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FGF-23 Levels before and after Renal transplantation

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*I dedicate this work to my family, especially to My dear **Mother** for her continuous support and her great effort through out my life .To my loving **Father** and to my loving **sisters** who always show so much Care, aid and patience.*

Contents

Page

LIST OF ABBREVIATIONS

1,25(OH)2D	**1,25dihydroxy vitamin D**
25 (OH)D	**25dihydroxy vitamin D**
ACEI	**Angiotensin converting enzyme inhibitor**
ADHR	**Autosomal dominant hypophosphatemic rickets**
ADMA	**Asymmetrical diethylarginine**
ADYN	**Adynamic bone disease**
ANP	**Atrial naturietic peptide**
ARF	**Acute renal failure**
ARHR	**Autosomal recessive hypophosphatemic rickets**
ASARM	**Acidic, serine and aspartate rich motif**
AKI	**Acute kidney injury**
ARHR1and 2	**Autosomal recessive hypophosphatemic rickets,type 1and 2**
BMP	**Bone morphogenetic protein**
BMD	**Bone mineral denisty**
CAR12	**Carbonic anhydrase 12**
CaCO3	**Calcium carbonate**
CaXPO4	**Calciumxphosphate**
CKD	**Chronic Kidney disease**
CKD-MBD	**Chronic kidney disease:Mineral and bone disorder**
CrCl	**Creatinine clearance**
CRF	**Chronic renal failure**
CRP	**C-reactive protein**

CSF	**Cerebrospinal fluid**
CSR	**Calcium-sensing receptor**
CV	**Cardiovascular**
CaSR	**Calcium-sensing receptor**
cKL	**Shedded full-length Klotho**
CYP24A1	**1,25dihydroxyvitamin D 24-hydroxylase**
CYP27B1	**25-dihydroxyvitamin D 1-alpha-hydroxylase**
DM	**Diabetes mellitus**
DMP1	**Dentin matrix protein 1**
ECF	**Extracellular fluid**
ENPPI	**Ectonucleotide pyrophosphate/phosphdiesterase**
ESRD	**End stage renal disease**
FDA	**Food and drug administration**
FGF23	**Fibroblast growth factor 23**
FSGS	**Focal and segmental glomerulosclerosis**
FGF	**Fibroblast growth factor**
Fgf23-/-	**Fibroblast growth factor 23 knockout mice**
FGFR	**Fibroblast growth factor receptor**
FD	**Fibrous dysplasia**
GALNT3	**Polypeptide N-acetylgalactosaminyltransferase 3**
GFR	**Glomerular filtration rate**
GPCR	**G-protein coupled receptor**
HD	**Hemodialysis**
HFTC	**Hyperphosphatemic familial tumoral calcinosis**

HPTH	**Hyperparathyroidism**
HPT	**High turnover osetodystropy**
HRH	**Hyperparathyroidism**
IL	**Interleukin**
Klotho-/-	**Klotho knockout mice**
Ksp -KL-/-	**Distal tubule-specific Klotho knockout mice**
KDIGO	**Kidney Disease Improving Global Outcomes**
LAV	**Left atrial volume**
LDL	**Low density lipoprotein**
LTOM	**Low turnover osteomalacia**
LVH	**Left ventricular hypertrophy**
LVMI	**Left ventricular mass index**
MEPE	**Matrix extracellular phosphoglycoprotein**
MGP	**Matrix G1a protein**
MUO	**Mixed uremic osteodystropy**
NaPi- II	**Sodium phosphate cotransporters**
NKF	**National kidney foundation**
NO	**Nitric oxide**
NPT2a	**Sodium phosphate cotransporters**
OGD	**Osteoglophonic dysplasia**
OPN	**Osteopontin**
PHEX	**Phosphate-regulating endopeptidase homolg,X-linked**
PHPT	**Primary hyperparathyroidism**
PRMT	**Protein methyltransferase**

PTG	**Parathyroid gland**
PTH	**Parathyroid hormone**
PTHrP	**Parathyroid hormone-related peptide**
PTH-KL-/-	**Parathyroid-specific Klotho knockout mice**
PTH1R	**Parathyroid hormone 1 receptor**
RAAS	**Renin-Angiotensin-Aldosterone system**
RCT	**Randomized controlled study**
RGD	**Arginine glycine aspartate**
RVR	**Renal vascular resistance**
sFRP4	**Secreted frizzled-related protein 4**
SIBLING	**Small integrin binding ligand N-linked glycoprotein**
sHPT	**Secondary hyperparathyroidism**
TGF-B	**Transforming growth factor -3**
TIO	**Tumor induced osteomalacia**
TNF	**Tumor necrosis factor**
US	**United states**
USRDS	**The united states Renal Data System**
VC	**Vascular calcification**
VDR	**Vitamin D receptor**
VDRE	**Vitamin D receptor element**
VSMC	**Vascular smooth muscle cell**
XLHR	**X linked hypophosphatemic rickets**

List of tables

List of figures

Introduction and aim of the work

- Our understanding of the dramatic changes in bone and mineral metabolism that occur in patients with chronic kidney disease (CKD) has increased with the discovery of the bone-derived hormone fibroblast growth factor 23 (FGF-23) .

(Silver J et al,2013)

-The discovery of fibroblast growth factor 23 (FGF23), a novel bone-derived hormone that inhibits phosphate reabsorption and calcitriol production by the kidney, has uncovered primary regulatory pathways and new systems biology governing bone mineralization, vitamin D metabolism, parathyroid gland function and renal phosphate handling. ***(Evenepoel P et al,2007)***

-As our knowledge expands regarding the regulation and functions of FGF23, the assessment of FGF23 will become an important diagnostic marker as well as a therapeutic target for management of disordered mineral metabolism in a variety of acquired and hereditary disorders. ***(Stubbs J et al ,2007)***

-Disordered phosphate homeostasis with elevated circulating levels of fibroblast

growth factor 23 (FGF23) is an early and pervasive complication of CKD. CKD is

likely the most common cause of chronically elevated FGF23 levels, and the

clinical condition in which levels are most markedly elevated.***(Wolf M,2012)***

-Recently, FGF-23 has been suggested to be responsible for the hypophosphatemia and inappropriately low calcitriol levels observed after renal transplantation .

(Evenepoel P et al,2007)

Aim of work

The aim of the present prospective study was therefore to investigate FGF-23 levels in patients with end-stage renal disease before and after a successful renal transplantation and their probable association with markers of bone and mineral metabolism.

Chapter One

Bone and Mineral Metabolism in Chronic Kidney Disease

Bone and Mineral Metabolism in Chronic Kidney Disease

The chronic kidney disease-bone and mineral disorders (CKD-MBD) represents a dynamic area of research. Recently, new factors such as FGF-23 have been added to the classic list of regulators of bone metabolism, which include calcium, phosphorus, PTH and calcitriol. ***(Mejía N et al,2011)***

Vascular calcification, one of the most important complications of CKD-MBD is regulated by a complex variety of promoters and inhibitors. The relationship between vascular calcification, bone loss and mortality, together with the existence of likely common signaling pathways are subject of interesting investigations. ***(Mejía N et al,2011)***

In healthy individuals kidneys regulate calcium and phosphorus homeostasis through tubular reabsorption mechanisms. Patients with chronic kidney disease have seriously compromised homeostatic mechanisms, giving rise to different adaptive changes in calcium (Ca), phosphorus (P), parathyroid hormone (PTH), vitamin D and fibroblastic growth factor (FGF-23) levels. ***(Torregrosa JV et al ,2011)***

There are various clinical signs, although secondary hyperparathyroidism (SHPT), fractures, bone pain, vascular calcification and cardiovascular events are highlighted as causing lower quality of life with a high morbidity and mortality.***(KBS,2005) (Mejía N et al,2011)***

Disturbances of mineral metabolism are common if not ubiquitous during the course of chronic kidney disease (CKD) and lead to serious and debilitating complications unless these abnormalities are addressed and treated.

The spectrum of disorders includes abnormal concentrations of serum calcium, phosphate, and magnesium and disorders of parathyroid hormone (PTH) and vitamin D metabolism. These abnormalities as well as other factors related to the uremic state affect the skeleton and result in the complex disorders of bone known as renal osteodystrophy; it is now recommended that this term be used exclusively to define the bone disease associated with CKD. ***(Kevin J et al,2007)***

The clinical, biochemical, and imaging abnormalities heretofore identified as correlates of renal osteodystrophy should be defined more broadly as a clinical entity or syndrome called chronic kidney disease–mineral and bone disorder (CKD-MBD***).(Moe S et al,2006)***

The spectrum of skeletal abnormalities seen in renal osteodystrophy (fig 1.1) includes the following:

1- **Osteitis fibrosa**, a manifestation of hyperparathyroidism characterized by increased osteoclast and osteoblast activity,peritrabecular fibrosis, and increased bone turnover.

2- **Adynamic bone disease**, a condition characterized by abnormally low bone turnover.

3- **Osteopenia or osteoporosis**.

4-**Osteomalacia,** a manifestation of defective mineralization of newly formed osteoid most often caused by aluminum deposition; bone turnover is decreased

5- Combinations of these abnormalities termed **mixed renal osteodystrophy**.

6- **Other abnormalities** with skeletal manifestations (e.g.,chronic acidosis, b2-microglobulin amyloidosis).

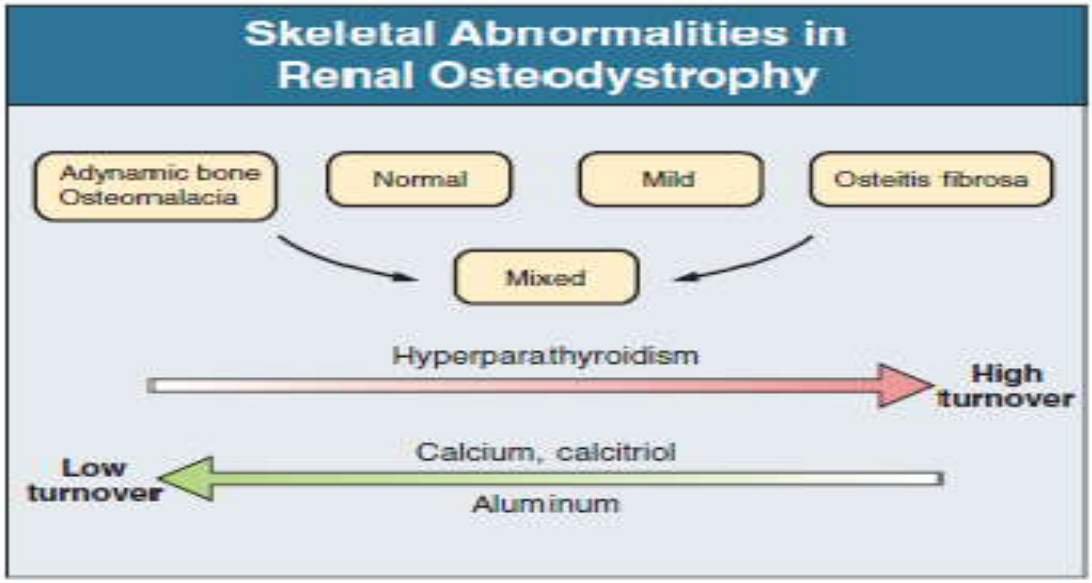


Figure 1.1 The spectrum of renal osteodystrophy. *The range of skeletal abnormalities in renal bone disease encompasses syndromes with both high and low bone turnover.*

EPIDEMIOLOGY

The prevalence of the various types of renal bone disease in patients with end-stage renal disease (ESRD) is illustrated in **Fig 1.2.** In patients on hemodialysis, osteitis fibrosa and adynamic bone disease now occur with almost equal frequency.

In contrast, in patients on peritoneal dialysis, the adynamic bone lesion predominates. Osteomalacia represents only a small fraction of cases in either group but is more common in certain ethnic groups, particularly Indo-Asians. The abnormalities of the skeleton start relatively early in the course of CKD. ***(Malluche HH et al,2008)***

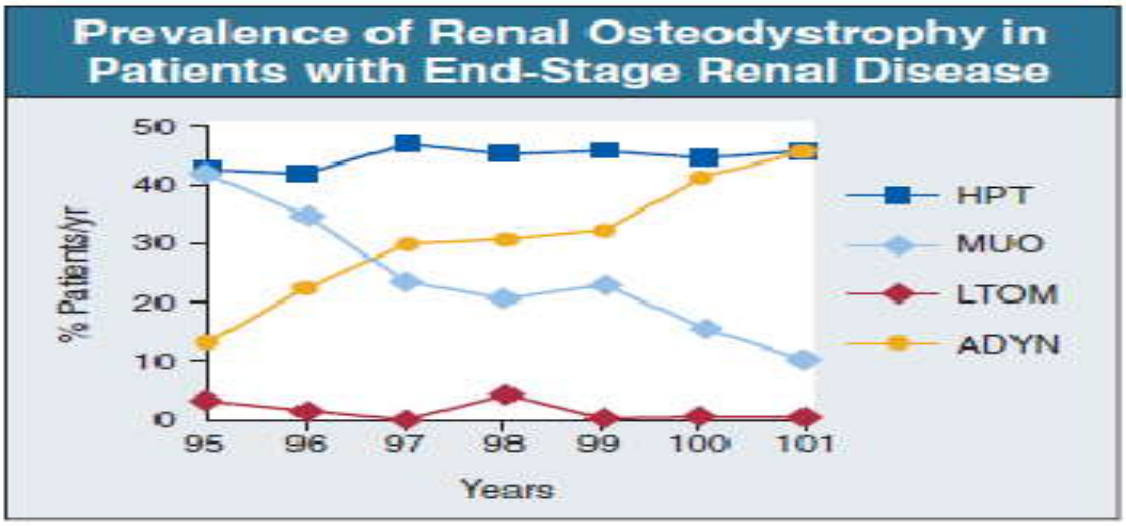


Figure 1.2 Prevalence of renal osteodystrophy in patients with end-stage renal disease.
HPT, high-turnover renal osteodystrophy; MUO, mixed uremic osteodystrophy; LTOM, low-turnover osteomalacia;ADYN, adynamic bone disease.

PATHOGENESIS

The major factors that are operative in early CKD may vary as CKD progresses. Similarly, the predominance of one particular pathogenetic mechanism over another may contribute to the heterogeneity of bone disorders. ***(Wolf M,2012)***

1- Osteitis Fibrosa: Hyperparathyroidism:High-Turnover Renal Bone Disease:

It begins quite early in the course of CKD, frequently when kidney function declines to less than 60 mL/minute/1.73m. Elevated levels of PTH in blood and hyperplasia of the parathyroid glands are seen early in CKD. Whereas the level of free (non–protein bound) calcium in blood is normally the principal determinant of PTH secretion, during the course of CKD, several metabolic disturbances also alter the regulation of the secretion of PTH. ***(Keith DS , 2007)***

Numerous factors that lead to the over activity of parathyroid glands. These factors include the retention of phosphorus, decreases in the levels of calcitriol, intrinsic alterations within the parathyroid gland that give rise to increased PTH secretion as well as increased parathyroid growth, skeletal resistance to the actions of PTH, and hypocalcemia. ***(Moe S et al,2006)***

A-*Abnormalities of Calcium Metabolism*

There are three main body pools of calcium: the bony skeleton (mineral component), the intracellular pool (mostly protein bound) and the extracellular pool.

The calcium in the extracellular pool is in continuous exchange with that of bone and cells and is altered by diet and excretion. Calcium metabolism depends on the close interaction of two hormonal systems: PTH and vitamin D. Perturbations of both of these systems occur during the course of CKD, with adverse consequences on the skeleton. ***(Kevin and Esther, 2007)***

Total serum calcium tends to decrease during the course of CKD as a result of phosphate retention ,decreased production of 1,25-dihydroxyvitamin D (calcitriol) from the kidney ,decreased intestinal calcium absorption and skeletal resistance to the calcemic action of PTH, but the levels of free calcium remain within the normal range in most patients as a result of compensatory hyperparathyroidism. ***(Kevin and Esther, 2007)***

Because calcium is a major regulator of PTH secretion, persistent hypocalcemia is a powerful stimulus for the development of hyperparathyroidism and also contributes to parathyroid growth***.(Yilmaz MI et al,2010)***

B-*Abnormalities of Phosphate Metabolism*

With progressive CKD, phosphate is retained by the kidney. However, hyperphosphatemia usually does not become evident before CKD stage 4 until then compensatory hyperparathyroidism and increases in circulating fibroblast growth factor 23 (FGF-23) result in increased phosphaturia, maintaining serum phosphate levels in the normal range. ***(Wesseling-Perry K et al,2010)***

One mechanism by which phosphate retention may lead to hyperparathyroidism **Fig 1.3** is by a decrease in serum free calcium, which in turn stimulates the secretion of PTH. Thus, a new steady state is achieved in which serum phosphate is restored to normal at the expense of a sustained high level of PTH. This cycle is repeated as renal function declines until sustained and severe hyperparathyroidism is present. ***(Viaene L et al,2012)***

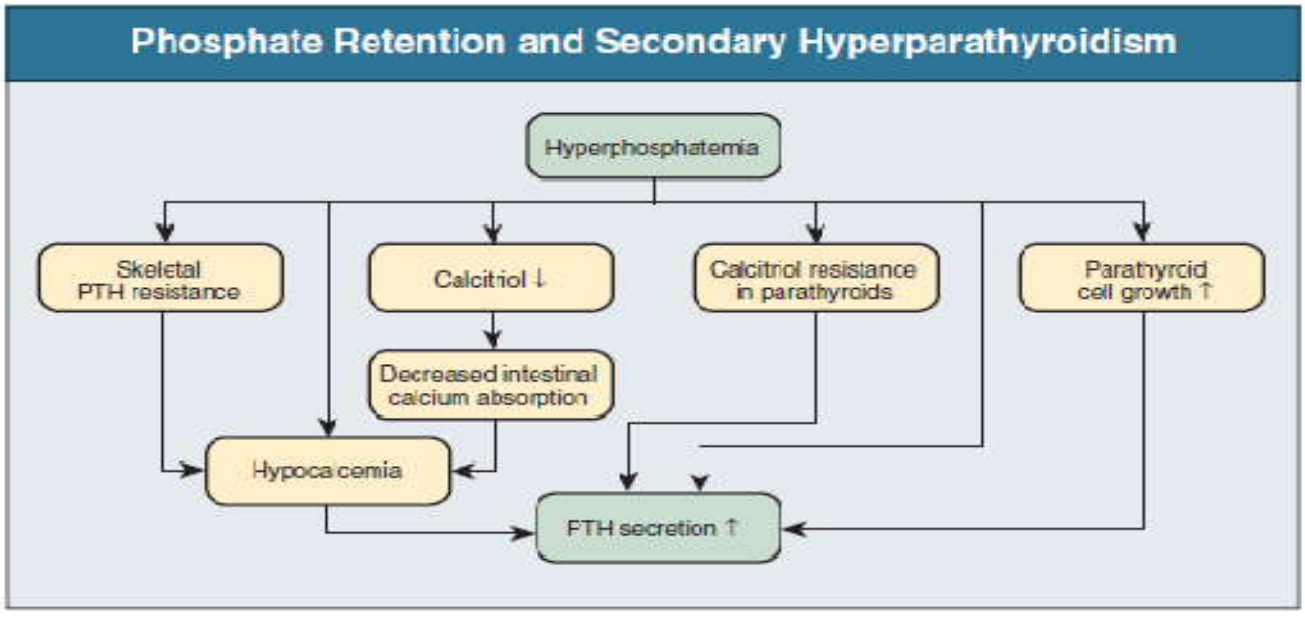


Figure 1.3 Role of phosphate retention in the pathogenesis of secondary hyperparathyroidism. *Hyperphosphatemia stimulates parathyroid hormone (PTH) secretion indirectly by inducing hypocalcemia, skeletal resistance to PTH, low levels of calcitriol, and calcitriol resistance. Hyperphosphatemia also has direct effects on the parathyroid gland to increase PTH secretion and parathyroid cell growth.*

Second, Phosphate retention leads to decreased production of calcitriol by the kidney, either directly or by increasing the levels of FGF-23 (which decreases the activity of 1α-hydroxylase). The decrease in calcitriol allows increases in PTH gene transcription by direct action and also decreases intestinal calcium absorption, leading to hypocalcemia, which in turn stimulates PTH secretion. ***(Wahl P et al,2012)***

Third, hyperphosphatemia is associated with resistance to the actions of calcitriol in the parathyroid glands, which also favors the development of hyperparathyroidism and also induces resistance to the actions of PTH in bone. ***(Taylor EN et al,2011)***

Finally, phosphate *perse* appears to affect PTH secretion independently of changes in serum calcium or serum calcitriol. Phosphate may also have an effect on parathyroid growth independent of serum calcium. ***(Vervloet MG et al ,2012)***

Current evidence suggests that FGF-23 also acts directly on the parathyroid gland and has inhibitory effects on PTH secretion and parathyroid growth.

This would suggest that the main effects of FGF-23 on the pathogenesis of hyperparathyroidism appear to be indirect as a result of the potent effect of FGF-23 to decrease calcitriol production. These various actions may explain the association of the levels of FGF-23 with patient outcome*.(**Titan SM et al,2011)***

C-*Abnormalities of Vitamin D Metabolism*

The conversion of 25-hydroxyvitamin D to its active metabolite 1,25-dihydroxyvitamin D occurs mainly in the kidney by the enzyme 1- alpha-hydroxylase. Renal calcitriol production progressively declines during the course of CKD as a result of several mechanisms (Fig. 1.4). ***(Gutierrez OM and Wolf M,2010)***

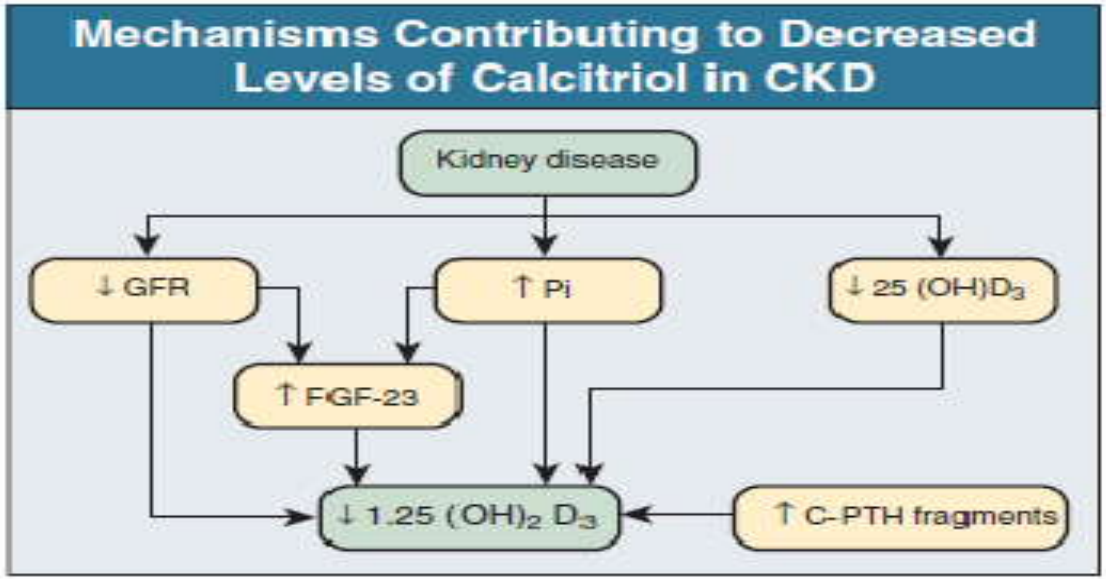


Figure 1.4 Mechanisms contributing to decreased levels of calcitriol in CKD

Calcitriol production is compromised in the setting of CKD by a reduction in 25-hydroxyvitamin D levels and the decrease in GFR, which further limits the delivery of 25-hydroxyvitamin D to the site of the 1 alpha hydroxylase in the proximal tubule. Phosphate retention either directly or by inducing an increase in FGF-23 also decreases the activity of 1 alpha hydroxylase. **(*Hansen D et al,2011)***

Finally, it appears that circulating PTH fragments may also directly decrease calcitriol production. The resultant decreased levels of calcitriol contribute to the pathogenesis of hyperparathyroidism by several direct and indirect mechanisms (Fig.1.5***).(Isakova T et al,2011)***

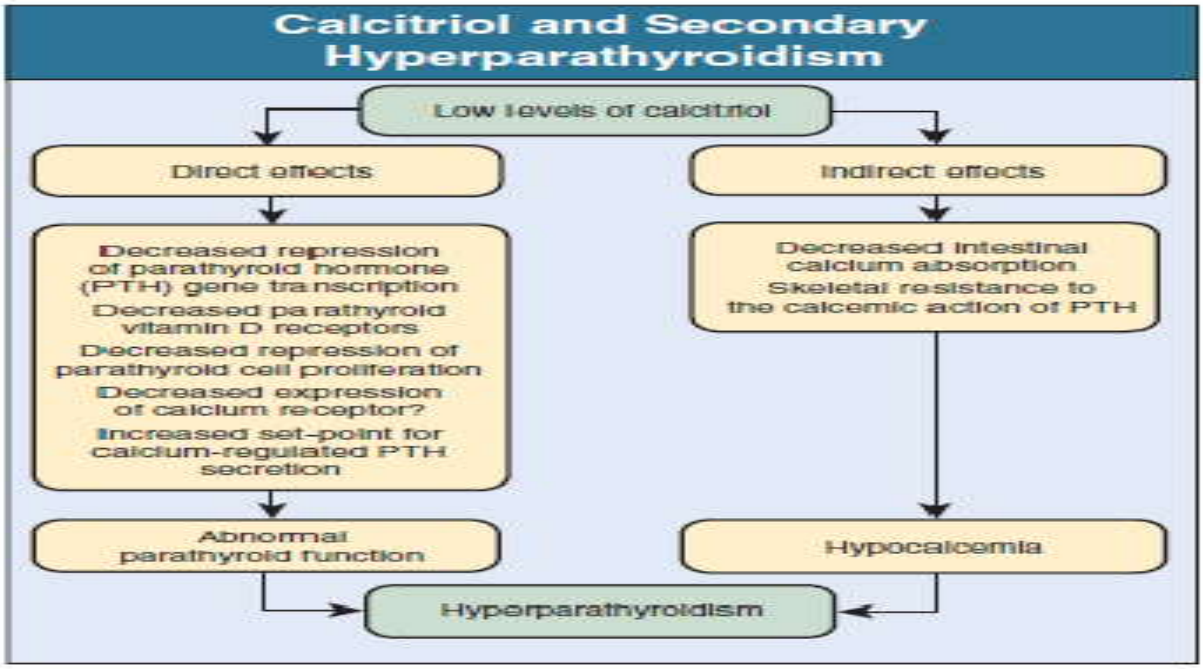


Figure 1.5 Role of low levels of calcitriol in the pathogenesis of secondary hyperparathyroidism.

