A STUDY OF METACARPOCORTICAL INDEX TO PREDICT RENAL OSTEODYSTROPHY IN CHRONIC RENAL FAILURE

Dissertation Submitted to

THE TAMILNADU Dr.M.G.R. MEDICAL UNIVERSITY

CHENNAI- 600 032

With Partial fulfillment of the requirement

for the award of degree of

M.D. DEGREE IN GENERAL MEDICINE

BRANCH – 1

Register Number :201711204

THANJAVUR MEDICAL COLLEGE AND HOSPITAL

THANJAVUR

MAY 2020

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This is to certify that the dissertation entitled **"A STUDY OF METACARPOCORTICAL INDEX TO PREDICT RENAL OSTEODYSTROPHY IN CHRONIC RENAL FAILURE"** is the bonafide work of **Dr. GOVINDARAJ V** in partial fulfillment of the University regulations of the Tamil Nadu Dr. M.G.R Medical University, Chennai for M.D General Medicine Branch I examination to be held in MAY 2020.

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A STUDY OF METACARPOCASUAL INDEX TO PREDICT RENAL OSTEODYSTROPHY IN CHRONIC RENAL FAILURE

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PLACE:THANJAVUR **(Dr.GOVINDARAJ V)**

ACKNOWLEDGEMENT

I would like to thank our respected dean **Prof.Dr.KUMUDHA LINGARAJ MD DA.,** Thanjavur Medical college for permitting me to utilize the facilities of Thanjavur Medical college and Hospital for this dissertation. I wish to express my respect and sincere gratitude to my beloved teacher, Unit chief and Head of the Department, **Prof.Dr.K.NAMASIVAYAM**M.D., Professor of Medicine for his valuable guidance, support and encouragement during the study and also throughout my course period.

I am greatly indebted to the Assistant Professors, **Dr.R.T.SHRIRAM GANESH M.D DA., Dr.S.HEMA AKILANDESWARI M.D, Dr.N.RAMESH M.D,**fortheir valuable suggestions throughout the course of the study.

I am extremely thankful to **Prof. Dr. RAJ KUMAR MD. DM., Department of Nephrology** for their constant support, guidance, cooperation and to complete thisstudy**.**

I sincerely thank all the staffs of department of medicine for the timely help rendered to me whenever and wherever needed.

I am extremely thankful to my beloved father **Mr. V.VELUSAMY** and mother **Mrs.V.KALAISELVI**, my sister for their constant support throughout my study and course period.

I extend my thanks to all my friends, batch mates, my juniors and senior colleagues who have stood by me and supported me throughout my study and course period.

Finally, I thank all the patients, who form the most vital part of my work, for their extreme patience and co-operation without whom this project would have been a distant dream and I pray God for their speedyrecovery.

CONTENTS

INTRODUCTION

Chronic renal failure is a common renal problem, with several etiologies resulting in progressive destruction of nephron number and function and often leads to End stage renal disease(ESRD). Uremia is a complex state reflecting all organ systems dysfunction with distinctive signs and symptoms that result from chronic renal failure. In Chronic renal disease, when GFR falls below 60ml/min, Bone disease is seen in 75% of patients. Renal osteodystrophy is common in CKD patients, where kidneys unable to maintain appropriate calcium and phosphorus levels in blood. The changes in bone may begin early before symptoms appear and is usually called as silent crippler.

Normally, the process of replacement and removal of bone is tightly coupled. Osteoblasts are responsible for production of constituents such as ground substances, collagen which later mineralised. Osteoclasts in contact with bone surfaces reabsorb bone.

ESRD often result in abnormal bone turnover, coupling and mineralisation. When GFR falls below 60 ml/min, there is rise in PTH and decline in 1,25dihydroxyvitamin D levels leads to retention of phosphate. As PTH rise, phosphate and calcium balance restored. With further reduction to 20-40 ml/min, normal calcium and phosphate haemostasiscannot be maintained. With worsening Uraemia, skeletal PTH resistance increases and abnormal bone

histology is seen in almost all patients. With disease progress, skeletal resistance to PTH occurs. PTH levels and Bone specific alkaline phosphatise (ALP) is helpful in evaluating bone disease, but bone biopsy and histiomorphometry still remains as gold standard test. Calcium salts and calcitriol is used to improve osteomalacia by suppressing PTH but it predispose patient to vascular calcification, cardiovascular mortality, fracture. Newer options can be used like calcemic vitamin D analogues, calcimimetics and biphosphanates and sevelamer for phosphate control. To improve Bone mineral density, calcitriol and hormone replacement therapy(HRT) can be used.

With the use of combined modalities like biochemical markers, histology, bone densiometry, early intervention & effective management, morbidity associated with CRF has been reduced.

One of the earliest changes seen in radiologically in CRF is metacarpocortical index. This study is done to measure bone density by thickness of second metacarpal bone by X-ray which is an easy and effective method to predict bone changes in association with biochemical parameters like urea , creatinine, calcium, ALP, phosphorous and uric acid.

AIM OF STUDY

1. Detecting renal osteodystrophy in early stages
2. Calculating metacarpocorticalindex(MCI) and predict quantitative bony changes in chronic kidney disease.
3. Comparing MCI in chronic kidney disease with biochemical parameters like blood urea, serum creatinine, serum calcium, serum phosphorous, serum alkaline phosphatase, serum uric acid.

REVIEW OF LITERATURE

GLOBAL DISEASE BURDEN OF CKD:

Chronic kidney disease (CKD) is becoming a major global disease affecting 10% of population and causing one million deaths. It is a growing cause of mortality which made this condition as 13th leading cause of death in 2013, comparing to its 27th place in 1990. It is closely related with heart and blood vessel disease, as 7% of all cardiovascular mortality is related to reduced GFR, a principal marker of chronic kidney disease(CKD). CKD remains a important cause of mortality and non – fatal outcomes. In GBS study, CKD is the 20th leading cause for people living with disability.

Kidney diseases are growing more and more nowadays due to many factors like aging population and growing prevalence of diabetes. Chronic kidney disease(CKD) is a widespread disease with high mortality and morbidity, directly and indirectly causing many kidney and cardiovascular diseases. The time has come for CKD screening by general practitioners, at least in high risk populations. The good news is that many treatment protocols and guidelines have been developed for protecting kidneys. Giving the available cost effective treatment is very helpful in delaying the complications developed by CKD. Kidney disease is very common and dreadful, yet treatable.

The kidney is one of the highly differentiated organs in the body. In development of kidneys, nearly 30 different cell types from filtering capillaries and nephron surrounding a interstitium. This diversity modulate a variety of complex physiologic processes, endocrine function, water and soluble transport, acid base balance, blood pressure regulation, and intraglomerularhemodynamics, removal of drug metabolites are all accomplished by intricate mechanisms of renal response.

DISEASE BURDEN OF CKD IN INDIA:

There is a growing burden of chronic disease in developing countries. Previously considered to be a health problem in developed countries, 4 out of 5 chronic disease deaths nowadays occur in low income countries. The projected death due to chronic disease is 3.18 million in 1990(40.4% of all death) and is 7.63 million expected in 2020(66.7% of all death). Usually, main focus is on diseases like hypertension, diabetes mellitus, cardiovascular disease. Nowadays there is increase in CKD progressing to end stage renal disease(ESRD).There is a heavy financial burden of renal replacement therapy(RRT).There is rapid worldwide increasing trend in chronic renal failure. By 2020, there are 17,83,000patients received treatment of chronic kidney disease worldwide. This number is increasing by 7% each year.

The exact disease burden cannot be estimated as there is no proper registry available. Based on data available, the CKD-stage 3 and beyond

prevalence easy 0.79% at of 4,972 subjects. But the study was based on patient's serum creatinine alone, which underestimate the disease prevalence. This study also evaluated risk factor like diabetes, hypertension, renal stone disease. Another study in south Indiasuggests a prevalence of 8.6 per thousand population. The commonest cause in the study was diabetic nephropathy.

In June 2005, a project started named "Indian CKD registry" to find out many complications related to CKD. It started as a pilot project with 10 centres. They have 30,000 subjects in database. Mean age of population in the study is 40-50 yrs with 70% of them are males. The major population belongs to stage CKD 4-5. The diabetes mellitus is found to be important cause in 30% of patients. Cardiovascular disease is seen in CKD patients as disease progress with 0.7% in stage 3 to 43% to stage 5.

"Screening and early evaluation of kidney disease(SEEK)" started in 2006 by nephrologists generate data in CKD patients to determine prevalence of CKD in India. Its aim is to find out the prevalence of cases and complications relevant to Indian population. This study shows the disease has high prevalence of 17.4% using egger formulae.

RENAL OSTEODYSTROPHY:

Renal osteodystrophy is bone morphology abnormalities present in patients with chronic renal failure. It is a measurement of skeletal component of End stage renal disease and CKD-MBD (chronic kidney disease- mineral bone disease). The term renal osteodystrophy coined in 1943. National kidney foundation defined renal osteodystrophy in 2004 as constellation of bone disorders in chronic renal failure which leads to fractures and altered mineral metabolism and extraskeletal manifestations. However this definition lacks bone specificity. “Kidney disease improving global outcome(KDIGO)” committee proposed renal osteodystrophy as , term to various bone pathology in patients with CKD.

Another recently used broader term is CKD-MBD(CKD- mineral and bone disorders) which refer to clinical, biochemical, imaging abnormalities associated with renal osteodystrophy. CKD-MBD is defined as disorder of bone and mineral metabolism due to CKD, and is associated with features like

1. Abnormalities of calcium, phosphorous, vitamin D metabolism.
2. Abnormalities of bone mineralisation, parathormone turnover, volume, strength, linear growth.
3. Vascular or other soft tissue calcification.

KDIGO recommend that CKD-MBD should be initially evaluated with biochemical testing (PTH, calcium, ALP, phosphate and imaging). However at present bone biopsy is the gold standard test but because of its invasive nature, we rely on other lab parameters for renal osteodystrophy. Bone biopsy is recommend, when there are inconsistencies in biochemical values, unexplained bone fractures or unexplained bone pain.

INCIDENCE AND PREVALANCE OF RENALOSTEODYSTROPHY:-

The worldwide population is aging. In the United States alone, the number of persons aged 65 years and over is expected to rise from 32 to 69 million between 1990 and 2050, and the number over the age of 85 years will increase from 3 to 15 million. The global population is demonstrating similar trends as the number of persons aged 65 years and over is expected to rise from 323 to 1555 million between 1990 and 2050. These demographic trends have raised great concern about the burden of several common diseases associated withaging.

Osteoporosis, defined by the World Health Organization as a disorder of bone resulting in decreased bone strength, is an extremely common disorder of aging that currently affects 10–12 million people in the United States alone. Future projections, based on the aging of the United States population, indicate that the number of people with

osteoporosis will increase exponentially during thefirsthalfofthiscentury.Forexample,theestimated7.8millionwomenand 2.3 million Men affected with osteoporosis at the hip today is expected to increase to 10.5 and 3.3 million, respectively, by2020.

PATHOGENISIS OF RENAL OSTEODYSTROHY:

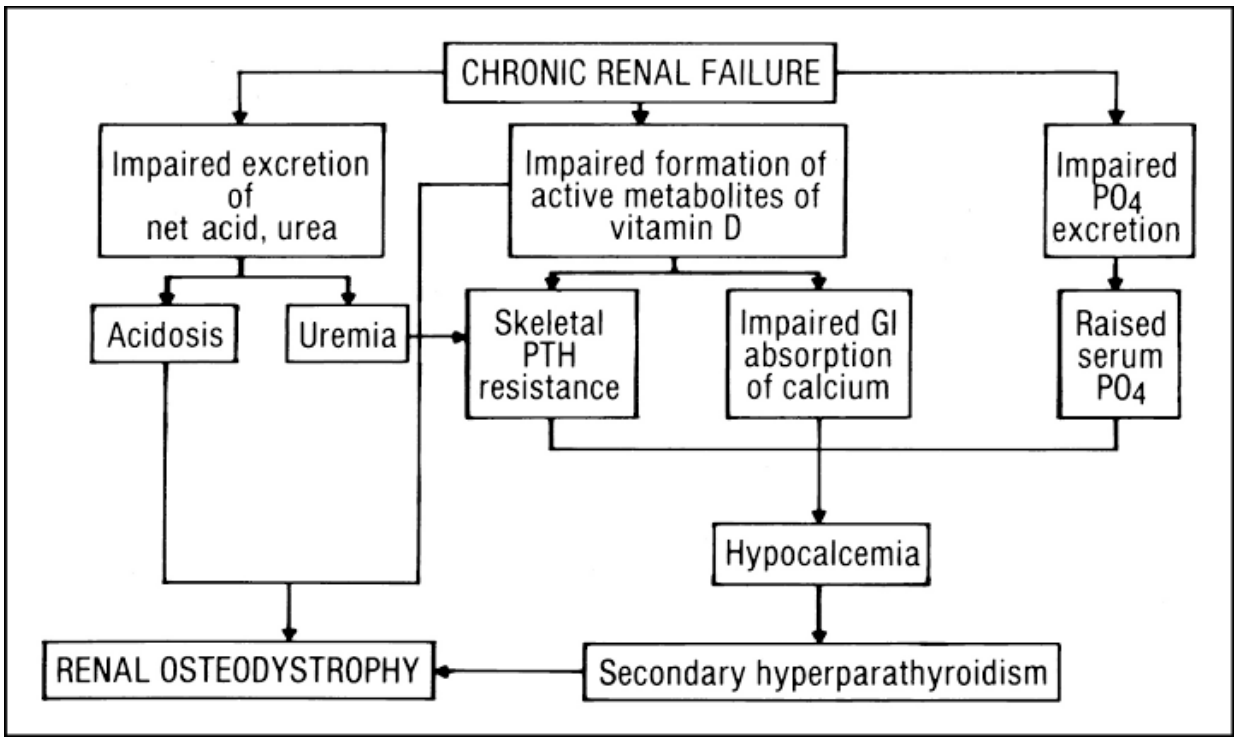
Renal osteodystrophy is described as a result of secondary hyperparathyroidismdue to hyperphosphatemia, associated withhypo-calcaemia.

In chronic renal failure, the kidneys unable to convert vitamin D3 to its active form calcitriol. Thus low activated vitamin D3 level results in hypocalcaemia. High level of fibroblast growth factor 23(FGF23) which is seen in CKD patients also found to be an important cause in pathogenesis of renal osteodystrophy.

In CKD, GFR reduction leads to decreased phosphate (PO4) excretion and increase in serum phosphate levels. Low attenuated vitamin D3 levels leads to decreased calcium absorption and increased parathorme (PTH) which causes bone demineralisation and increased serum calcium and PO4. Both increased serum PO4 and calcium leads to metastatic calcification.

In chronic renal failure, excess PTH production increases bone resorption rate and it leads to secondary hyperparathyroidism. However it is

influenced by multitude of factors like age, sex, ethnic origins, treatment such as calcium salts, calcimimetics, vitamin D, steroids. Thus it can leads to either low bone turn over disease or adynamic bone disease.



TYPES OF RENAL OSTEODYSTROPHY:

In chronic renal failure, renal osteodystrophy can be either low bone turnover disease or high bone turn over disease or mixture of both. It is broadly classified into osteomalacia (OM), osteofibrosa (OF). Though patients with CKD did not experience symptoms usually, skeletal changes develop years before symptoms rise. Low turnover bone disease is common lesion in patients

on peritoneal dialysis(48-62%) and predialysis (27-48%). Osteitisfibrosa occur in 32-37% of hemodialysis. With hemodialysis markers correlation, high turnover bone disease is most common in pre and post dialysis. Most common variant on peritoneal and hemodialysis is low bone turn over disease.

SIGNS AND SYMPTOMS:

The concept of CKD-MBD is not only associated with fractures, but also with poor quality of life, cardiovascular complications, increased morbidity and mortality in CKD patients.

