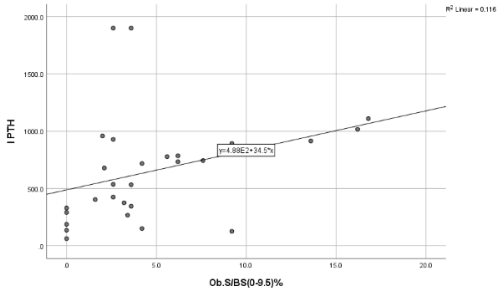


FIG NO: 33 I PTH and OcS/BS Correlation

The value of R is 0.577. This is a moderate positive correlation, which means there is a tendency for high X variable scores go with high Y variable scores (and vice versa).The value of R^2, the coefficient of determination, is 0.334.The P-Value is .000769.The result is significant at p<.05.

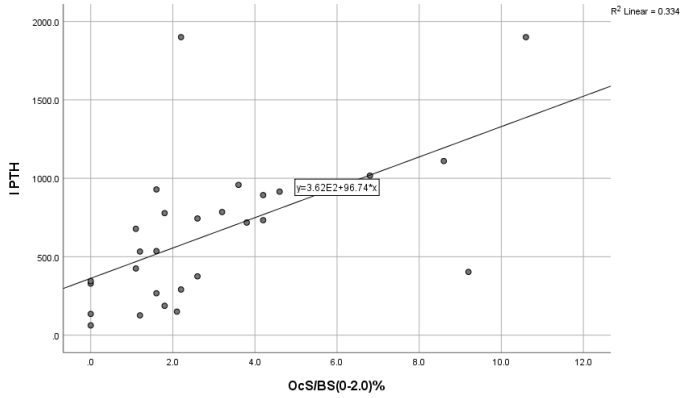


FIG NO: 34 I PTH AND ES/BS Correlation

This is a moderate positive correlation, which means there is a tendency for high X variable scores go with high Y variable scores (and vice versa).The value of R^2, the coefficient of determination, is 0.290.R value is 0.538.The P-Value is .0013.The result is significant at p<.05.

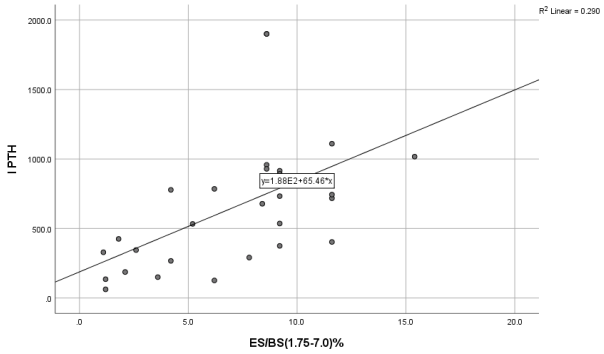


FIG NO: 35 I PTH Fibrosis/tissue area correlation

The value of R is 0.396.Although technically a positive correlation, the relationship between variables is weak. The value of R^2, the coefficient of determination, is 0.157.The P value is .020. the result is significant at p<.05.

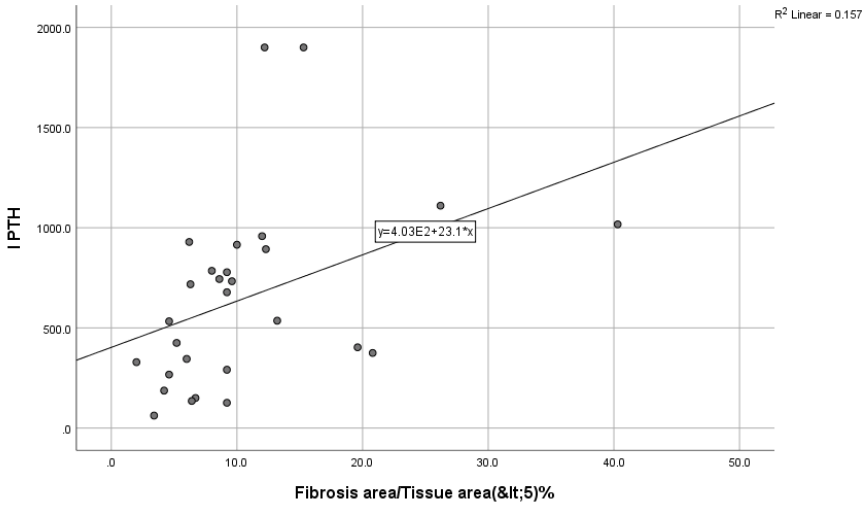


FIG NO: 36 ALP and OS/BS Correlation

The value of R is 0.414.Although technically a positive correlation, the relationship between variables is weak. The value of R^2, the coefficient of determination, is 0.172.the P value is 0.022.the result is significant at p<0.05