Low levels of calcitriol directly release the gene for PTH from suppression by the vitamin D receptor and allow increased PTH secretion. In many tissues, vitamin D regulates its own receptor by positive feedback;the vitamin D receptor content is decreased in parathyroid tissue in CKD. ***(Hasegawa H et al ,2010)***

Administration of calcitriol has been shown to increase the vitamin D receptor content in the parathyroid glands coincident with the suppression of PTH secretion. Studies *in vitro* have shown that calcitriol is a negative growth regulator of parathyroid cells; therefore, calcitriol deficiency in CKD may facilitate parathyroid cell proliferation***. (Isakova T et al,2011)***

Other direct consequences of low levels of calcitriol that contribute to the pathogenesis of secondary hyperparathyroidism include increasing the parathyroid set point for calcium-regulated PTH secretion and, possibly, decreasing the expression of calcium receptors.*(**Gutierrez OM et al,2009)***

Low levels of calcitriol may also promote the development of hyperparathyroidism indirectly as decreased calcitriol production can lead to progressive reductions in intestinal absorption of calcium and implicated in skeletal resistance to the calcemic actions of PTH leading to hypocalcemia and stimulation of PTH release. **(*Hansen D et al,2011)***

D-*Abnormalities of Parathyroid Gland Function*

There are intrinsic abnormalities in parathyroid gland function in the course of CKD in addition to those caused by hypocalcemia, low levels of calcitriol, and skeletal resistance to the actions of PTH. ***(Kawarazaki H et al,2011)***

Parathyroid hyperplasia is an early finding in CKD, there is decreased expression of vitamin D receptors as well as of calcium receptors.The parathyroid calcium receptor is centrally involved in the regulation of PTH secretion by calcium. ***(Komaba H et al,2010)***

Its expression and synthesis are decreased in parathyroid glands from hyperparathyroid subjects, leading to altered calcium-regulated PTH secretion. Increased concentrations of calcium are required *in vitro* to suppress PTH release from the parathyroid cells of uremic patients compared with those of normal controls. Thus,the set point for the concentration of calcium required to decrease PTH release by 50% appears to be increased. ***(Lopez I et al,2011)***

E-*Abnormal Skeletal Response to Parathyroid Hormone*

In patients with CKD, there is an impaired response of serum calcium to the administration of PTH and a delay in the recovery from induced hypocalcemia in the presence of larger increments in PTH levels.

Thus, in CKD, the skeleton is relatively resistant to the calcemic actions of PTH. The resultant decrease in serum calcium levels stimulates PTH secretion and contributes to the pathogenesis of secondary hyperparathyroidism. ***(Manghat P et al,2010)***

Factors involved in the skeletal resistance to PTH in CKD include decreased levels of calcitriol, downregulation of the PTH receptor, and phosphate retention. ***(Marks J et al,2010)***

2- *Low-Turnover Metabolic Bone Disease in CKD (Adynamic Bone Disease):*

Low-turnover bone disease commonly is observed in patients with kidney disease, especially in patients who are on dialysis, and is characterized by an extremely slow rate of bone formation. Some cases demonstrate osteomalacia, which is characterized by defective bone mineralization in addition to the very slow bone formation rate.***(Moe SM et al,2011)***

The osteomalacic lesion is due mostly to aluminum accumulation and is less common nowadays with decreased use of aluminum-based phosphorus binders ***(Malluche HH et al, 2008).***

The adynamic bone of kidney disease has been described in some cases even before dialysis. The pathogenesis of adynamic bone is not well defined, but it seems that a number of factors might be involved.

A number of these factors contribute to a relative state of hypoparathyroidism such as the administration of high calcium loads from calcium-containing phosphate binders or the use of high-dialysate calcium concentrations, as well as the use of potent vitamin D sterols. ***(Couttenye MM et al, 1999)***

Age also may be a factor because many elderly patients may have low bone turnover on the basis of postmenopausal osteoporosis or osteopenia in association with systemic disease.

Several other complications of the uremic state can lead directly lead to decreases in bone formation, undefined uremic toxins, acidosis, decreased expression of PTH receptors, alterations in concentrations of growth factors and cytokines that affect bone turnover ***(Gonzalez EA et al, 2004)***

Clinical manifestation:

1-Musculoskeletal symptoms:

Clinical manifestation of hyperparathyroidism and Metabolic bone disease in patient with kidney disease often is asymptomatic, and symptoms appear only late in its course. Many of the symptoms are nonspecific in nature mainly in the lower back, hips, and legs and include pain and stiffness in joints, spontaneous tendon rupture, predisposition to fracture, and proximal muscle weakness. ***(Kevin and Esther, 2007)***

Bone deformity are common in patients with severe hyperparathyroidism , in adult , deformities arises as consequence of fracture with the axial skeleton being most commonly affected leading to kyphoscoliosis or chest wall deformities , in children, slipped epiphysis , occasional frank rachitic features and growth retardation may occur .***(Moe SM et al,2011)***

2-Metastatic calcification and calciphylaxis

Extraskeletal calcifications are encountered in patients with advanced CKD and aggravated by persistent elevation of calcium-phosphate product, particularly involving the vasculature and calcification may also occur in other sites such as the lung, myocardium and periarticular areas and calciphylaxis also may be seen.

Cardiovascular calcification is extremely common and important in patients with kidney disease causes left ventricular hypertrophy, congestive heart failure, and coronary ischemia, in whom it develops and progresses rapidly and predicts a variety of adverse outcomes. ***(Shanahan CM , 2005)(Oliveira RB et al,2011)***

Studies suggest that the normal vessel wall expresses proteins that inhibit calcification such as matrix Gla protein and fetuin-A are produced at remote sites and act to inhibit soft tissue calcification systemically. However, alterations of these proteins may lead to a seeming transformation of vascular smooth muscle cells into osteo/ chondrocytic-like cells that then facilitate calcification**.** ***(Ketteler M, 2005)***

The syndrome of calciphylaxis is characterized by vascular calcification in the tunica media of peripheral arteries. These calcifications induce painful violaceous skin lesions that progress to ischemic necrosis. This syndrome has been linked to serious complications and often death. Calciphylaxis has been associated with high serum calcium phosphate product and severe secondary hyperparathyroidism. ***(Sharon M and Neal X , 2008)***

Diagnosis:

In addition to the clinical manifestation , a variety of biochemical and radiographic techniques are helpful not only to establish the specific diagnosis but also to serve as guide for the therapeutic intervention'

1- Serum biochemistry:

The level of calcium and phosphate in serum are normal in patients with mild to moderate CKD, but with advanced CKD hypocalcemia and hyperphospatemia will occur. Hypercalcemia may be observed in cases of severe hyperpara-thyroidism or adynamic bone disease, especially with vitamin D therapy. ***(Eknoyan et al,2003)***

Hyperphosphatemia is an indication of noncompliance with phosphate binders or severe hyperparathyroidism secondary to increased release of phosphorus from bone. ***(Wesseling-Perry K et al,2010)***

The level of alkaline phosphates offers an index of osteoblast activity in patients with CKD. High level are commonly present in hyperparathyroid bone disease, and low values are present in patients with low turnover osteodystrophy. ***(Kevin and Esther, 2007)***

Serum PTH levels are better indicators of bone turnover. We should measure intact PTH (iPTH). Elevated iPTH levels are characteristic of patients with osteitis fibrosa , and low levels are found in patients with low bone turnover syndromes. ***(Martin A et al., 2004)***

Accumulation of aluminum (Al^{+3}) in bone and other organs such as the parathyroid glands may occur in patients with moderate or severe renal disease or in those undergoing dialysis. Aluminum accumulation in the parathyroid glands results in decreased secretion of PTH and suppression of bone turnover. In addition, Al^{+3} inhibits renal and intestinal 25-hydroxycholecalciferol 1α-hydroxylase activity, and thus Al^{+3} may further contribute to reduced levels of calcitriol. ***(Keith A, 2007)***

2- Radiology of the skeleton:

Routine skeletal x-ray is not recommended as it is insensitive for the diagnosis of renal osteodystrophy. In secondary hyperparathyroidism, there are subperiosteal erosions in the hands, clavicles and pelvis. ***(Yazgan P et al, 2008)***

Skull may show focal radiolucencies and ground-glass appearance known as "pepper pot" skull.Osteosclerosis of the vertebrae is responsible for the "rugger-jersey" appearance of the spine. Looser zones or pseudofractures are characteristic of osteomalacia .***(Oliveira RB et al,2011)***

3- Bone biopsy:

a) Bone biopsy is not routinely performed in clinical practice because of the invasive nature of the procedure, however bone biopsy is the recognized gold standard for the diagnosis and evaluation of renal bone disease.***(Martin JK et al, 2004) (Quarles LD,2011)***

b) Predominant High-Turnover Renal Osteodystrophy (Osteitis Fibrosa): is characterized by increase bone turnover, increase number and activity of osteoblast and osteoclasts, and variable amounts of peritrabecular fibrosis. ***(Coen G et al,2002) (Quarles LD,2011)***

c) Low-Turnover Bone Disease (Adynamic Bone Disorder): is characterized by normal or decrease osteoid volume and reduced bone formation rate. few osteoclasts and osteoblasts cover the majority of trabecular bone. ***(Quarles LD,2011)***

Two histologic subgroups can be identified in this type, depending on the cause of events leading to the decline in osteoblast activity: adynamic bone disorder and low-turnover osteomalacia from Al^{+3} intoxication. ***(Keith A , 2007)***

Osteomalacia is characterized by the presence of increased osteoid seam width increase in the trabecular surface covered with osteoid and decrease bone mineralization . **(Coen G et al,2002)** ***(Quarles LD,2011)***

d) Mixed uremic osteodystrophy is caused primarily by hyperparathyroidism (osteitis fibrosa) and defective mineralization with or without increased bone formation ***(Kevin and Esther, 2007) (Quarles LD,2011)***

Treatment:

Prevention is the primary goal in the management of renal osteodystrophy . Therapy should be initiated early in the course of CKD (GFR, 50-80 ml/min) so that parathyroid gland hyperplasia can be prevented. ***(William G, 2003)(Sanderson SR et al,2011)***

Hypocalcaemia, hyperphosphataemia and impaired renal 1,25 –dihydroxy vitamin D synthesis with attendant reductions in serum calcitriol concentrations and decreases in vitamin D receptor expression in the parathyroid glands each contribute to excess parathyroid hormone (PTH) secretion in patients with CRF.

All represent targets for therapeutic interventions aimed at preventing the development and controlling the progression of secondary hyperparathyroidism ***(Sanchez M et al, 2000)(Sanderson SR et al,2011)***

A- Control of hypocalcaemia:

Hypocalcaemia must be avoided, to prevent several compensatory responses that can lead to excess PTH synthesis and secretion and, ultimately, to overt secondary hyperparathyroidism in patients with CR F. ***(William G, 2003)***

The initial therapy of hypocalcaemia in mild to moderate CKD is the administration of calcium carbonate taken between meals with increasing doses as required.

Dietary calcium intake is typically only 500 –600 mg/day. A combined intake totally 1500 to 1800 mg of elemental calcium from dietary sources and oral calcium supplements should be sufficient to achieve this objective ***(Goodman, 2001),*** also active vitamin D sterols are often required, and assessment of efficacy of therapy is by follow up determinations of serum calcium and PTH.

The goal is to achieve levels of intact PTH of 150 to 300 pg/ml in CKD V, 70 to 110 pg/ml in CKD IV and 35 to 70 pg/ml in CKD III ***(Gonzalez EA et al, 2004)***

B- Control of phosphate:

Control of phosphate is the cornerstone of effective management of secondary hyperparathyroidism. Phosphorus levels are usually within normal range until the GFR falls below approximately 30 ml/min, or stage IVCKD. ***(Block G A et al.,2004)***

1-Dietary Restriction: Dietary phosphate restriction has been shown to prevent the development of HPTH early in the course of disease, as well as increase plasma calcitriol levels and inhibit parathyroid cell proliferation. Furthermore, it may be instrumental in preventing progressive renal failure and soft tissue calcification, although the need for adequate dietary protein limits this approach. ***(Glenn M , 2003) (Barreto FC et al,2008)***

Restriction of phosphorous in the diet to 800-1200mg/day **Fig 1.6** is the keystone of control of serum phosphorus as it present in dairy product and other foods as soft drinks, liver, meat, beans, nuts, whole grain breads and cereals ***(Adeney KL et al,2009)***

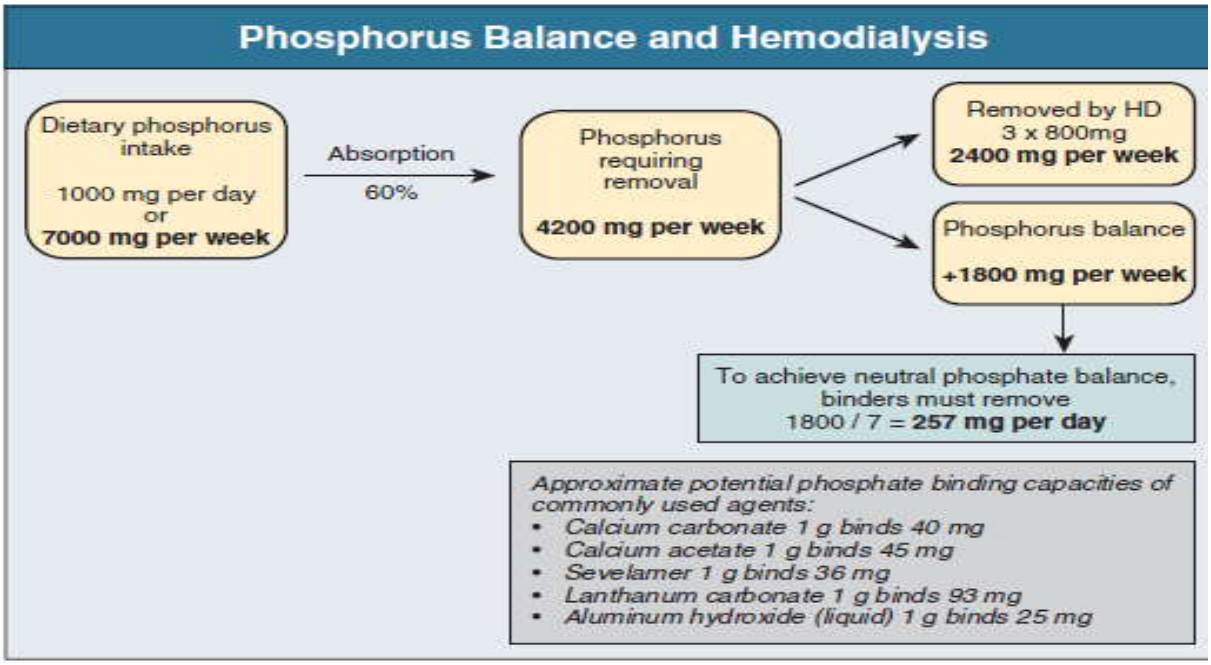


Figure 1.6 Phosphate balance and phosphate binders used in hemodialysis patients.

Aggressive dietary phosphate restriction among patients with CKD is impractical and could compromise overall nutrition, particularly protein intake. For preventing malnutrition among patients with CKD, the NKF–K/DOQI guidelines recommend a minimum protein intake of 1.2 g/kg per d (approximately 800 to 1000 mg/d phosphorus*)(**KDIGO,2009)***

2-Dialysis:

The clearance of phosphorus varies among the different modalities of dialysis. Ideally, adequate dialysis in any form would remove adequate amounts of all uremic toxins, including phosphorus. conventional, thrice-weekly hemodialysis (4h duration) removes approximately 900 mg of phosphorus each treatment (an average of only 300 mg/d). ***(Gotch FA et al,2003)(Kovesdy CP et al,2008)***

Short, daily hemodialysis utilizes blood flow rates (Qb) of 450 ml/min, dialysate flow rates (Qd) of 800 ml/min, a duration of 1.5 to 2.5 h, and a frequency of six to seven treatments per week. Alternatively, slow nocturnal hemodialysis (NH) entails Qb of 150 to 300 ml/min, Qd of 300 ml/min, duration of 6 to 8h, and a frequency of six to seven nights per week. ***(Kovesdy CP et al,2008)***

3-Phosphate Binders:

Restriction of dietary phosphate may be sufficient to prevent hyperpara-thyroidism in early CKD. As renal function deteriorates it became difficult and it is necessary to use agents that bind ingested phosphate including: aluminum hydroxide, calcium carbonate, calcium acetate, magnesium salts and non-calcium, non-aluminum phosphate binders. ***(Joseph A , 2005) (Domrongkitchaiporns S et al,2010)***

i-Aluminum Hydroxide: Aluminum hydroxide was a very efficient phosphate binder, long- term use was associated with aluminum accumulation and toxicity, manifesting itself as encephalopathy, osteomalacia, microcytic anemia, and myopathy. ***(Joseph A , 2005)***

ii-Calcium Carbonate and acetate: calcium salts taken with meals effectively bind phosphate limit their absorption and became the preferred agents. It has been shown to be effective phosphate binders in 60% to 70% of patient in HD. The dose requirement to prevent hyperphosphatemia range from 3 to 12gm (calcium carbonate) or 1.5 to 9 gm (calcium acetate) daily.***(Indridason OS et al, 2000)***

Hypercalcemia, however, has been associated with both calcium carbonate and calcium acetate ingestion. The progression of cardiovascular calcifications and the increased cardiovascular mortality among patients with ESRD . Calcium citrate potentiates aluminum uptake and should be avoided in CKD. ***(Kevin and Esther, 2007)***

iii-Magnesium Salts: are also effective phosphate binders for patients who become hypercalcemic on calcium-containing phosphate binder, but they should be used with caution in patients with renal dysfunction before dialysis due to hypermagnesemia . ***(Coladonato JA et al, 2002)***

iv-Sevelamer

Sevelamer hydrochloride is a novel nonaluminum, noncalcium phosphate-binding polymer. Sevelamer is a hydrogel of cross-linked poly (allylamine) and is completely resistant to digestive degradation and, therefore, not absorbed from the GI tract. It is an exchange resin that binds dietary phosphorus and releases chloride. ***(Ketteler M et al,2008)***

Multiple clinical studies have demonstrated that sevelamer lowers serum phosphorus levels among patients with ESRD and is generally well tolerated. Furthermore, sevelamer binds bile acids and thereby reduces fecal bile acid excretion and lowers LDL cholesterol. ***(Delmez J et al,2007)***

Sevelamer hydrochloride in a dose range of 2.4 to 4.8 gm\day provides effective phosphate control without hypercalcemia. It may be combined with both calcium and magnesium- containing phosphate binders if necessary. It also associated with decrease progression of vascular calcification .***(Hutchison AJ,2009)***

The replacement of the chloride with carbonate provides bicarbonate ions that may be a benefit to patients who have CKD and are not receiving dialysis, who are prone to acidosis and do not receive the benefits of renal replacement therapy**.** ***(Delmez J et al., 2007)***

<u>v-Lanthanum Carbonate:</u>

Lanthanum carbonate is rare earth element that was approved recently for the treatment of hyperphosphatemia among patients with ESRD. Lanthanum belongs to a group known as the "lanthanides" and has a low solubility. In the acid environment of the stomach and upper small intestine, lanthanum dissociates sufficiently to become available for phosphate binding **.*(Gonzalez-Parra E et al,2011)***

Overall, lanthanum carbonate was well tolerated, and the most common adverse events were GI, such as nausea and vomiting, which abated over time. Lanthanum was supplied as a chewable tablet in two dosage strengths, 250 and 500 mg. ***(Isakova T et al,2011)***

The total daily dose ranged between 1500 and 3000 mg. Despite encouraging results and need for alternative phosphate binders, the widespread use of lanthanum may be limited by concerns of long-term exposure and cost.***(Isakova T et al,2011)***

C- Use of vitamin D and its metabolites:

In patients with CKD stages II to V, clinicians have to rely on the use of calcitriol, its analoug 1-alpha 25 OH vitamin D (alfacalcidol), or other active vitamin D sterols, either alone or in combination with oral calcium supplements and/or non– calcium containing phosphate binders, to arrest or retard the steady increase in plasma PTH levels associated with the progression of chronic renal failure**. (*Tilman B and Eberhard , 2009)(Isakova T et al,2012)***

In patients with very high levels of PTH and very enlarged glands that may have sever nodular hyperplasia, the effectiveness of vitamin D metabolites may be limited because the levels of vitamin D receptor are low ***(Mehrotra R et al,2008)***

Vitamin D has an indirect effect on PTH through facilitating the intestinal absorption of calcium, and it may induce episodes of hyperphosphatemia and/or hypercalcemia. ***(Angel LM et al, 2006)***

Vitamin D in CKD patients is associated with an increased prevalence of vascular calcification and arterial stiffness, as well as with higher mortality. ***(Shroff et al, 2008)***

The administration of excessive amounts of calcitriol or alfacalcidol may induce adynamic bone disease and arterial calcification, as do excessive oral doses of calcium salts ***(Andress DL,2008)***

In recent years, there are analogues of calcitrol that have less calcemic activity than the parent compound and retain the ability to suppress PTH release. Such analogues of vitamin D are 22-oxacalcitriol, 1-alpha hydroxyvitamin D, and paricalcitol. It has been shown to decrease the level of PTH without aggravating hypercalcemia or hyperphosphatemia. **(*Teng M et al, 2003) (Yilmaz MI et al,2012)***

D- Role of calcimimetic agents:

A recently introduced approach to the treatment of refractory hyperpara-thyroidism is use of calcimimetics that increase CaR sensitivity to extracellular calcium, thus maximizing the suppressive effect of calcium on PTH secretion and production ***(Angel LM et al, 2006)(Koizumi M et al ,2012)***

Calcimimetics are positive allosteric modulators of the calcium-sensing receptor that increase its sensitivity to extracellular calcium by lowering the threshold for activation by extracellular calcium ions so inhibit the release of parathyroid hormone, and lower parathyroid hormone levels within a few hours after administration**.** ***(Block G A et al., 2004))(Koizumi M et al ,2012)***

Cinacalcet , is a safe and effective treatment for secondary HPT in PD and HD patients Once-daily oral treatment with cinacalcet at doses up to 180 mg effectively reduced iPTH levels, regardless of dialysis modality or disease severity. Furthermore, cinacalcet significantly improved serum calcium and phosphorus levels, leading to reductions in Ca X P values**.** ***(Wetmore JB et al,2010)***

Alternatively, cinacalcet has also been shown to downregulate mRNA expression levels encoding proteins that are involved in active transcellular calcium reabsorption in the intestine. ***(Yilmaz MI et al,2012)***

E-Role of parathyroidectomy

Despite aggressive efforts to control PTH levels , surgical parathyroidectomy continues to be necessary in those patient with sever hyperparathyroidism ***(Foley RN et al, 2005)***

1. Indications:

(1) Severe hyperparathyroidism : With persistent hypercalcemia, unresponsive to calcitriol and calcium ,with hypercalcemia , in renal transplant candidate,or with evidence of metastatic calcification.

(2) Progressive and symptomatic soft tissue calcification with high bone turnover (including calciphylaxis),

(3) Refractory severe pruritus, only if additional evidence of hyperparathyroidism.***(Kevin and Esther, 2007)***

2. Relative contraindication:

Accumulation of aluminum on the bone mineralizing surface increase markedly after parathyroidectomy that might predispose to osteomalacia so that parathroidectomy should not be done in patients who are aluminum loaded and if there is a history of long term aluminum exposure , a bone biopsy should be performed prior to parathyroidectomy **(*Ubara Y et al, 2003)***

3. Surgical strategy:

The choice of surgical procedure for parathyroidectomy are subtotal removal of parathyroids by resection of three glands and 75% of the fourth or total parathroidectomy with autotransplantion of parathyroid tissue in the forearm. ***(Ahmad R and Hammond JM , 2004)***

4. *Postoperative care* :

Within several hours after parathyroidectomy but especially during the first postoperative days hypocalcaemia ocurr.