Usually renal osteodystrophy is symptomless, it may show symptoms which include:

- Joint pain
- Bone pain
- Bone fracture
- Bone deformation
- Short stature
- Growth retardation

ALUMINIUM- RENAL OSTEODYSTROPHY:

Although major cause of bone disease in CKD patients is hyperparathyroidism. In patients receiving exogenous aluminium by dialysis, aluminium related bone disease is identified. Both hyperparathyroidism and aluminium toxicity patients have similar features, so diagnosis is often difficult.

Patients will present with elevation in parathyroid hormone levels, hypercalcemia, bone pain, fracture and bone resorption.

DIAGNOSIS OF RENAL OSTEODYSTROPHY:

Chronic kidney disease- mineral bone disease (CKD-MBD) usually starts in early course of CKD. However renal osteodystrophy is diagnosed after ESRD begins. In initial stages, serum calcium and phosphate levels are normal with high parathyroid hormone and FGF23 levels.

X-ray features of renal osteodystrophy include subperiostic bone resorption, chondrocalcinosis, osteopenia and bone fracture. Since current clinical, biochemical and imaging methods cannot diagnose correctly, bone biopsy still remains gold standard analysis for assessing renal

osteodystrophy. Doing parathyroidectomy for hyperthyroidism may exacerbate the development of aluminium toxicity.

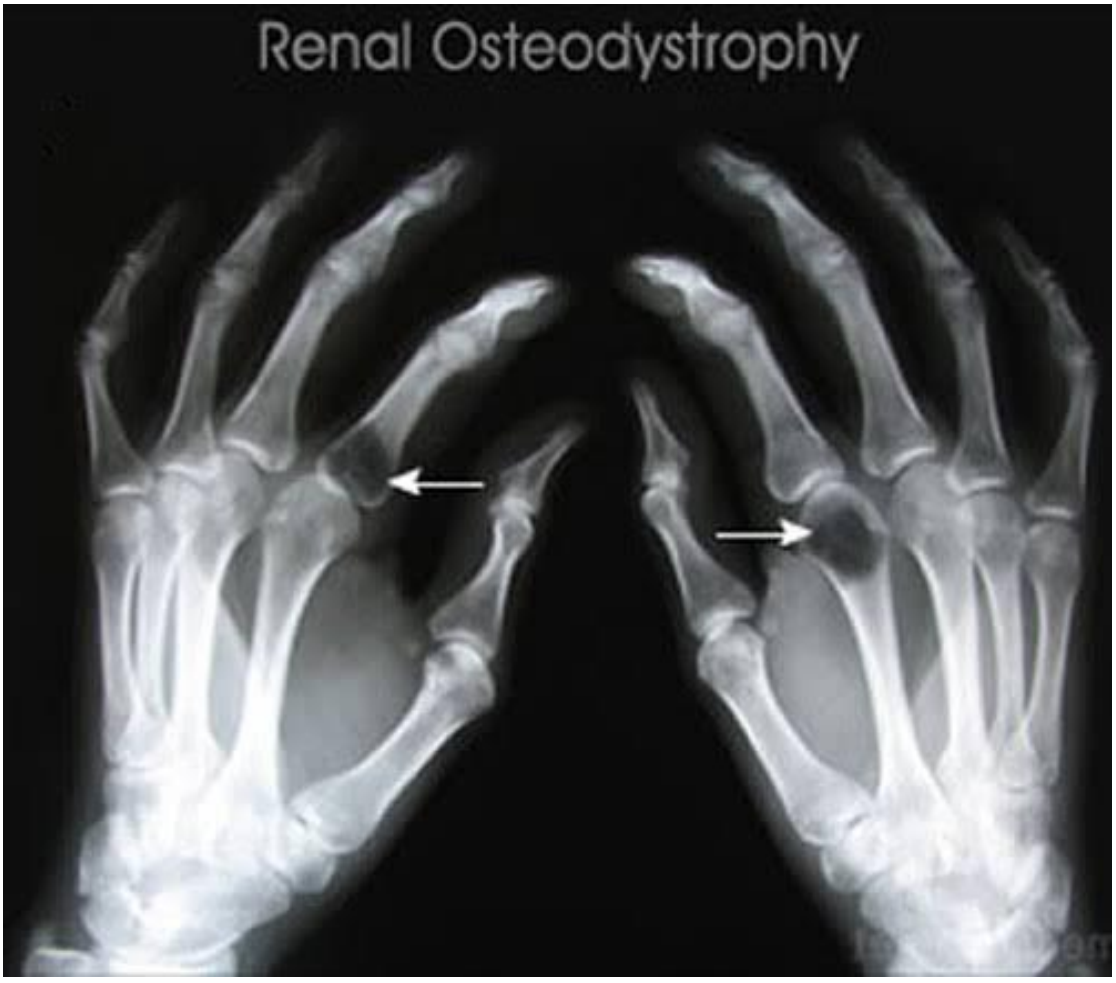
Usually high bone turnover rate is seen in hyperparathyroidism associated with increased number of bone cells, abnormal “woven” osteoid. Low bone turnover rate is seen in aluminium toxicity with paucity of bone cells, maintenance of a laminar osteoid and significant aluminium deposition. Diagnosis of aluminium toxicity is serum aluminium level measurements.

OTHER ABNORMALITIES IN CKD:

- Amyloid deposition
- Tendon rupture
- Crystal deposition
- Infection
- Destructive spondyloarthropathy
- Avascular necrosis

NON INVASIVE BONE IMAGING TECHNOLOGIES IN CKD:

- DXA
- Conventional and peripheral QCT
- Micro- magnetic resonance imaging
- Ultra high resolution peripheral QCT



DISORDERS OF CALCIUM AND PHOSPHOROUS METABOLISM:

Calcium:

In the case of Ca^{2+}, a typical daily diet will contain 1000 mg of elemental Ca^{2+}. Approximately 200 mg will be absorbed and 800 mg excreted. Ca^{2+} is absorbed throughout the intestine, but the low luminal pH (5 to 6) associated with the duodenum and jejunum promotes the ionization of Ca^{2+} and its efficient absorption. When the paracellular route is utilized (generally in jejunum/ileum), Ca^{2+} exit from the intestinal lumen is likely driven by a transepithelial electrochemical gradient. Although most studies on Ca^{2+} diffusion have been done on renal epithelium, it is not unrealistic to assume a similar process also occurs in the intestine. As such, the gradient will be generated by the action of brush border NKCC2 (Na-K-Cl-2 transporter) and ROMK (ATP-sensitive K^+ channel), and basolateral CLCNKB (Cl- channel) and the Na/K ATPase. Na^+, K^+, and 2 Cl- ions first leave the intestinal lumen (entering the cell) via NKCC2. This is offset by a flux of K^+ via ROMK. On the basal side Na^+ leaves the cell, and K^+ enters the cell via Na/K ATPase, while Cl- leaves the cell via CLCNKB channels. On balance, this creates an electrochemical gradient that drives Ca^{2+} out of the lumen and into the extracellular fluid via a paracellular route. Although paracellular diffusion can be pictured as simple diffusion between cells, this is likely not the case. As would be expected for the epithelium, tight junctions exist between cells, linking them physically and

creating a barrier to free intercellular diffusion. In kidney, a unique junctional complex molecule has been found that regulates both $Mg2^{+}$ and Ca^{2+} transit through tight junctions. Termed paracellin-1, it is a member of the claudin superfamily of junctional molecules. It forms a trimeric complex with occludin and JAM (junctional adhesion molecule). Although this claudin is restricted to kidney, other claudins, such as claudin-2, -15 and -20, are found in small intestine and likely serve as regulators of divalent cation transit

Facilitated, or transcellular Ca^{2+} transport (in duodenum/jejunum) actually utilizes a different set of ion transporters/channels (Figure 1A). Again, and principally based on renal epithelial studies, there are both luminal and basolateral transporters/channels. The hallmark of transcellular transport is its sensitivity to levels of active $1,25(OH)_2$ vitamin D3 (abbreviated in this article as VitD). On the luminal side there is a transient receptor potential-vanilloid 6 channel (TRPV6). This is a six-transmembrane domain channel protein that exists as either a homotetramer, or heterotetramer with TRPV5. It is upregulated by low cytosolic Ca^{2+}, opens in the presence of low luminal Ca^{2+}, is inactivated by both phosphorylation and a Ca^{2+}-calmodulin complex, and its levels are increased in response to VitD. Ca^{2+} enters the cell due to an electrochemical gradient. Once inside, its transit through the cell is mediated by Ca^{2+}-binding proteins (calbindins) whose synthesis is upregulated by VitD. In the intestine, calbindin-D9K is used, which has two Ca^{2+}-binding sites. Calbindin-D9K

delivers Ca^{2+} to one of two basolateral transporters/pumps, 120 kDa NCX1 (a Ca^{2+}-Na^{+} exchanger) or 138 kDa PMCA1b (plasma membrane Ca^{2+}-dependent ATPase). The NCX1 exchanger internalizes three Na^{+} for one Ca^{2+}, while PMCA1b pumps Ca^{2+} out at the expense of ATP. In the intestine, PMCA1b represents the dominant pathway for Ca^{2+} extrusion. As with TRPV6, PMCA1b gene expression is positively regulated by VitD. PMCA1b is also positively regulated by estrogen, which may be critically important during pregnancy and lactation.

Three things should be noted about the TRPV system. First, in the intestine, both TRPV6 and TRPV5 exist. It is suggested however, that TRPV6 predominates in intestine, while TRPV5 predominates in kidney. Second, in kidney, TRPV5 expression is regulated by klotho, a transmembrane, multifunctional protein that exhibits beta-glucuronidase activity. Renal TRPV5 is glycosylated and constitutively active. De-glycosylation by membrane-associated klotho blocks TRPV5 turnover, retaining TRPV5 in the membrane and prolonging its activity. It is not known if the same phenomenon occurs in intestine, but this would seem a possibility. Finally, in kidney, parathyroid hormone (PTH) is known to positively regulate all of the molecules involved in cellular Ca^{2+} transport. The exact effects of PTH on intestinal molecules is uncertain, but presumably parallel those in kidney.

Phosphorus:

Phosphorus is remarkably abundant and derived from natural sources such as dairy products, cereals and meat, and unnatural sources such as carbonated beverages. Total daily intake varies, depending on the study, but a representative range is 1000 mg (in women) to 1500 mg (in men). Approximately 70% of dietary phosphorus is absorbed, principally in the jejunum. Again this occurs through one of two ways; a passive intercellular route and a facilitated transport intracellular route. Phosphorus absorption is described as being minimally regulated. At issue is whether most of the absorption is passive or facilitated.

Paracellular/intercellular transport is favored electrochemically because the phosphorus concentration of the intestinal lumen exceeds that of the extracellular fluid underlying the epithelium, and the extracellular fluid is electropositive relative to the intestinal lumen. In counterpoint, intercellular junctions are highly impermeable to phosphate ions, and this seems to be the overriding consideration. Thus, on balance, paracellular transport appears to contribute only modestly to phosphate absorption.[2]

Transcellular/facilitated diffusion is a function of at least three components; a luminal Na^+/P_i co-transporter, a basolateral Na^+/K^+ ATPase, and an as yet unidentified, but hypothesized, basolateral P_i transporter (Figure 1B).

The Na^+/P_i transporter (or NPT2b) is an 80 kDa, 8-transmembrane domain protein that simultaneously transports one Na^+ and one P_i ion into the cell. Once internalized, P_i exits the cell on the basolateral side. NPT2b is reported to be positively regulated by VitD, and NPT2b likely responds to differences in intracellular Na^+ concentration. The exact role VitD plays is not clear. While it seems to increase NPT2b expression or "activity," it must act at a posttranscriptional level given the NPT2b gene has no VitD response element. It may indirectly impact NPT2b by promoting the activity of the Na^+/K^+ ATPase, reducing intracellular Na^+ and pulling in luminal Na^+ accompanied by P_i. Another molecule reported to increase P_i uptake is STC-1/stanniocalcin-1. STC-1 is a secreted, dimericphosphoglycoprotein that is made in kidney. The mechanism of uptake is unclear, although it undoubtedly involves recently discovered STC receptor(s). STC-2, a related protein, has been shown to regulate NPT2a expression in the kidney.

Non-hormonal factors that impact both Ca^{2+} and P_i absorption include high luminal levels of both Ca^{2+} and P_i that generate insoluble CaHPO4 complexes, and the use of antacids that contain aluminum, which renders P_i unabsorbable.

Serum Calcium & Phosphorus:

Once absorbed, both Ca^{2+} and phosphorus circulate in multiple forms. Approximately 50% of serum Ca^{2+} is freely ionized, while 45% is bound to

protein and 5% exists in poorly-defined complexes. Phosphorus is either inorganic (30%) or organic (70%). Of the 30% inorganic phosphorus, 10% is ionically bound to protein (and thus is not filtered by the kidney), while 90% is ionic and freely filtered by the kidney. Within the "ionic 90%," approximately 5% exists as a divalent phosphate salt (Mg or Ca), 30% exists as a Na^{+} salt, and 65% is free phosphate ion. Approximately 80% of free phosphate ion is HPO_4^{-2}, while 20% is $H_2PO_4^{-1}$; thus, the designation "P_i" is generally taken to mean HPO_4^{-2}. Organic phosphorus may take many forms including phospholipids, phosphate esters, phosphoproteins, phosphonucleotides, etc

Molecules Regulating Calcium & Phosphorus Metabolism

Vitamin D:

A number of hormones circulate that impact Ca^{2+} and P_i metabolism. The first is $1,25(OH)_2$ vitamin D3 (VitD). The precursor for VitD, 7-dehydrocholesterol, occurs naturally in basal keratinocytes. It is the last step in the synthesis of cholesterol (Figure 2). 7-dehydrocholesterol (7DHC; also known as provitamin D), in the presence of sterol D7-reductase, forms cholesterol. Following exposure to UV-B radiation (290-319 nm), 7DHC is cleaved in its B ring and undergoes spontaneous isomerization to form vitamin

D3. This is bio-inactive, but will bind to endothelia-produced, 53 kDa vitamin D-binding protein (VDBP). Each VDBP-vitamin D3 complex transits first to the liver, where -OH is added at position #25, and then to the kidney, where a second -OH is added at position #1. While 1,25 $(OH)_2$ vitamin D3/VitD is considered the active form, 25(OH) vitamin D3 is also suggested to have select bioactivity, particularly in promoting Ca^{2+} uptake in intestine. The second, or position #1 hydroxylation, is performed by 1 alpha-hydroxylase, an enzyme that shows considerable regulation by a number of factors. PTH, IFN-gamma, and IGF-I increase 1 alpha-hydroxylase activity, while Ca^{2+}, P_i and klotho depress 1 alpha-hydroxylase activity.

The most notable aspect of the second, or kidney-based hydroxylation, is the circuitous route taken by the VDBP-25(OH) vitamin D3 complex. Rather than binding to basolateral VDBP receptor(s) on proximal tubule cells, it is first filtered through the glomerulus, and then binds to luminal, 550 kDamegalin on proximal tubule cells. This induces internalization with apparent complex dissociation. Newly freed 25(OH) vitamin D3 now binds to a new intracellular VDBP, termed IDBP-1, which directs it to mitochondrial CYP1/1 alpha-hydroxylase. Following its formation, VitD (1,25$(OH)_2$ vitamin D3) diffuses freely out of the cell to interact with either the 48 kDa vitamin D receptor/VDR or an incompletely characterized 60 kDa membrane receptor that induces rapid, non-transcriptional responses in cells. Although VitD has significant effects on

bone and renal metabolism (see below), it also likely has the notable effect of down-regulating its own activity. It does so by up-regulating the activity of 24-hydroxylase, an enzyme that replaces the hydroxide at the #1 position with a hydroxide at position #24, inactivating the vitamin.