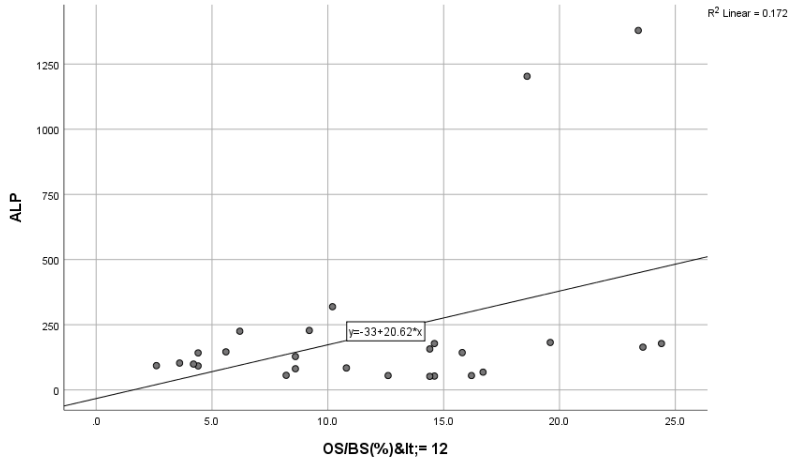
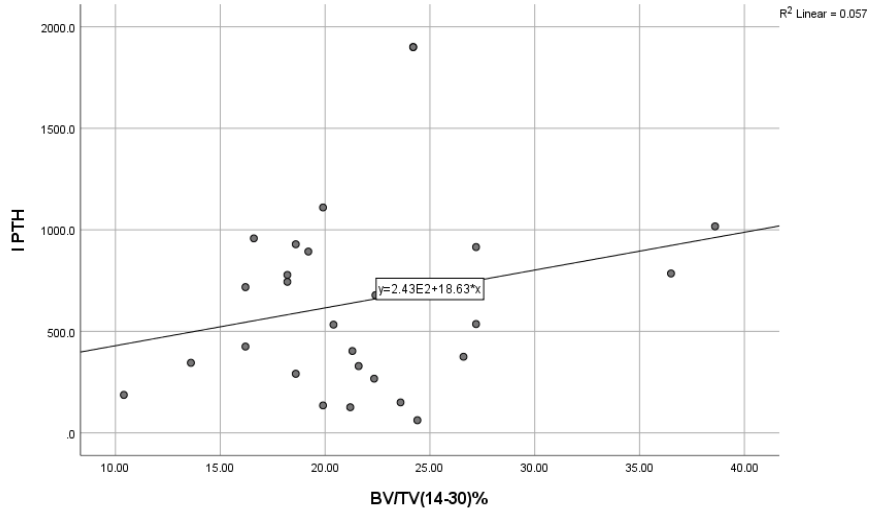


FIG NO: 37 I PTH and bone volume correlation

The value of R is 0.238.Although technically a positive correlation, the relationship between the variables is weak. The value of R^2, the coefficient of determination, is 0.057.The P-value is 0.082.the result is not significant at p<.05



DISCUSSION

In this study, a total of 28 patients with CKD in the age group of 18-55 years were enrolled. Almost all the continuous variables in this study were non parametric based on tests of normality.

Among the 28 patients in this study, the median age was 33 years and the mean age was 33.07 ± 10.42 years. Median age for male patients was 36 years and the mean age was 35.10 ± 10.12 years. The median age for female patients was 29 years and the mean age was 29.3 ± 9.41 years. Most studies of epidemiology of CKD were done in CKD patients which included diabetics and hypertensive patients.In these studies mean age was higher compared to this study. In Indian Registry by Mohan M[1] et al (2012), the mean age was 50.1 ± 14.6 years, with M:F ratio of 70:30. In our study, DM patients were excluded and most patients were 20-40 years of age. Common causes of CKD in these age group were Glomerulonephritis(IgA Nephropathy, FSGS and secondary Autoimmune GN),CAKUT. Out of the total 28 patients, 20(71%) were males and 8 (29%) were females. Male: female ratio in this study was 2.5%. Using data from National Health and Nutrition Examination Survey from 1988–1994 through 2011–2012, prevalence of CKD in women was higher in 2003–2004 than in 1988–1994 but has since largely remained unchanged, with rates of 7.8% (6.3–9.4%) in women and 5.9% (4.5–7.2%) in men in 2011–2012[81]. United States Renal Data System (USRDS) data on CKD prevalence in 2011–2014 also reported a higher prevalence of CKD in women 16.5 versus 13% in men[82].