Hypocalcemia after parathyroid surgery may be due to hungry bone syndrome where calcium and phosphorus are rapidly deposited in the bone. This is characterized by hypoparathyroidism and transient, but occasionally severe hypocalcemia , so that oral calcium supplementation (2-4 gm/day) and large dosages of intravenous calcium (0.5-5gm/day) and oral or intravenous calcitriol (2-6 mcg/day) may be required to maintain serum calcium levels in an acceptable range . ***(Mazzaferro S et al,2000).***

The use of calcitriol may minimize the need for large doses of calcium salts; however, its use may interfere with successful function of the transplanted gland. A reasonable approach would be the use of intravenous calcitriol administered at the end of each dialysis treatment for two to three treatments before parathyroidectomy, followed by the lowest dose of oral calcitriol needed. ***(Keith A, 2007)***

Treatment of Adynamic Bone Disease:

No specific treatment is available, effective measures include a reduction in calcium-containing phosphate binders because it increase the frequency of hypercalcemia , also the dialysate calcium content should reduce and vitamin D metabolites should discontinue to avoid over suppression of PTH secretion, but others phosphate binders that do not contain calcium may be beneficial ***.(Mathew S et al.,2007)***

Stepped approach to treatment in predialysis patient:

Serum calcium, phosphate, 25(OH) vitamin D and intact PTH levels should be measured initially. We suggest attaining KDIGO treatment goals for PTH levels, serum phosphate and calcium levels. KDIGO 2009 guidelines suggest the following stepped treatment approach in patients with CKD grades 3 through 5 not yet on dialysis with PTH levels higher than target level:

Step 1: Our initial focus in managing secondary hyperparathyroidism in the predialysis patient is the treatment of hyperphosphatemia. Among patients with serum phosphate levels greater than target levels, they suggest first restricting dietary phosphate intake, limit phosphate intake to 900 mg per day

Step 2: Among patients with serum phosphate levels greater than target levels despite dietary phosphorus restriction **after two to four months**, we suggest the administration of phosphate binders. The two principal options are calcium and non-calcium based phosphate binders:

- For patients with an initial serum calcium levels less than 9.5 mg/dL (<2.37 mmol/L), a calcium containing phosphate binder may be administered as long as hypercalcemia does not develop.
- For patients with an initial serum calcium level greater than 9.5 mg/dL (<2.37 mmol/L), we recommend a non-calcium based phosphate binder rather than a calcium-containing phosphate binder.

Among predialysis patients with stage 3 to 5 CKD and elevated plasma intact PTH, we also suggest that treatment with ergocalciferol be initiated if nutritional vitamin D deficiency exists, as demonstrated by a 25(OH)-vitamin D (calcidiol) level of less than 30 ng/mL.

After initiating treatment, serum calcium and phosphorus should be monitored quarterly, and continued need for supplementation with ergocalciferol can be re-evaluated annually. If the serum level of corrected total calcium exceeds 10.2 mg/dL (2.54 mmol/L), ergocalciferol therapy should be discontinued.

Some experts also consider initiating phosphate binders among patients with elevated PTH levels and normal phosphate levels based upon the observation that compensatory mechanisms occur that help maintain serum phosphate in the normal range until the late stage of CKD. If this performed, small doses of phosphate binders may be administered with meals.

Step 3: If elevated PTH levels remain despite optimal ergocalciferol and phosphate binder therapy over **a six-month period**, we suggest administering a low dose active oral vitamin D analog. Any one of the available active oral agents (calcitriol, alfacalcidol, doxercalciferol, or paricalcitol) may be administered using cost and formulary availability as guides

Treatment with a vitamin D analog should not be given to predialysis patients with stage 3 to 5 CKD with elevated serum phosphate and calcium (>9.5) levels. If the serum level of corrected total calcium exceeds 10.2 mg/dL (2.54 mmol/L), ergocalciferol therapy and all forms of vitamin D therapy should be discontinued.

Step 4 : Among predialysis patients with secondary hyperparathyroidism that is refractory to therapy with vitamin D analogues, calcium supplements, and phosphate binders, cinacalcet may be useful. However, the use of cinacalcet in early stages of CKD is highly controversial. Some experts and the KDIGO working group recommend NOT giving cinacalcet given the paucity of data concerning efficacy and safety in predialysis patients with CKD.

Alternatively, parathyroidectomy could be considered for patients with refractory hyperparathyroidism and hypercalcemia not responsive to medical therapy.

Stepped approach to treatment in dialysis patient:

Steps 1 and 2: The initial focus in managing secondary hyperparathyroidism should be the prevention and management of hyperphosphatemia.

As a first step, a dietary restriction of 900 mg/day of phosphorus is appropriate. There should be an emphasis on high biologic sources of phosphorus (meats, eggs) and avoidance of lower nutritional sources (certain vegetables, colas).

They suggest the following interventions based upon serum phosphate and calcium levels.

- Phosphate <5.5 mg/dL (<1.78 mmol/L) and calcium <9.5 mg/dL (<2.37 mmol/L) — Calcium-based phosphate binders should be administered.(in the presence of concurrent therapy with active vitamin D analogues).
- Phosphate <5.5 mg/dL (<1.78 mmol/L) and calcium >9.5 mg/dL (>2.37 mmol/L) — No phosphate binder is necessary in most patients. Among those with vascular calcifications, we suggest treatment with a non-calcium containing phosphate binder.
- Phosphate >5.5 mg/dL (>1.78 mmol/L) and calcium >9.5 mg/dL (>2.37 mmol/L) — We recommend the administration of a non-calcium containing phosphate binder rather than calcium containing binders.
- Phosphate >5.5 mg/dL (>1.78 mmol/L) and calcium <9.5 mg/dL (<2.37 mmol/L) — We suggest first titrating a calcium-based phosphate binder (up to 1,500 mg of elemental calcium from binders alone if there is concurrent use of active vitamin D analogues). If phosphate remains

above 5.5 mg/dL (>1.78 mmol/L), we then add a non-calcium containing phosphate binder.

Step 3 : The next step is to decide whether phosphate binder therapy is sufficient or whether a calcimimetic or vitamin D analogue should be added. This is based upon calcium, phosphate, and PTH levels that are measured If calcium supplementation and phosphate binders are effective in controlling PTH (ie, level between 150 and 300 pg/mL), no additional therapy may be needed. Serial follow up of PTH levels should be performed at three month intervals to assess the continued control of disease.

If PTH levels remain greater than 300 pg/mL with optimal binder therapy, the choice is either cinacalcet or vitamin D analogues. The decision to use vitamin D or cinacalcet as the next step, without additional data on outcomes, should be based upon the calcium and phosphate levels that are measured when administering optimal phosphate binders:

- If the calcium and phosphate levels are both toward the upper limit of target levels, we suggest administering cinacalcet. This is because cinacalcet lowers both these parameters, while vitamin D therapy has the potential to further increase calcium and phosphorus levels.
- If the calcium level is near or below the lower limit of normal and the phosphate is well within the normal range, we suggest the administration of vitamin D, given that cinacalcet would further lower the serum calcium.

3-OSTEOPOROSIS IN CHRONIC KIDNEY DISEASE

Whereas abnormal bone is common and fracture risk is increased in CKD patients, the relative contribution of classic osteoporosis (as defined by World Health Organization criteria) to the CKD-MBD complex is not well defined. Data from studies of anti-osteoporosis agents are available only for patients in CKD stages 1 to 3. Nevertheless, postmenopausal women and elderly men nowadays are highly prevalent in late-stage CKD populations, and it is thus likely that classic osteoporosis also contributes to their bone disease. ***(Kevin and Esther, 2007)***

Pathogenesis of Osteoporosis in Chronic Kidney Disease

Osteoporosis may be associated with low, normal, or high bone turnover and is characterized by thin and disconnected trabeculae and the loss of the plate-like bone structure. Many patients with CKD have abnormal mineralization and increased osteoid, which is quite untypical for osteoporosis. ***(Quarles LD,2011)***

Typical pathogenetic factors of osteoporosis including hypoestrogenemia, immobilization, and corticosteroid use are frequent in CKD patients, although some postmenopausal women in late-stage CKD may have relatively normal estrogen levels. However, the sum of CKD-MBD–related biochemical disturbances probably represents the decisive factors as to which bone phenotype predominates. Secondary hyperparathyroidism, relative hypoparathyroidism (as in ABD), and 25-hydroxyvitamin D as well as 1,25-dihydroxyvitamin D deficiencies may dominate . ***(Quarles LD,2011)***

Diagnosis :

In patients with advanced CKD, bone turnover biomarkers and measurements of bone mineral density by dual-energy x-ray absorptiometry are useless tools in the differential diagnosis of classic osteoporosis from other CKD-MBD–related bone disease. Bone mineral density does not predict fracture risk in CKD patients as it does in the general population, implying that abnormal bone quality rather than density is the major disturbance in such patients. The only reliable methodology to diagnose osteoporosis and to discriminate it from other bone manifestations in CKD patients is bone biopsy. ***(Jamal SA et al,2007)***

Treatment of Osteoporosis in Chronic Kidney Disease

Post hoc analyses of large prospective treatment studies using anti-osteoporotic medications demonstrated that it seems safe and efficacious to treat postmenopausal women in stages CKD 1 to 3 if they have a high risk of fractures (according to World Health Organization criteria) and no features of CKD-MBD. In such populations, bisphosphonates, raloxifene, and teriparatide appear to be feasible therapeutic options. (***Ishani A et al,2008)***

In contrast, for patients in CKD stages 3 to 5 with features of CKD-MBD, there are no data available on the safety and efficacy of any of these anti-osteoporotic medications. In CKD patients with ABD, bisphosphonates may aggravate osteoclast paralysis. In CKD patients with secondary hyperparathyroidism, bisphosphonates may upregulate PTH secretion. None of these anti-osteoporotic compounds can thus be recommended in patients with CKD-MBD to date, unless bone biopsy proves the exclusive presence of osteoporosis.(***Ishani A et al,2008)***

4-β2-MICROGLOBULIN–DERIVED AMYLOID

β2-Microglobulin–derived (Aβ2M) amyloidosis, also termed dialysis-associated amyloidosis, exclusively affects patients with stage 5 CKD. It is a systemic amyloidosis. Clinical manifestations are largely confined to the musculoskeletal system. In recent years, the disease has become notably infrequent. ***(Kevin and Esther, 2007)***

Pathogenesis

Fibrils of Aβ2M amyloid are derived from the circulating precursor protein β2-microglobulin, the nonvariable light chain of the HLA class I complex. The pathogenesis appears to involve three events:

(1) pronounced renal retention of β2-microglobulin (11.8 kd), leading to plasma levels that can be elevated up to 60-fold in dialysis patients.

(2) modifications of the β2-microglobulin molecule that render it more amyloidogenic, such as limited proteolysis or the formation of different sugar-protein cross-links

(3) local factors that contribute to and determine the particular spatial localization of the amyloidosis. ***(Floege J et al,2004)***

Clinical Manifestations and Diagnosis

Aβ2M amyloidosis mainly is manifested at osteoarticular sites, particularly synovial membranes.Carpal tunnel syndrome typically worsens at night and during hemodialysis. It is often bilateral and usually requires surgery. ***(Floege J et al,2004)***

Osteoarthropathy of peripheral joints, resulting from amyloid deposition in periarticular bone and the synovial capsule is characterized by recurrent or persistent arthralgias, stiffness of large and medium-sized joints, and swelling of capsules and adjacent tendons. ***(Jamal SA et al,2007)***

Recurrent joint effusions and synovitis, often in the shoulders and knees, may occur. The clinical presentation may vary from frank, acute arthritis to slow, progressive destruction of the affected joints. Destructive spondylarthropathy resulting from Aβ2M amyloidosis can be manifested as asymptomatic deposits, radiculopathy, stiffness and, finally, medullary compression with resulting paraplegia or cauda equina syndrome. ***(Floege J et al,2004)***

Other manifestations include camptodactyly (a flexion deformity resulting in bent fingers that cannot completely extend or straighten) resulting from amyloid deposits along the flexor tendons of the hands . Patients undergoing dialysis can also have subcutaneous tumorous deposits of Aβ2M amyloid; however, diffuse infiltration of the subcutaneous fat or skin has not been observed. . ***(Kevin and Esther, 2007)***

Diagnosis

Plasma levels of Aβ2m do not distinguish between patients with the amyloidosis and those without. Ultrasound can detect synovial Aβ2M amyloidosis as thickening of the joint capsules of the hip and knee, biceps tendons, and rotator cuffs as well as the presence of echogenic structures between muscle groups and joint effusions. . ***(Kevin and Esther, 2007)***

On radiologic examination, affected joints may present with single or multiple juxta-articular, "cystic" (i.e., amyloid-filled) bone radiolucencies. Such bone defects are prone to pathologic fractures. ***(Floege J et al,2004)***

Diagnostic criteria for Aβ2M amyloid–induced cystic bone radiolucencies include (1) diameter of lesions more than 5 mm in wrists and more than 10 mm in shoulders and hips
(2) normal joint space adjacent to the bone defect
(3) exclusion of small subchondral cysts in the immediate weight-bearing area of the joint and of defects of the "synovial inclusion" type
(4) increase of defect diameter of more than 30% per year
(5) presence of defects in at least two joints.

The definitive diagnosisof Aβ2M amyloidosis relies on histology. Fat aspiration and rectal biopsy are not helpful in Aβ2M amyloidosis, but diagnostic material can be obtained from synovial membranes, synovial fluid, or bone lesions. ***(Floege J et al,2004)***

Treatment and Prevention

Therapy for established Aβ2M amyloidosis is symptomatic. NSAIDs and physical and surgical measures such as carpal tunnel decompression, and bone stabilization in areas of cystic destruction are all used. ***(Kevin and Esther, 2007)***

Renal transplantation is the preferred treatment because it leads to rapid symptomatic improvement and halts further progress of the disease, but it is controversial whether this can actually lead to regression of established Aβ2M amyloid deposits. ***(Floege J et al,2004)***

A number of strategies exist for prevention of the clinical manifestations of Aβ2M amyloidosis. The risk of carpal tunnel syndrome is reduced by 40% to 50% in patients treated with high-flux hemo(dia)filtration and minimal in patients receiving online hemodiafiltration. ***(Floege J et al,2004)***

Chapter THREE

FGF23 IN CHRONIC KIDNEY DISEASE

FGF23 IN CHRONIC KIDNEY DISEASE

Chronic kidney disease (CKD) is a growing public health epidemic that is associated with a markedly increased risk of cardiovascular mortality. Mineral metabolism disordered and particularly, disordered of phosphorus metabolism appears to be a contributing factor. ***(Wolf M,2012)***

Fibroblast growth factor 23(FGF23) regulates phosphorus and vitamin D metabolism. Its levels increase progressively beginning in early CKD, presumably as a physiological adaptation to maintain normal serum phosphate levels or normal phosphorus balance. ***(Isakova T et al,2011)***

FGF23 promotes phosphaturia and decreases production of calcitriol. Recent studies suggest that increased FGF23 is associated with mortality, left ventricular hypertrophy, endothelial dysfunction and progression of CKD. ***(Wolf M,2012)***

These results were consistently independent of serum phosphate levels. FGF23 is emerging as a novel biomarker that may help identify which CKD patients might benefit most from aggressive management of disordered phosphorus metabolism. ***(Wolf M,2012)***

It is also possible that markedly increased FGF23 levels in CKD could contribute directly to tissue injury in the heart, vessels and kidneys, an exciting question that is sure to be the topic of intense investigation in the near future. ***(Wolf M,2012)***

INTRODUCTION: BURDEN OF CHRONIC KIDNEY DISEASE

Chronic kidney disease (CKD) is a growing public health epidemic that is estimated to affect 3% of the US adult population, or approximately 26 million Americans and far more patients worldwide. ***(Snyder JJ et al,2009)***

The disease accounts for more than 24% of annual Medicare expenditures. The growing burden of CKD reflects the impact of the rapidly increasing prevalence of diabetes and hypertension, which account for more than 50% of all adult cases of end stage renal disease requiring dialysis **.** ***(Coresh J et al,2007)***

Indeed, the 30% increase in prevalence of CKD over the past decade has prompted the U.S. Renal Data System (USRDS) to issue for the first time a separate report documenting the magnitude of CKD in addition to its annual dialysis report: The USRDS 2009 Annual Data Report (Fig 3.1): Atlas of Chronic Kidney Disease and End Stage Renal Disease.***(USRDS ,2010)***

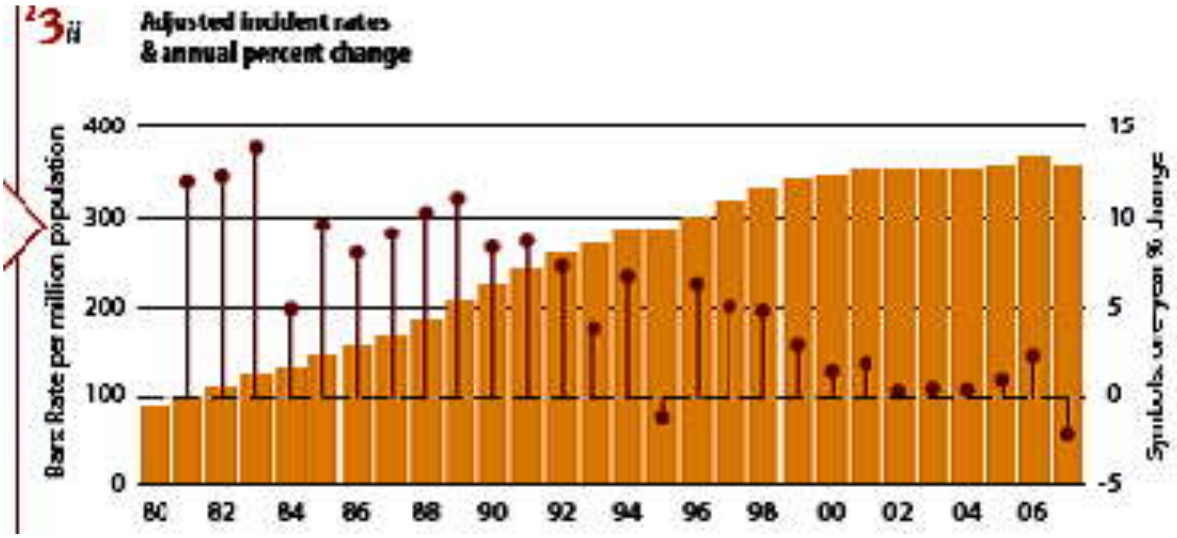


FIGURE 3.1*: Adjusted U.S. incidence rates of ESRD and annual percent change. (U.S. Renal Data System: USRDS **2009**)*

Although the overwhelming economic and medical impact of the growing dialysis population (Stage *5* CKD: >350,000) is widely recognized, >13 million people suffer from CKD Stages *3* or 4 which are risk factors for cardiovascular disease (CVD) mortality. ***(SnyderJJ et al,2009)***

Indeed, besides progression of renal failure to the point that dialysis or kidney transplantation is required for survival, the most common and morbid adverse outcomes of CKD is CVD, including high rates of atherosclerosis, myocardial infarction, peripheral vascular disease, stroke, extensive arterial calcification with increased vascular stiffness, left ventricular hypertrophy and congestive heart failure. ***(Schiffrin EL et al,2007*)**

The tight relationship between CVD and CKD stems partly from their sharing a common set of risk factors such as diabetes and hypertension. New-onset CVD events are more common in all stages of CKD than in the general population and the outcomes related to these events are worse in CKD patients than in the general population. ***(Weiner DE et al,2004)***

Furthermore, the presence of CVD is associated with more progressive CKD. In addition to these shared pathways, CKD appears to be an independent risk factor for CVD: the presence of CKD confers even greater risk than would be expected after taking into account the high frequency of traditional CVD risk factors. ***(El sayed EF et al,2007)***

The observation of dramatically increased risk of CVD in patients with CKD has fueled renewed emphasis on earlier diagnosis of CKD through widespread screening of at risk populations, earlier intervention against traditional CVD risk factors in CKD patients and the search for new, CKD specific risk factors to target for future interventions aimed at improving renal and cardiovascular outcomes.***(Komaba H and Fukagawa M ,2010)***

Definition and Staging of Chronic Kidney Disease (CKD)

CKD is ***defined as*** the presence, for at least **3** months, of evidence of kidney damage with an abnormal GFR **or**, alternatively, by a GFR below **60** mL/min/1.73 m^2 body surface area *(**National Kidney Foundation**, **2002**).*

Kidney damage is defined as pathologic abnormalities or markers of damage, including abnormalities in blood or urine tests or imaging studies. ***(National Kidney Foundation, 2002).***

The **KDIGO 2012** guidelines use a **five**-stage schema *(Table 2-1)* based on the reduction in GFR to help classify the severity of CKD. An international position statement added modifiers for noting whether a patient is treated with dialysis or transplantation

The presence of ***hypertension*** should be noted independently of the CKD stage. *(***KDIGO,2012***).*

Stages of Chronic Kidney Disease (Table 3-1).

GFR categories in CKD

GFR category	Description	GFR (mL/min/1.73 m^2)
G1	Kidney damage with normal or ↑ GFR	≥ 90
G2	Kidney damage with mild ↓ GFR	60–89
G3	3A moderate ↓ GFR 3B moderate ↓GFR	59-45 44-30
G4	Severe ↓ GFR	15–29
G5	Kidney failure	< 15 (or dialysis)

Complications of CKD

CKD is a systemic disease that affects multiple organ systems during its course. With progressive loss of kidney function, patients with CKD develop multiple hemodynamic, hematologic and metabolic complications. The clinical impact of these consequences has been reviewed **to** detail recently elsewhere. ***(Abboud H and Henrich WE,2009)***

In the current chapter, focused attention is paid to the mineral metabolism abnormalities that are common in CKD, with specific emphasis to the role of FGF23 in CKD. ***(Wolf M ,2012)***

OVERVIEW OF NORMAL MINERAL METABOLISM AND ITS DISORDERS IN CKD:

Mineral metabolism disorder is among the earliest and most common complications of CKD***.(Voormolen N et al,2007)***

In addition to its well-known effect on skeletal complications, the statute of disordered mineral metabolism in CKD has grown dramatically in recent years with the publication of a series of large epidemiological studies of dialysis and subsequently.

Earlier stage CKD patients that demonstrated various components of disordered mineral metabolism to be independently associated with kidney disease progression, cardiovascular disease and mortality. ***(Bhuriya R et al,2009)***

Understanding these observations requires a brief overview of mineral metabolism in health and in CKD.

Under normal conditions, calcium and phosphorus balance, their serum levels and skeletal integrity and growth are tightly regulated by the interrelationships between parathyroid hormone (PTH), vitamin D and most recently, fibroblast growth factor 23 (FGF23). ***(Kovesdy CP et al,2008)***

Collectively, these hormones contribute to the regulation of mineral absorption by the intestine, mineralization of bone and renal excretion of excess minerals. ***(Bhuriya R et al,2009)***

1) Parathyroid hormone (PTH)

PTH is a polypeptide containing 84 amino acids that is secreted by the parathyroid glands after cleavage from preproparathyroid hormone (115 amino acids) to proparathyroid hormone (90 amino acids) to the mature hormone 84-amino acid, full-length protein (PTH 1-84). ***(Goodman WG and Quarles LD,2007)***

The major target end organs for parathyroid hormone (PTH) action are the kidneys, skeletal system, and intestine. It has a very short half-life (minutes) in the circulation and is degraded by the liver and kidney**.** ***(Garth F et al, 2008)***

The secretion of PTH is regulated by the extracellular ionized calcium concentration. The parathyroid cells regulate PTH secretion by having the ability to sense changes in extracellular calcium concentration. ***(Juppner H,2011)***

This is accomplished through a G protein–coupled receptor (GPCR) known as the calcium-sensing receptor (CaR).

The calcium ion is a ligand for this receptor. Ligand binding to the CaR activates downstream signaling pathways in response to increases in extracellular calcium concentration. ***(Jono S et al ,2008)***

This process, in turn, suppresses PTH secretion. If calcium levels are persistently elevated, activation of this receptor also reduces PTH mRNA levels and inhibits parathyroid cell proliferation.

In addition to parathyroid cells, the CaR is also prominently expressed in the kidney, where it regulates calcium handling by the renal tubules**.** ***(Jono S et al ,2008)***

Phosphorus retention promotes PTH mRNA translation and enhances PTH synthesis; however, both vitamin D and calcium negatively regulate the rate of gene transcription for pre-pro-PTH. ***(Garth F et al, 2008)***

The various vitamin D analogues, including calcitriol, or 1,25-dihydro-xyvitamin D3, act by binding to the vitamin D receptor (VDR) in target tissues, including the parathyroid glands. After binding to its ligand, the VDR interacts with a vitamin D response element (VDRE) and diminishes gene transcription. ***(William G, 2003)***

PTH exerts direct effects on kidney and bone cells and indirect effects on enterocytes. The effects of parathyroid hormone on mineral metabolism are initiated by the binding of parathyroid hormone to the type 1 parathyroid hormone receptor in the target tissues. ***(Koizumi M et al,2012)***

Parathyroid hormone regulates large calcium fluxes across bone, kidneys, and intestines. Another parathyroid hormone receptor (type 2) has been found in the brain and the intestines. ***(Juppner H et al ,2010)***

Parathyroid hormone–related peptide is a distant homologue of parathyroid hormone and is not a true hormone. It is synthesized in cartilage and in many more tissues than is parathyroid hormone and its secretion is not regulated by serum calcium.