Simply put, it maintains serum Ca^{2+} concentrations in the normal range. Basically by up-regulating elements associated with the intestinal Ca^{2+} absorption process such as TRPV6 and calbindin-D9K. It does play a role in immunity, reproduction, and phosphate metabolism, and it does have a complex relationship with other crucial hormones associated with bone metabolism. But, in summary, its target is intestinal.

It is said that four hours of intense sun exposure per week on either the face or upper extremeties will generate adequate vitamin D3 levels. During winter, or under sunless conditions, either nutritional supplements, fatty fish, or also fortified milk are required to supply needed vitamin D3. Dietary vitamin D3 is absorbed by the gut, transported to the liver by chylomicrons, and either stored in fat or converted to 25(OH) vitamin D3. Nutritional supplements may contain either vitamin D2 or vitamin D3. The difference is only in the source (D2/ergocalciferol from plants; D3/calciferol from animals). Both are convertible into active $1,25(OH)_2$ vitamin D. Although vitamin D supplementation is often recommended for "healthy bones," some studies strongly recommend a dual supplement composed of vitamin D2/3 and vitamin

K. As will be shown later, adequate Ca^{2+} absorption is only part of the story. It must also be successfully incorporated into bone mineral, a process strongly impacted by vitamin K.

Parathyroid Hormone (PTH):

PTH, or parathyroid hormone, is a 9.4 kDa polypeptide product of the Chief cells of the parathyroid gland (Figure 3). In contrast to VitD, which insures adequate total body Ca^{2+} stores, PTH regulates the distribution of total body Ca^{2+}. Its release results in a rapid mobilization of Ca^{2+} from bone. As such, it is the principal regulator of minute-to-minute circulating Ca^{2+} levels, and its secretion is quite sensitive to prevailing Ca^{2+} concentration. The receptor for circulating Ca^{2+} is a 140 kDa, 7-transmembrane domain receptor (CaSR) that, when activated, represses PTH release from Chief cells. The CaSR is suggested to easily detect a 200 μM fluctuation in extracellular Ca^{2+}. When circulating levels fall below a threshold, CaSR signaling is reduced and PTH is released.

When released, PTH would seem to exist in a bewildering number of isoforms. It is initially synthesized as a 115 amino acid (aa) prepropeptide that contains a 25 aa signal sequence and a six aa N-terminal pro-segment. The C-terminal 84 aa make up the mature, circulating form of PTH. Only the first 34 aa of the mature polypeptide are necessary for bioactivity, and this fact serves as

the basis for PTH pharmacological analogs. Normally, 20% of circulating PTH is full-length, while 80% shows some N-terminal truncation (C-PTH). There are fragments that start at aa position # 4, 7, 8, 10, 15, 34 35, 37, 41, and 43, and perhaps more differing at the C-terminus. All seem to be targets of proteases such as cathepsins. Some are generated by Chief cells and some by hepatocytes. Cleavage of the first six aa appears to render the molecules inactive toward the PTH receptor (PTH1R). Remarkably, N-terminally truncated PTH molecules seem to have their own receptor, currently referred to as C-PTHR. It has yet to be characterized. C-PTHs often show activity antagonistic (or anti-calcemic) to that of PTH. The ratio of full-length to truncated forms varies with the ambient level of Ca^{2+}. At low Ca^{2+} concentrations, additional Ca^{2+} is needed from mineral stores and full-length PTH represents 30%-40% of total PTH (~18 pM). By contrast, under high Ca^{2+} conditions, total PTH falls to 5 pM and full-length PTH only represents 5% of this amount. In addition to Ca^{2+}-induced variability, PTH shows a circadian rhythm. There is a 30% baseline difference between peak (10 PM-3 AM) and trough (10 AM-Noon) release. Notably, osteoporosis patients seem to lose this rhythm.

PTH1R/PTHR1, the receptor for PTH, is a 7-transmembrane domain G protein-coupled receptor (GPCR) found on select cell types, including osteoblasts, osteoclasts, hematopoietic stem cells, and renal tubule cells (proximal and distal epithelium). As noted above, there is also a hypothesized

receptor for C-PTH. This receptor is apparently highly expressed by osteoblasts and osteocytes, and when ligated increases intracellular Ca^{2+} but not cAMP. Functional outcomes attributed to C-PTHR include a decrease in circulating Ca^{2+} and P_i, the promotion of bone formation, and an increase in osteoclast formation and activity.

PTH, by definition, is a normo-calcemic hormone. That is, it exists to maintain blood/extracellular fluid Ca^{2+} levels within a narrow range. It does so by inducing Ca^{2+} release from bone, reducing Ca^{2+} loss through the urine, and promoting VitD production through the upregulation of renal 1a-hydroxylase. It has an indirect, but important effect on phosphorus. By promoting phosphorus excretion and thereby reducing overall phosphorus load, PTH fulfills its principle function, facilitation of Ca^{2+} release from bone.

FGF-23:

Fibroblast growth factor-23 (FGF-23) is the newest member of a diverse and large FGF family of proteins. As with other members, FGF-23 shows a typical beta-trefoil structure. Unlike most members, however, FGF-23 contains a signal sequence, an atypical intrachain disulfide bond, and an extended C-terminus/pro-segment. This places it in the small FGF-19 subfamily. FGF-23 is a 30 kDa, secreted glycoprotein that undergoes post-translational processing. Following removal of the signal sequence and pro-segment between

Arg179 and Ser180, mature FGF-23 is generated that is 155 aa in length. This mature form, however, is bio-inactive; it appears that the C-terminal pro-segment is essential for bioactivity. It is believed to be synthesized by osteoblasts in response to VitD. Although its receptor was unknown for some time, it now appears to bind to FGF R1c, 2c, 3c, and FGF R4. It is reported that klotho, with its associated carbohydrate moiety, is likely to be the physiological co-receptor for FGF-23.

Full-length FGF-23 is considered to be a phosphatonin. Among other things, phosphatonins decrease plasma phosphate by promoting phosphate excretion. They are considered analogous to calcitonin, which decreases serum Ca^{2+} levels. FGF-23 has two principal actions: promoting phosphate excretion in the urine and suppressing VitD synthesis. Its effect on phosphate resorption is mediated by its ability to downregulate phosphorus transporters on the luminal side of renal epithelium. Its effect on VitD synthesis is mediated by blocking 1a-hydroxylase activity in kidney.

The relationships between VitD, PTH, and FGF-23 are complex and perhaps not intuitive. It could be said that PTH regulates the Ca^{2+}-VitD axis, while FGF-23 regulates the P_i-VitD axis. One model suggests that under conditions of low circulating/extracellular fluid Ca^{2+} (a simple Ca^{2+} deficiency), PTH is released from Chief cells. This withdraws Ca^{2+} from the bone for a short-term effect. It also induces 1 alpha-hydroxylase activity in kidney to

create active VitD. VitD does two important things. First, it promotes Ca^{2+} absorption, leading to an increase in total body stores. Second, it increases intestinal phosphorus absorption, presumably for the purpose of providing the mineral counterpart to Ca^{2+} needed during mineralization (hydroxyapatite/$Ca^{2+}{}_{10}(PO_4)_6(OH)_2$). Under the conditions of simple Ca^{2+} deficiency, there is no concomitant phosphorus deficit. With increased VitD activity, however, phosphorus is now in excess. PTH, in the short term, can influence P_i excretion in a manner identical to that of FGF-23; that is, promote excretion rather than reabsorption. On balance, this should eliminate excess phosphorus, but with an increase in circulating Ca^{2+} due to VitD-mediated absorption, we know PTH release comes to a halt due to the Ca^{2+}-CaSR actions on Chief cells. What is needed longer-term is an additional phosphatonin that will bring phosphorus levels back to normal. That molecule is FGF-23 (and perhaps MEPE and/or sFRP-4). In order to "catch up" with continuous Ca^{2+} and P_i absorption, FGF-23 will downregulate 1a-hydroxylase activity and upregulate 24-hydroxylase activity, thereby removing the stimulus for excess phosphorus uptake.

Although FGF-23 seems to be made by osteoblasts, what induces its expression? It would appear that VitD induces its expression.It has also been suggested that circulating phosphorus drives FGF-23 release, particularly since

FGF-23 is supposed to protect against excess phosphorus; i.e.-stimulus-response.Indeed, extracellular P_i by itself may independently upregulate a number of genes, including $beta_5$ integrin, STAT5, and osteopontin, and an upregulation of the FGF-23 gene would be consistent with this observation. However, in healthy subjects phosphorus intake does not influence FGF-23 levels. High phosphorus diets in renally uncompromised individuals does lead to increased excretion, but without a change in FGF-23. Curiously, in rats a high phosphorus diet does lead to increased serum phosphorus and a subsequent increase in FGF-23. In mice, dietary phosphorus is also reported to affect FGF-23 synthesis. Thus, the system may show some species specificity.

Calcitonin:

Calcitonin is a 3 kDa, 32 aa peptide that belongs to the calcitonin gene-related peptide family. It is made by C cells of the thyroid gland purportedly in response to elevated blood Ca^{2+} levels. It is synthesized as a 141 aaprepreproprecursor that is processed into a 32 aa mature peptide. Calcitonin has a potent inhibitory action on osteoclasts mediated by its GPCR, termed the calcitonin R. The molecule seems to have an ontogenic component to it, as it is highly active in the young of species and loses its potency with age. In adult humans, it may act as a stress-related molecule.

Soluble Frizzled-related Protein-4 (sFRP-4):

sFRP-4 is a member of a small family of secreted proteins that structurally resemble the extracellular domain of the frizzled family of receptors. The mature sFRP-4 molecule is 328 aa in length, contains a 120 aa frizzled/cysteine-rich domain, and a 100 aanetrin-like region. In rat, the molecule is highly spliced, with variants occurring at the C-terminus. Similar variants may exist in human. sFRP-4, like other sFRPs, binds to both Wnt ligands and frizzled receptors-1 and -4, and the family is generally acknowledged to be inhibitory to Wnt signaling. There may be additional activities. For instance, sFRP1 is reported to bind to RANK L, an inducer of osteoclast formation.

Like FGF-23, sFRP-4 is reportedly expressed by osteoblasts and unidentified cells in kidney. sFRP-4 is also considered to be a phosphatonin. FGF-23 induces internalization of renal phosphate transporters, likely through FGF R signaling. sFRP-4, however, seems to antagonize Wnt signaling, not initiate it. Although highly conjectural, there are at least three possible mechanisms of action. First, FGF-23, when present as a full-length molecule, shows phosphatonin activity. When cleaved into mature N- and C-termini, it loses its activity. It has been suggested that a matrix-metalloproteinase (MMP)-like molecule is responsible for cleavage, and MMP-inhibition by a tissue inhibitor of metalloproteinase (TIMP) would ensure

continuing FGF-23 activity. sFRP-4 has a TIMP-like domain and has the theoretical potential to neutralize MMPs, thereby, guaranteeing the integrity of FGF-23 and promoting its phosphatonin activity. It should be noted that furin-type convertases have also been reported to cleave FGF-23. Second, an absence of Wnt signaling in osteoblasts can lead to apoptosis. Since osteoblasts initiate bone formation, a reduction in osteoblast number would translate into a reduction in mineralization rate that would be accompanied by a reduced need for phosphate. This would translate into a reduction in kidney P_i resorption due to reduced demand. Finally, sFRP-4 inhibits VitD production via 1a-hydroxylase. Since VitD is associated with increased P_i uptake, removal of the VitD effect would translate into increased P_i excretion.

MEPE (Matrix Extracellular Phosphoglycoprotein):

MEPE is a third phosphatonin. It is a 45-65 kDa, secreted glycoprotein that belongs to the SIBLING (short integrin-binding ligand interacting glycoprotein) family of molecules. The mature molecule is 508 aa in length, serine-rich, contains an RGD motif for cell attachment, and a C-terminal Ser-Asp-Gly-Asp motif associated with glycosaminoglycans. MEPE is synthesized by osteoblasts and osteocytes, particularly during mineralization. It is associated with the extracellular matrix (ECM) and with PHEX, a type II transmembrane metalloproteinase on the surface of osteoblasts. PHEX has no proteolytic activity on MEPE, but instead protects MEPE from cleavage by cysteine-

proteases such as cathepsin B. This is likely due to PHEX acting as a pseudosubstrate for cathepsin(s). When cathepsins have functional access to MEPE, they cleave the molecule between Arg507 and Asp508, generating an 18 aa C-terminal peptide called ASARM. This peptide seems to perform two functions; an inhibition of mineralization and the promotion of urinary phosphate loss. The regulation of mineralization may be the principal function for MEPE (or its cleavage product). The phosphatonin effect, while material, may be complementary except under unregulated conditions.

OTHER MARKERS OF UNDETERMINED SIGNIFICANCE:

OsteocalcinorGLAprotein(proteincontainingγcarboxyglutamicacid)is the most abundant noncollagenic protein of the bone matrix. The physiological roleofosteocalcinisstillnotcompletelyunderstood.Osteocalcinsurelyplaysan important role in bone formation, perhaps favoring or preventing it. It was generally thought that osteocalcin stimulates bone formation, although recent

studies have shown an impressive augmentation of bone formation and bone density in transgenic animals lacking its gene. This has therefore led to the provoking hypothesis that osteocalcin might actually be an inhibitor of bone formation. The synthesis of osteocalcin by mature osteoblastic cells is probably one of the signals used by PTH to slow osteoblastic activity and thereby bone formation

Numerous studies have shown the degree of interest in the use of plasma osteocalcin measurements in several metabolic bone disease. It has always been considered to be a useful marker of the rate of bone formation. However, its use inthecontextofrenalosteodystrophyisstillhamperedbyseveralproblems.First, many osteocalcin fragments of yet unknown function are retained in the plasma of uremic patients. Second, at least three forms of intact osteocalcin can be measuredintheplasma:total,carboxylated,anddecarboxylated.Third,theintact molecule is rapidly degraded at room temperature. Thus, the concentration measureddependsonthecharacteristicsoftheantibodiesusedintheassay.Many oftheseantibodiesrecognizetheintactmoleculebutalsosomeofthefragments. As already mentioned, osteocalcin fragments are also liberated during bone matrix degradation. It is possible that in the future there could be one or more of these fragments measurable in the plasma that is specific for bone resorption. Finally, perhaps because of the limitations previously described, there is a great intra-analysis variability in the dosage of OC with most of the current assays. In

patients with chronic renal failure, circulating intact osteocalcin represents 26% of total osteocalcin. The remaining 74% comprises mainly four fragments: N-terminal, midregion, midregion C-terminal, and C-terminal..

Using the ELSA-OSTEO IRMA kit (Cis Bio Int.), which uses two antibodies, one recognizing the end N-terminal fragment and the other recognizing a midregion amino acid sequence, we and others have shown osteocalcin values four to six Z-scores higher in hemodialysis patients than in normal individuals. In spite of this accumulation, the plasma osteocalcin concentration demonstrated good sensitivity in making the distinction between patients with hyperparathyroidism and those with normal or low bone turnover. The values were 555 versus 198 ng/ml, respectively. As with otherbiochemicalmarkers, diagnostic sensitivity was low when the aim was to differentiate patients with adynamic bone disease from those with normal bone turnover. Although weaker than bAP and iPTH, the correlations of plasma osteocalcin with bone histomorphometric parameters in hemodialysis patients were quite good. The results of these studies demonstrate the limitation of the use of plasma osteocalcin as a biochemical marker of bone remodeling in patients with impaired renal function and in patients under hemodialysis treatment. Obviously, a clear understanding of the physiological role of osteocalcin and its fragments is still lacking. The development of new assays will certainly increase its sensitivity in the evaluation of renal

osteodystrophy.