Amarpali Brar et al[83]. Showed population-based studies indicated a higher prevalence of CKD in women; however, there are fewer women on renal replacement therapy than men. Men may progress to end-stage kidney disease more rapidly. Gender differences in rates of CKD progression may be influenced by potential antifibrotic and antiapoptotic effects of estrogen or proinflammatory deleterious effects of testosterone. Women are referred later for kidney replacement therapy and receive fewer arteriovenous fistulas than men receive, irrespective of race. Women are also less likely to receive kidney transplants as compared with men but are more likely to donate a kidney

All 28 patients taken in this study were CKD 5 stage in which 27 patients were in ESRD and 1 patient was not in ESRD. The mode of RRT in ESRD patients are HD. In 78 % HD was initiated first time and 18 % patients were on MHD.

The other biochemical parameters were correlated with the predominant types. The serum calcium was deranged in the study population and was lower than normal. However no significant correlation was seen between the intact PTH and S Calcium. Intact PTH was significantly correlated with bone turnover. In study Moore[84] and Malluche[85] no correlation was seen between the bone histology and serum calcium .

The mean serum phosphate in the HPT subtype was slightly higher than in the MUO group. Positive correlation was seen between the serum phosphate and intact PTH level but was statistically not significant(p=.2). Coen G. Spasovski[86] however did not find any correlation between the bone histologic type and the phosphate levels .

The mean ALP level in MUO was higher (316) than in HPT(219.10). This finding corroborated with the findings of Spasvoski[86]. Weak positive non significant(p=.22) correlation was found between the histological type and the S. ALP levels. Eastwood[87] did not find any correlation between the ALP and the histologic types. Spasvoski however did find a correlation between histologic types and ALP levels. Malluche et al[85] . found the levels to be higher in HPT than MUO but there was no correlation between histology and ALP levels. The ALP is usually raised in high turnover bone diseases. The two major subgroups in our study population MUO and HPT being a high turnover the ALP levels were comparable. Hence no significant correlation could be ascertained.

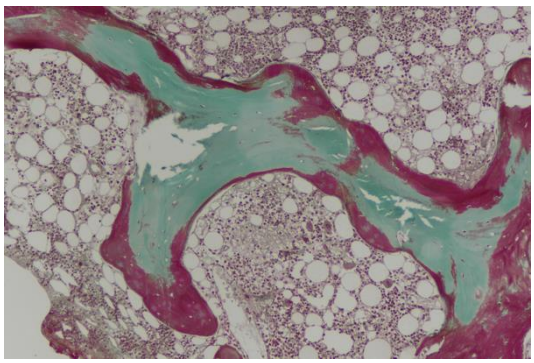


Fig No 38.MUO increased osteoid thickness(pink colour) with increased eroded areas