Its local release activates the type1 parathyroid hormone receptor and its affinity for this receptor is similar to that of parathyroid hormone. ***(*Lopez I et al, *2011)***

In the kidney, PTH acts on proximal tubular cells to inhibit the reabsorption of phosphate.

Phosphate transport depends on the actions of sodium-phosphate cotransporters 2a and 2c (NPT2a, NPT2c), which are located in the brush border membranes of proximal tubular cells.

PTH acts to inhibit phosphate reabsorption by causing NPT2a to be removed from the luminal membrane and be degraded. ***(Mohammed S et al , 2007)***.

PTH also stimulates the activity of the renal 1α-hydroxylase in proximal tubular cells. This is a microsomal cytochrome P-450 enzyme that leads to the formation of biologically active $1,25(OH)_2$ vitamin D from its circulating precursor 25(OH) vitamin D **.**

PTH also inhibits 24-hydroxylase, thus preventing the formation of the inactive metabolite, $24,25(OH)_2$ vitamin D***. (Mori S et al, 2008).***

Both these actions increase the circulating concentrations of $1,25(OH)_2$ vitamin D, an increase that, in turn, stimulates intestinal calcium absorption**.** ***(Yamashita T et al,2003)***

PTH acts to increase the reabsorption of calcium by the kidney. The majority of calcium is reclaimed from the glomerular filtrate in the proximal tubule through a PTH-independent paracellular process linked to the reabsorption of sodium.

PTH helps to promote some calcium reabsorption in the cortical thick ascending loop of Henle by increasing the net positive charge on the luminal side of the tubule, which enhances paracellular calcium and magnesium reabsorption. ***(Garth F et al, 2008)***

However, the distal tubule is the primary target of PTH's actions to promote calcium transport. In these cells, PTH leads to insertion of calcium channels into the apical membrane of the cell and stimulates the activity of basolateral sodium/calcium exchangers.

The net result is to stimulate directional, transcellular calcium transport from the tubule lumen, across the cell and into the ECF.

Parathyroid hormone (PTH) promotes absorption of calcium from the bone in 2 ways.

The rapid phase brings about a rise in serum calcium within minutes and appears to occur at the level of the osteoblasts and osteocytes. ***(Goodman WG and Quarles LD,2007)***

Although it may seem counterintuitive that the cells that promote deposition of bone are involved in resorption, these cells form an inter-connected network known as the osteocytic membrane overlying the bone matrix, but with a small layer of interposed fluid termed bone fluid.***(Quarles LD,2011)***

When parathyroid hormone (PTH) binds to receptors on these cells, the osteocytic membrane pumps calcium ions from the bone fluid into the extracellular fluid. ***(GarthF et al, 2008)***

The slow phase of bone resorption occurs over several days and has 2 components. First, osteoclasts are activated to digest formed bone, and second, proliferation of osteoclasts occurs.

Interestingly, mature osteoclasts lack parathyroid hormone (PTH) membrane receptors; activation and proliferation appear to be stimulated by cytokines released by activated osteoblasts and osteocytes or by differentiation of immature osteoclast precursors that possess parathyroid hormone (PTH) and vitamin D receptors. ***(Rhee Y et al, 2011)***

So that PTH does not act directly on osteoclasts to stimulate bone resorption. PTH increases the number and activity of osteoclasts by acting on osteoblasts which release cytokines such as interleukin-6 (IL-6).

Recently, a study reported that serum circulating levels of IL-6 and IL-6 soluble receptors were increased significantly in untreated patients with Primary hyperparathyroidism (PHPT) and fell into the normal range after surgery ***(Oliveira RB et al,2010).***

2) FGF23

FGF23 is a produced mainly by osteocytes, through binding to FGF receptor-klotho complexes that are primarily expressed in the kidneys and the parathyroids***.(Quarles LD,2011)***

FGF23 exerts its physiological functions which include: inducing phosphaturia by down regulating renal sodium—phosphate cotransport in the proximal tubule ***.(*** **Stubbs JR** ***et al,2012)***

Similar to the effect of PTH; inhibiting calcitriol production by down regulating the renal 1 –alpha hydroxylase and stimulating the catabolic 24-hydroxylase; and inhibiting PTH secretion. ***(Ben-Dov IZ et al 2007)***

In healthy individuals, when phosphorus intake is high, FGF23 levels increase, which promote phosphaturia and inhibit calcitriol production thereby limiting the efficiency of dietary phosphorus absorption***.(Antoniucci DM et al,2006).***

Conversely, on a low phosphorus diet, FGF23 levels fall, tubular conservation of filtered phosphate increases as does the renal production of 1,25D, which increases the efficiency of dietary phosphorus absorption . ***(Burnett SM et al,2006)***

The net result of these compensatory mechanisms is the maintenance of a normal **serum** phosphate level despite day-to-day fluctuation in dietary phoshorus intake.

The effect of FGF23 to reduce PTH secretion can be understood as an additional mechanism for reducing calcitriol production***(Wolf M,2010)***

Indeed, PTH and FGF23 complete two separate but interrelated, counter—regulatory, negative endocrine feedback loops with vitamin D: FGF23 inhibits renal calcitriol production while calcitriol stimulates FGF23 secretion, whereas PTH stimulates renal calcitriol production while calcitriol inhibits PTH secretion. ***(White KU et al,2005)***

FGF23 was originally discovered in rare hereditary and acquired hypophosphatemic rachitic disorders in which primary FGF23 excess causes hypophosphatemia and inhibits the appropriate calcitriol response that would be expected in states of such severe hypophosphateniia***(Tencza AL et al,2009)***

In contrast, primary deficiency of biologically active FGF23 or resistance to its renal effects as in states of Klotho deficiency result in a physiological syndrome characterized by hyperphosphatemia, increased calcitriol and extensive soft-tissue calcifications and premature death***. (Kuro OM,2011)***

CKD can be viewed as the most common state of secondary FGF23 excess in which serum phosphate levels are normal to high, fractional excretion of phosphate is high, calcitriol level slow and FGF23 levels are markedly increased to a far greater extent that in the primary FGF23 syndromes **(Fig. 3.2).** .***(Wolf M,2012)***

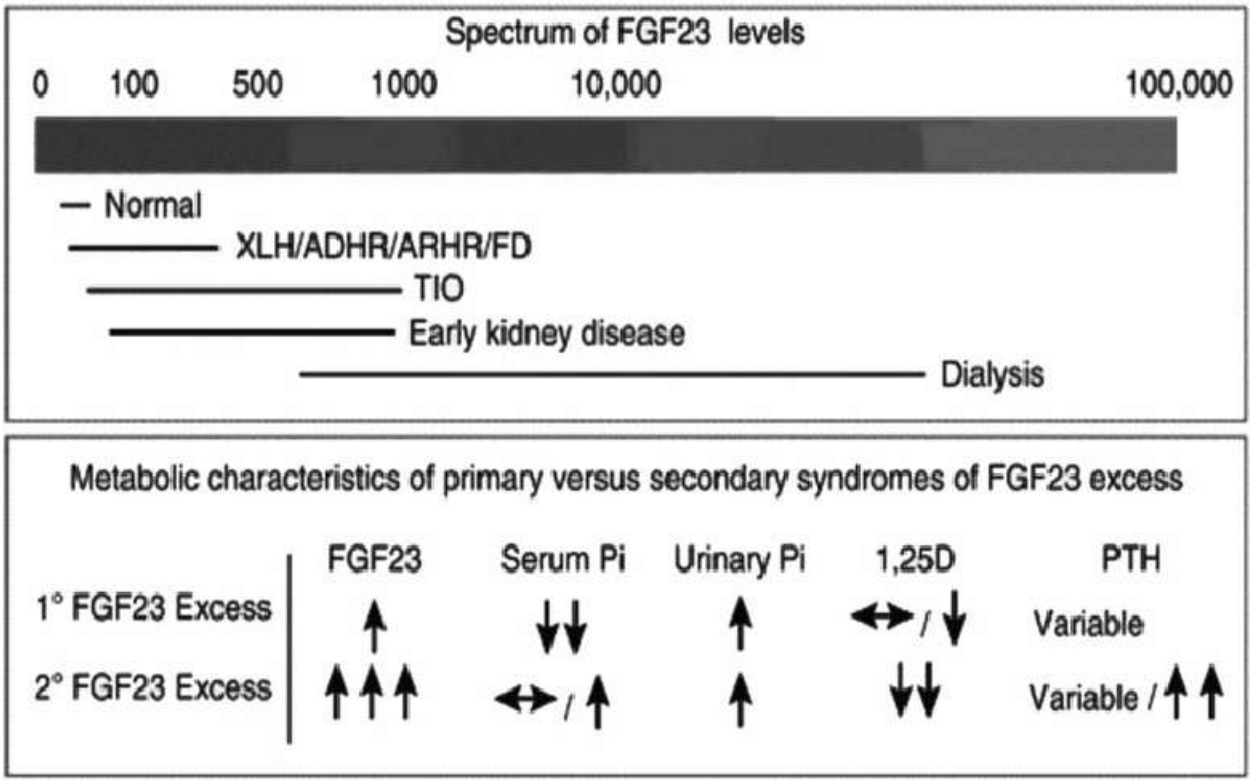


Figure3.2. Spectrum of FGF23 level **in** CKD: The tipper insert illustrates the spectrum of FGF23 levels that can be observed under normal conditions and in a variety of syndromes of FGF23 excess. Circulating FGF23 levels are 10- to 20-fold above normal (30-60 RU/mL using a C-terminal FGF23 assay) in patients with hereditary hypophosphatemic rickets syndromes, including X -l inked hypophosphatemia (XLI I), autosomal dominant hvpophosphatemic (ADHR), autosomal recessive hypophosphatemic rickets (ARHR), and fibrous dysplasia (FD).

Although FGF23 levels are often even higher in patients with tumor—induced osteomalacia (TIO), the highest levels are encountered in patients with kidney disease and especially in those on dialysis, in whom levels can reach concentration s more than 1000—fold above the normal range.

The lower insert illustrates the differences in the metabolic characteristics of primary syndromes of FGF23 excess, such as the hereditary diseases and TIO, versus 'secondarv' syndromes of FGF23 excess, such as kidney disease.

In addition to the severity of the FGF23 increase, the primary difference is normal to high serum phosphate (Pi) levels in patients with kidney disease compared to those with hypophosphatemia which is the sin qua non of the hereditary syndromes.

Although variable. l,25D levels tend to be lower and the PTH levels higher in patients with kidney disease than in those with the hereditary syndromes. Urinary fractional exeretion of phosphate is high in both predialysis kidney disease and genetic hypophosphatemic disorders.

Disturbed Mineral Metabolism in Chronic Kidney Disease: Historical View

Patients with CKD develop several disturbances in mineral metabolism that begin early in the course of disease frequently when kidney function declines to less than 60 mL/minute/1.73m. Levin A,

The historic perspective of disordered mineral metabolism and resulting secondary hyperparathyroidism in CKD was largely developed through animal models of end stage disease before FGF23 was discovered and highlighted 3 main factors: hyperphosphatemia,hypocalcemiaand I-25D deficiency-(Fig.3.3**).(*Slatopolsky Fand Delmez JA,1996)***

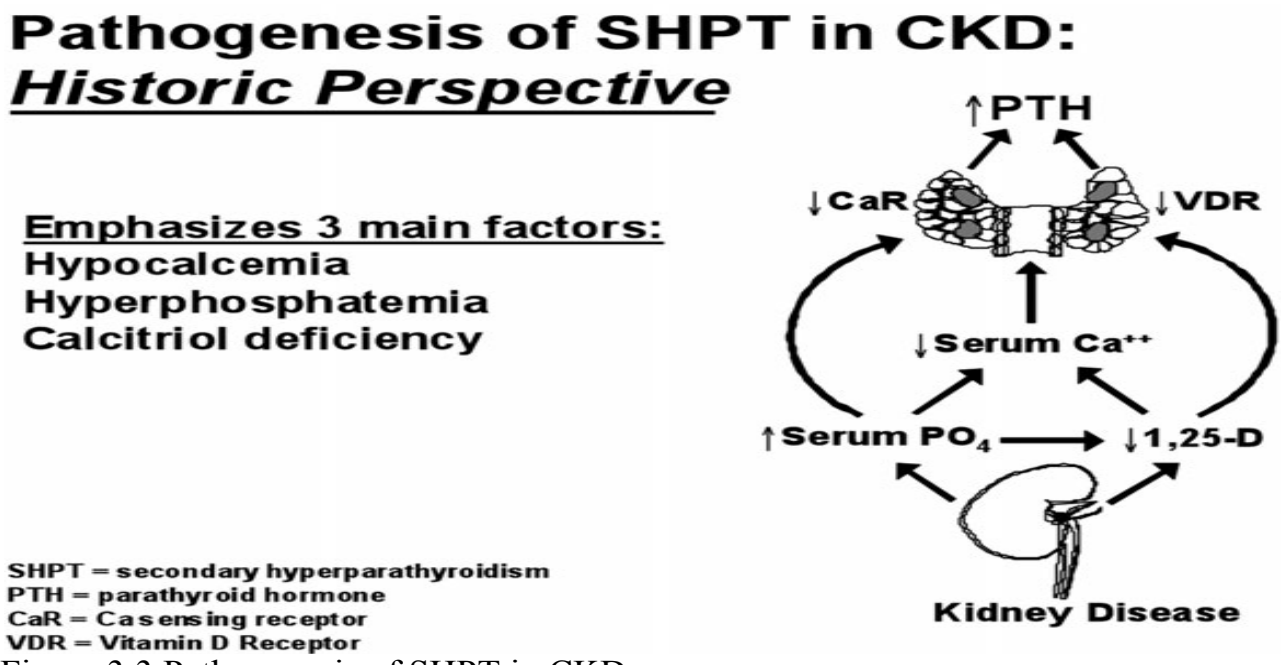


Figure 3.3 Pathogenesis of SHPT in CKD

It proposed that hyperphosphatemia develops when the ailing kidneys cannot sufficiently excrete phosphate loads while 1,25D deficieney was thought to develop when insufficient renal mass limited renal 1-alpha hydroxylase activity. ***(Denda M et al,1996)***

Hyperphosphatemia stimulates excessive PTH secretion (fig 3.4), progressive parathyroid hyperplasia and exacerbates 1,25D deficiency by inhibiting 1-alpha hydroxylase. ***(Slatopolsky Fand Delmez JA,1996)***

Hyperphosphatemia and 1,25D deficiency contribute to hypocalcemia, which stimulates PTH further**.**

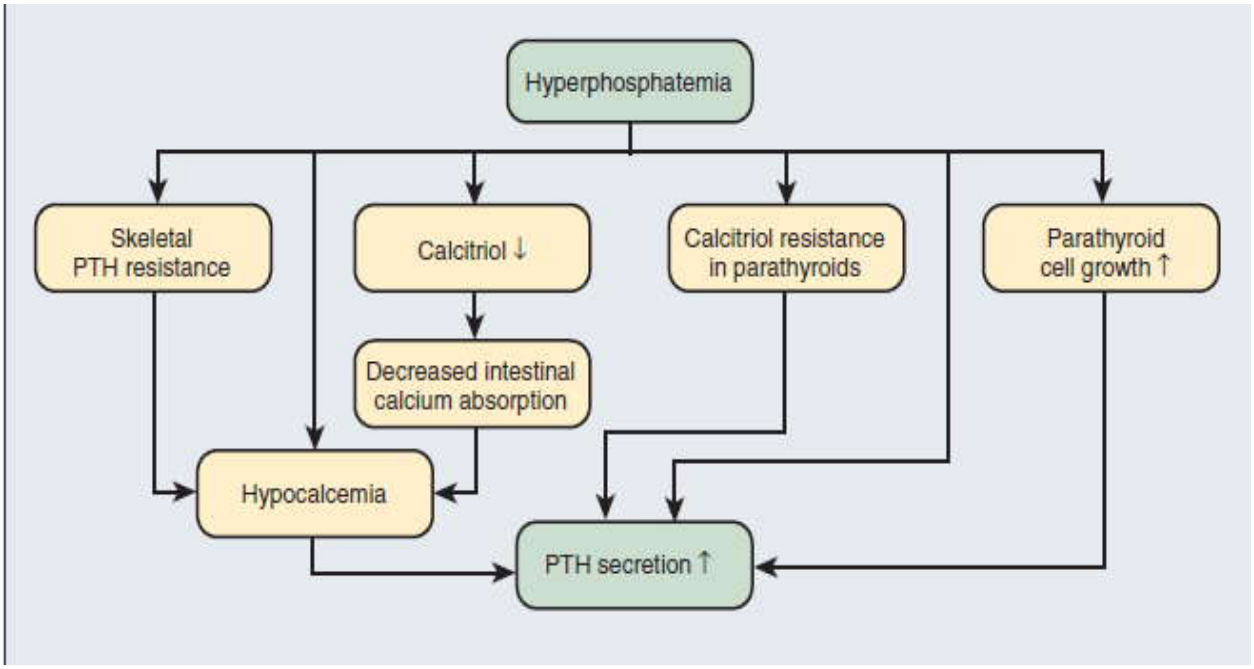


Fig3.4: effect of hyperphosphatemia in CKD

***Calcitriol production decresed in the setting of CKD due to: fig 3.5 (*Rodriguez M et al ,2005)**

- Decrese GFR limits the delivery of 25 hydroxyvitamin D to the site of the 1α hydroxylase in the proximal tubule.
- Phosphate retention either directly or by inducing an increase in FGF-23 also decreases the activity of 1α-hydroxylase.
- Circulating PTH fragments may also directly decrease calcitriol production. ***(Levin A et al,2007)***

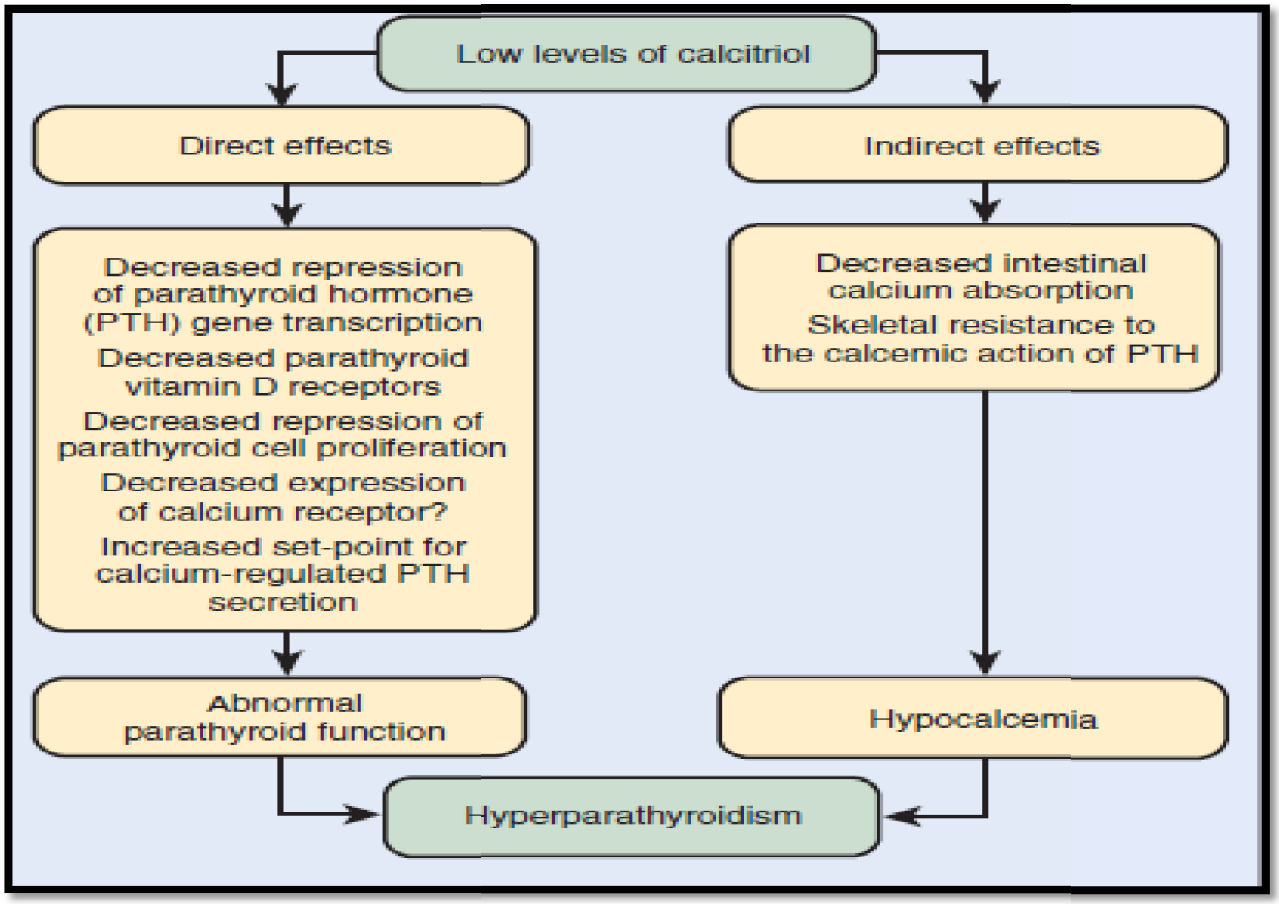


Figure 3.5 : Role of calcitriol in CKD

At the cellular level, parathyroid expression of the vitamin D and calcium sensing receptors declines progressively, leading to parathyroid resistance to inhibition by 1,25D and calcium The net result is constitutive PTH secretion and progressive parathyroidhyperplasia**.** ***(LaClair RE et al,2005).***

Disturbed Mineral Metabolism in Chronic Kidney Disease: The Post-FGF23 View

Circulating levels of FGF23 rise progressively as renal function declines with levels already elevated as early as CKD Stages 2-3. Several of these discrepancies now appear to be explained by the excessive production of FGF23. ***(Wolf M,2012)***

Importantly, the elevation of FGF23 levels is detectable long before hyperphosphatemia first appears***(Gonzalez EA et al,2004)***

The latter highlights both the vital compensatory role of FGF23 to help maintain normal serum phosphate levels in CKD and the potential of FGF23 elevation as a more sensitive biomarker to identify disordered phosphorus metabolism in CKD before there is evidence of overt hyperphosphatemia.***(Larsson T et al,2003)***

CKD patients who maintain their usual phosphorus intake recruit the same physiological response as normal subjects fed a high phosphorus diet: increased FGF23 secretion which helps augment urinary phosphate excretion but in the process leads to decreased 1,25D levels. ***(Shigematsu T et al,2004)***

While this FGF23 response appears to help maintain a normal serum phosphate levels it does so at the cost of an early reduction in 1,25D levels. ***(Komaba H and Fukagawa M,2009)***

By directly inhibiting calcitriol production and thereby releasing the parathyroids from its feedback inhibition (Fig. 3.6), early FGF23 excess may be one of the key upstream steps in the pathogenesis of secondary hyperparathyroidism. ***(Komaba H and Fukagawa M,2009)***

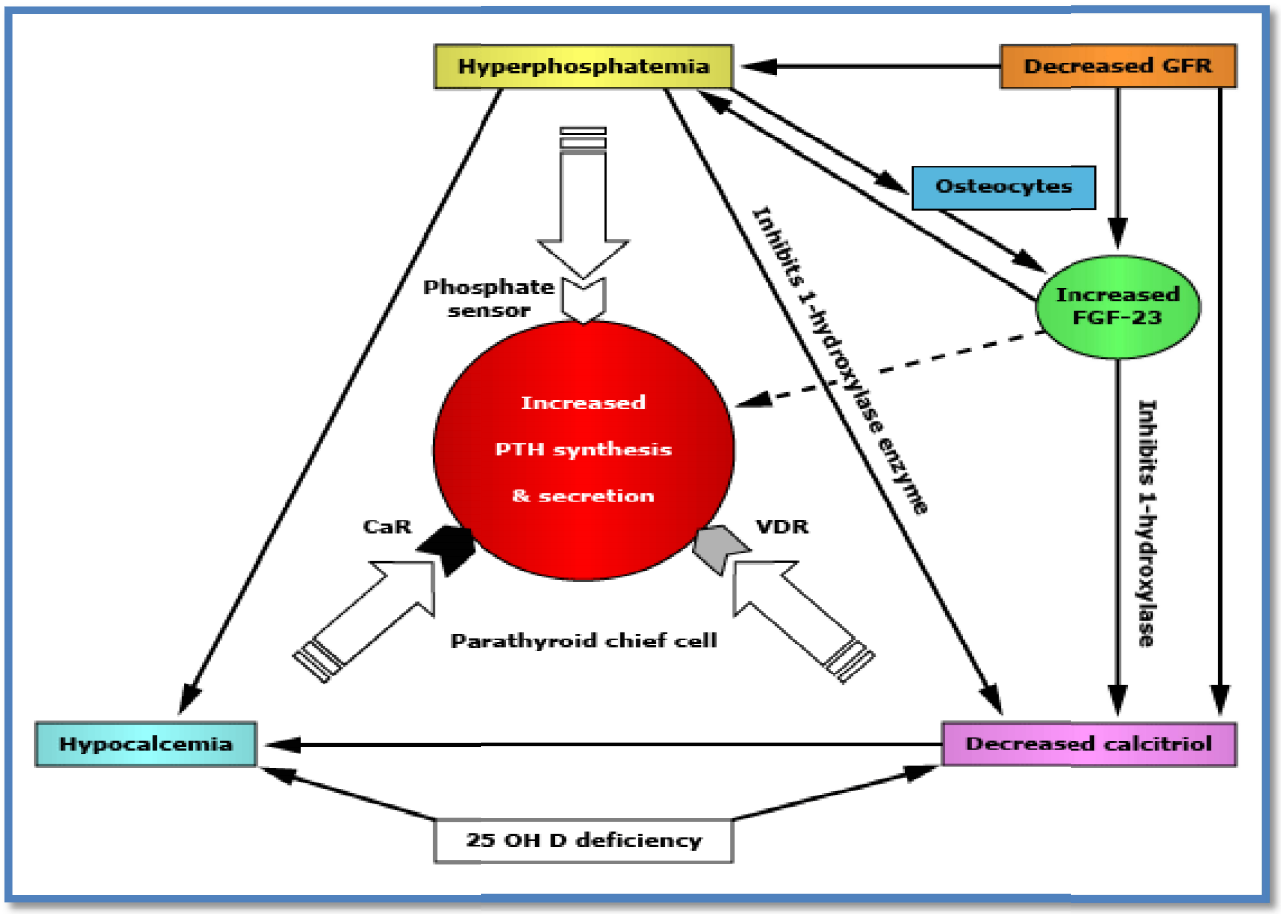


Fig. 3.6 *emphasizes the degree of phosphate intake relative to the degree of renal dysfunction and deemphasizes the need for overt hyperphosphatemia. Indeed, maintaining a ''usual'' phosphorus intake in the face of' decreased renal function may be an initiating trigger in the pathogenesis of secondary hyperparathyroidism in CKD.*

A number of observations support this view. Uremic animals fed a phosphorus restricted diet do not develop secondary hyperparathyroidism and secondary hyperparathyroidism in vitamin D receptor null mice can be rescued by a low phosphorus diet. ***(Denda M et al,1996)***

Human studies are corroborative. Restricting phosphorus intake in CKD patients is associated with increased 1,25D and decreased PTH levels.***(Koizumi T et al,2002)***

Although the mechanism was unknown. It now appears that differences in FGF23 levels could explain these findings.