PYRIDINOLINES:

In the last two decades, intense research has focused on the development of methods capable of accurately assessing urinary and plasma concentrations of PYD and DPD, as well those found in a diversity of tissues including bone, cartilage, tendon, dentin, skin, cornea, and vessels.The first methods were based on conventional chromatography after borohydride reduction of collagen cross- links, high-performance liquid chromatography (HPLC) using aminopropyl- silica column and radioactivity monitoring, followed by HPLC and fluorescence detection techniques. Last, reproducible results have been obtained using reversed-phase, ion-pair quantitative HPLC (abstract; Robins *et al*, ibid). Certainly,HPLC-based techniques are highly quantitative and reliable;however, they are too time consuming and cumbersome to become a routine clinical application.

Fortunately, specific polyclonal and monoclonal antibodies have recently been successfully developed for the direct measurement of PYD and DPD (abstract; Robins *et al*, ibid). The concentrations of PYD and DPD measured by ELISA methods are usually 4to10 times lower than the value obtained by HPLC. Nevertheless, the values obtained with either method are excellently correlated (r = 0.85 to 0.90). In addition, some authors have even

reported that the measurement of DPD by ELISA is more reliable than by HPLC. Moreover, measurement of PYD and DPD, released after hydrolysis of the urine sample,either by HPLC or ELISA, has been shown to serve as an excellent marker of bone resorption rate in malnourished children, osteoporotic and postmenopausal women, primary and secondary hyperparathyroidism, rheumatoid arthritis and osteoarthritis, Paget's disease, acromegaly, hyperthyroidism, in patients with tumor-associated hypercalcemia, and in uremic patients. In addition, an Italian group has recently demonstrated the increase in urinary excretion of PYD and DPD in normocalcemic patients with kidney transplantation was more sensitive than plasma measurement of iPTH, bAP, and tAP for the diagnosis of secondary hyperparathyroidism.Inanotherstudydealingwithpatientstreatedbyperitoneal dialysis, PYD and DPD concentrations in serum, urine, and dialysis fluid were well correlated with plasma iPTH and bAP levels. Moreover, the authors observed that circulating PYD and DPD levels progressively increased with the duration of renal failure and with the time spent on peritoneal dialysis.

TARTARATE RESISTANT ACID PHOSPHATASES:

In some studies, the amount and the activity of TRAP seems to correlate with bone resorption rate (abstract; Lam *et al*, *ClinChem*30:457, 1984). However, few studies have been performed in uremic patients. A decade ago, a

correlation between serum TRAP and BAP levels was demonstrated using electrophoreticmethods.Anothergroup,usinganenzymaticmethod,hasrecently shownacorrelationbetweenserumTRAPactivityandthenumberofosteoclasts andthepercentageoferodedbonesurface(abstract;Malluche*etal,JAmS Nephrol*2:337, 1991). Similarly, serum TRAP appears to correlate with plasma tAP and iPTH.

Specific antibodies against TRAP have been successfully developed and appliedinanELISA.WiththismethodelevatedlevelsofTRAPhavebeenfound in the serum of patients with primary hyperparathyroidism. Surprisingly, however, it was found that normal subjects had measurable amounts of serum TRAP. It is likely, therefore, that the specificity of this ELISA assay may not be perfect,inthatitmightberecognizingotheracidphosphatasesorthesameTRAP produced by other cells. Significant TRAP activity has recently been detected in osteoblasts andosteocytes.

To date, the value of serum TRAP measurement in uremic hemodialysis patients still remains to be established. The sensitivity of enzymatic methods and the few available immunological assays are still low. Certainly, progress in understanding its physiological role as well as its biochemistry and immunochemistry should lead to the development of more sensitiveassays.(77)

PROCOLLAGEN TYPE I CARBOXY-TERMINAL EXTENSION PEPTIDE:

Nondialyzed patients with chronic renal failure have significantly increasedplasmaPICPlevels.However,thisincreasedoesnotcorrelatewith other humoral markers of bone turnover or with bonehistomorphometricalaparameters. In the same study, it was observed that patients receiving $1,25OH_2D_3$ had higher plasma PICP levels than patients without treatment. In patients already treated by hemodialysis, the results are contradictory. Some workers have found that plasma PICP concentration provides useful information regarding the degree of bone formation. They also observed that PICP levels correlate quite well with plasma bAP, osteocalcin, iPTH, and boneformationrate (BFR).

By analogy with plasma bAP, PICP levels increased significantly during the first two weeks after surgical PTX, probably as a consequence of diminished osteoclast activity following the reduction of iPTH levels and an increase in mineral deposition. However, our own observations have not demonstrated any great value of plasma PICP in the diagnosis of the type of bone remodeling in hemodialysis patients. Additional studies remain to be performed in order to define its value in renal osteodystrophy.

PROCOLLAGEN TYPE I CROSS-LINKED CARBOXY- TERMINALTELOPEPTIDE:

Several observations have demonstrated a good correlation between plasma ICTP and histomorphometric parameters of bone resorption in patients withadiversityofmetabolicbonediseases.However,otherclinicalstudieshave notsupportedthismethodasaspecificindicatorofboneresorptionrate.Inthe caseofpatientswithchronicrenalfailure,ICTPtendstoaccumulateintheserum with the decline in renal function and even further in hemodialysis patients. The few studies performed in hemodialysis patients have not supported its use as a humoral marker of bone remodeling.

β2-MICROGLOBULIN, ADVANCED END-GLYCATION PRODUCTS, AND BONE SIALOPROTEIN:

β-2Microglobulin(β_2m)accumulatesintheserumofchronicrenalpatients andcanleadtoβ_2m-relatedamyloiddepositsinosteoarticulartissues.Inaddition to being an indicator of an inflammatory state, it has been suggested that β_2m couldserveasaboneremodelingmarkeraswell.Serum$\beta_2$mconcentrationshave been found to correlate with serum TRAPin postmenopausal women. Moreover,inarecentstudy,wehaveobservedthatpatientswithseveresecondary hyperparathyroidism showed significantly higher serum β_2m levels than

patients with normal bone turnover. In addition, serum β_2m correlated with serum bAP, PYD, and osteocalcinlevels.

Regarding AGEs, these also have a tendency to accumulate in the serum of uremic patients. Recent studies have shown that AGEs were able to stimulate bone resorption probably through the stimulation of IL-6production.Because IL- 6 is one important mediator of the action of PTH on bone cells, it is possible that AGE scould influence bone metabolism in hemodialysis patients.Finally,bone sialoprotein, a glycoprotein of the bone matrix with a molecular weight of 70 to 80 kDa, has been shown to be a bone indicator of bone resorption in cancer patients and in menopausal women.

SCLEROSTIN:

One of the earliest changes in response to renal injury is the increased osteocyte production of secreted factors including the anti-anabolic protein, sclerostin.ElevatedsclerostinisassociatedwithreducedWnt/β-cateninsignaling in bone and decreased osteoblast differentiation/activity. Agents that directly or indirectly inhibit β-catenin signaling have differential skeletal effects suggesting additional mechanisms contribute to the diversity of renal osteodystrophies.

OSTEOPOROSIS:

Osteoporosis is a disease that is characterized by low bone mass, deterioration of bone tissue, and disruption of bone microarchitecture: it can lead to compromised bone strength and an increase in the risk of fractures .

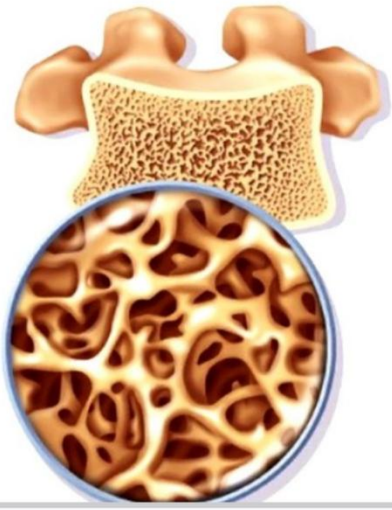
Osteoporosis is the most common bone disease in humans, representing a major public health problem. It is more common in Caucasians, women, and older people. Osteoporosis is a risk factor for fracture just as hypertension is for stroke. Osteoporosis affects an enormous number of people, of both sexes and all races, and its prevalence will increase as the population ages. It is a silent disease until fractures occur, which causes important secondary health problems and even death

There are some other factors that increase fracture risk and osteoporosis, independent of bone mineral density (BMD) :

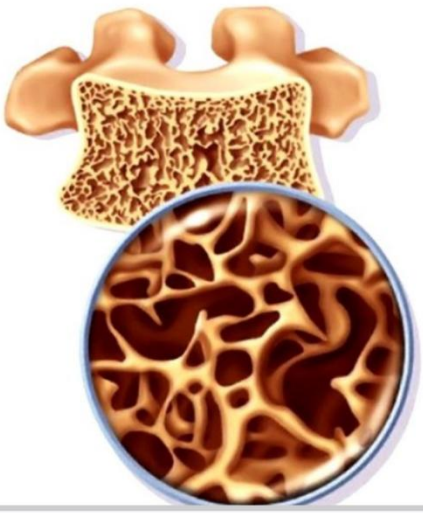
- - Age of the patient
- - A low body mass index (BMI<21 kg/m^2) is a significant risk factor for hip fracture .
- - A history of a previous osteoporotic fracture is another important factor for further fracture risk and almost doubles the risk of spinal fractures.
- - Parental history of hip fracture
- - Smoking

- - Oral glucocorticoids ≥5 mg/d of prednisone for >3 months (ever)
- - There is a dose-dependent relationship between alcohol intake and fracture risk. Daily intake of 3 or more units of alcohol is associated with fracture risk
- - Rheumatoid arthritis increases fracture risk independently of BMD, as well as the use of glucocorticoids
- - Falls are an important risk factor for osteoporotic fractures

NORMAL BONE



OSTEOPOROSIS



STUDIES OF FRACTURE RISK ASSOCIATED WITH CKD:-

Studies of fracture risk associated with CKD

Study	Definition of kidney function	Fracture site	Fracture risk (95% CI)
Dukas*et al.* (2005)	<65 ml/min	Hip	OR 1.57 (1.18–2.09)
		Wrist	OR 1.79 (1.39–2.31)
		Vertebral	OR 1.31 (1.19–1.55)
Nickolas *et al.* (2006)	<59 ml/min	Hip	OR 2.32 (1.13–4.74)
Ensrud*et al.* (2007)	45–59 ml/min	Hip	HR 1.24 (0.60–2.56)
	<45 ml/min		HR 1.41 (0.59–3.36)
	45–59 ml/min	Trochanteric	HR 3.69 (1.21–11.24)
	<45 ml/min		HR 5.04

Study	Definition of kidney function	Fracture site	Fracture risk (95% CI)
			(1.38–18.45)
Jamal *et al.* (2007)	<45 ml/min	Any fracture	OR 1.3 (1.0–1.6)
		Vertebral	OR 2.5 (1.6–3.9)
Fried *et al.* (2007)	<60 ml/min	Hip	HR 1.38 (0.99–1.94)
	Per s.d. increase in cystatin C	Hip	HR 1.16 (1.01–1.33)

CI, confidence interval; CKD, chronic kidney disease; HR, hazard ratio; OR, oddsratio.

METACARPOCORTICAL INDEX

Calculation of metacarpocorticalindex(MCI), can be used as a easy and simple reliable method of predicting Renal osteodystrophy in CRF patient.

CALCULATION OF MCI:

X-ray right hand – AP view should be taken.

MCI= Lateral + Medial cortical thickness at midpoint of second metacarpal/

Total thickness at midpoint of second metacarpal.

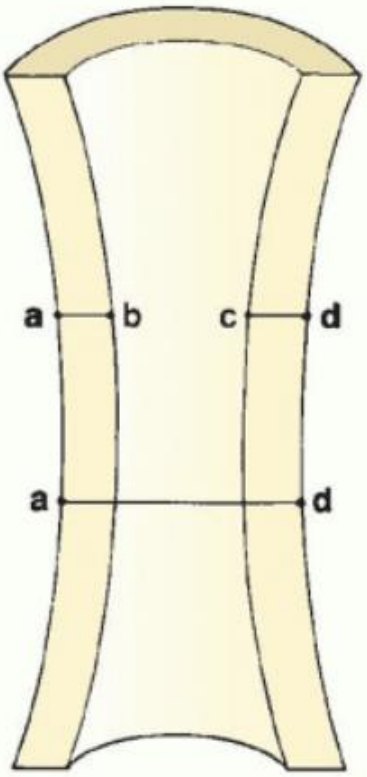
It is helpful in preventing dreadful complication of CKD as it predicts Renalosteodystrophy as early in asymptomatic stage and helps in early intervention and treatment.

The mineral bone disease in chronic kidney disease is usually diagnosed based on Indirect evaluation by radiologic and biochemical values. Trabecular bone structure evaluation by Radiologic method is often difficult. On the other hand, cortical bone permits radiologic evaluation of bone resorption. The width of bone marrow cavity is increased in endosteal bone resorption and measurement of the width may indicate degree of resorption. Cortical thickness is reduced by periosteal resorption. The bone mass changes by endosteal or subperiostealresorption in chronic kidney disease patients can be assessed by cortical bone mass measurement. Metacarpal bone mass measurement radiologically is an easy procedure for quantitating the cortical

bone mass. It is very effective in documenting changes in bone mass during hemodialysis and in transplant patients.

In 1975, Horsemen and simpson first described the measurement of metacarpal cortical thickness, which was very useful in assessing the loss of bone. On this Non-Invasive modality, only very few studies available till now. In UK, a study done in 1978 which shows metacarpal thickness was done in CKD patients by measuring at mid point of index, middle and ring metacarpals. The results were not significant in that study. Adams et al (1970) found that this measurement had no help in predicting the fracture risk. Another study was done in 1981, for evaluating the bone loss in Chronic Kidney disease patients. Similar study was done in 2015, found significant association of calcium, uric acid, phosphate, alkaline phosphatise (ALP) with metacarpal bone thickness and is helpful in predicting early changes in renal osteodystrophy.

Cortical-Thickness Measurement



$ab + cd$ = combined cortical thickness

$\frac{ab + cd}{ad}$ = index of bone mass

$ab + cd \cong \frac{ad}{2}$

(the sum of the cortices approximates one-half the bone's diameter)

MATERIALS AND METHODS

STUDY POPULATION :

This study was conducted on 50 patients of chronic renal failure admitted in Thanjavur medical college, thanjavur in the department of General medicine and Nephrology. A control group of 50 persons were studied.

INCLUSION CRITERIA:

STUDY GROUP:

- CRF of anycause
- Both male andfemale
- Age between 18 to 50 yrs in both male and female.

CONTROL GROUP:

- No evidence ofCRF
- Apparentlyhealthy
- Age between 18 to 50 yrs in both male and female.

EXCLUSION CRITERIA:

a) Acute renal failure

b) Bones changes other thanCRF

c) Rickets

d) Drug intake(steroids)

e) Patient refusal.

ANTICIPATED OUTCOME:

Right hand X-ray of second metacarpal bone for calculating MCI can predict quantitative bone changes which is useful in preventing complications of renal osteodystrophy (ex :fractures).

DATA COLLECTION:

A detailed medical history, clinical examination was collected and relevant laboratory investigations was done as indicated in each patient**.**

LABORATARY INVESTIGATIONS:

- Serumurea,
- serum creatinine
- serumcalcium,
- serum phosphorus,

- serum uricacid,
- serum alkaline phosphatase
- serum vitaminD3
- x ray AP view of right hand

DESIGN OF STUDY:

Hospital based cross sectional observational study

PERIOD OF STUDY: January 2018 to October 2019

COLLABORATING DEPARTMENTS:

Department of Radiology

Department of Biochemistry

Department of Nephrology

ETHICAL CLEARANCE:

Obtained (certificate enclosed)

CONSENT: Informed and written consent obtained

ANALYSIS: STATISTICAL ANALYSIS

CONFLICT OF INTEREST: NIL

FINANCIAL SUPPORT: NIL.