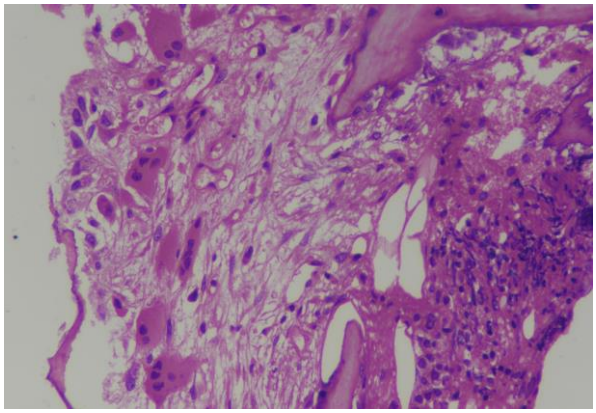


Fig No 39.High turnover with increased osteoclast activity

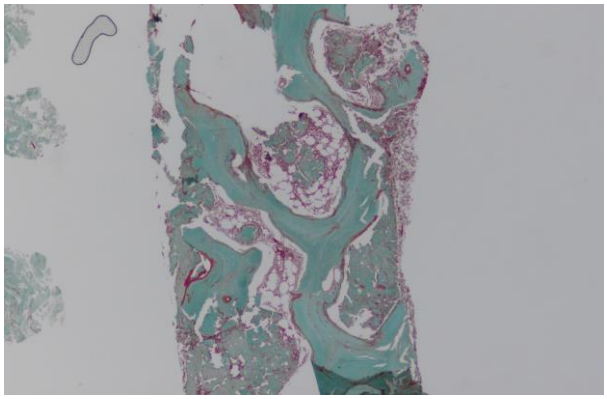


Fig No 40.ABD without osteoid and without cellular activity

The mean PTH in HPT group was at lower (388.33) than the MUO group(996.67). Kazama[88] found that the MUO group had higher levels than HPT. This finding was not similar to the Coen G[89]. Malluche also not found the levels of PTH to be higher in HPT than in MUO. Coen et al.[90] found that the predictive value of intact PTH in the noninvasive diagnosis of renal bone disease is higher in hemodialysis patients than in predialysis patients. We found correlation between the PTH levels and osteoblastic and osteoclastic activity and the 2 major histologic types, HPT and MUO subtypes. PTH has been found to be raised in high turnover diseases viz. HPT and MUO. Probably that is the reason why we got comparable results in the study group. The iPTH levels may also not be a good marker for bone turn over in mild renal failure[91].

The Vit D3 (25 OH) mean was higher in MUO group(16.9) than the HPT group(11.01). We did not found a significant correlation(p=0.35) between intact PTH and the Vitamin D levels. Levin had found that the Vitamin D levels are associated with PTH levels[92]. It was noted that the Vit D3 levels declines early in CKD even before the PTH begins to rise. The Vit D3 levels have an inverse relationship with serum PTH levels. In our group, the PTH was higher in the MUO group. Other causes could be because of the decreased sunlight exposure or malnutrition[92].

Anaemia developing in the course of chronic kidney disease is an established finding. Lower haemoglobin may result from the loss of erythropoietin synthesis in the kidneys and/or the presence of inhibitors of erythropoiesis. Numerous articles by Fisher, Erslev and Besarab and others have documented the association of anaemia with kidney failure and describe its various causes[93-95]. The severity of anaemia in chronic kidney disease is related to the duration and extent of kidney failure. In our study most of patients (96%) were anaemic, of these 7% were mildly anemic,50% were moderately anemic,21% were severely anemic and 18% had very severe anemia. Four percent of patients had no anaemia.

The incidence of mixed uremic osteodystrophy was 46% in the present study. Its frequency is comparable to the study conducted by Faugere M C, Malluche H and Coen G who found MUO in 54.54%, 45–48% and 63.1% respectively (Table 9) discussion table. In the latest study by Kazama, the hyperparathyroid and mixed uremic osteodystrophy have similar incidence and in our study the findings are comparable if not similar. This study and various other studies as given in Table 9 have shown low turnover renal osteodystrophy to be one of the predominant sub-type. However adynamic bone disease was the lowest in the study by Faugere[96] and Malluche[85].

Table No:9.Comparison of the various studies based on bone marrow biopsy along with our study

	NORMAL(%)	ABD(%)	MUO(%)	HPT(%)
Faugere et al.	-	16.35	54.54	29.09
Kazama	-	38.6	18.6	42.6
Spasovski et al.	32	35	18	9
Sherrard et al.	-	53.66	6.9	39.37
Shin et al.	8.6	34.8	12.1	42.8
Coen et al.	13.1	21	63.1	26
Our Study	29	14	46	11

Normal histology has also been also documented in similar studies by Coen[89], Shin[97] and Spasvoski et al.[86] The finding by Shin as the lowest group in the study was comparable. In our study normal histology present in 29%.It may due to incidentally diagnosed CKD 5D without any intervention to CKD MBD.

The difference in the spectrum of ROD reported by the various study groups could be due to the difference in the criteria for patient recruitment (selected vs unselected populations), cohort size, genetic and dietary factors, referral rates and use of phosphate binders.

Therapeutic implications of knowing the different types of ROD are as follows:- in case of severe hyperparathyroidism with marked bone marrow fibrosis, aggressive treatment with IV calcitriol at high doses is indicated. In patients with MUO, Vitamin D analogs are to be given. Low turnover patients require a reduction in therapy aimed at suppression of PTH using Phosphate binders and avoidance of vitamin D over treatment[85].