These studies support the pathogenic primacy of phosphorus intake in the development of 1,25D deficiency and secondary hyperparathyroidism, **(fig 3.7)** even in the absence of hyperphosphatemia. ***(Kusano K et al,2008)***

Indeed, total phosphorus intake seems to be more important than the serum phosphate levels as rats with experimental CKD develop progressively increased FGF23 and phosphaturia and decreased 1, 25D levels before hyperphosphatemia appears***.(Hasegawa T et al,2003)***

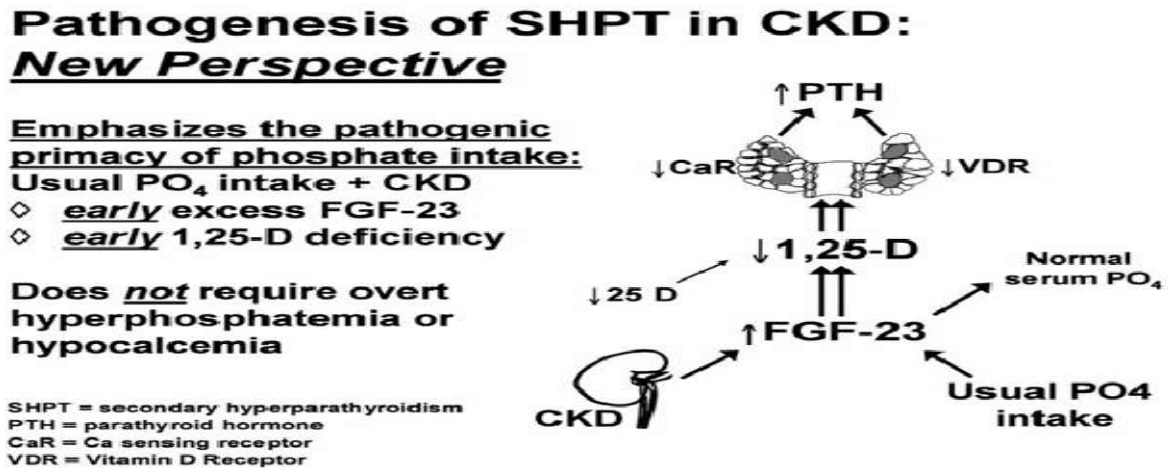


Figure 3.7. *Pathogenesis of SHPT in CKD. The new perspective in CKD emphasizes the degree of phosphate intake relative to the degree of renal dysfunction and de emphasizes the need for overt hyperphosphatemia. Early FGF23 excess may be a key upstream event of increased Pill in CKD.*

Indeed, the univariate association between decreased GFR and decreased 1 ,25D was completely extinguished when FGF23 was added to the model, suggesting that FGF23 rather than reduced GFR may be the key factor that underlies 1,25D deficiency in early CKD. ***(Oliveira RB et al,2009)***

To date, a handful of small pilot studies have suggested that dietary phosphorus binders can effectively reduce FGF23 levels in CKD which mirror similar results in animal models of CKD and in healthy volunteers. ***(Nagano N et al,2006)***

I) DISORDERED PHOSPHORUS METABOLISM AND ADVERSE CLINICAL OUTCOMES

***Phosphorus Excess and Adverse Clinical Outcomes in CKD** :*

In addition to its well known adverse effects on bone and the parathyroids, hyperphosphatemia has also emerged as a novel risk factor for kidney disease progression and cardiovascular disease in CKD(fig 2.8).*(* **Isakova T,** ***et al,2011)***

When mildly uremic rats were administered diets with high phosphorus content they experienced accelerated kidney disease progression, and epidemiological studies suggest that higher serum phosphate levels predict more rapid CKD progression.

In support of these findings, animal and human studies found that phosphorus restricted diets slow the decline in kidney function. ***(Kusano K et al,2008)***

Arterial calcification causing arterial stiffening is an important phenotype of vascular injury in CKD that is independently associated with mortaity. ***(Kendrick J et al,2011)***

Hyperphosphatemia is independently associated with greater burden of arterial calcification in dialysis and predialysis CKD and even in patients with normal renal function.*(* ***Seiler S et al,2011)***

A potential mechanism for arterial calcification induced by phosphorus excess is phosphorus-dependent transformation of vascular smooth muscle cells into bone forming osteoblast like cells in the arterial media leading to ectopic ossification in the walls of the large conduit arteries. ***(Shalhoub V et al,2012)***

Another cardiovascular phenotype that is a risk factor for mortality and also may be associated with phosphorus excess is left ventricular hypertrophy. ***(Achinger SG et al,2006)***

Left ventricular hypertrophy is common in CKD patients and contributes to high rates of congestive heart failure and sudden cardiac death, which are leading causes for CVD related mortality in CKD. ***(Stevens KK et al,2011)***

In animal studies, excess dietary phosphorus loading led to hyperphosphatemia and left ventricular hypertrophy, whereas intensive daily hemodialysis that can rapidly reverse hvperphosphatemia is associated with regression of LVH, suggesting a potential effect of phosphate. ***(Culleton BF et al,2007)***

Finally, many studies have identified hyperphosphatemia as an independent risk factor for mortality among dialysis patients**.** ***(Taylor EN et al,2011)***

Thus, it is important to note that subsequent studies of predialysis and non—CKD populations confirmed an increased future risk of mortality is association with subtle increases in serum phosphate levels that were well within the normal range**.*(Kalantar—Zadeh K et al,2006)***

These findings in healthy population indirectly support the analogous results in CKD, further strengthening the body of data that promote phosphorus excess as a novel risk factor for cardiovascular disease and mortality in CKD. ***(Vervloet MG et al,2011)***

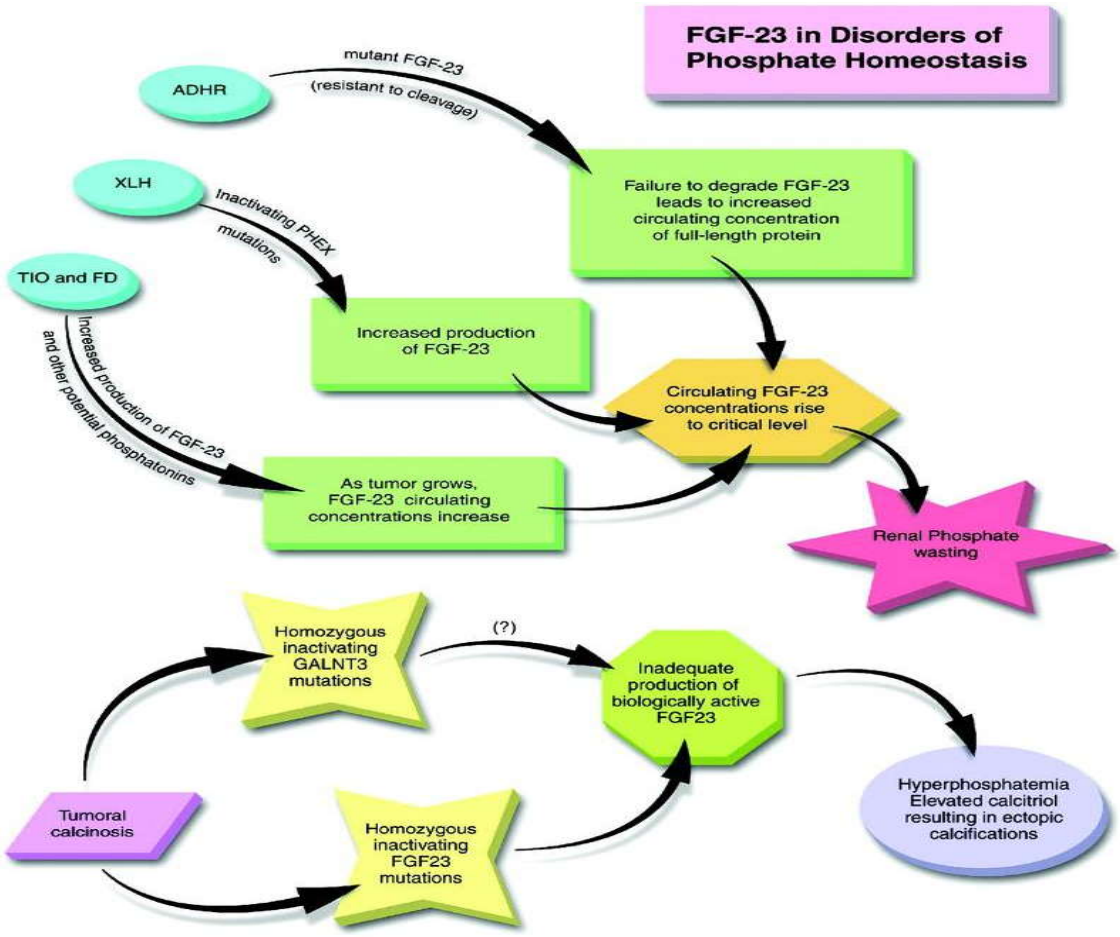


Fig 3.8: FGF23 and disorders of phosphate homeostasis

Limitations of Serum Phosphate for Clinical Management of Early CKD

Despite the considerable excitement surrounding hyperphosphatemia as a risk factor for mortality and novel candidate for therapeutic intervention in CKD, there are major limitations that will likely preclude its use as a biomarker for clinical practice in the vast majority of predialysis patients. ***(Isakova T et al,2011)***

For example, the absolute differences in serum phosphate levels that conferred increased risk of mortality are too small to be used reliably in the clinical management of individual patients given that these differences are actually smaller than the diurnal and postprandial variability in serum levels across the hours of the day**.** ***(Markowitz M et al, 1981)***

Thus, the discovery of FGF23 has not only revolutionized our understanding of mineral metabolism physiology, it has also presented a novel potential biomarker to stratify phosphorus—related risk with far greater resolution than the serum phosphate itself, especially patients with early CKD in whom phosphate levels are routinely normal. ***(Isakova T et al,2011)***

II) FGF23 Excess and Adverse Clinical Outcomes in CKD

Increased FGF23 levels have been shown to be independently associated with a variety of adverse renal and cardiovascular outcomes. ***(Viaene L et al,2012)***

However, in most cases, the results were independent of serum phosphate levels and the reported associations were stronger for FGF23 compared to serum phosphate, highlighting the potential superiority of FGF23 as a biomarker compared to phosphate. ***(Gutierrez 0M et al,2005)***

In the early 1980s, a link between dietary phosphate intake in CKD patients and the rate of progression to renal failure was established and more recent evidence suggests increased levels of FGF23 potentially reflecting chronically increased dietary phosphate loads may also predict a more rapid deterioration of renal function***. (Haut LL et al,1980)***

Consistent with previous reports, in a cross sectional evaluation of nondiabetic adult patients with CKD Stages 1—4, higher serum values of the Ca x P product, PTH and FGF23 were observed with progressive CKD stages. ***(Wahl P et al,2012)***

In a subsequent 53 month prospective analysis of 177 patients from this cohort, older age, higher protein excretion rates, lower glomerular filtration rates, higher serum phosphate.

PTH and FGF23 levels were all associated with an increased rate of CKD progression defined as a doubling of serum creatinine or the need for renal replacement therapy during the follow up period. However, in multivariable analyses, only baseline eGFR and FGF23 were independent predictors of progression. ***(Fliser D et al,2007).***

The etiology of the association between increased FGF23 and renal failure progression is not understood but if confirmed could reflect detrimental effects of phosphate itself, or an uncharacterized toxic effect of FGF23 on the renal parenchyma. ***(Jono S et al,2008)***

Importantly, the effect of FGF23 was independent of serum phosphate levels suggesting that FGF23 is superior to isolated serum Phosphate measurements as a biomarker of phosphorous—related toxicity. ***(Gutierrez OM et al,2009)***

Several recent studies examined FGF23 and common cardiovascular phenotypes in CKD, While the results for vascular calcification are conflicting, several reports linked higher FGF23 levels to increased left ventricular mass index and Jell ventricular hypertrophy in dialysis patients and earlier stage CKD. ***(Stevens KK et al,2011)***

Additional confirmation of an FGF23 vascular disease link came from studies of healthy non-CKD patients, in which higher FGF23 levels were independently associated with impaired vascular reactivity and with increased arterial stiffness. ***(Mirza MA et al,2009)***

The association between increased FGF23 and Left ventricular hypertrophy in predialysis CKD patients was independent of traditional risk factors and serum phosphate levels, which were not associated with left ventricular mass index. ***(Srivaths PR et al, 2011)***

In these studies, the effect of FGF23 was independent of serum phosphate levels suggesting again that FGF23 is superior to isolated serum phosphate measurements as a biomarker of phosphorous related toxicity. ***(Hsu HJ and Wu MS,2009)***

III) FGF23 Excess and Mortality on Dialysis

One of the largest studies to date of FGF23 and hard clinical outcomes was a prospective study of FGF23 levels at the initiation of dialysis and risk of subsequent mortality on dialysis (Fig 3.9**) .*(Shalhoub V et al,2012)***

By helping to prevent or attenuate the severity of hyperphosphatemia, increased FGF23 levels should appear to be a protective compensation in CKD.

However, the increased FGF23 also inhibits renal production of 1,25D leading to severe prolonged 1,25D deficiency which itself is a risk factor for mortality. ***(Silver J and Naveh T,2010)***

Where as hyperphosphatemia and low 1,25D levels are independent risk factors for mortality in CKD, whether increased FGF23 is protective or harmful in terms of mortality was unknown.***(Shimada T et al,2009)***

In a prospective study of 10,044 incident hemodialysis patients, the association between FGF23 and phosphate with mortality was assessed using measurements of these analytics from samples that were collected at the first outpatient hemodialysis session among patients new to dialysis. ***(Fliser D et al,2007)***

All patients were included in the analysis of phosphate and mortality while the FGF23 analyses used a nested, prospective case—control sampling.

Cases were patients who died during the first year on dialysis and controls were those who survived the first year. ***(Oliveira RB et al,2009)***

To minimize confounding by hyperphosphatemia, frequency matching ‘as used to randomly select 50 cases and 50 controls within each quartile of baseline serum phosphate so that a final sample of 200 cases and 200 controls with balanced serum

phosphate levels are selected. **Figure 3.9 : Increased** FGF23 levels are associated with mortality in hemodialysis .***(Shimada T et al,2009)***

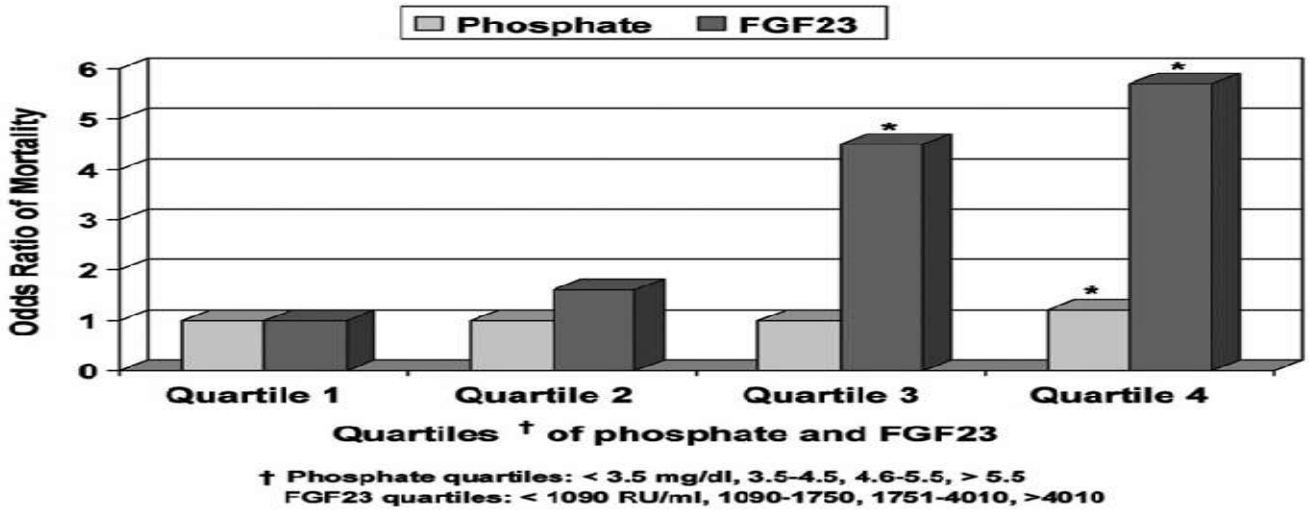


Figure 3.9 : Relation between FGF23 levels and mortality in hemodialysis

Several important observations emerged from this study. Increased FGF23 levels were for the first time associated with mortality and the results were independent of serum phosphate levels and other known risk factors. There was a strong "dose response" relationship and virtually no confounding. ***(Moshayoff V et al,2011)***

Furthermore, the magnitude of risk associated with FGF23 was dramatically larger than the analogous results for serum phosphate, which were comparable to previous reports.

These results indicate that serum phosphate levels provide partial assessment of risk associated with disordered phosphorus metabolism, especially when serum levels are relatively normal.***(Shimada T et al,2009)***

In contrast, FGF23 was most informative when serum phosphate was normal. This is of great relevance to early CKD Stage 3 CKD is the most common cause of disordered phosphorus metabolism but since the vast majority of the estimated 15 million affected

in the US has normal serum phosphate levels, few are treated with phosphorus reduction strategies such as dietary phosphorus restriction and phosphorus binders that appear capable of lowering FGF23 levels. ***(Burnett SM et al,2006)***

These data suggest that FGF23 may be a more sensitive biomarker to help identify which normophosphatemic patients might benefit from phosphorus reduction strategies and how to titrate these therapies. Another important aspects of this study was the tight correlation between FGF23 levels measured using either assay strategy. ***(Wolf M ,2012)***

This is important because it has been assumed that intact FGF23 assays must be preferable because C-terminal fragments could theoretically accumulate in CKD as is the case for PTH.***(Shimada T et al,2009)***

FGF23 fragments do not accumulate to a significant extent in CKD and thus, both assays measured the same dramatically increased levels of biologically active FGF23.

(Manghat P et al,2010)

This was also the first report of racial and ethnic differences in FGF23 levels.

While further validation is needed, these results are plausible as they appear to explain other known racial differences in mineral metabolism: compared to whites, blacks demonstrate decreased urinary phosphate excretion and increased serum phosphate levels despite increased PTH and significantly higher 1,25D despite significantly decreased 25D substrate. ***(Fliser D et al,2007)***

Through its effects on phosphaturia and one alpha-hydroxylase, decreased FGF23 levels could account for these discrepancies. ***(Gutierrez GM et al,2008)***

Finally, the strong association between FGF23 and adverse outcomes raises an important question: is FGF23 excess a biomarker of another true risk factor or is it a direct uremic toxin. .***(Lopez I et al,2011)***

For example, it is possible that any impact of FGF23 on mortality may reflect effects of I ,25D deficiency, toxicity due to high phosphorus load, or klotho deficiency.***(Lopez I et al,2011)***

Alternatively, it is possible that at markedly elevated levels, as observed in CKD, FGF23 could induce direct tissue injury that contributes to mortality, presumably through Klotho-independent mechanisms. Further research is needed to dissect these possibilities. ***(Oliveira RB et al,2009)***

Therapeutic Implications of FGF23 Research

The growing understanding of FGF23 physiology and its potential role as a biomarker in CKD has potentially important clinical implications: earlier institution of phosphorus—related therapies in predialysis CKD patients with normal serum phosphate but elevated FGF23 levels. ***(Wolf M,2012)***

Thus, it could be envisioned that FGF23 screening of early CKD patients with normal serum phosphate levels could be used to identify candidate patients for early dietary phosphorus restriction and phosphate binder therapy, just as Pill screening is justified in patients with normal calcium levels to identify those who may nevertheless benefit from initiation of active vitamin D therapy. ***(Oliveira RB et al,2009)***

This physiological-based approach would replace the current standard of delaying therapy for disordered phosphorus metabolism until the serum level is abnormally elevated.

However, before such a strategy could be brought to clinical practice, it must be demonstrated that FGF23 levels can be safely lowered in early CKD patients with elevated levels. ***(Oliveira RB et al,2009)***

A handful of recent studies examined the effect of phosphate binders on FGF23 levels**.** A short term 6 wk dose titration study evaluated the effect of calicum acetate versus sevelamer hydrochloride on PTH and FGF23 levels in forty patients with CKD Stages **3_4.** ***(Juppner H,2011)***

Treatment was associated with improved control of secondary hyperparathyroidism without corresponding changes in serum phosphate levels. Sevelamer but not calcium acetate also lowered FGF23 levels*.(**Koizumi M et al,2012)***

In another study, forty six patients undergoing maintenance hemodialysis therapy were randomly treated with 3gm sevelamer hydrochloride and 3 gm of calcium bicarbonate (CaCO3), or 3 g of CaCO3 alone*.(**Desjardins L et al,2011)***

Although the serum phosphate levels were comparable before treatment, the levels were significantly lower in the patients treated with sevelamer hydrochloride ÷ CaCO3 than those with CaCO3 alone after 4 weeks of treatment. .*(**Koizumi M et al,2012)***

FGF23 levels significantly decreased after 4 weeks of the treatment with sevelamer hydrochloride CaCO3 from the pretreatment levels, while no changes were observed in the patients treated with CaCO3 alone. Thus, more aggressive treatment with hinders reduced FGF23 levels in dialysis patients, presumably by blocking intestinal phosphorus absorption*.(**Isakova T et al,2011)***

In predialysis patients, placebo controlled studies are justified and should be performed. Studies that focus **on** the effects of dietary phosphorus restriction should also be performed, in comparison to or in combination with binders*.(**Hasegawa H et al,2010)***

If FGF23 can be successfully lowered in this population, a potential landmark randomized trial of binders and diet versus placebo with mortality as the outcome could be envisioned on the not too distant horizon. ***(Gonzalez-Parra E et al,2011)***

Other therapies commonly used to treat secondary hyperparathyroidism in CKD also impact FGF23 levels, although the data are limited, Vitamin D sterol therapy, which is commonly used to treat elevated PTH levels in CKD patients, would also be expected to increase FGF23 levels, but in the two studies that examined this, the effect was modest, albeit in relatively short—term studies .***(Nishi H et al,2005)***

In contrast, cinacalcet hydrochloride, an allosteric activator of the calcium-sensing receptor that is also used to lower PTH levels in dialysis patients, had a modest FGF23 reducing effect in one study. ***(Wetmore JB et al,2010)***

FGF23 in end-stage renal disease

In patients with end-stage renal disease, serum FGF23 levels markedly increase in response to chronic phosphate load and active vitamin D therapy. ***(Isakova T et al ,2011).***

However,this pronounced increase in FGF23 fails to compensate for increased phosphate retention due to decreased nephron mass, leading to overt hyperphosphatemia.