STATISTICAL ANALYSIS

The data were coded and entered in MS-excel office 2010. The data were analyzed using Graph Pad Prism version 5. The categorical data were represented as n(%) and numerical data in mean with SD. Fisher's exact test was used to compare the proportions between the groups for sample less than 30.Unpaired 't' test was used to compare the means between the two groups. One-way ANOVA with Tukey's post hoc test was used to compare the means for more than two groups. Pearson's correlation was used to find the degree of correlation between the parameters. $P<0.05$ was considered statistically significant.

RESULTS OF STUDY

A cross sectional prospective observational study was carried out in thanjavur medical college hospital from January 2018 to October 2019. 100 patients were included in the study after an informed and written consent was obtained. Patients included in study were divided into two groups. Study group were people with chronic renal failure age between 18 to 50 years in both male and female. Control group were people were apparently healthy individuals without chronic renal failure age between 18 to 50 years in both male and female. History and clinical examination was collected from each patients. Then X ray right hand AP view done . Relevant laboratory investigations were done. Metacarpocortical index was calculated from right hand X ray and it was statistically analyzed with other laboratory parameters and results were obtained. The statistical results of the study of the 100 patients were summarized as follows:

AGE DISTRIBUTION

Table1: Frequency distribution of age in various categories in the study.

S.No	Age in years	n	%
1	≤20 years	4	4
2	21 – 30 years	22	22
3	31 – 40 years	40	40
4	41 – 50 years	32	32
5	>50 years	2	2

Data are expressed in frequency (n) with proportions. The total sample (N) =100. The mean age in the study was 36.9 years with SD of 9.1 years and range of 19 to 55 years.

32% of patients were between 41-50 years, 40 % of patients were 31-40 years, 22% of patients were between 21-30 years and 4 % were less than 20 years.

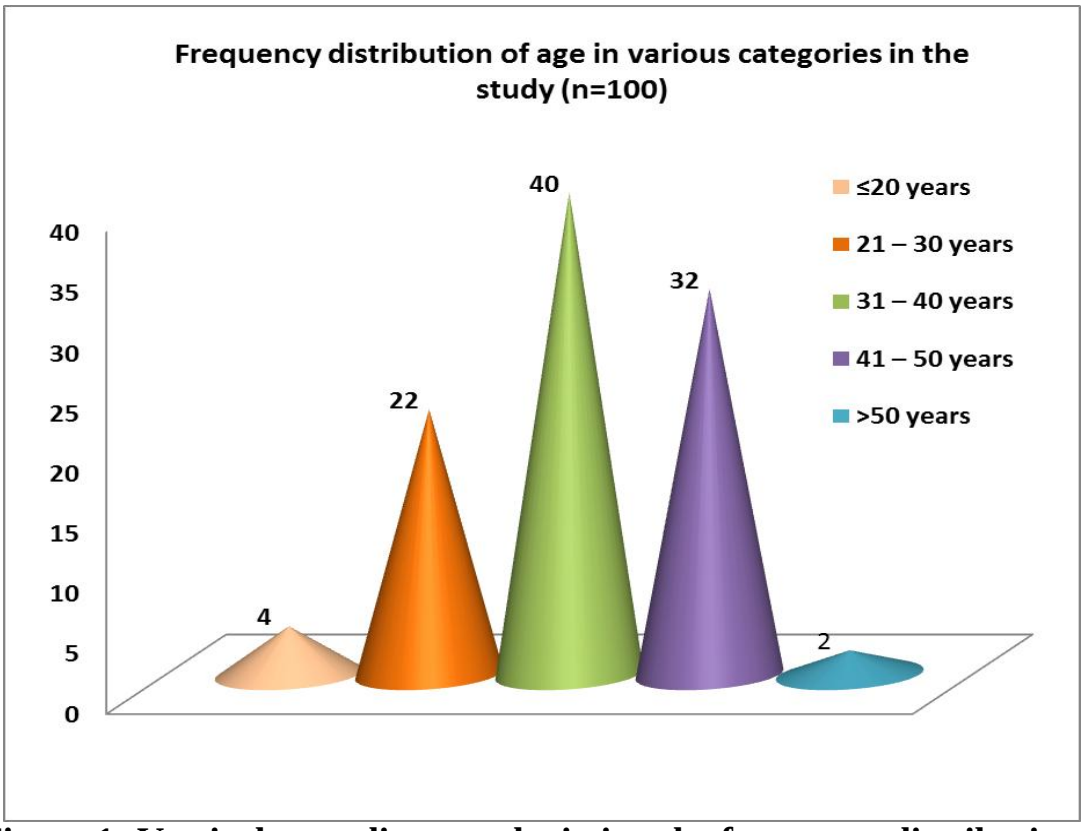


Figure 1: Vertical cone diagram depicting the frequency distribution of age in various categories in the study. Total N=100. Data represents n.

Majority of the population for study were contributed by people of age more than 40 years and people of less than 20 years contributed least.

SEX DISTRIBUTION

Table 2: Frequency distribution of gender in the study population.

S.No	Gender	n	%
1	Male	60	60
2	Female	40	40

Data are expressed in frequency (n) with proportions.

The total sample (N) = 100.

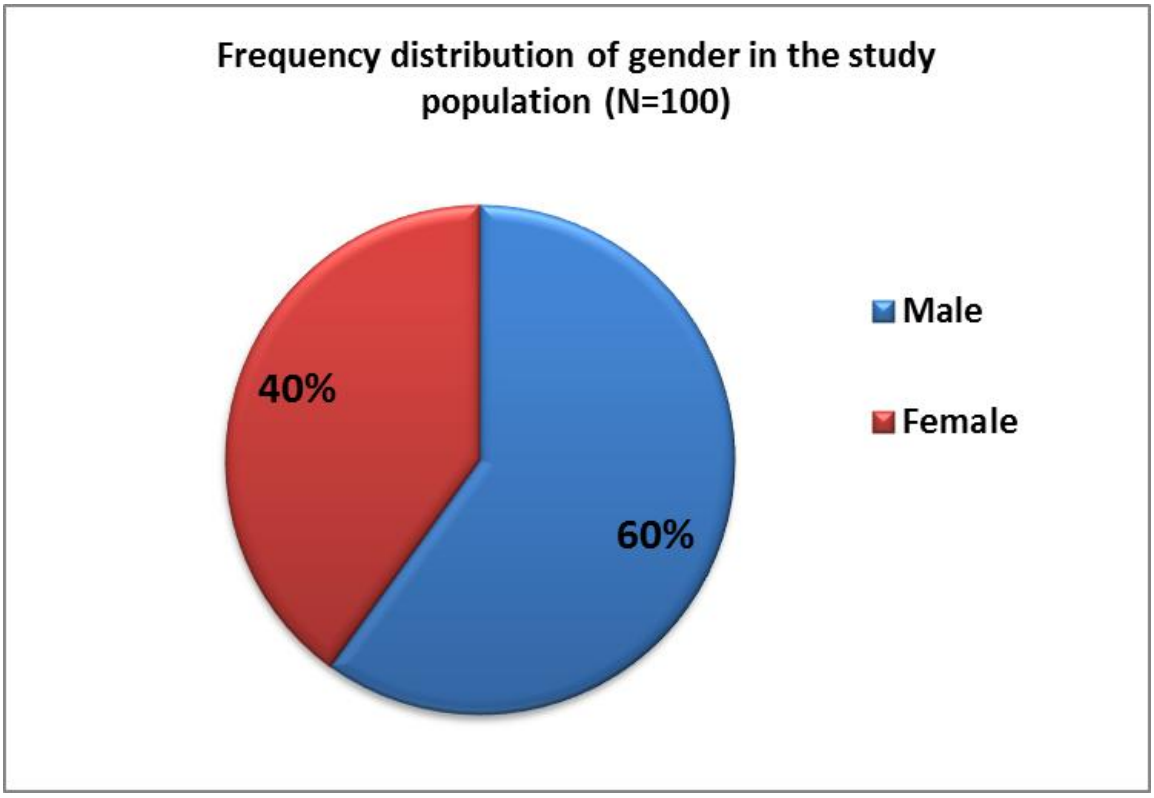


Figure 2.Pie chart depicting the frequency distribution of gender in the study population.

Males formed the major population of study.

Table 3. Comparison of age distribution between the case and control groups in the study.

S.No	Age in years	Case (n=50)		Control (n=50)		Chi-square value	df	P value
		n	%	n	%			
1	≤20 years	0	0	4	8	7.082	4	0.132 (NS)
2	21 – 30 years	0	0	2	4			
3	31 – 40 years	10	20	12	24			
4	41 – 50 years	22	44	18	36			
5	>50 years	18	36	14	28			

Data are expressed as n(%). Fisher's exact test was used to compare the proportions. NS= Not significant.

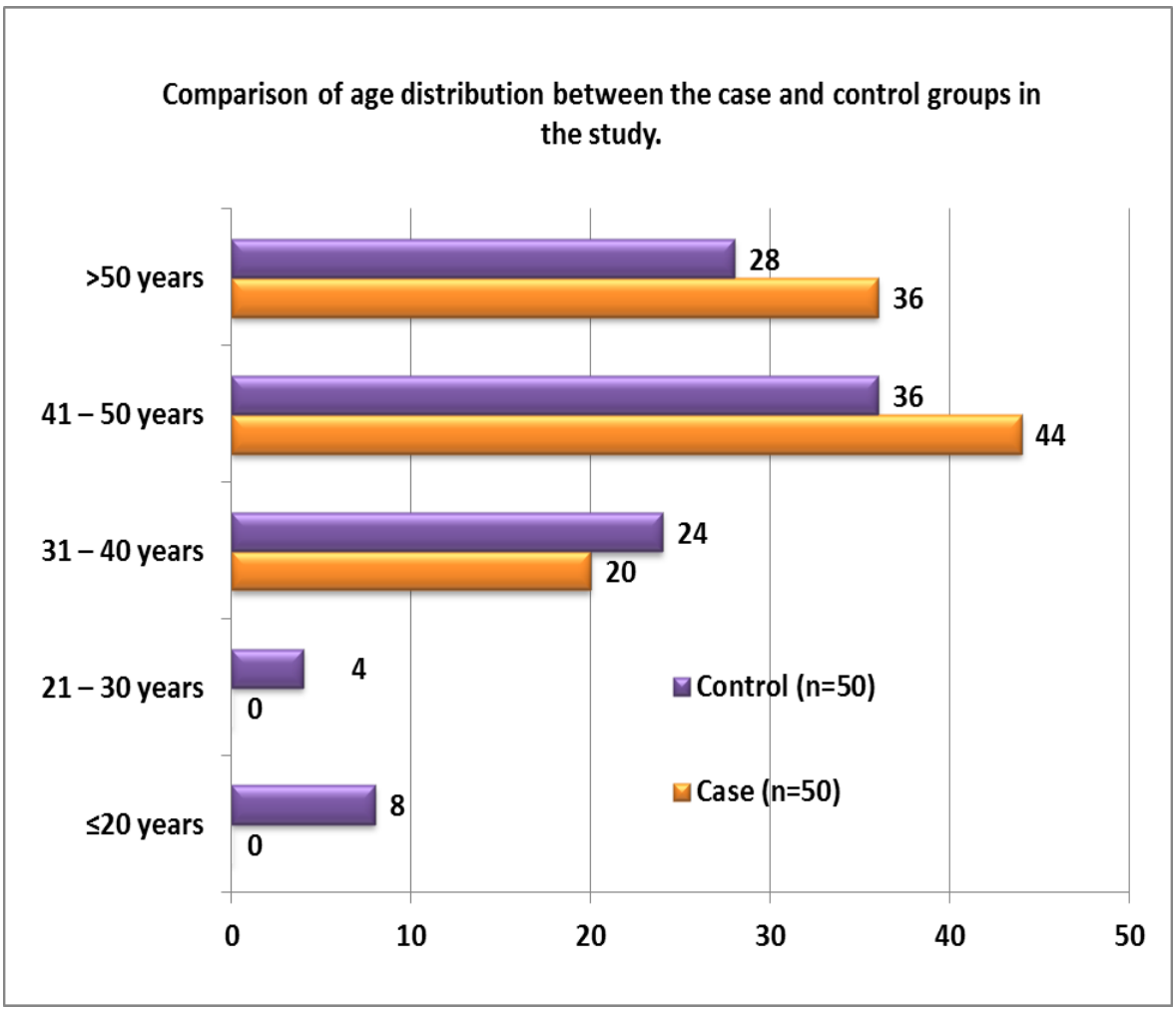


Figure 3. Horizontal bar diagram depicting the comparison of age distribution between the case and control groups in the study. Data represents %.

Table 4. Comparison of gender distribution between the case and control groups in the study.

S.No	Gender	Case (n=50)		Control (n=50)		Chi-square value	df	P value
		n	%	n	%			
1	Male	30	60	30	60	<0.001	1	>0.999 (NS)
2	Female	20	40	20	40			

Data are expressed as n(%). Fisher's exact test was used to compare the proportions. NS= Not significant.

AVERAGE MCI BETWEEN CASE GROUP AND CONTROL GROUP:

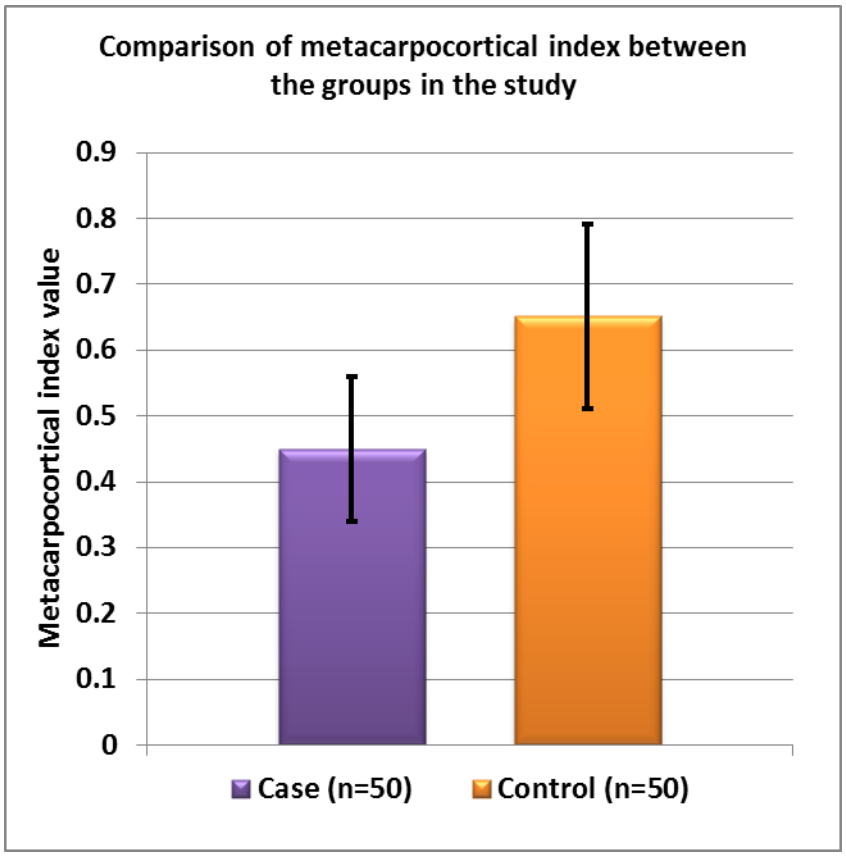


Figure 4. Vertical bar diagram depicting the comparison of metacarpocortical index between the groups in the study. Height of the bar represents the mean and error bar represents the SD.

Metacapocortical index in study group is 0.44

Metacapocortical index in study group is 0.65

Table 5.Comparison of metacarpocortical index between the groups in the study.

S. No	Parameter	Case (n=50)		Control (n=50)		t value	df	P value
		Mean	SD	Mean	SD			
1	Metacarpo-cortical index	0.449	0.11	0.651	0.14	7.674	98	<0.0001*

Data are expressed as mean with SD. Unpaired 't' test was used to compare the means between the groups. *indicates $p<0.05$ and considered statistically significant.