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Conclusion:

Intact PTH is the main determinant of the type of bone histomorphometry. The level of intact PTH depend not only on one biochemical parameter but multiple factors which influence it's levels. KDIGO guidelines suggested that the intact PTH levels should be 2 to 9 times of the upper limit of normal along with other biochemical parameters monitored. In most of the studies there were no significant correlation between biochemical parameters and bone histomorphometry. It may be due to different population groups like dialysis and non dialysis patients, stage of CKD, calcium supplements prescribed and type of phosphate binders. In our study also, the most common ROD is Mixed Uremic Osteodystrophy. Our study suggests that bone biopsy is desired to ascertain CKD-MBD renal osteodystrophy profile, with existent indications of bone biopsy, as a related diagnostic tool in providing guide for diagnosis and treatment of mixed pattern of renal osteodystrophy not delineated by biochemical parameters alone and otherwise missed in CKD patients who are on chronic HD/PD.

ETHICAL CONSIDERATION

The purpose of the present study is to find out the the prevalence of type of bone disease in patients of CKD attending OPD/IPD, diagnosed by bone biopsy which is gold standard. The 2017 KDIGO clinical practice guidelines state that, in patients with CKD stage 3-5D, it is reasonable to perform a bone biopsy if knowledge of type of ROD will impact treatment decisions. Currently, biochemical markers and imaging tests are not accurate predictors of bone histology[9].

Thus, the gold standard for the diagnosis and specific classification of renal osteodystrophy (ROD) remains the histomorphometric analysis of the bone biopsy.

The safety of bone biopsy is studied since many year, there is some morbidities but no mortality. there is data lacking of types of renal ostodystrophy in CKD patient in the india. All tests will be done after informing patients and their attendants that they are a part of the study and after taking full informed consent. There are no potential risks for the patients participating in the study.

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अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर
All India Institute of Medical Sciences, Jodhpur
संस्थागत नैतिकता समिति
Institutional Ethics Committee

No. AIIMS/IEC/2020/2118 **Date: 01/01/2020**

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number: **AIIMS/IEC/2019-20/1027**

Project title: **"Study of association between biochemical parameters of mineral bone disease and bone histomorphometry in chronic kidney disease patients."**

Nature of Project:	**Research Project**
Submitted as:	**D.M. Dissertation**
Student Name:	**Dr.Santosh Kumar Maurya**
Guide:	**Dr.Manish Chaturvedy**
Co-Guide:	**Dr. Praveen Sharma, Dr.Abhay Elhence, Dr.Poonam Elhence & Dr.Nitin Kumar Bajpai**

This is to inform that members of Institutional Ethics Committee (Annexure attached) met on **17-01-2020** and after through consideration accorded its approval on above project. Further, should any other methodology be used, would require separate authorization.

The investigator may therefore commence the research from the date of this certificate, using the reference number indicated above.

Please note that the AIIMS IEC must be informed immediately of:

- Any material change in the conditions or undertakings mentioned in the document.
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

The Principal Investigator must report to the AIIMS IEC in the prescribed format, where applicable, bi-annually, and at the end of the project, in respect of ethical compliance.

AIIMS IEC retains the right to withdraw or amend this if:

- Any unethical principle or practices are revealed or suspected
- Relevant information has been withheld or misrepresented

AIIMS IEC shall have an access to any information or data at any time during the course or after completion of the project.

On behalf of Ethics Committee, I wish you success in your research.

Dr. Praveen Sharma
Member Secretary
Institutional Ethics Committee
AIIMS,Jodhpur

Enclose:
1. Annexure 1

Basni Phase-2, Jodhpur, Rajasthan-342005, **Website:** www.aiimsjodhpur.edu.in, **Phone:** 0291-2740741 Extn. 3109
Email: ethicscommittee@aiimsjodhpur.edu.in

Annexure I

Institutional Ethics Committee
All India Institution of Medical Sciences, Jodhpur

Meeting of Institutional Ethics committee held on **17-01-2020 at 10:00** AM at Committee Room, Admin Block AIIMS Jodhpur.