In this context,recent clinical studies have shown that high FGF23 level was an independent predictor of mortality in both incident and prevalent dialysis patients ***(Kendrick J et al ,2011).***

Interestingly, in both studies, the results were independent of serum phosphate,and the association between high FGF23 and mortality remained significant even in patients with normophosphatemia.***(Nakanishi S et al,2010)***

Several recent studies also demonstrated that elevated FGF23 levels were associated with arterial stiffness, increased left ventricular mass index and increased prevalence of left ventricular hypertrophy in patients with CKD **(Mirza MA et al ,2009).**

These data indicate that FGF23 screening may serve to measure a novel biomarker to guide treatment of disordered phosphate metabolism. Whether FGF23 reduction strategies would prolong survival of CKD patients is worthy of further investigation . ***(Isakova T et al, 2011).***

Recent clinical studies also showed an association between serum FGF23 and the severity of secondary hyperparathyroidism.

Several studies, though not all, reported that serum FGF23 levels significantly correlated with serum PTH levels ***(Rhee Y et al,2011).***

Interestingly, it has also been shown that serum FGF23 levels predict the effectiveness of active vitamin D therapy as well as the future development of refractory hyperparathyroidism in dialysis patients with secondary hyperparathyroidism.***(Shalhoub V et al,2012).***

The mechanisms underlying this association are not clear; however, given that FGF23 is a more sensitive biomarker of disordered phosphate metabolism . ***(Isakova T et al, 2011).***

It can be deduced that chronic phosphate retention as reflected by elevated FGF23 levels may contribute to further progression of parathyroid hyperplasia.

Otherwise, it is also possible that high levels of FGF23 at baseline may be a consequence of prolonged active vitamin D administration for severe secondary hyperparathyroidism,which may lead to future resistance to this therapy. (***Nakanishi S et al, 2010)***

The association between high FGF23 levels and the severity of secondary hyperparathyroidism may seem at odds with the inhibitory effect of FGF23 on PTH secretion. In these patients, increased FGF23 should act on the parathyroid as a negative regulator but fails to suppress PTH secretion***. (Seiler S et al,2010)***

Theoretically, this fact suggests the presence of resistance to FGF23 action. A similar paradox has been observed in refractory secondary hyperparathyroidism, in which parathyroid glands do not respond to calcium supplementation and calcitriol therapy, which should decrease PTH secretion. ***(Canalejo R et al,2010)***

In the past, such resistance to medical treatment was explained by a decrease in the expression of calcium-sensing receptors (CaSRs) and vitamin D receptors (VDRs), particularly in glands with nodular hyperplasia, which is a more severe form of parathyroid hyperplasia ***(Tokumoto M et al, 2002).***

In this context, we have recently shown that Klotho and FGFR1c expression decreased significantly in uremic parathyroid hyperplasia, particularly in glands with nodular hyperplasia ***(Komaba H et al,2010).***

Similar finding were also reported by another study.These findings suggest that the depressed expression of the Klotho- FGFR1c complex in hyperplastic glands may explain, at least in part, the resistance to extremely high FGF23 levels that would be expected to decrease serum PTH levels fig. ***(Kumata C et al,2010)***

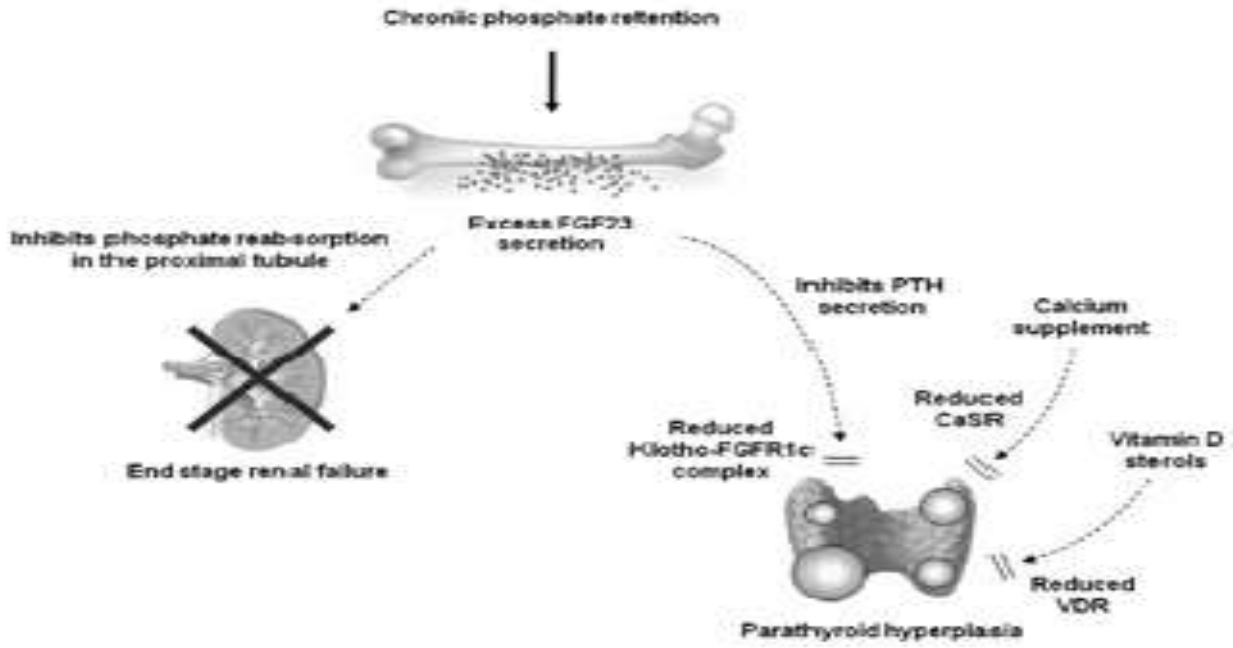


Fig. 3.10 - Role of FGF23 in dialysis patients. In dialysis patientswithout functioning kidneys, FGF23 secretion is markedly elevated in response to chronic phosphate retention and active vitamin D therapy. In this setting, increased FGF23 should act on the parathyroid as a negative regulator, but fails to suppress PTH secretion. This parathyroid resistance to FGF23 may be due to down-regulation of the Klotho-FGFR1c complex.
CaSR = calcium-sensing receptor; FGF23 = fibroblast growth factor 23; FGFR1c = fibroblast growth factor receptor 1c; PTH = parathyroid hormone; VDR = vitamin D receptor

Indeed, recent preliminary data also showed that FGF23 does not inhibit PTH secretion in vivo in CKD rats or in vitro in parathyroid gland cultures from CKD rats.

Further studies are needed to determine whether downregulation of the Klotho-FGFR1c complex plays a role in the pathogenesis of secondary hyperparathyroidism.***(Canalejo R et al,2010)***

Chapter FOUR

FGF-23 and Renal Transplantation

FGF-23 and Renal Transplantation

Kidney transplantation is the preferred treatment for the growing number of patients with ESRD. ***(Pascual M et al,2002)***

A successful kidney transplant restores renal function to near normal, frees patients from the rigors of a relentless dialysis schedule, and dramatically prolongs survival. ***(Jardine AG et al ,2011)***

Allograft and patient survival have improved steadily over past decades as a result of advances in operative techniques, immune suppression regimens, and prophylaxis against opportunistic infections. ***(Jardine AG et al ,2011)***

An increased circulating level of fibroblast growth factor 23 (FGF23) is an independent risk factor for mortality, cardiovascular disease, and progression of chronic kidney disease (CKD), but its role in transplant allograft and patient survival is unknown. ***(Wolf M et al,2011)***

As a result, death and disability caused by cardiovascular disease and late graft loss caused by chronic allograft nephropathy have surpassed infection and early allograft loss caused by rejection as the primary threats to the health of kidney transplant recipients. ***(Howard RJ et al,2002)***

Bone Disease after Renal Transplantation

Accelerated bone mineral density (BMD) loss is an almost universal complication in transplant recipients, it is mainly considered a side effect of immunosuppressive drugs, especially glucocorticoids. ***(Atsumi K et al, 1999).***

It has been well established that a rapid decrease in bone mineral density (BMD) occurs in the first 6 to 12 mo after a successful renal transplantation and persists, albeit at a lower rate, for many years.***(Weisinger JR et al, 2006)***

This rapid BMD loss significantly increases the fracture risk of these patients to levels that are even higher than those of patients who have chronic kidney disease stage 5 and are on dialysis.

It is established that the fracture risk is higher in renal transplant recipients compared with recipients of other organs. This point to specific risk factors among preexisting renal osteodystrophy, hypogonadism, and metabolic acidosis undoubtedly play an important role. ***(Kunzendorf U et al, 2008).***

The presence of low BMD in renal transplant patients as a predictor of risk fracture is controversial.

Indeed, as has been suggested also for patients with postmenopausal osteoporosis, there is not a compelling correlation between the decline in BMD and skeletal fractures. ***(Wolf M etal,2011)***

However, bone disease after renal transplantation probably represents a unique bone disorder that must encompass underlying renal osteodystrophy.***(Sirilak S et al,2012)***

In fact, this syndrome results from multiple factors that include pretransplantation bone status, use of glucocorticoids and other immunosuppressive drugs, hypophosphatemia, and alterations of the calcium–vitamin D axis.***(Kawarazaki H et al,2011)***

Recent studies have demonstrated decreased osteoblast number, reduced bone formation rate, delayed mineralization, and increased osteoblast and osteocyte apoptosis. **.*(Weisinger R et al, 2006)***

Pathogenesis of Post transplantation Bone Disease:

Although some early reports have linked post transplantation bone disease mainly to glucocorticoid excess. ***(Tataranni T et al,2011)***

It has become clear that it rather comprises a spectrum of metabolic alterations of bone remodeling that include the status of bone metabolism during dialysis (secondary hyperparathyroidism, adynamic bone disease, osteomalacia, and mixed bone disease), as well as new factors that occur after transplantation (Table 3.1). ***(Weisinger JR et al,2006)***

Thus, post transplantation bone disease represents a complex disarray that could encompass the variable preexisting renal osteodystrophy alterations.

It is interesting that these bone lesions are observed frequently in patients who have relatively normal kidney function and often are independent of serum PTH levels.***(Bhattacharyya N et al,2012)***

It is evident that posttransplantation bone disease is a complex problem that ranges from low to high turnover , indicating that the pathogenesis of post transplantation bone disease is multifactorial and could include the following contributing factors (Table 3.1). ***(Ball AM et al,2002)***

Table 4.1: **Contributing factors of posttransplantation bone disease**
(Weisinger JR et al,2006)

Pretransplantation factors	**Posttransplantation factors**
Preexisting bone disease Osteitis fibrosa Mixed bone disease Adynamic bone disease Osteomalacia	Immunosuppressive drugs PTH status Hypophosphatemia Renal function

Contributing factors of posttransplantation bone disease :

1-Pre transplantation Renal Osteodystrophy:

In the past several years, the spectrum of renal osteodystrophy in dialysis patients has changed considerably. .***(Sirilak S et al,2012)***

The incidence and the prevalence of low bone turnover, particularly adynamic bone disease, has increased steadily, becoming the main type of bone alteration in many centers.

Therefore, it seems reasonable to consider low pre transplantation bone turnover as a risk factor for development or aggravation of the characteristics of adynamic bone lesions in the early posttransplantation period favored by the use of relatively high doses of glucocorticoids. ***(Tataranni T et al,2011)***

2- Parathyroid hormone PTH

There seems to be no doubt that pre transplantation renal osteodystrophy plays an important role in the maintenance or development of post transplantation alterations of bone remodeling.

Indeed, most transplant patients have various forms of preexisting bone disease that may persist after transplantation. ***.(Bhattacharyya N et al,2012)***

The prevalence of hyperparathyroidism among transplant patients is approximately 30 to 50% (23–26).

In patients with nonsuppressible nodular parathyroid hyperplasia, the persistently elevated PTH levels after restoration of normal renal function may play a primary role in maintaining a high bone turnover.***(Kawarazaki H et al,2011)***

Likewise, in patients with less severe secondary hyperparathyroidism, restoration of normal serum PTH levels may take several years. In addition, not infrequently,patients may develop *de novo* secondary hyperparathyroidism that results from progressive functional alterations of the transplanted kidney ***(Vautour LM et al,2004).***

Therefore, at least some of the skeletal and mineral abnormalities can be attributed to persistently elevated PTH levels.
However, in many studies, the bone histopathologic findings are heterogeneous, without apparent correlation with posttransplantation serum PTH levels, suggesting that other factors that start operating after transplantation could play a central role in the development of these bone alterations. ***(Ball AM et al,2002)***

Finally, we found a positive correlation between osteoblast surface and the serum levels of pre- and posttransplantation PTH, suggesting an important role of the hormone in preserving osteoblast number and activity after transplantation ***(Rojas E et al,2003).***

PTH increases the lifespan of mature osteoblasts by preventing apoptosis. These findings are in agreement with the fact that posttransplantation apoptosis was rare in patients with pretransplantation secondary hyperparathyroidism ***(Rojas E et al,2003).***

There is no evidence in the literature as to the optimal PTH values to be reached after renal transplantation, and it is not clear at whether these changes in bone cell morphology relate to the drop in PTH level or its absolute value. ***(Connolly GM et al,2009)***

The high prevalence of persistent or *de novo* hyperparathyroidism after transplantation limits the feasibility of establishing these values.

However, we could speculate that the PTH levels after transplantation, in patients with relatively normal renal function, should approach normal values to reestablish normal bone turnover, counteracting the effects of glucocorticoids and other immunosuppressant agents. ***(Tataranni T et al,2011)***

3- Immunosuppressive Therapy:

i) Role of Calcineurin inhibitor in posttransplant BMD:

Several studies suggest that post transplantation immunosuppressive therapy constitutes a major factor in the pathogenesis of post transplantation bone disease ***(Rix M et al,2003).***

The possible role of the calcineurin inhibitor cyclosporine remains controversial. Studies in animals and humans have shown that cyclosporine causes high bone turnover ***(Saab G et al,2003).***

In the rat, cyclosporine causes bone loss that is associated with increased bone resorption and formation; however, other studies have failed to demonstrate an effect of the drug on mineral and bone metabolism in renal transplant recipients. ***(Carlini RG et al,2000).***

It should be considered, however, that the role of cyclosporine in transplant patients has been difficult to evaluate because its effects on bone turnover may be masked by glucocorticoids.

Indeed, some reports indicated that, in the absence of glucocorticoids, cyclosporine does not seem to induce bone loss ***(Sprague SM et al,2004).***

Tacrolimus, another calcineurin inhibitor, also causes trabecular bone loss in the experimental animal , but the information on the skeletal effect of this drug in humans is limited. ***(Monegal A et al,2001).***

In liver transplant recipients, tacrolimus has been associated with a significant higher femoral neck BMD 2 yr after transplantation as compared with patients who were treated with cyclosporine . ***(Monegal A et al,2001).***

Limited information is available regarding rates of bone mass loss and fractures with newer immunosuppressive agents such as mycophenolate mofetil, sirolimus, basiliximab, or daclizumab.***(Mikuls TR et al,2003)***

ii) Role of Glucocorticoids in posttransplant BMD:

However, with these new regimens that also reduced glucocorticoid requirements, the risk of bone alterations may be reduced.

Many studies in kidney transplant patients have shown a correlation between glucocorticoid cumulative dose and BMD. ***(Tataranni T et al,2011)***

In some biopsy studies, glucocorticoids also seem to be the sole determinant of bone volume and turnover . Thus, the cumulative and mean prednisone doses correlated negatively with bone turnover, whereas there was no correlation with cyclosporine cumulative dose or serum PTH . ***(Monier-Faugere MC et al,2000)***

Because neither immunosuppressive therapy nor biochemical and hormonal parameters, including PTH, calcitriol, and serum phosphorus, correlated with delayed mineralization, they concluded that post transplantation bone disease is mainly a consequence of glucocorticoid therapy . ***(Monier-Faugere MC et al,2000)***

The mechanisms whereby glucocorticoids may affect bone metabolism are multifactorial. These drugs increase osteoclastic resorption and decrease osteoblastic activity ***(Manolagas SC ,2000).***

Similarly,they also may affect indirectly bone metabolism by decreasing intestinal calcium absorption, leading to increased PTH secretion. There is evidence suggesting that under normal conditions, an important number of osteoblasts undergo apoptosis . ***(Rojas E et al,2003).***

Moreover, studies in mice indicate that glucocorticoids promote osteoblast and osteocyte apoptosis and inhibit osteoblastogenesis, resulting in the defective bone formation that is observed in glucocorticoid-induced osteoporosis. (***Sprague SM et al,2004).***

Therefore,it seems possible that continuous use of glucocorticoids represents an important pathogenic factor in the development and maintenance of posttransplantation bone disease.

It is interesting that we found early after transplantation a negative correlation between cumulative dose of glucocorticoids and posttransplantation osteoblastic surface ***(Rojas E et al,2003).***.

Because the study was performed during a period of maximal glucocorticoid use, it seems possible that glucocorticoids may have played a role in the increased osteoblast apoptosis. However, we could not demonstrate a correlation between glucocorticoids and the number of osteoblast apoptosis. ***(Rojas E et al,2003).***

Localized osteonecrosis is another important long-term bone alteration that is associated with glucocorticoid use and represents the most debilitating of the musculoskeletal complications after renal transplantation. It is usually multifocal, with 50 to 70% of affected patients having more than one joint involved***.(Lopez-Ben R et al,2004)***

It usually begins at the weight-bearing surface of the femoral head with collapse of surface bone and cartilage. Previous studies suggested an incidence of approximately 15% within 3 yr of transplantation; however, the risk decreased after the introduction of cyclosporine with consequent decreases in glucocorticoid dosage .***(Lopez-Ben R et al,2004)***

4-Role of the Hypophosphatemia and Phosphatonins:

Hypophosphatemia is a frequent finding in the early post-transplant period, occurring in up to 90% of the renal transplant recipients. Renal phosphate wasting is the main mechanism contributing to this complication. It is

recognized that both hypophosphatemia and renal phosphate wasting may have a detrimental impact on bone mineralization. ***(Ghanekar H et al, 2006).***

Many studies reported that renal phosphate wasting in the post-transplant period is caused partly by the persistence of inappropriately high levels of fibroblast growth factor 23 (FGF-23) ***(Evenepoel P et al, 2007).***

This condition is often referred to as "tertiary hyperphosphatoninism." As opposed to serum parathyroid hormone (PTH) levels, FGF-23 levels return to normal by 1 year after transplantation in the majority of the patients. This evolution goes along with the regression of hypophosphatemia and renal phosphate wasting in these patients. ***(Evenepoel P, et al 2008).***

Pre transplant FGF-23 levels are the most important determinant of FGF-23 levels up to 1 year after transplantation, So that a high pre transplant FGF-23 level is a risk factor for accelerated BMD loss in this period. ***(Evenepoel P, et al 2008).***

Hypophosphatemia related to decreased renal tubular reabsorption is a common complication following kidney transplantation, and is usually limited to the early post-transplant period ***(Ghanekar H et al,2006).***

Persistently elevated parathyroid hormone (PTH)level have long been considered to be the cause of post-transplant hypophosphatemia, but hyperparathyroidism does not appear to be the only mechanism. Decreased renal tubular reabsorption may occur, despite low levels of PTH, and hypophosphatemia can persist even after elevated PTH levels have normalized. ***(Green J et al,2001).***

Furthermore, even if hypophosphatemia and hyperparathyroidism stimulate calcitriol (1,25-(OH)2-D3) synthesis, calcitriol levels are often inappropriately low following renal transplantation, despite normal or mildly impaired allograft function this occur because FGF-23 decreases renal tubular reabsorption of Pi and inhibits renal 1a-hydroxylase leading to decreased calcitriol synthesis.*(Marks J et al,2010).*

Phosphatonin fibroblast growth factor-23 (FGF-23) is involved in phosphate (P) excretion and vitamin D metabolism. Recently,FGF-23 has been suggested to be responsible for the hypophosphatemia and inappropriately low calcitriol levels observed after renal transplantation. ***(Marks J etal, 2010).***

Recent studies suggest that high level of FGF-23 encountered in terminal renal failure persist after kidney transplantation and may contribute to early post-transplant hypophosphatemia ***(Evenepoel P etal,2006).***

It is unclear whether FGF-23 and PTH act alone or together in the development of hypophosphatemia. On the other hand, factors other than PTH and FGF-23 – such as renal function and dietary phosphorus intake – may modulate serum phosphate (sPi) levels ***(Marks J et al, 2010).***

Prospective studies to investigate FGF-23 levels in patients with end-stage renal disease before and after renal transplantation at 3, 6, and 12 months post-transplantation and their probable association with markers of bone and mineral metabolism. ***(Economidou D ,et al 2009)***

FGF-23 levels decrease dramatically after successful renal transplantation. Pre-transplantation FGF-23 correlate with P levels 3 months post-transplantation. ***(Economidou D ,et al 2009)***

As a result disorders of FGF-23 excess are characterized by hypophosphatemia with increased renal phosphate wasting and inappropriately low calcitriol levels for the degree of hypophosphatemia ***(Fukagawa M, et al 2005).***

Still though, its role in the hypophosphatemia observed after renal transplantation remains largely unknown.

The aims of many prospective studies were therefore to investigate FGF-23 levels in patients with end-stage renal disease before and after a successful renal transplantation and their probable association with markers of bone and mineral metabolism. ***(Evenepoel P et al, 2007).***

The major findings of these studies were the following:

(i) intact FGF-23 levels decrease dramatically after successful renal transplantation and remain within normal limits when graft function is good.

(ii) TmPO4/GFR and P levels at month 3 correlate significantly with FGF-23 levels before transplantation

(iii) No association between FGF-23 and 1,25(OH)2VitD levels was found.