This study compares MCI between case and control group, which shows it is less in case compared to control group indicating bone loss in chronic renal failure. Results were statistically significant.

SERUM UREA LEVELS AND METACARPOCORTICAL INDEX OF BOTH CASES AND CONTROLS

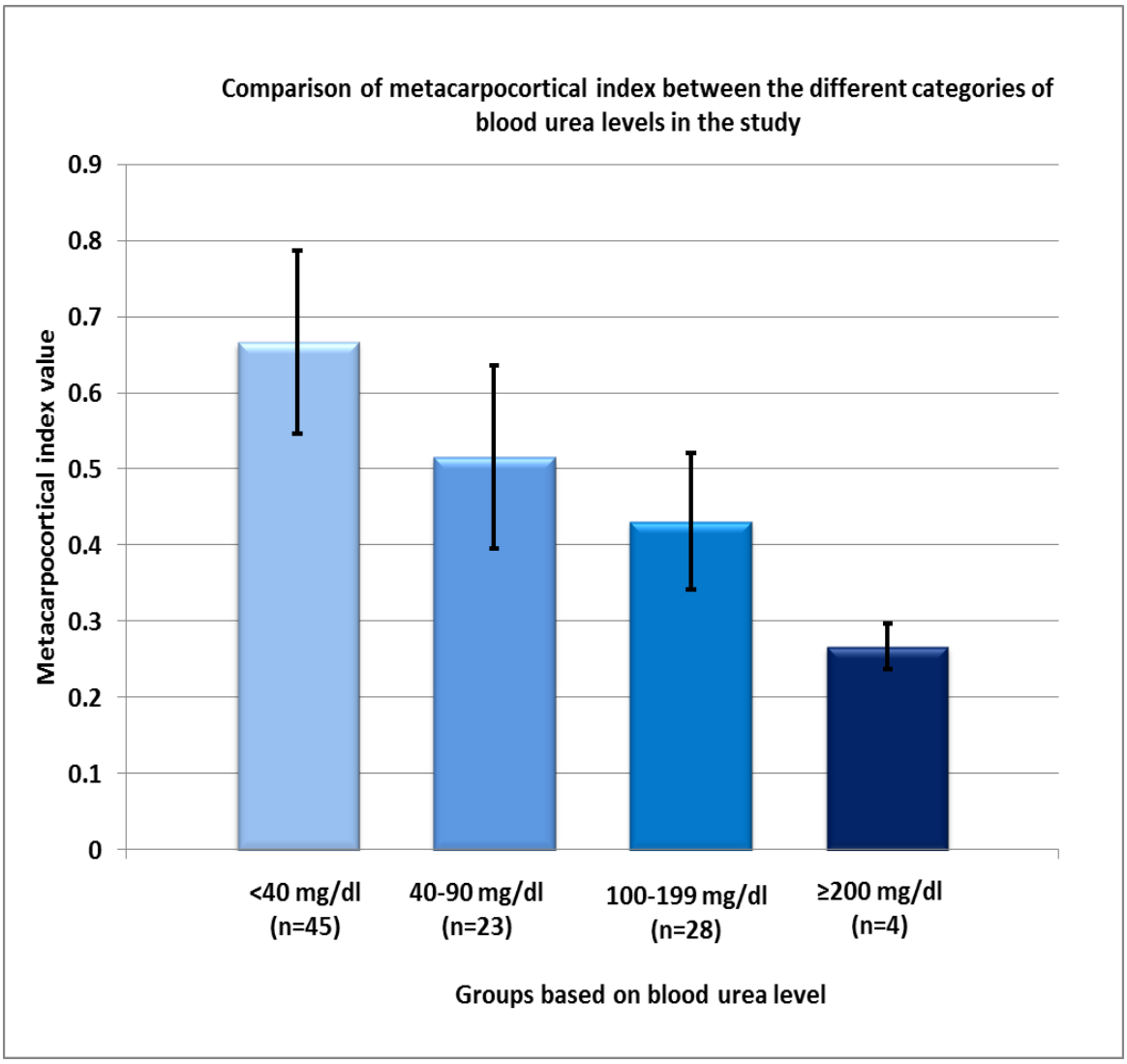


Figure 5. Vertical bar diagram depicting the comparison of metacarpocortical index between the different categories of blood urea levels in the study. Height of the bar represents the mean and error bar represents the SD

Table 6. Comparison of metacarpocortical index between the different categories of blood urea levels in the study.

S. No	Category of urea levels	Metacarpocortical index value			Range		F value	df	P value
		n	Mean	SD	Min	Max			
1	<40 mg/dl	45	0.667	0.12	0.42	0.88	34.78	df1 =3 df2=96	<0.0001*
2	40 – 99 mg/dl	23	0.516	0.12	0.32	0.88			
3	100 – 199 mg/dl	28	0.431	0.09	0.28	0.70			
4	≥200 mg/dl	4	0.267	0.03	0.22	0.31			

Data are expressed as mean with SD. One-way ANOVA with Tukey's post hoc test was used to compare the variance of means between the groups. *indicates $p<0.05$ and considered statistically significant

This table shows there is decline in metacarpocortical index with increase in serum urea levels and it is statistically significant.

SERUM CREATININE LEVELS AND CORRESPONDING METACARPOCORTICAL INDEX:

Table 7. Comparison of metacarpocortical index between the different categories of serum creatinine levels in the study.

S. No	Category of creatinine levels	Metacarpo cortical index value			Range		F value	df	P value
		n	Mean	SD	Min	Max			
1	<1.4 mg/dl	49	0.648	0.14	0.32	0.88	22.46	df1=4 df2=95	<0.0001*
2	1.4 – 5.9 mg/dl	23	0.541	0.09	0.37	0.77			
3	6 – 10.9 mg/dl	17	0.423	0.08	0.28	0.7			
4	11 – 15 mg/dl	4	0.361	0.06	0.31	0.44			
5	>15 mg/dl	7	0.304	0.06	0.22	0.42			

Data are expressed as mean with SD. One-way ANOVA with Tukey's post hoc test was used to compare the variance of means between the groups. *indicates $p<0.05$ and considered statistically significant.

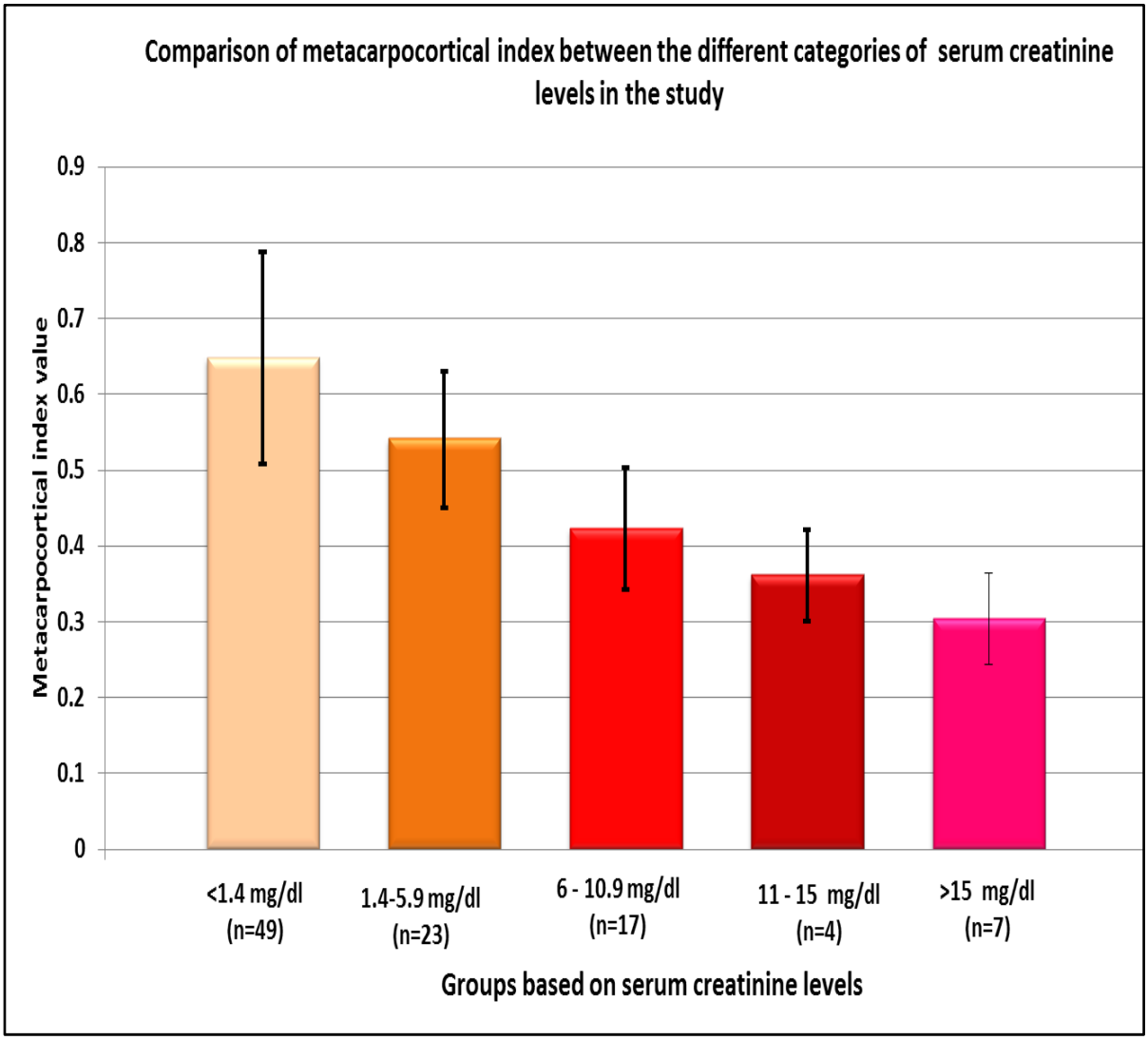


Figure 6. Vertical bar diagram depicting the comparison of metacarpocortical index between the different categories of serum creatinine levels in the study. Height of the bar represents the mean and error bar represents the SD.

This clearly shows with increase in serum creatinine, there is fall in metacarpocortical index and it is statistically significant.

SERUM CALCIUM LEVELS AND CORRESPONDING METACARPOCORTICAL INDEX:

Data are expressed as mean with SD. One-way ANOVA with Tukey's post hoc test was used to compare the variance of means between the groups. *indicates $p<0.05$ and considered statistically significant.

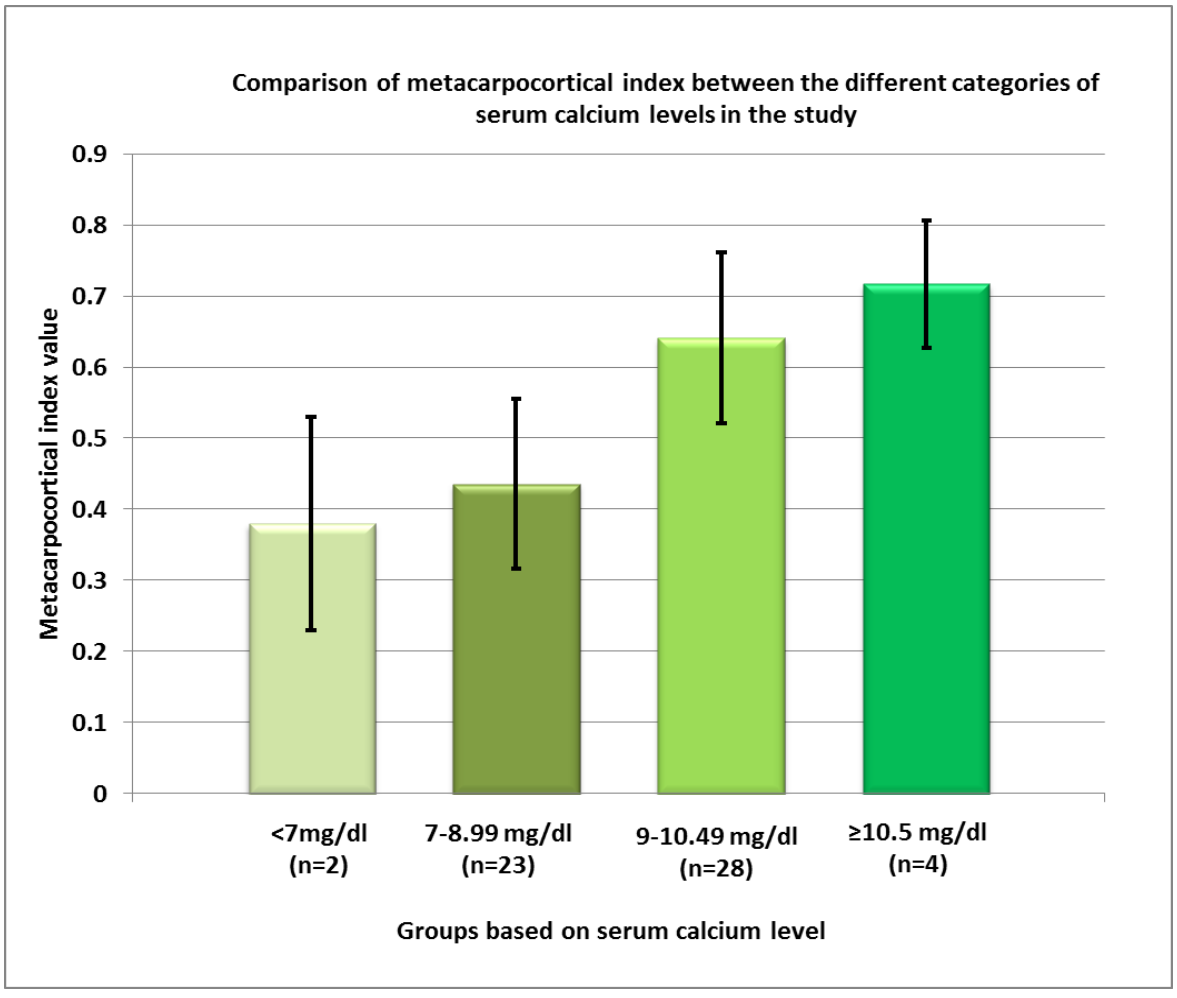


Figure 7. Vertical bar diagram depicting the comparison of metacarpocortical index between the different categories of serum calcium levels in the study. Height of the bar represents the mean and error bar represents the SD.

Table 8. Comparison of metacarpocortical index between the different categories of serum calcium levels in the study

S. No	Category of calcium levels	Metacarpocortical index value			Range		F value	df	P value
		n	Mean	SD	Min	Max			
1	<7 mg/dl	2	0.38	0.15	0.27	0.49	31.96	df1=3 df2=96	<0.0001 *
2	7-8.99 mg/dl	47	0.436	0.12	0.22	0.72			
3	9– 10.49 mg/dl	39	0.641	0.12	0.46	0.88			
4	≥ 10.5 mg/dl	12	0.717	0.09	0.55	0.88			

It shows that as calcium level decreases, metacarpocortical levels decreased and the results are statistically significant.