Following members were participated in the meeting:-

S/No.	Name of Member	Qualification	Role/Designation in Ethics Committee
1.	Dr. F.S.K Barar	MBBS, MD (Pharmacology)	**Chairman**
2.	Justice N.N Mathur	LLB	Legal Expert
3.	Dr. Varsha Sharma	M.A (Sociology)	Social Scientist
4.	Mr. B.S.Yadav	B.Sc., M.Sc. (Physics), B.Ed.	Lay Person
5.	Dr. K.R.Haldiya	MD (General Medicine)	Clinician
6.	Dr. Arvind Mathur	MBBS, MS (General Medicine)	Clinician
7.	Dr. Sneha Ambwani	MBBS, MD (Pharmacology)	Basic Medical Scientist
8.	Dr. Kuldeep Singh	MBBS, MD (Paediatric), DM (General Medicine)	Clinician
9.	Dr. Abhinav Dixit	MBBS, MD (Physiology), DNB (Physiology)	Basic Medical Scientist
10.	Dr. Pradeep Kumar Bhatia	MBBS, MD (Anaesthesiology)	Clinician
11.	Dr. Tanuj Kanchan	MBBS, MD (Forensic Medicine)	Basic Medical Scientist
12.	Dr. Pankaj Bhardwaj	MBBS, MD (CM&FM)	Clinician
13.	Dr. Praveen Sharma	M.Sc., Ph.D. (Biochemistry)	Member Secretary

Dr. Praveen Sharma
Member secretary
Institutional Ethics Committee
AIIMS,Jodhpur

ANNEXURE-1

GOVERNMENT OF INDIA

ALL INDIA INSTITUTE OF MEDICAL SCIENCES,

JODHPUR-342005, INDIA

INFORMED CONSENT FORM

I _____ s/d/w of _____ , a resident of _____, hereby declare that I give informed consent to participate in the Thesis study labelled "study of prevalence of mineral bone disease in chronic kidney disease patients with correlation between biochemical parameters and bone histomorphometry" **.** Dr.Santosh kumar Maurya has informed me to my full satisfaction, in the language I understand, about the purpose, nature of study and various investigations to be carried out for the study. I have been informed about the duration of the study and possible complications caused by study.

I give full consent for being enrolled in the above study and I reserve my rights to withdraw from the study whenever I wish without prejudice of my right to undergo further treatment at this hospital and its associated hospitals.

_____ _____

Name of Subject Date Signature of subject

We have witnessed that the patient signed the above form in the presence of his/her free will after fully having understood its contents.

_____ _____

Name of Witness Date Signature of witness

_____ _____

Name of Investigator Date Signature of Investigator

ANNEXURE-2

अखिल भारतीय आयुर्विज्ञान संस्थान

जोधपुर, राजस्थान

सूचित सहमति प्रपत्र

थीसिस का शीर्षक: "पुराने गुर्दे के मरीजों में अस्थि रोग और रक्त के रसायन में सम्बन्ध और अस्थि रोग के सम्भावना का अध्ययन "

dm छात्र का नाम- डॉ संतोष कुमार मौर्य

रोगी / स्वयं सेवी पहचान संख्या.: _____

मैं,_____पुत्र /पुत्री_____

निवासी_____मेरी पूर्ण, नि: शुल्क, स्वैच्छिक सहमति देता हु निम्नलिखित अध्ययन का हिस्सा बनने के लिए . : "पुराने गुर्दे के मरीजों में अस्थि रोग और रक्त के रसायन में सम्बन्ध और अस्थि रोग के सम्भावना का अध्ययन", जिस की प्रक्रिया और प्रकृति मेरी पूरी संतुष्टि के लिए मेरी अपनी भाषा में मुझे समझाया गया है। मैं पुष्टि करता हूं कि मेरे पास प्रश्न पूछने का अवसर था। मैं समझता हूं कि मेरी भागीदारी स्वैच्छिक है और किसी भी कारण के बिना, किसी भी समय अध्ययन से बाहर निकलने के मेरे अधिकार से अवगत हूं. मैं समझता हूं कि मेरे और मेरे किसी भी मेडिकल रिकॉर्ड के बारे में एकत्र की गई जानकारी एम्स जोधपुर से या नियामप्राधिकरणों से जिम्मेदार व्यक्ति द्वारा देखी जा सकती है। मैं इन व्यक्तियों के लिए अपने रिकॉर्ड तक पहुंचने की अनुमति देता हूं।