They observed a significant reduction, already from the first trimester post-transplantation that reached 90% of pre-transplant values. ***(Marks J et al, 2010)***

In patients with chronic renal failure and secondary hyperparathyroidism that have undergone renal transplantation, it is expected that there is a greater production in the first trimester after transplantation, until osteoblasts become inactive and bone metabolism is suppressed. .***(Krocker D,et al 2006)***

Also, It has been reported that corticosteroids, calcineurin inhibitors, and mTOR inhibitors stimulate FGF-23 production .***(Krocker D,et al 2006)***

Even though in the first months after transplantation, with the use of higher doses of these drugs we as well as others observed the greater reduction of Phosphorous**.** ***(Evenepoel P et al, 2007).***

Intact FGF-23 contributes to the hypophosphatemia observed in the first month after transplantation, but after the first 6 months possible causes are persistent secondary hyperparathyroidism, tubular damage, and/or immunosuppressive drugs. ***(Wolf M et al,2011)***

FGF-23 level reduction as early as the fifth post transplant day. ***(Pande S et al, 2006)***

A significant reduction of FGF-23 levels. In this study, there are patients in the first trimester after transplantation with higher than normal intact FGF-23 levels but with lower glomerular filtration rate. ***(Evenepoel P et al, 2007)***

iPTH concentrations and FGF-23 decrease progressively after renal transplantation. However, resolution of secondary hyperparathyroidism (SHPT) is incomplete 1 year after transplantation in about 50% of the recipients.***(Torres A et al, 2002)***

Patients that developed hypophosphatemia tended to have higher pre- and post transplantation iPTH levels as well as higher pretransplantation FGF-23 levels. These findings suggest that FGF-23 and iPTH could act synergistically to cause phosphaturia, as has been previously suggested ***(Naesens M et al, 2007).***

The role of iPTH in post transplantation hypophosphatemia is further supported by the observation that renal transplant recipients that had undergone parathyroidectomy before transplantation had lower Ca and higher P levels 12 months post transplantation. TmPO4/GFR was also higher in patients with a history of parathyroidectomy.***(Ghanekar H et al, 2004).***

Pretransplantation but not post transplantation FGF-23 levels correlated with P levels as well as TmPO4/GFR at 3 months post transplantation. This finding is consistent with previous studies that implicate FGF-23 in postrenal transplantation hyposphosphatemia ***(Evenepoel et al, 2007).***

Up to 91% of our patients had sufficient 1,25(OH)2VitD levels between 3 and 12 months after renal transplantation, but there was no association was found between FGF-23 and 1,25(OH)2VitD levels. ***(Naesens M et al., 2007)***

Recent studies have also highlighted the critical role of FGF23 in the pathogenesis of post-transplant hypophosphatemia. This complication occurs in up to 93% of patients receiving renal transplants, and has long been attributed to persistently increased levels of PTH due to secondary hyperparathyroidism .***(Wolf M etal,2011)***

In the pre transplant period, FGF23 secretion is extremely up-regulated to counteract chronic phosphate retention, but fails to exert its hormonal effects in the absence of functioning kidneys. After kidney transplantation, however, this excess FGF23 acts on the allograft to promote phosphaturia and suppress 1,25(OH)2D production (Fig. 4.1). ***(Sato T et al,2009).***

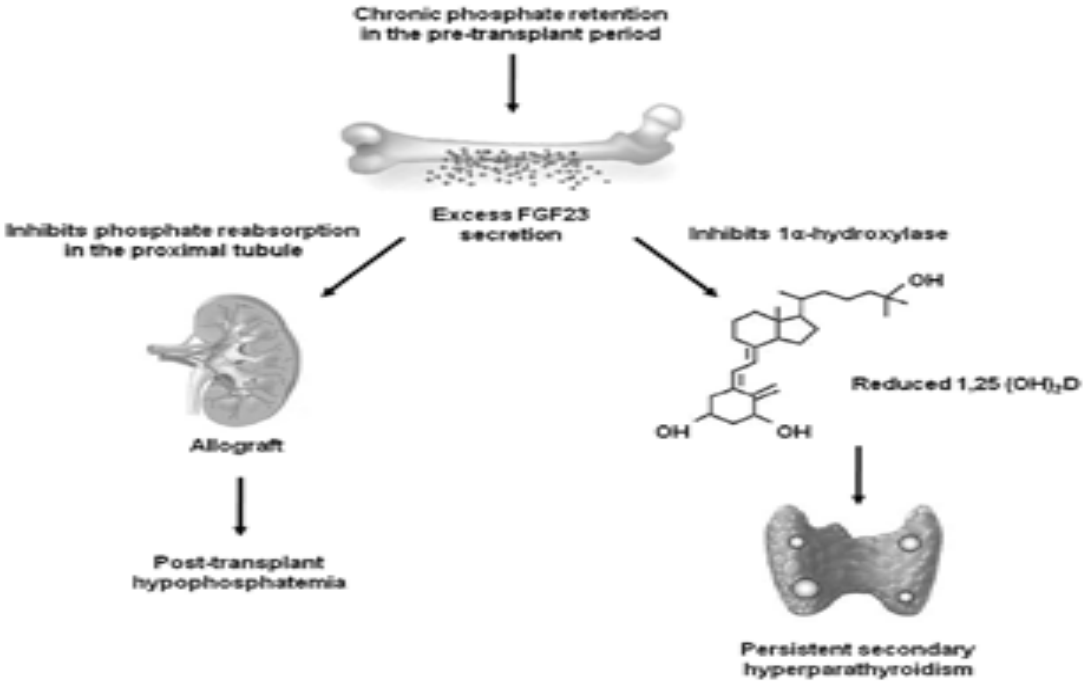


Fig. 4.1 - *Role of FGF23 in renal transplant patients. In patients undergoing kidney transplantation, elevated FGF23 due to chronic phosphate retention in the pretransplant period acts on the allograft to promote phosphaturia and suppress 1,25(OH)2D production. This results in post-transplant hypophosphatemia and persistent secondary hyperparathyroidism.*

However, this PTH-centric view cannot explain the concurrent 1,25(OH)2D deficiency or the development of urinary phosphate wasting even in the absence of elevated PTH levels. In this context, recent data suggest that FGF23 is the primary pathogenic factor that accounts for the syndrome of post-transplant hypophosphatemia and concomitant vitamin D deficiency ***(Sato T et al,2009).***

It is still not clear why autonomic FGF23 secretion can persist for months following transplantation, even in the presence of hypophosphatemia. It is possible that the use of immunosuppressive drugs contributes to this situation. ***(Krocker D et al, 2006)***

Indeed, it has recently been shown that FGF23 mRNA expression in human osteoblasts increases in response to dexamethasone. ***(Mirams M et al,2004).***

However, given the absence of clinically relevant hypophosphatemia following nonrenal organ transplantation, it is unlikely that immunosuppressive drugs are the primary cause of persistently elevated FGF23 ***(Evenepoel P et al,2008).***

Another possibility is that uremic bone may develop resistance to feedback inhibition of FGF23, perhaps caused by the preceding years of chronic phosphate retention that stimulates FGF23 secretion. ***(Bhan I et al,2006)***

Although the precise mechanism underlying excessive FGF23 secretion following kidney transplantation remains unclear, this FGF23-centric view poses questions for current strategies used to manage post-transplant hypophosphatemia because both high-dose phosphate supplements and active vitamin D sterols further stimulate FGF23 secretion.***(Bhan I et al,2006)***

In this regard, it is promising that anti-FGF23 monoclonal antibodies are effective to treat Hyp mice, a homologue of human XLH , This insight may present new strategies to manage persistent hypophosphatemia in kidney transplant recipients. ***(Aono Y et al,2009).***

5- Role of Deteriorating Renal Function.

The level of renal function that is achieved as a result of transplantation is a critical determinant of whether secondary hyperparathyroidism will be present.

Patients who do not achieve a GFR _70 ml/min per 1.73 m2 are at greater risk for progression of renal bone disease ***(Rix M et al,2003).***

In addition, as mentioned previously, some patients may develop *de novo* secondary hyperparathyroidism that results from progressive functional alterations of the transplanted kidney ***(Weisinger JR et al,2004).***

6- Role of Hypogonadism.

In addition to the physiologic decrease in gonadal steroids that is associated with aging and menopause, endocrine dysfunction that leads to premature hypogonadism or impaired gonadal function is a common feature of CKD.

Therefore, the role of hypogonadism and postmenopausalosteoporosis should not be overlooked when evaluating skeletal health in CKD and transplant patients ***(Weisinger JR et al,2006).***

In conclusion, although the alterations of bone remodeling after transplantation are heterogeneous, most studies reflect a decreased bone formation in the face of persistently elevated bone resorption.

This imbalance in bone remodeling that favors resorption leads to a progressive loss of bone mass and increased fracture risk.***(Sirilak S et al,2012)***

As summarized in Figure 4.2, the mechanisms that are involved in these alterations include preexisting conditions such as the predominant state of bone turnover before transplantation, but posttransplantation events such as the effects of glucocorticoids, the occurrence of hypophosphatemia, and perhaps other biochemical factors seem to be fundamental for the alterations of bone remodeling. ***(Wolf M et al,2011)***

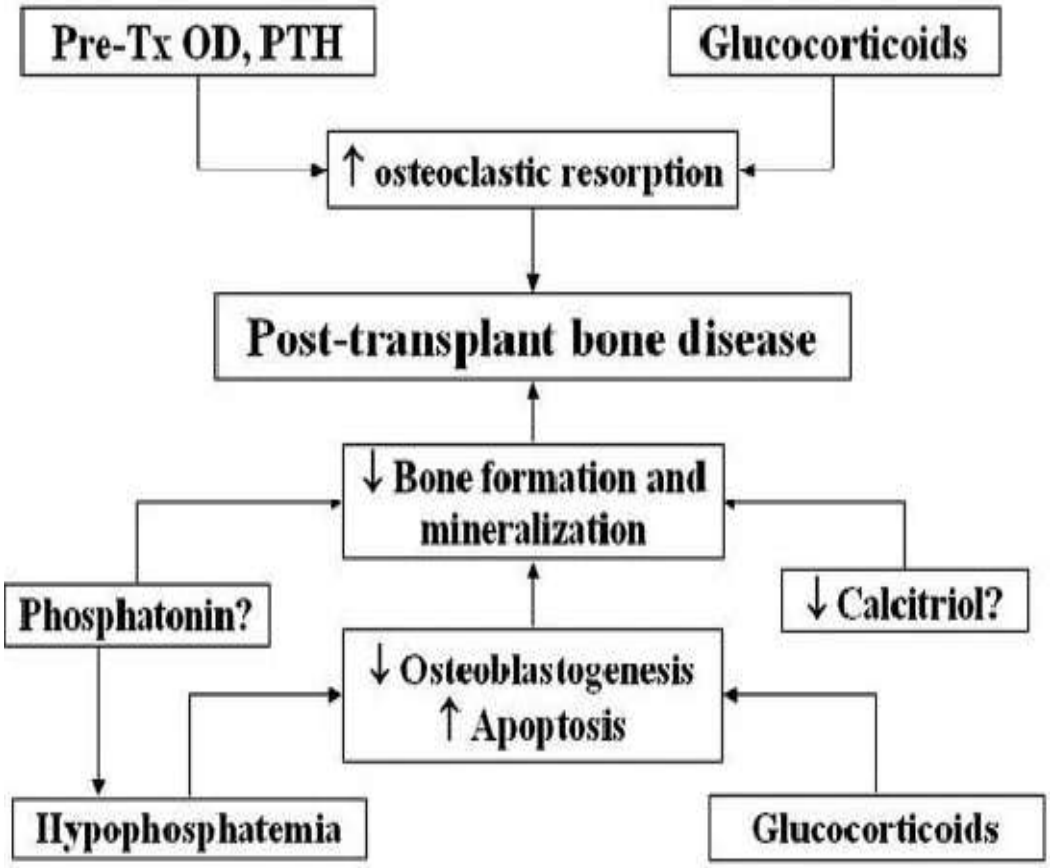


Figure 4.2 **Factors that contribute to posttransplantation bone disease**. *Renal osteodystrophy during dialysis and glucocorticoid administration after transplantation play an important role in the increased posttransplantation bone resorption. In addition, the majority of the patients show decreased bone formation and mineralization in which low calcitriol levels, glucocorticoids, and, probably, other immunosuppressive drugs play important roles. Hypophosphatemia and possibly substances with phosphatonin activity may affect this mechanism either by a direct effect on bone cells or by altering calcium-phosphate metabolism, particularly during the early posttransplantation period. The final result of this multifactorial process that determines an increase in osteoclastic resorption and decrease in bone formation is an imbalance in bone turnover that leads to bone mass loss that may persist long after transplantation .*

Fibroblast Growth Factor 23 and Cardiovascular Mortality after Kidney Transplantation

Circulating fibroblast growth factor 23 (FGF23) is associated with adverse cardiovascular outcomes in CKD.
Whether FGF23 predicts cardiovascular mortality after kidney transplantation, independent of measures of mineral metabolism and cardiovascular risk factors, is unknown. ***(Leandro C et al,2013)***

The risk of premature death due to cardiovascular disease remains greatly increased in kidney transplant recipients compared with the general population. ***(Jardine AG et al,2012).***

Deregulation of calcium/phosphate metabolism is common in CKD and in kidney transplant recipients with impaired renal function ***(Carlsson AC et al,2013).***

In patients with CKD, circulating levels of fibroblast growth factor 23 (FGF23), parathyroid hormone (PTH), and phosphate have been identified as independent risk factors for cardiovascular disease and all-cause mortality ***(Tonelli M et al,2009).***

Experimental studies demonstrated that FGF23 may be directly involved in the development of left ventricular hypertrophy (LVH) . Whether FGF23 predicts cardiovascular mortality in renal transplant recipients is unknown. ***(Faul C et al,2011)***

Plasma FGF23 is a risk factor for cardiovascular mortality in renal transplant recipients, independent of Framingham risk factors (recipient age and sex, systolic BP, antihypertensive treatment use, smoking status, diabetes mellitus,

plasma total cholesterol, and HDL cholesterol), known correlates of FGF23 including estimated GFR [eGFR] and proteinuria , and factors related to phosphate metabolism. ***(Vervloet MG et al,2012)***

They also addressed whether FGF23 is associated with the left ventricular wall strain markers mid regional fragment of pro–A-type natriuretic peptide (ANP) and N-terminal-pro brain natriuretic peptide (NT-proBNP) and with copeptin, the stable C-terminal portion of the precursor of vasopressin , and whether these cardiac markers influence the association between FGF23 and cardiovascular mortality. ***(Jardine AG et al,2012).***

Leandro C et al, study identified high plasma FGF23 levels as an independent risk factor for cardiovascular mortality in kidney transplant recipients. The association was consistent in regression models adjusted for renal function, measures of mineral metabolism, and cardiovascular risk factors.

The significantly improved NRI suggests that FGF23 levels may have an additional value to Framingham risk factors to predict the risk of cardiovascular mortality in renal transplant recipients. ***(Leandro C et al,2013)***

Their findings are in line with previous data linking high FGF23 levels with incident cardiovascular disease and mortality in the CKD population, and with a higher risk of all-cause mortality after kidney transplantation ***(Wolf M et al,2011).***

Furthermore, FGF23 levels have been associated with established cardiovascular risk factors and with a higher risk of cardiovascular events in the general population . ***(Carlsson AC et al,2013).***

Although patients in the highest FGF23 tertile were characterized by an overall high cardiovascular risk profile, the associations between FGF23 and cardiovascular and all-cause mortality remained significant after adjustment for several major cardiovascular risk markers, including Framingham risk factors, suggesting that FGF23 is a strong and independent risk marker. ***(Leandro C et al,2013)***

The potential clinical relevance of FGF23 in addition to established risk markers is further supported by improved reclassification (NRI and IDI). ***(Leandro C et al,2013)***

Furthermore, FGF23, but not serum phosphate, PTH, or vitamin D, was independently associated with cardiovascular mortality (Figure 4.3), suggesting a specific role for FGF23. ***(Leandro C et al,2013)***

The observed association between elevated FGF23 levels and a higher cardiovascular risk initially positioned FGF23 as a biomarker of phosphate toxicity. ***(Leandro C et al,2013)***

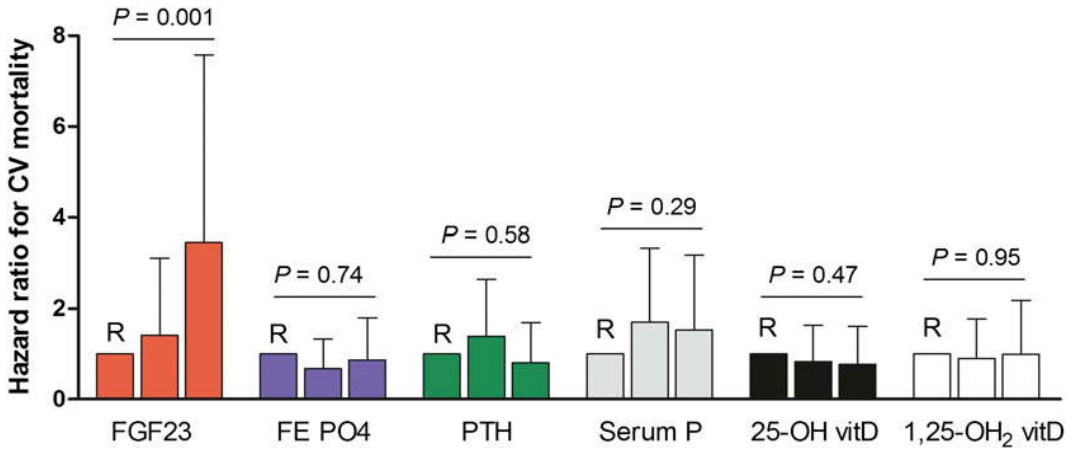


Figure 4.3: | Comparative analysis of fibroblast growth factor 23 (FGF23), fractional phosphate excretion (FE PO4), parathyroid hormone,phosphate (P), 25(OH)-vitaminD, or 1,25(OH)2 vitaminDas independent risk factors for cardiovascular (CV)mortality. The model was adjusted for age, sex, cardiovascular history, estimated GFR, proteinuria, and Framinghamrisk factors. For each exposure, the lowest tertile served as the reference group (R).

More recently, animal studies demonstrated that FGF23 has "off-target" effects, directly contributing to the development of LVH .***(Faul C et al,2011)***

It was found that FGF23 was independently associated with MR-proANP and NTproBNP, both markers of left ventricular wall strain, and with copeptin, a stable peptide derived from the vasopressin precursor ***(Abbasi A et al,2012).***

All three cardiac markers are used as markers of heart failure. Although these associations are in line with a potential role for FGF23 in LVH, we could not demonstrate modulation of the association between FGF23 and cardiovascular mortality by these cardiac markers. ***(Sabatine MS et al,2012)***

Furthermore, only a small amount of FGF23 was explained by copeptin, MR-proANP, and NT-proBNP , suggesting that FGF23 is independently related to cardiovascular and all-cause mortality. ***(Leandro C et al,2013)***

Ix JH et al, the association between FGF23 and cardiovascular events was modified by sex . We could not confirm this effect modulation, which could be explained by differences in renal function between the Heart and Soul study (eGFR, 71623 ml/min per 1.73 m2) and our cohort (eGFR, 47616 ml/min per 1.73 m2). ***(Leandro C et al,2013)***

Data from 24-hour urine collections allowed us to include phosphate intake (assessed by 24-hour urinary phosphate excretion) and excretion (fractional phosphate excretion) in our analyses. Neither measure interacted with the association between FGF23 levels and cardiovascular mortality. ***(Vervloet MG et al,2012)***

The use of 24-hour urine collections, which enabled us to adjust for proteinuria and phosphate excretion, the availability of multiple markers of cardiovascular risk, and the complete follow-up. ***(Leandro C et al,2013)***

FGF23 was also associated with a higher risk of graft failure in univariate analysis, in line with a previous study. ***(Wolf M et al,2011).***

The association was no longer significant after adjustment for eGFR and proteinuria in addition to known risk factors for graft failure .
It was hypothesized that renal function after transplantation has a stronger effect on the risk of graft failure than on the risk of mortality. ***(Schnitzler MA et al,2012)***

FGF23 as an independent risk factor for cardiovascular mortality after kidney transplantation. Although it may be relevant to consider therapies reducing FGF23 levels to improve cardiovascular outcomes, it should be kept in mind that high FGF23 levels may serve an important physiologic goal, namely to keep phosphate balance. ***(Wolf M,2012)***

A recent study demonstrated that specific FGF23 blockade with a neutralizing antibody did reduce secondary hyperparathyroidism but increased serum phosphate, aortic calcification, and mortality . ***(Shalhoub V et al,2012)***

Plasma FGF23 is independently associated with cardiovascular and all-cause mortality after kidney transplantation. The association remained significant afteradjustment for measures of mineral metabolism and cardiovascular risk factors. ***(Leandro C et al,2013)***

Patients and Methods

Patients

This is a cohort - prospective, observational study was carried out during the period between 2011 and 2013 on 40 patients with ESRD on maintenance hemodialysis (21 males, 19 females).

- **Group A**: a group of 20 HD patients on maintenance HD.
- **Group B** : a group of 20 HD patients scheduled for renal transplantation and follow up of patients 3 and 6 months after successful renal transplantation.

The study protocol was approved by the ethical committee of our faculty.
All the patients were recruited from the Dialysis Unit in Kasr El-Aini Hospital, Cairo University.
The patients with ESRD had been undergoing hemodialysis 3 times weekly on standard bicarbonate dialysis with low-f lux dialyzers.

All patients will be subjected to the following:

- Detailed history taking and physical examination to exclude any disease which might affect the parameters to be investigated
- Body mass index (BMI) as calculated by weight in kg/square of height in meters
- Quantification of FGF-23 (pg/mL) Concentration by ELISA
- Serum calcium, phosphate ,PTH ,Alb and creatinine was done by the use of colormetry in the routine clinical laboratory.

All Laboratory investigations measured again in GROUP B : 3 months and 6 months after renal transplantation to compare the results.

INCLUSION CRITERIA*:*

- Patients of both sex with ESRD on regular hemodialysis.
- Age more than 18 years and less than 65years
- Renal transplant patients with normal kidney functions post transplant.

The allograft types in renal transplanted patients were living donor allograft with local ethical committee approval.
They received triple drug-immunosuppressive treatment consisting of cyclosporine (5 mg/kg initially, maintenance dose based on blood trough level), prednisolone (200 mg initially, tapered to 5 mg), and mycophenolate mofetil (2 x 1,000 mg per day).

EXCLUSION CRITERIA*:*

- Renal impairment after transplantation
- Age less than 18 years and more than 65 years old

Parathyroid hormone test

Test principle

PTH-EASIA is a solid phase Enzyme Amplified Sensitivity immunoassay performed on a microtiter plate. It allows the determination of intact human parathyroid hormone (PTH) in serum or plasma.

***N.B**; the kit developed and manufactured by Roche Diagnostics, Indianapolis in USA*

Sampling

Blood samples promptly separated from the blood cells and temporarily kept on ice or refrigerated at 4°C,Serum samples were used as recommended.

Assay procedure

1. We select sufficient strips to accommodate standards, controls and all test samples.
2. The strips are fitted into the holding frame and unused strips stored in the foil pouch with desiccant, at 2 to 8°C.
3. 50 µL of Incubation Buffer dispensed into all wells.
4. 200 µL of each standard, control or sample dispensed into the appropriate wells. time between distribution of first standard and last sample can be up to 30 minutes without affecting the results.
5. Then we incubate for 2 hours at room temperature on a horizontal shaker set at 700 ± 100 rpm.
6. The plate was washed 4 times by:

a) Aspirating the liquid from each well;

b) Dispensing 0.4 mL of Wash Solution into each well;

c) Aspirating the contents of each well.

7. 100 µL of anti-PTH HRP conjugate dispensed into all wells.
8. We incubated 1 hour at room temperature on a horizontal shaker set at 700 ± 100 rpm.
9. The plate washed 4 times by:

a) Aspirating the liquid from each well;

b) Dispensing 0.4 µL of wash solution into each well;

c) Aspirating the contents of each well.

10. 100 µL of the Chromogenic Solution dispensed into each well within 15 minutes following the washing step.

11. We incubate 30 minutes at room temperature on a horizontal shaker set at 700 ± 100 rpm.

12. 200 µL of Stop Solution dispensed into each well. Mix.

13. Finally we read the absorbances within 1 hour and calculate the results as follows:-

We read the micro titer plate at 450 nm (reference filter: 650 nm). Then we construct a standard curve using all standard points.

We plot the OD on the ordinate against the standard concentrations on the abscissa using either linear-linear or semi-log graph paper and we draw the curve by connecting the plotted points with straight lines.

We determine PTH concentrations of samples or controls for which absorbance is no greater than those of the last standard plotted at 450 nm.

If any control or sample has an absorbance greater than the absorbance of the last standard read at 450 nm, a second reading at 490 nm (reference filter: 650 nm) is needed. then we proceed to construct

a second standard curve at 490 nm using all the standard points. the segment of the curve drawn between the last standard read at 450 nm and the most concentrated standard will be considered at 490 nm. the

concentrations of samples and controls for which absorbances are included in this segment, are read at 490 nm.

Note:

The same equipment must be used for both readings at 450 nm and 490 nm.2. the readings at 490 nm are only for off-scale values at 450 nm (above 1.5 OD units) and should not replace the reading at 450 nm for values below 1.5 OD units.

FGF23 TEST

Test Principle

The Human Intact FGF-23 ELISA Kit is a two-site enzyme-linked immunosorbent assay (ELISA) for the measurement of FGF-23 in plasma or cell culture media. two affinity purified goat polyclonal antibodies have been isolated and selected to detect epitopes within the amino terminal and the carboxyl terminal portions of FGF-23. one antibody is immobilized onto the microtiter plate wells for capture. the other antibody is conjugated with horseradish peroxidase (HRP) for detection.a sample containing FGF-23 is incubated simultaneously with the immobilized capture antibody and the HRP conjugated detection antibody in a microtiter well. intact FGF-23 contained in the sample is immunologically bound by the capture antibody and the detection antibody to form a “sandwich” complex:

Well/Anti-Human FGF — Human Intact FGF-23 — HRP/Anti-Human FGF (C-terminal) (NH2-terminal). at the end of this incubation period, the well is washed to remove any unbound antibody and other components. this immobilized sandwich complex is then incubated with substrate solution in a timed reaction and then measured in a spectrophotometric microtiter plate reader.

The enzymatic activity of the antibody complex bound to the well is directly proportional to the amount of intact FGF-23 in the sample. a standard curve is generated by plotting the absorbance versus the respective intact FGF-23 concentration for each standard on linear or logarithmic scales. the concentration of human intact FGF-23 in the samples is determined directly from this curve.

***N.B**; the kit developed and manufactured by immutopics, inc san clementa*

Specimen collection

The intact FGF-23 molecule appears to be highly unstable resulting in decreased immunoreactivity over time. specimen collection and assay or storage procedures carried out in an expeditious manner. measurement of the intact FGF-23 concentration made by using EDTA plasma or cell culture media. three hundred microliters of plasma or culture media collected to assay the sample in duplicate.

The sample was collected while patients were fasting for 12 hours and in the morning (which is recommended). the sample was centrifuged and the plasma or media separated from the cells. samples assayed immediately or stored frozen at -20^0C or below.

Procedure notes

1. All standards, controls and samples be assayed in duplicate. the average absorbance reading of each duplicate used for data reduction and the calculation of results.

2. Light sensitive reagents (i.e. HRP Conjugated Antibody, the working HRP Antibody solution consisting of combined HRP Conjugated antibody and HRP antibody diluting buffer, and ELISA HRP substrate) are kept in the

original amber bottles or other suitable container which was well protected from light.

3. Any unused antibody coated strips are stored in the resealable aluminum pouch with desiccant to protect from moisture.

4. The sample and all reagents were pipetted carefully to minimize air bubbles in the wells.

5. The sequence and timing of each reagent addition is important as both the immunological and enzymatic reactions are in kinetic modes. the washing step is also an important part of the total assay procedure.

6. Samples with values greater than the highest standard were diluted 1:10 with saline and reassayed. multiplying the result by 10.

7. Plasma or cell culture media samples may contain fibrin clots or cellular debris. Freeze and thaw of plasma samples may accelerate clot formation. these samples were centrifuged and decanted prior to assay to remove all particulate material which can cause random high non-specific binding on well surface

Calculation of results

The two absorbance readings taken before and after the addition of the ELISA stop solution allow for the construction of two standard curves using the human intact FGF-23 standards contained in the kit.