SERUM PHOSPHORUS LEVELS AND CORRESPONDING METACARPOCORTICAL INDEX:

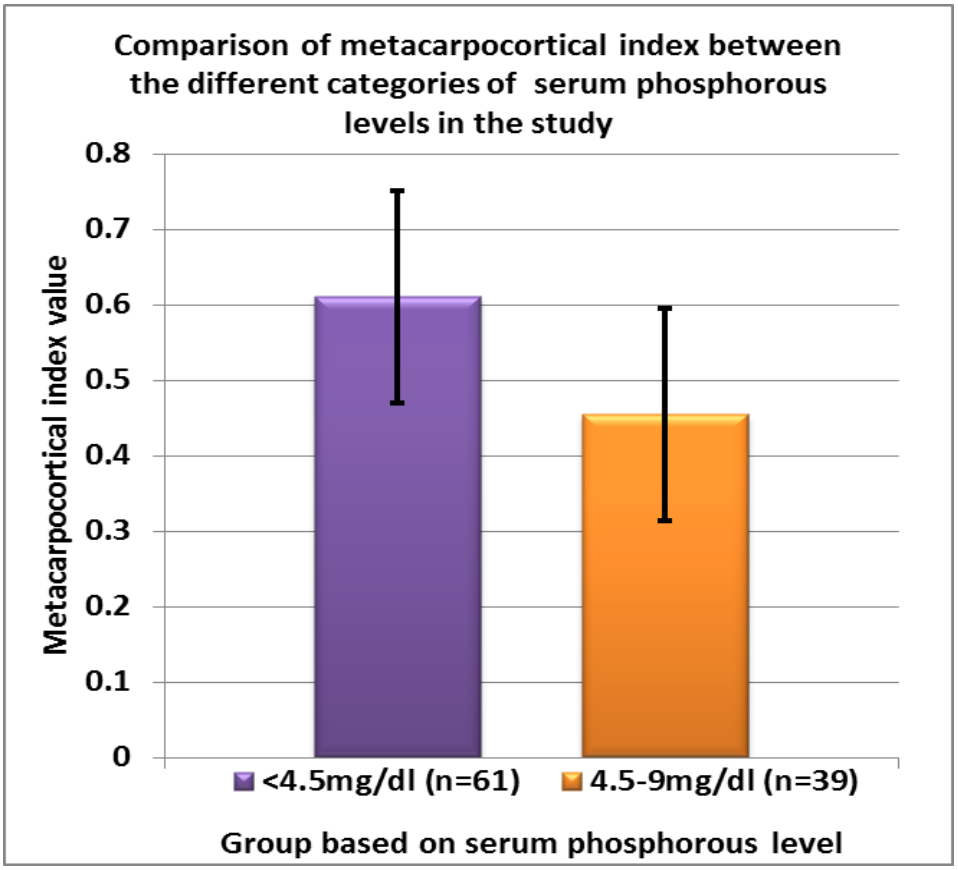


Figure 8. Vertical bar diagram depicting the comparison of metacarpocortical index between the different categories of serum phosphorous levels in the study. Height of the bar represents the mean and error bar represents the SD.

Table 9. Comparison of metacarpocortical index between the different categories of serum phosphorous levels in the study

S. No	Category of phosphorous levels	Metacarpocortical index value			Range		t value	df	P value
		n	Mean	SD	Min	Max			
1	<4.5 mg/dl	61	0.611	0.14	0.28	0.88	5.145	95	<0.0001*
2	4.5 – 9 mg/dl	39	0.455	0.14	0.22	0.78			

Data are expressed as mean with SD. Unpaired 't' test was used to compare the means. *indicates $p<0.05$ and considered statistically significant.

This table shows that with decrease in metacarpocortical index, there is increase in serum phosphorus and it is statistically significant.

SERUM ALKALINE PHOSPHATASE LEVELS AND CORRESPONDING METACARPOCORTICAL INDEX:

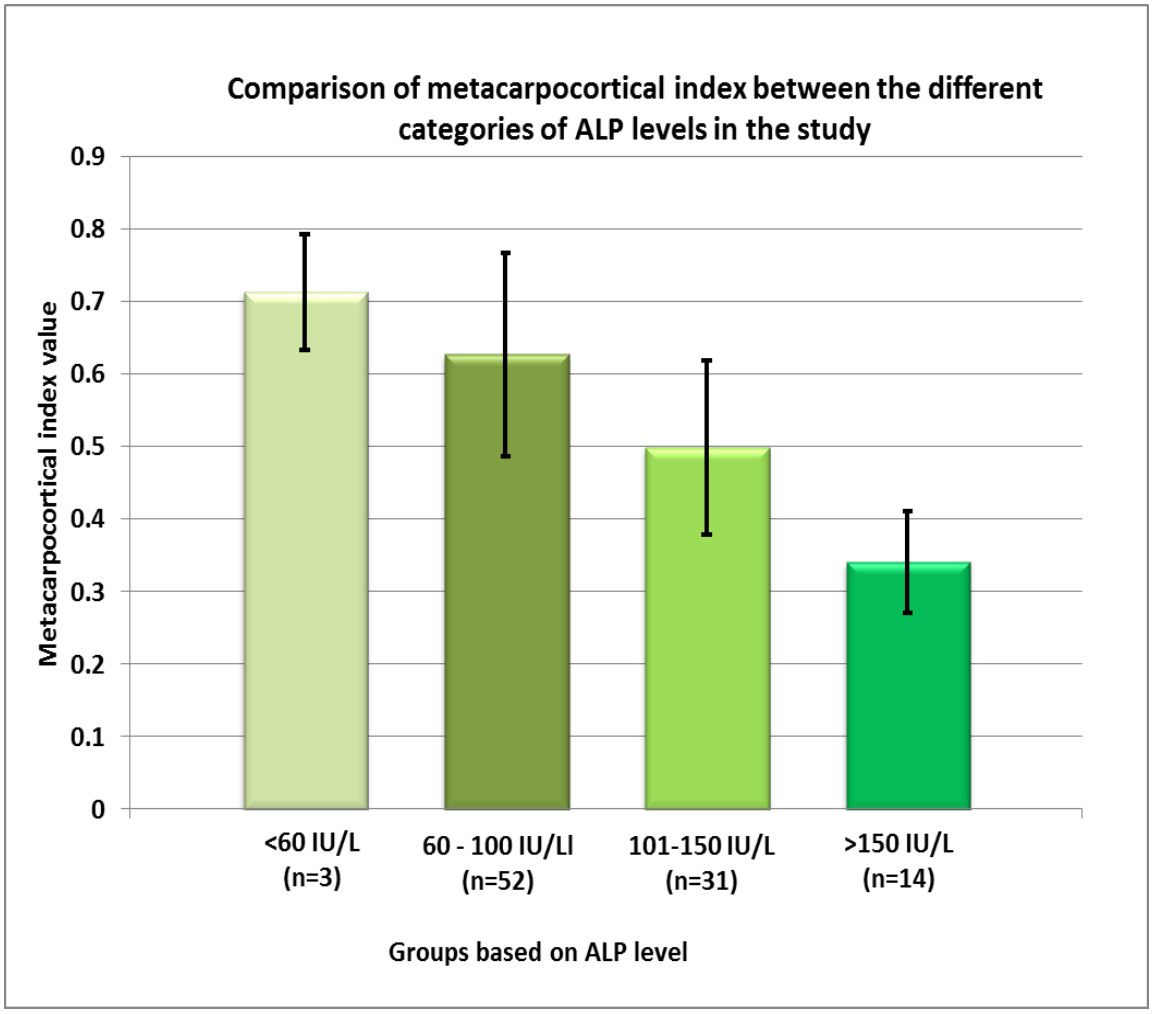


Figure 9. Vertical bar diagram depicting the comparison of metacarpocortical index between the different categories of ALP levels in the study. Height of the bar represents the mean and error bar represents the SD.

Table 10. Comparison of metacarpocortical index between the different categories of ALP levels in the study

S. No	Category of ALP levels	Metacarpo cortical index value			Range		F value	Df	P value
		n	Mean	SD	Min	Max			
1	<60 IU/L	3	0.713	0.08	0.64	0.8	4.26	df1=3 df2=96	0.0071*
2	60-100 IU/L	52	0.627	0.14	0.32	0.88			
3	101-150 IU/L	31	0.499	0.12	0.32	0.78			
4	>150 IU/L	14	0.341	0.07	0.22	0.45			

Data are expressed as mean with SD. One-way ANOVA with Tukey's post hoc test was used to compare the variance of means between the groups. *indicates $p<0.05$ and considered statistically significant.

This table shows there is decrease in metacarpocortical index with increase in serum alkaline phosphatase and it is statistically significant.

SERUM ALKALINE PHOSPHATASE LEVELS ANDCORRESPONDING METACARPOCORTICAL INDEX:

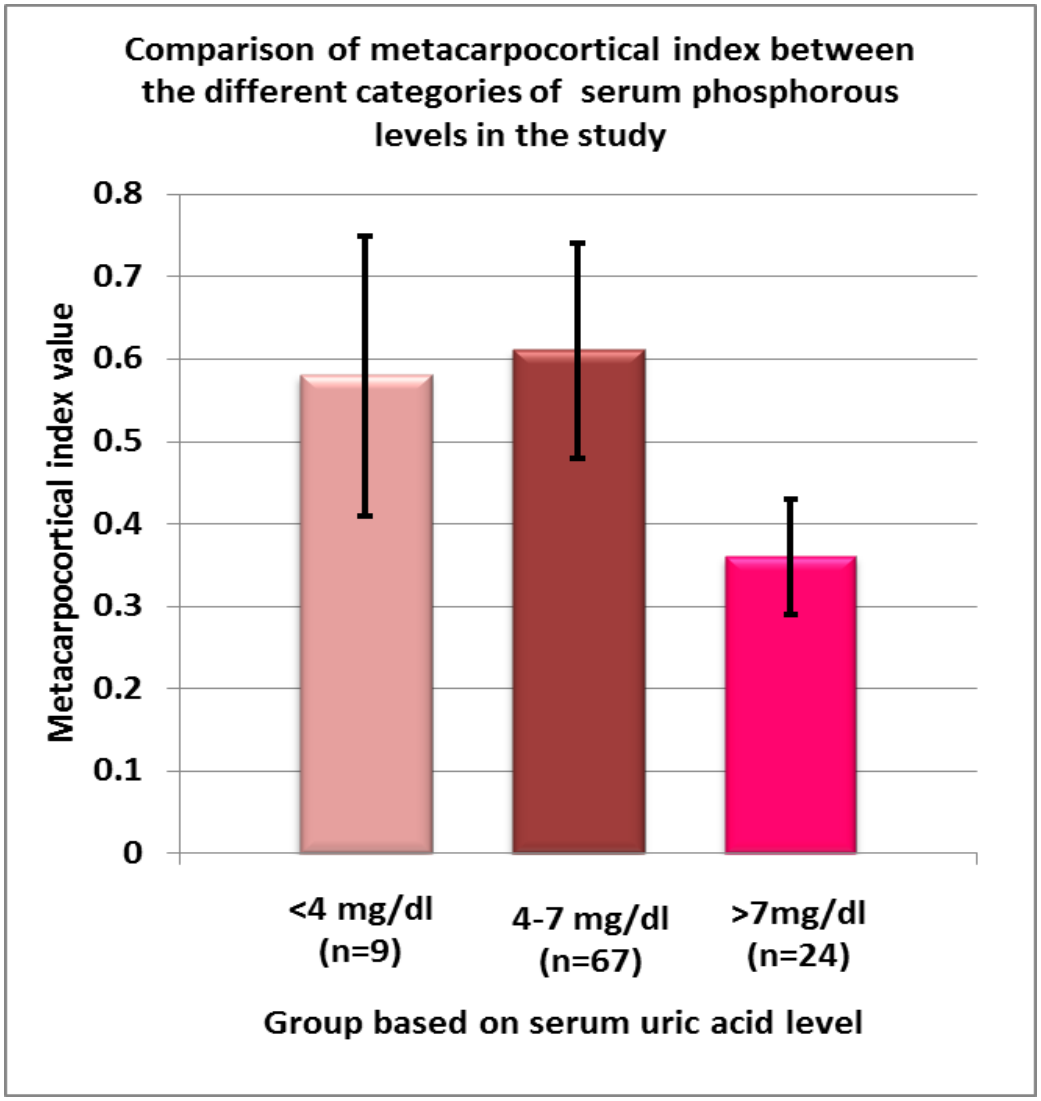


Figure 10. Vertical bar diagram depicting the comparison of metacarpocortical index between the different categories of uric acid levels in the study. Height of the bar represents the mean and error bar represents the SD.

Table 10. Comparison of metacarpocortical index between the different categories of uric acid levels in the study

S. No	Category of ALP levels	Metacarpo cortical index value			Range		F value	df	P value
		n	Mean	SD	Min	Max			
1	<4 mg/dl	9	0.58	0.17	0.37	0.88	35.19	df1=2 df2= 97	<0.0001 *
2	4 – 7 mg/dl	67	0.61	0.13	0.32	0.88			
4	>7 mg/dl	24	0.36	0.07	0.22	0.60			

Data are expressed as mean with SD. One-way ANOVA with Tukey's post hoc test was used to compare the variance of means between the groups. *indicates $p<0.05$ and considered statistically significant.

This table shows that metacarpocortical index decreases with increase in serum uric acid. It is statistically significant.

Table 11. Correlation between the metacarpocorticalindex with the various biochemical parameters measured in the study.

S.No	Correlation of MCI with	Pearson's r value	R square	P value	Interpretation
1	Serum urea	-0.71	0.504	<0.00001*	Negative association with strong strength
2	Serum creatinine	-0.56	0.313	<0.00001*	Negative association with moderate strength
3	Serum calcium	0.72	0.518	<0.00001*	Positive association with strong strength
4	Serum phosphorous	-0.55	0.302	<0.00001*	Negative association with moderate strength
5	Serum alkaline phosphate	-0.65	0.422	<0.00001*	Negative association with moderate strength
6	Serum uric acid	-0.47	0.221	<0.00001*	Negative association with moderate strength

Correlation was done using Pearson's correlation test. * indicates $p<0.05$ and considered statistically significant.

DISCUSSION

This study was done in chronic renal failure patients by measuring metacarpocortical index (MCI) of second metacarpocortical bone of right hand to document renal osteodystrophy and to correlate blood values of calcium, phosphorous, urea, creatinine, uric acid, alkaline phosphatase (ALP). MCI was calculated in 50 healthy individuals (control group) in both male and female of age group between 18-50 years and this mean MCI index was taken as reference and it is compared with MCI calculated in study group (CRF). This study mainly focus on younger population of age between 18-50 yrs in comparison with previous studies like R.G.Henderson et al which had elder population predominantly.

The results showing the MCI value in study group(CRF) (0.44) is declined in comparison with control group (0.65), indicating bony changes. Previous famous study conducted by D.Anil kumar et al shows it was 0.38 in case group and 0.57 in control group.

The study proves that MCI index declines as serum creatinine value increases. In patients with creatinine value of more than 15, MCI was 0.30. Whereas it is 0.34 in Dr.D.Anil kumar et al study

This study comparing blood urea levels and MCI index, clearly shows that as blood urea level raises MCI index decreases proportionately. Patients of blood urea levels more than 200 mg% has MCI of 0.26. It was 0.37 in Dr.D.Anil kumar et al study

This study shows correlation of serum calcium levels and MCI index. We can see a proportional elevation of serum calcium with elevating MCI index. Patients with calcium of less than 7 shows MCI of 0.38. Whereas it is 0.38 in Dr.D.Anil kumar et al study

This study compared CRF patients blood levels of serum phosphorus and found that MCI decreases with elevated levels of serum phosphorus. Patients with phosphorus more than 4.5 had MCI of 0.45. It was 0.39 in Dr.D.Anil kumar et al study

This study also has correlation between serum uric levels and MCI. We can see a trend of declining MCI index as uric acid levels elevated. Patients with uric acid level more than 7 has MCI of 0.36. It was 0.40 in a study conducted by Dr.D.Anil kumar et al study

This study finally also has a correlation between blood levels of alkaline phosphatase (ALP) and MCI index. We can see clearly a decrease in MCI, when serum alkaline phosphatase (ALP) increases.

Patients with alkaline phosphatase levels more than 150 IU/L has mean MCI index of 0.341. Dr.D.Anil kumar et al study shows MCI of 0.35 in serum ALP more than 150 IU/L.

CONCLUSION

This study concludes that

1) Measurement of metacarpocortical index from second metacarpal bone of right hand by X-ray technique is a simple and cost effective method to predict quantitative bone changes in chronic renal failure

   .