दिनांक: _____ _____

स्थान : _____ हस्ताक्षर/बाएं अंगूठे की छाप

यह प्रमाणित करने के लिए कि उपर्युक्त सहमति मेरी उपस्थिति में प्राप्त की गई है।

तारीख :_____ _____

स्थान: _____ हस्ताक्षर

साक्षी1 साक्षी2

हस्ताक्षर: _____ हस्ताक्षर: _____

नाम: _____ नाम: _____

स्थान : _____ स्थान : _____

ANNEXURE-3
PATIENT INFORMATION SHEET

Name of the patient: **Patient ID.:**

STUDY OF PREVALENCE OF MINERAL BONE DISEASE IN CHRONIC KIDNEY DISEASE PATIENTS WITH CORRELATION BETWEEN BIOCHEMICAL PARAMETERS AND BONE HISTOMORPHOMETRY"

Aim of the study:

- To identify the prevalence of type of osteodystrophy among CKD patient stage 3-5 including on maintainance hemodialysis.
- To identify Vascular calcification, calciphylaxis and soft tissue calcification.

Study site- IPD/OPD patients at Dept. of Nephrology AIIMS, Jodhpur.

Study procedure- patients attending OPD and admitted in IPD with diagnosis of CKD stge 3b to stage 5ND and 5D included in study.base line investigation with specific investigation for bone turn over will be do.

Likely benefit- study to help prevalence of bone disease in CKD patient.

Confidentiality- All the data collected from each study participant will be kept highly confidential.

Risk -Enrollment in above study poses no substantial risk to any of the study participant.

Withdrawl from study- You are free to decide whether to participate or not in the study or withdraw from the study anytime. If you choose not to participate in the study or withdraw from the study, you will continue to receive the same amount of care and treatment at AIIMS, Jodhpur.

अनुलग्नक 4

रोगी सूचना पत्रक

रोगी का नाम: रोगी आईडी :

"पुराने गुर्दे के मरीजों में अस्थि रोग और रक्त के रसायन में सम्बन्ध और अस्थि रोग के सम्भावना का अध्ययन "

1. अध्ययन साइट: नेफ्रोलॉजी विभाग, ऑल इंडिया इंस्टीट्यूट ऑफ मेडिकल साइंसेज, जोधपुर, राजस्थान।

2. अध्ययन प्रक्रिया: विस्तृत इतिहास, नैदानिक परीक्षा और आवश्यक बेसलाइन प्रयोगशाला

3. गोपनीयता: प्रत्येक अध्ययन प्रतिभागी से एकत्र किए गए सभी डेटा को अत्यधिक गोपनीय रखा जाएगा।

4. जोखिम: उपर्युक्त अध्ययन में नामांकन किसी भी अध्ययन प्रतिभागी को कोई बड़ा जोखिम नहीं है।

5. अध्ययन से निकासी: आप यह तय करने के लिए स्वतंत्र हैं कि अध्ययन में भाग लेने या अध्ययन में या किसी भी समय अध्ययन से वापस लेना है या नहीं। यदि आप अध्ययन में भाग लेने या अध्ययन से वापस लेने का चयन नहीं करते हैं, तो आपको एम्स, जोधपुर में समान देखभाल और उपचार प्राप्त करना जारी रहेगा।

अधिक जानकारी / प्रश्नों के लिए, निम्नलिखित कर्मियों से संपर्क किया जा सकता है:
डॉ संतोष कुमार मौर्य, नेफ्रोलॉजी विभाग, ऑल इंडिया इंस्टीट्यूट ऑफ मेडिकल साइंसेज, जोधपुर, राजस्थान। पीएच: 7668182401

ANNEXURE 5

PROFORMA

Name: **Study Serial No.:**

Age: **Sex:** **Reg No.:**

Address:

Phone No.:

Diagnosis: Serum creatinine (stable for 3 month)

e GFR

stage of CKD

Duration of ESRD_____HD_____

PBF

HB … blood urea …. uric acid…....

Calcium… phosphorous … Serum albumin…

alkaline phosphatase (ALP) ……. S.PTH ….

25-hydroxycholecalciferol……..

Biopsy report-

Turnover

bone formation rate

ObS/BS
OcS/bs
ES/BS

osteoblast and osteoclast surface and number

Mineralization

OS/BS

osteoid thickness

Bone volume

BV/TV

Impression of bone histology

Lateral abdominal radiograph – aortic calcification - yes/no

Echocardiogram - valvular calcification- yes/no