2) Comparing MCI in CRF with biochemical parameters found that there is decline in MCI with elevated levels of serum creatinine, urea, phosphorous, alkaline phosphatase and uric acid levels and MCI has been in found to be increased with elevated serum calcium levels

3) X-ray right hand for calculating MCI from second metacarpal bone can predict quantitative bone changes which is useful in preventing complications of renal osteodystrophy.

4) Thus MCI is simple, non invasive, accessible, reliable method to predict renal osteodystrophy in early stages and helps in preventing grave complications of osteodystrophy by early intervention and treatment.

LIMITATIONS OF STUDY

1) This is a single centre study.
2) In X-ray reporting there may be an inter-observer variation.
3) This study was done in less number of patients.
4) In this study serum parathormone, vitamin D3 levels were not assessed.

BIBLIOGRAPHY

1) Metacarpal Bone Mass in Normal Adults and in Patients with Chronic Renal Failure J. Andresen, H. E. Nielsen, T. E. Hansen, L.Bolvig

2) First Published November 1, 1981 Hutchison AJ, Whitehouse RW, Boulton HF, Adams JE, Mawer EB, Freemont TJ, Gokal R 1993 Correlation of bone histology with parathyroid hormone, vitamin D3, and radiology in end- stage renal disease. Kidney Int44:10711077 | ||

3) Rix M, Andreassen H, Eskildsen P, Langdahl B, Olgaard K 1999 Bone mineral density and biochemical markers of bone turnover in patients with predialysis chronic renal failure. Kidney Int 56:10841093.|

4) Loss of metacarpal and iliac bone in chronic renal failure: influence of hemodialysis , parathyroid activity ,type of renal disease, physical activity and heparin consumption Clinical science(1979),56,317-324

5) GRIFFITHS, H.J. & ZIMMERMAN, R.E. (1973) In: International Conference on Bone Mineral Measurement, p. 337. Ed. Mazess, R.B. US. Department of Health, Education and Welfare, D.H.E.W. Publication no. (NIH) 75-683.

6) INGHAM, J.P., STEWART, J.H. & POSEN, S. (1973) Quantitative skeletal histology in untreated end-stage renal failure. British Medical

Journal, ii, 745-148.

7) HORSMAN, A. & SIMPSON, M. (1975) the measurement of sequential changes in cortical bone geometry. British Journal of Radiology,48,411-476.

8) VISSER, W.J., NIERMANS. H.J., ROELOFS, J.M.M.,RAYMAKERS,J.A. & DUURSMA, S.A. (1977) In: Bone Histiomorphometry, p. 79. Ed. Meunier, P.J. Societe de la Nouvelle ImprimeFournie, Toulous

9) DrD.Anil Kumar, DrB.Srinivas, Dr.S.PremSagar A Study of Metocarpocortical Index (MCI) in Chronic Renalfailure Indian Journal of Applied Research, Vol.5, Issue : 7 July2015

10) Stehman-Breen CO, Sherrard DJ, Alem AM, et al. Risk factors for hip fracture among patients with end-stage renal disease. *Kidney Int.* 2000; **58**:2200–2205.

11) Tejwani NC, Schachter AK, Immerman I, Achan P. Renal osteodystrophy. *J Am AcadOrthop Surg.*2006; **14**:303–311.

12) M.A. Fontaine, A. Albert, B. Dubois, *et al.*Fracture and bone mineral density in hemodialysis patients ClinNephrol, 54 (2000), pp.218-226

ANNEXURES

PROFORMA

PARTICULARS OF THE PATIENT:

NAME:

AGE/SEX:

IP NO:

CASE/CONTROL:

ADDRESS:

DATE OF ADMISSION:

DATE OF DISCHARGE:

FINAL DIAGNOSIS:

COMPLAINTS WITH DURATION:

PAST HISTORY:

DM Y/N

HTN Y/N

CAD Y/N

CVA Y/N

CKD Y/N

MALIGNANCY Y/N

TB Y/N

PERSONAL HISTORY:

SMOKING Y/N:

ALCOHOL Y/N:

FAMILY HISTORY:

ON EXAMINATION:

VITAL SIGNS:

Pulse:

B.P:

R.R:

sPo2:

Temperature:

CVS:

RS:

ABDOMEN:

CNS:

INVESTIGATIONS:

Hb:

RBS:

UREA:

CREATININE:

SERUM CALCIUM:

SERUM PHOSPHORUS:

SERUM URIC ACID:

SERUM ALP:

XRAY RIGHT HAND AP VIEW

:METACARPOCORTICAL INDEX:

ANNEXURE

CONSENT FORM

Yourself Mr/Miss/Mrs ..... is being asked to be a participant in the *"**A STUDY OF METACARPOCORTICAL INDEX TO PREDICT RENAL OSTEODYSTROPHY IN CHRONIC KIDNEY DISEASE"***in Thanjavur Medical College Hospital, Thanjavur , conducted by DR.GOVINDARAJ V ., Post Graduate Student, Department of General Medicine, Thanjavur Medical College. You are eligible after looking into the inclusion criteria. You can ask any question you may have before agreeing to participate.

TOPIC OF RESEARCH

A STUDY OF METACARPOCORTICAL INDEX TO PREDICT RENAL OSTEODYSTROPHY IN CHRONIC KIDNEY DISEASE

PURPOSE OF RESEARCH

In this study, interventions that are both cost saving and feasible in developing countries, X-ray AP view is used to predict early changes in renal osteodydtrophy in CKD patients.

PROCEDURES INVOLVED IN THE STUDY:

- Fetching baseline characteristics of the patient like age, gender, etc.,
- Properly elicited medical history pertaining to the complaints
- Detailed general and systemic examination as guided by the medical history

- Blood examination, Xray AP view of right hand as guided by the clinical examination

- Treatment with standard protocol currently followed in our hospital.

- Continued follow up of patient.

- Recording all the above variants / events into the database and

analyzing them by statistical methods to arrive at our objectives.

DECLINE FROM PARTICIPATION

You are hereby made aware that participation in this study ispurely voluntary and honorary, and that you have all the rights to decline from participating in it.

PRIVACY AND CONFIDENTIALITY

Privacy of individuals will be respected and any information about you or provided by you during the study will be kept strictly confidential.

AUTHORIZATION TO PUBLISH RESULTS

Results of the study may be published for scientific purposes and/or presented to scientific groups, however you will not be identified.

STATEMENT OF CONSENT

I volunteer and consent to participate in this study. I have read the consent or it has been read to me. The study has been fully explained to me, and I may ask questions at any time.

---------- ----------

Signature /Left thumb impression Date

(volunteer)

---------- ----------

Signature of witness Date

MASTER CHART

S.NO	NAME	AGE	SEX	METACARPO CORTICAL INDEX	SERUM UREA (mg/dl)	SERUM CREATININE (mg/dl)	SERUM CALCIUM (mg/dl)	SERUM PHOSPHORUS (mg/dl)	SERUM ALP (IU/L)	SERUM URIC ACID (mg/dl)
1	PALANISAMY	44	M	0.33	170	16.4	7	8.5	168	8
2	MANIMARAN	37	M	0.5	64	2.8	9	3.5	116	6
3	SHANKAR	32	M	0.44	156	12.6	7.5	6.5	160	7
4	PANDIYA	29	M	0.41	170	7	8	5.5	142	7.5
5	MUTHAMARAJI	38	F	0.4	150	9.4	8	5.5	152	8
6	RENUGA	38	F	0.42	106	7.6	8.5	5.5	146	7.5
7	DHARMARAJ	36	M	0.45	144	9	8.5	6	154	6.5
8	MEENA	24	F	0.56	100	2.5	10	4.5	110	4
9	KRISHNAVENI	38	F	0.52	62	2.6	9.5	4	116	5
10	SKIKASUNDARAM	40	M	0.31	234	17.6	7	7.5	172	8
11	KARTHICK	26	M	0.31	176	15.3	7.5	7	164	7.5
12	VIDIVELLI	40	F	0.66	102	1.9	10.5	3.5	90	4.5
13	KUTHAIYAN	47	M	0.27	260	24	6.5	8	182	8.5
14	RAGUPATHI	47	M	0.27	222	21	7	8.5	170	8
15	BARATH MOHAN	29	M	0.47	86	5.1	8.5	4	101	6
16	RANI	45	F	0.45	106	8.9	8.5	6	148	6.5
17	KARUMARIYAMAL	29	F	0.41	182	9.4	8.5	6.5	154	7
18	ANANTHI	35	F	0.5	84	4.8	9	3.5	118	5
19	DHANAVALLI	45	F	0.37	140	12.9	7.5	6	166	8
20	SARASWATHI	24	F	0.5	80	2.6	8	4.5	106	6.5
21	LUTHER MARY	23	F	0.43	168	6.5	8	4	140	7.5
22	SANKAR	36	M	0.4	194	6.5	8	5.5	148	7.5
23	RENGANATHAN	40	M	0.31	172	14.3	7.5	6	154	8
24	PAKKIRISAMY	47	M	0.37	76	5.6	8.5	2.6	101	2.9
25	ANANTH	25	M	0.47	80	7.8	8	3.6	71	3.3
26	SRINIVASAN	49	M	0.49	142	7.8	6.9	2.7	140	3.9
27	BALATHANDAYUTHAPANI	39	M	0.55	96	2.6	8.5	2.2	90	2.6
28	ROSELIN MARY	38	F	0.46	134	7.6	9.2	2.6	120	4.9
29	GOVINDARAJ	36	M	0.6	82	3.9	9.1	2.8	116	3.5
30	MANGALAM	22	F	0.32	90	8.4	7	3.5	90	8
31	RAJIV GANDHI	32	M	0.4	130	5.6	8.5	4	112	7.5
32	PALANIVEL	45	M	0.28	140	10.6	7.5	3.5	152	7.5
33	MATHIYALAGAN	45	M	0.58	90	3.6	9	4.5	130	4.5
34	MARAN	48	M	0.7	110	6.5	8	5	80	6
35	SUDHA	33	F	0.6	54	1.8	10	3.5	96	4
36	KALAISELVI	39	F	0.44	118	5.4	8	4.5	136	8.5
37	PERIYASAMY	42	M	0.44	112	5.2	8	4	120	7
38	DHINAKARAN	45	M	0.32	162	12.2	7.5	6.5	140	7.5
39	CHITRA	47	F	0.54	48	1.5	9	3.5	92	6
40	ALAGAR	50	M	0.33	130	10.4	7	8	150	8.5
41	ANNAIKATTU	50	F	0.5	59	2.2	9.5	4	108	6.5
42	PAPPATHI	35	F	0.5	68	1.8	10	4	90	7
43	SIRANJEEVI	32	M	0.42	116	52	8.5	4.5	136	8
44	VICTOR	48	M	0.6	120	5.4	7.5	6.5	150	7.5
45	SAROJA	48	F	0.4	130	10.4	7.5	6	158	7.5
46	PARTHIBAN	50	M	0.41	160	8	7	5.5	116	8
47	SURESH	33	M	0.7	90	5.4	8	4.5	130	7
48	KARTHICK	29	M	0.46	80	5.2	8	4.5	100	6.5
49	SIVA	35	M	0.68	60	2.7	9.5	4.5	110	5
50	SENTHAMARI	32	F	0.22	210	19.5	7	8	170	8

S.NO	NAME	AGE	SEX	METACARPO CORTICAL INDEX	SERUM UREA (mg/dl)	SERUM CREATININE (mg/dl)	SERUM CALCIUM (mg/dl)	SERUM PHOSPHORUS (mg/dl)	SERUM ALP (IU/L)	SERUM URIC ACID (mg/dl)
51	BANUMATHI	40	F	0.7	33	1	9	3.5	72	5
52	JAYA	40	F	0.57	20	0.9	9	3	78	5.5
53	SHANTI	45	F	0.68	20	0.8	10.6	3	84	6
54	VIJAYLAKSHMI	37	F	0.56	16	0.8	10	3.5	78	4.5
55	SANTHI	43	F	0.6	18	0.8	11	3	92	5
56	MANIKANDAN	20	M	0.76	38	1.1	11	4	68	5
57	PRABAKARAN	22	M	0.8	26	1.1	11	3.6	90	7
58	VISU	55	M	0.75	36	1.2	9.5	3.5	90	7
59	NALINI	24	F	0.72	18	1	9.5	2.5	102	4
60	UTHIRAPATTI	51	M	0.78	28	1	9	4.6	108	6
61	ANUSYA	22	F	0.68	20	0.6	10	4	96	5.5
62	BHAVANI	45	F	0.77	30	1.4	9	3	106	6
63	BHUVANAMANI	38	F	0.87	38	1.3	10	3.5	82	7
64	KARPARAJ	43	M	0.86	20	0.5	10	4	92	5
65	DHANABAL	50	M	0.78	18	0.8	11	4	88	6.5
66	CHELLADURAI	50	M	0.85	24	1.1	9	3.5	82	5
67	MUTHULAKSHMI	23	F	0.66	22	0.8	10.6	2.6	78	6.5
68	SELVARAJ	35	M	0.58	32	1.1	9.5	4	82	4
69	KRISHNAMOORTHY	35	M	0.55	38	1.2	10	3	78	6
70	RAJAVEL	25	M	0.72	22	0.7	8.6	4	68	6
71	PRABAVATHY	20	F	0.68	28	1	9	3	72	6.5
72	SATHIYAMOORTHY	48	M	0.47	38	1	9	2.6	69	5.5
73	MANIKANDAN	38	M	0.64	30	0.8	8.6	3.1	54	2.7
74	GOKILA	20	F	0.76	22	1	11	4	86	4.6
75	SELVI	46	F	0.88	40	1.2	9.5	3.5	86	6
76	DINESH	26	M	0.62	26	0.7	7.8	4.5	70	6
77	VILLAYUDHAM	43	M	0.88	26	0.7	10.5	4	74	2.9
78	VEERAMANI	29	M	0.7	30	0.8	9.8	3.9	49	4.5
79	JHANSIRANI	32	F	0.5	22	0.6	10	3	98	6.5
80	VIGNESH	22	M	0.8	24	0.6	9.2	3.7	52	3.8
81	AMUTHARANI	37	F	0.42	26	0.7	7.5	3	62	3.5
82	SINTHAMANI	29	F	0.7	22	1.1	9	4.5	66	5
83	MUTHU	45	M	0.44	26	0.8	8	4	78	4.5
84	SAKTHIVEL	38	M	0.42	23	0.8	7	3.5	74	5
85	PANEERSELVAM	50	M	0.7	34	1.1	8.5	3.5	74	5
86	VANITHA	43	F	0.76	22	1.1	11	4	100	7
87	SAMIKANNU	49	M	0.66	20	0.8	10	3.5	88	5.5
88	AMANULLA	46	M	0.55	18	0.8	105	4	78	5
89	AYYAPAN	40	M	0.72	30	0.8	10	3	87	6
90	SARAVANAN	32	M	0.75	36	1	10	4.5	66	5
91	LAKSHMIPRIYA	26	F	0.72	30	0.9	10.6	3	87	6.6
92	MUTHAIYA	40	M	0.68	20	1.1	95	2.5	100	4
93	MURUGAN	22	M	0.42	30	1	8.5	5	90	6.5
94	KRISHNAVENI	40	F	0.76	32	0.8	9	2.6	82	5
95	MAIKALRAJ	32	M	0.33	58	1.1	7.2	6	95	8
96	DINESH	25	M	0.52	44	0.8	8.4	6	86	5
97	GOWRI	19	F	0.5	16	0.7	10	3.5	92	6
98	CHARLES	38	M	0.32	60	1.2	8	5.5	102	6.5
99	MURALI	36	M	0.54	38	1.1	9.5	3.5	88	5.5
100	KANDASAMY	40	M	0.46	50	1.3	7.5	4.5	98	5