We refer to the individual vial label for exact concentration. the primary curve used for calculation of results is the second reading taken after the addition of the ELISA Stop solution and we read at 450 nm. This data utilizes the absorbance values obtained with the first five standards.

The first reading taken before the addition of the ELISA Stop solution and read at 595 nm - 650 nm is intended to extend the analytical range to the value of the sixth (highest) standard provided in the kit. It should be utilized only if sample results extend beyond the value of the fifth standard. results obtained with the first reading should not replace the on-scale reading at 450 nm.

Each curve generated as follows:

Primary Procedure — we read at 450 nm

1. We calculate the average absorbance for each pair of duplicate assay wells.
2. Then we subtract the average absorbance of the 0 pg/mL standard from the average absorbance of all other readings to obtain corrected absorbance.
3. The standard curve is generated by plotting the corrected absorbance of the first five standard levels on the ordinate against the standard concentration on the abscissa using linear-linear or log-log paper.

Appropriate computer assisted data reduction programs may also be used for the calculation of results.

The intact FGF-23 concentration of the controls and samples are read directly from the standard curve using their respective corrected absorbance. If log-log graph paper or computer assisted data reduction programs utilizing logarithmic transformation are used, samples having corrected absorbance between the 0 pg/mL standard and the next highest standard should be calculated by the formula:

$$\text{Value of unknown} = \frac{\text{(unknown).}}{\text{Corrected Absorbance}} \times \frac{\text{Value of the 2nd Std}}{\text{(2nd Std.)}}$$

Secondary Procedure -we read at 595 nm - 650 nm

1. We calculate the average absorbance for each pair of duplicate assay wells.2. The standard curve is generated by plotting the absorbance of the three highest standards on the ordinate against the standard concentration on the abscissa using linear-linear or log-log graph paper.

3. The intact FGF-23 concentration of samples reading greater than the fifth standard are read directly from the standard curve.

Statistical analysis:

- All collected questionnaires were revised for completeness and consistency. Pre-coded data was entered on the computer using "Microsoft Office Excel Software" program (2010) for windows. Data was then transferred to the Statistical Package of Social Science Software program, version 21 (SPSS) to be statistically analyzed.
- Data was summarized using mean, and standard deviation for quantitative variables and frequency and percentage for qualitative ones.

- Comparison between groups was performed using independent sample t-test or one way ANOVA for quantitative variables and Chi square or Fissure exact test for qualitative ones

- Repeated measurements were analyzed by using repeated measures ANOVA test with post hoc Bonnferronitest (for pairwise comparisons).

- Pearson or Spearman correlation coefficients were calculated to get the association between parametric or non-parametric quantitative variables respectively.

- P values less than 0.05 were considered statistically significant, and less than 0.01 were considered highly significant.

- Graphs were used to illustrate some information.

Results

Group A = patients on dialysis, **Group B** = patients undergoing transplantation **B0**= before transplantation, **B3** = after 3 months of transplant, **B6** = after 6 months

Group A: is our control group whom are CRF patients on regular hemodialysis.

Group B0: are patients whom are CRF patients on regular hemodialysis will undergo renal transplantation.

Group B3 :are patients whom had renal transplantation for 3months duration.

Group B6 :are patients whom had renal transplantation for 6months duration.

Table 6.1: **Comparison between Group A & Group B0 with different parameters:**

	Group A	Group B0	P value
Age (years)	36.2 ± 12.6	24.1 ± 7.0	**0.001 HS**
Sex			
Male	10 (50.0%)	11 (55.0%)	1.0
Female	10 (50.0%)	9 (45.0%)	NS
BMI (Kg/m^2)	23.6 ± 2.9	22.2 ± 2.7	0.1 NS
Duration of Dialysis (years)	4.4 ± 2.7	0.9 ± 0.5	**<0.001 HS**
Creatinine (mg/dl)	10.5 ± 2.5	9.3 ± 2.0	0.1 NS
Calcium	7.2 ± 1.1	7.2 ± 0.5	0.8 NS
Phosphorus	6.2 ± 0.8	6.4 ± 0.8	0.6 NS
FGF-23	625.3 ± 18.2	604.5 ± 39.4	**0.04 S**
Albumin	3.3 ± 0.3	3.5 ± 0.4	0.09 NS
PTH	497.0 ± 228.8	447.6 ± 188.8	0.5 NS

Comparing of Group A and Group B0 at different parameters: there was a statistically significant (P< 0.001) with age of patients of both groups, there was a statistically significant P- value (< 0.001) with duration of dialysis, and also there was a statistically significant P- value (0.04) with comparing FGF-23 level of 2 groups.

Table 6. 2: Comparison between Group A & Group B3 with different parameters:

	Group A	Group B3	P value
BMI (Kg/m^2)	23.6 ± 2.9	22.9 ± 2.1	0.4 NS
Creatinine (mg/dl)	10.5 ± 2.5	0.96 ± 0.26	**<0.001 HS**
Calcium (mg/dl)	7.2 ± 1.1	8.5 ± 0.4	**<0.001 HS**
Phosphorus(mg/dl)	6.2 ± 0.8	4.3 ± 0.6	**<0.001 HS**
FGF-23(pg/ml)	625.3 ± 18.2	242.3 ± 9.5	**<0.001 HS**
Albumin (mg/dl)	3.3 ± 0.3	4.0 ± 0.2	**<0.001 HS**
PTH(pg/ml)	497.0 ± 228.8	214.0 ± 37.7	**<0.001 HS**

On comparing Group A and Group B3 with different parameters:

we found a statistically significant P- value (< 0.001) with level of creatinine (10.5 ± 2.5 to0.96 ± 0.26) , statistically significant P- value (< 0.001) with level of phosphorous (6.2 ± 0.8 to 4.3 ± 0.6) , statistically significant P- value (< 0.001) with level of FGF-23(625.3 ± 18.2 to242.3 ± 9.5) , statistically significant P- value (< 0.001) with level of PTH (497.0 ± 228.8 to214.0 ± 37.7) , , statistically significant P- value (< 0.001) with level of Albumin (3.3 ± 0.3 to 4.0 ± 0.2) , statistically significant P- value (< 0.001) with level of calcium (7.2 ± 1.1 to8.5 ± 0.4)

Table 6. 3: Comparison between Group A & Group B6 with different parameters:

	Group A	Group B6	P value
BMI (Kg/m^2)	23.6 ± 2.9	22.9 ± 2.1	0.4 NS
Creatinine (mg/dl)	10.5 ± 2.5	0.92 ± 0.21	**<0.001 HS**
Calcium	7.2 ± 1.1	8.6 ± 0.3	**<0.001 HS**
Phosphorus	6.2 ± 0.8	4.4 ± 0.5	**<0.001 HS**
FGF-23	625.3 ± 18.2	136.3 ± 9.8	**<0.001 HS**
Albumin	3.3 ± 0.3	4.2 ± 0.2	**<0.001 HS**
PTH	497.0 ± 228.8	92.5 ± 19.2	**<0.001 HS**

On comparing Group A and Group B6 with different parameters:

we found a statistically significant P- value (< 0.001) with level of creatinine (10.5 ± 2.5 to0.92 ± 0.21) , statistically significant P- value (< 0.001) with level of phosphorous (6.2 ± 0.8 to 4.4 ± 0.5) , statistically significant P- value (< 0.001) with level of FGF-23(625.3 ± 18.2 to136.3 ± 9.8) , statistically significant P- value (< 0.001) with level of PTH (497.0 ± 228.8 to 92.5 ±19.2) , , statistically significant P- value (< 0.001) with level of Albumin (3.3 ± 0.3 to 4.2 ± 0.2) , statistically significant P- value (< 0.001) with level of calcium (7.2 ± 1.1 to8.6 ± 0.3)

Changes of different parameters after transplantation

Table 6.4: Comparison between Group B0 & Group B3 with different parameters:

	Group B0	Group B3	P value
Creatinine (mg/dl)	9.3 ± 2.0	0.96 ± 0.26	**<0.001 HS**
Calcium	7.2 ± 0.5	8.5 ± 0.4	**<0.001 HS**
Phosphorus	6.4 ± 0.8	4.3 ± 0.6	**<0.001 HS**
FGF-23	604.5 ± 39.4	242.3 ± 9.5	**<0.001 HS**
Albumin	3.5 ± 0.4	4.0 ± 0.2	**<0.001 HS**
PTH	447.6 ± 188.8	214.0 ± 37.7	**<0.001 HS**

Step P value* = *P value of comparison of the measurement with its previous time measurement

On comparing Group B0 and Group B3 with different parameters:

we found a statistically significant P- value (< 0.001) with level of creatinine (9.3 ± 2.0 to 0.96 ± 0.26) , statistically significant P- value (< 0.001) with level of phosphorous (6.4 ± 0.8 to 4.3 ± 0.6) , statistically significant P- value (< 0.001) with level of FGF-23(6O4.5 ± 39.4 to242.3 ± 9.5) , statistically significant P- value (< 0.001) with level of PTH (447.6 ± 188.8 to 214.0 ±37.7) , , statistically significant P- value (< 0.001) with level of Albumin (3.5 ± 0.4 to 4.0 ± 0.2) , statistically significant P- value (< 0.001) with level of calcium (7.2 ± 0.5 to8.5 ± 0.4)

Table 6.5: Comparison between Group B0 & Group B6 with different parameters:

	Group B0	Group B6	P value
Creatinine (mg/dl)	9.3 ± 2.0	0.92 ± 0.21	**<0.001 HS**
Calcium	7.2 ± 0.5	8.6 ± 0.3	**<0.001 HS**
Phosphorus	6.4 ± 0.8	4.4 ± 0.5	**<0.001 HS**
FGF-23	604.5 ± 39.4	136.3 ± 9.8	**<0.001 HS**
Albumin	3.5 ± 0.4	4.2 ± 0.2	**<0.001 HS**
PTH	447.6 ± 188.8	92.5 ± 19.2	**<0.001 HS**

***Step P value** = P value of comparison of the measurement with its previous time measurement*

On comparing Group B0 and Group B6 with different parameters:

we found a statistically significant P- value (< 0.001) with level of creatinine (9.3 ± 2.0 to0.92 ± 0.21) , statistically significant P- value (< 0.001) with level of phosphorous (6.4 ± 0.8 to 4.4 ± 0.5) , statistically significant P- value (< 0.001) with level of FGF-23(604.5 ± 39.4 to136.3 ± 9.8) , statistically significant P- value (< 0.001) with level of PTH (447.6 ± 188.8 to 92.5 ±19.2) , , statistically significant P- value (< 0.001) with level of Albumin (3.3 ± 0.3 to 4.2 ± 0.2) , statistically significant P- value (< 0.001) with level of calcium (7.2 ± 0.5 to8.6 ± 0.3)

Table 6. 6: Comparison between Group B3 & Group B6 with different parameters:

	Group B3	Group B6	P value
Creatinine (mg/dl)	0.96 ± 0.26	0.92 ± 0.21	0.3 NS
Calcium	8.5 ± 0.4	8.6 ± 0.3	0.1 NS
Phosphorus	4.3 ± 0.6	4.4 ± 0.5	0.6 NS
FGF-23	242.3 ± 9.5	136.3 ± 9.8	**<0.001 HS**
Albumin	4.0 ± 0.2	4.2 ± 0.2	**0.004 HS**
PTH	214.0 ± 37.7	92.5 ± 19.2	**<0.001 HS**

On comparing Group B3 and Group B6 with different parameters:

we found a statistically significant P- value (< 0.001) with level of FGF-23(242.3 ± 9.5 to136.3 ± 9.8) , statistically significant P- value (< 0.001) with level of PTH (214.0 ± 37.7 to 92.5 ±19.2)

Changes of different parameters after transplantation

Table 6.7: Comparison between Group B0 & Group B3&B6 with different parameters:

	Group B0	Group B3	Group B6	0-6 P value
Creatinine (mg/dl)	9.3 ± 2.0	0.96 ± 0.26	0.92 ± 0.21	**<0.001**
Step P value	……	**<0.001**	0.3	
Calcium	7.2 ± 0.5	8.5 ± 0.4	8.6 ± 0.3	**<0.001**
Step P value		**<0.001**	0.1	
Phosphorus	6.4 ± 0.8	4.3 ± 0.6	4.4 ± 0.5	**<0.001**
Step P value	…..	**<0.001**	0.6	
FGF-23	604.5 ± 39.4	242.3 ± 9.5	136.3 ± 9.8	**<0.001**
Step P value	…..	**<0.001**	**<0.001**	
Albumin	3.5 ± 0.4	4.0 ± 0.2	4.2 ± 0.2	**<0.001**
Step P value	……	**<0.001**	**0.004**	
PTH	447.6 ± 188.8	214.0 ± 37.7	92.5 ± 19.2	**<0.001**
Step P value	…….	**<0.001**	**<0.001**	

***Step P value* = P value of comparison of the measurement with its previous time measurement**

Comparison between Group B0 & Group B3&B6 with different parameters: we found a statistically significant P- value (< 0.001) with level of creatinine (9.3 ± 2.0 to0.92 ± 0.21) , statistically significant P- value (< 0.001) with level of phosphorous (6.4 ± 0.8 to 4.4 ± 0.5) , statistically significant P- value (< 0.001) with level of FGF-23(604.5 ± 39.4 to136.3 ± 9.8) , statistically significant ces P- value (< 0.001) with level of PTH (447.6 ± 188.8 to 92.5 ±19.2) , , statistically significant P- value (< 0.001) with level of Albumin (3.3 ± 0.3 to 4.2 ± 0.2) , statistically significant P- value (< 0.001) with level of calcium (7.2 ± 0.5 to8.6 ± 0.3)

we found that there was a statistically significant P- value (< 0.001) with different parameters(calcium,phosphorous,FGF23,PTH, serum creatinine) on comparing between group B0 &B3 and group B0& B6.

Where there is statistically significant P- value (< 0.001) at level of FGF23 and PTH only on comparison of group B3 and groupB6 .

Table 6.8: Correlation of FGF-23 with other parameters before transplantation

		FGF-23
Age	**r**	0.238
	P value	0.139
BMI	**r**	-0.203
	P value	0.209
Duration of Dialysis	**r**	**0.338**
	P value	**0.033**
Creatinine	**r**	0.145
	P value	0.373
Calcium	**r**	**-0.446**
	P value	**0.004**
Phosphorus	**r**	**0.652**
	P value	**<0.001**
Albumin	**r**	**-0.362**
	P value	**0.022**
PTH	**r**	**0.111**
	P value	**0.007**

Correlation of FGF-23 with other parameters before transplantation:

We found a positive correlation between duration of dialysis and level of FGF23 P value (**0.033),** there is a negative correlation between level of calcium and level of FGF23 P value (**0.004), there is** a positive correlation between level of phosphorous and level of FGF23 P value (**<0.001),** there is a negative correlation between level of albumin and level of FGF23 P value (**0.022), there is** a positive correlation between level of PTH and level of FGF23 P value (**0.007**)

Table 6.9 :Correlation of FGF-23 with other parameters after 3 months of transplantation

		FGF-23
Age	**R**	0.362
	P value	0.117
BMI	**r**	0.073
	P value	0.761
Creatinine	**r**	0.025
	P value	0.918
Calcium	**r**	-0.168
	P value	0.478
Phosphorus	**r**	0.196
	P value	0.407
Albumin	**r**	**-0.476**
	P value	**0.034**
PTH	**r**	0.160
	P value	0.500

Table 6.10 : Correlation of FGF-23 with other parameters after 6 months of transplantation

		FGF-23
Age	**r**	0.085
	P value	0.722
BMI	**r**	-0.192
	P value	0.417
Creatinine	**r**	-0.019
	P value	0.936
Calcium	**r**	0.108
	P value	0.652
Phosphorus	**r**	0.201
	P value	0.397
Albumin	**r**	-0.144
	P value	0.544
PTH	**r**	-0.091
	P value	0.702

Figures

Fig 6.1: **Comparison between Group A & Group B0,B3,B6 with creatinine**

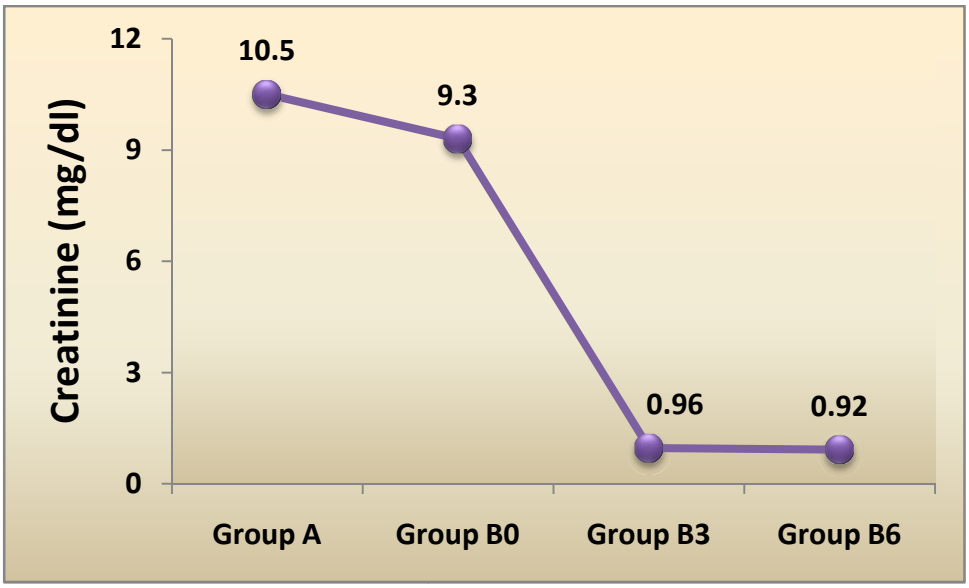


Fig 6.2: **Comparison between Group A & Group B0,B3,B6 with calcium**

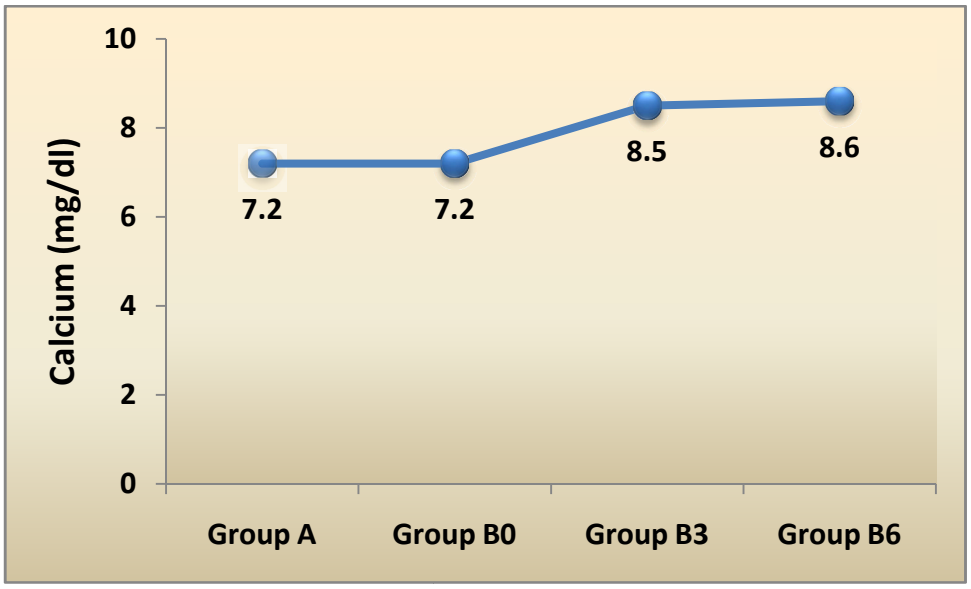


Fig 6.3: **Comparison between Group A & Group B0,B3,B6 with phosphorous**

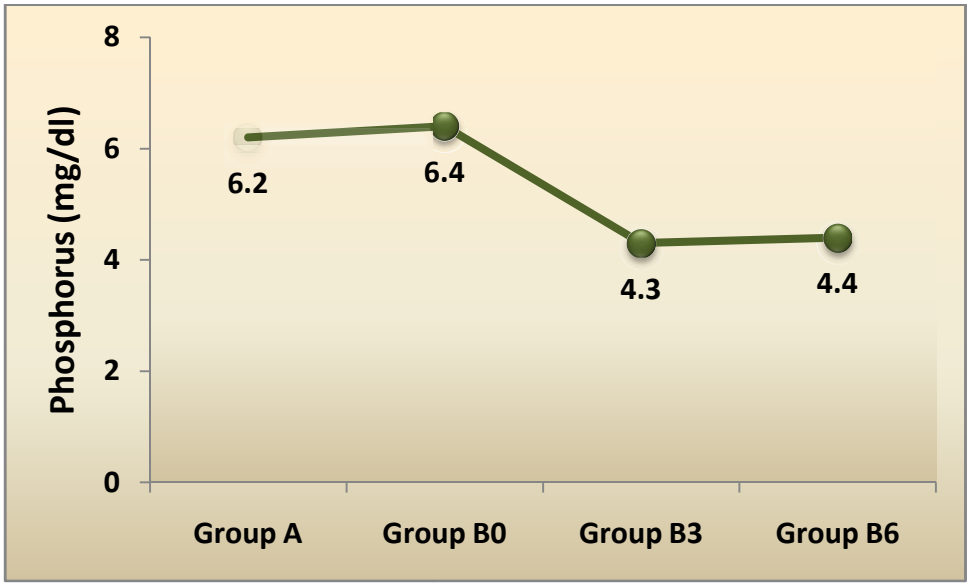


Fig 6.4: **Comparison between Group A & Group B0,B3,B6 with FGF23**

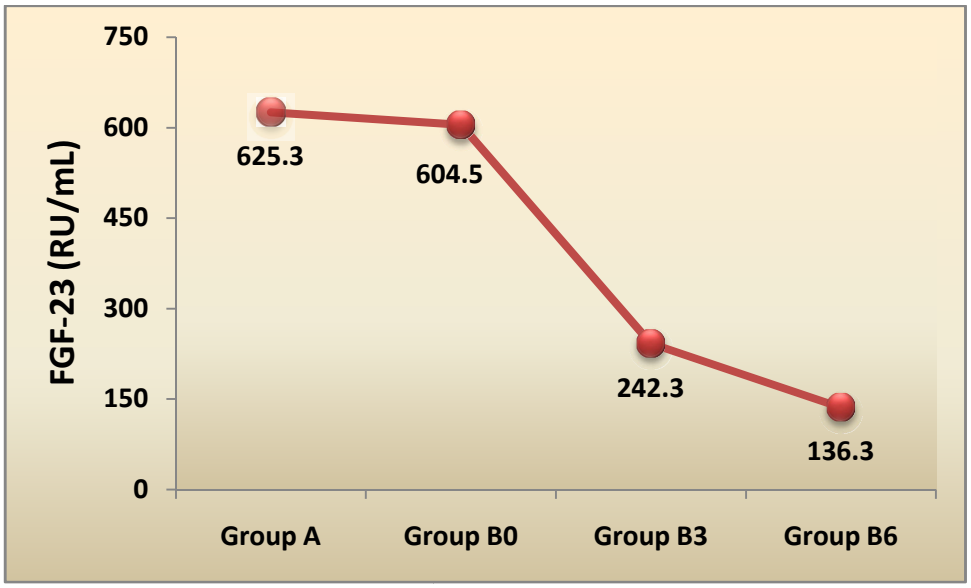


Fig 6.5: **Comparison between Group A & Group B0,B3,B6 with Albumin**

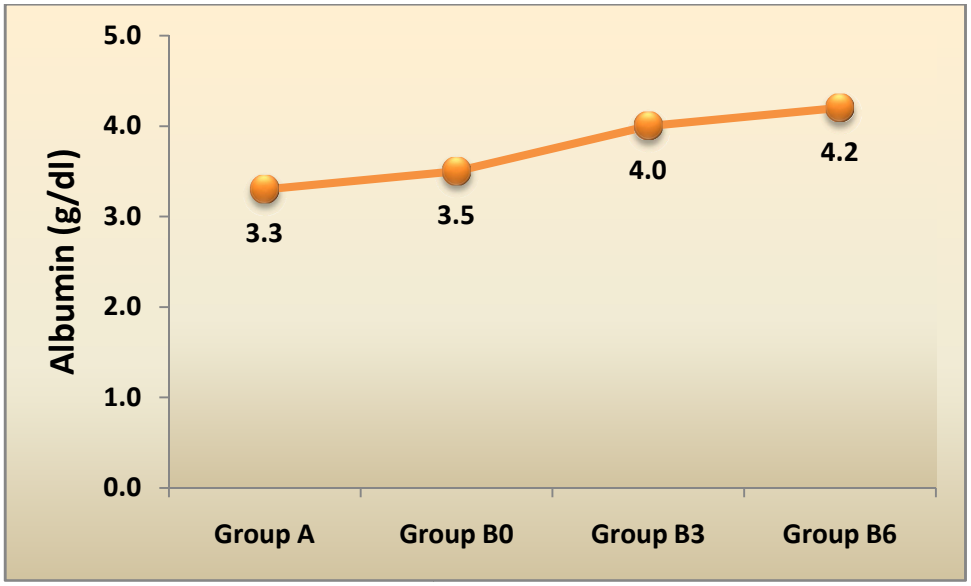


Fig 6.6: **Comparison between Group A & Group B0,B3,B6 with PTH**

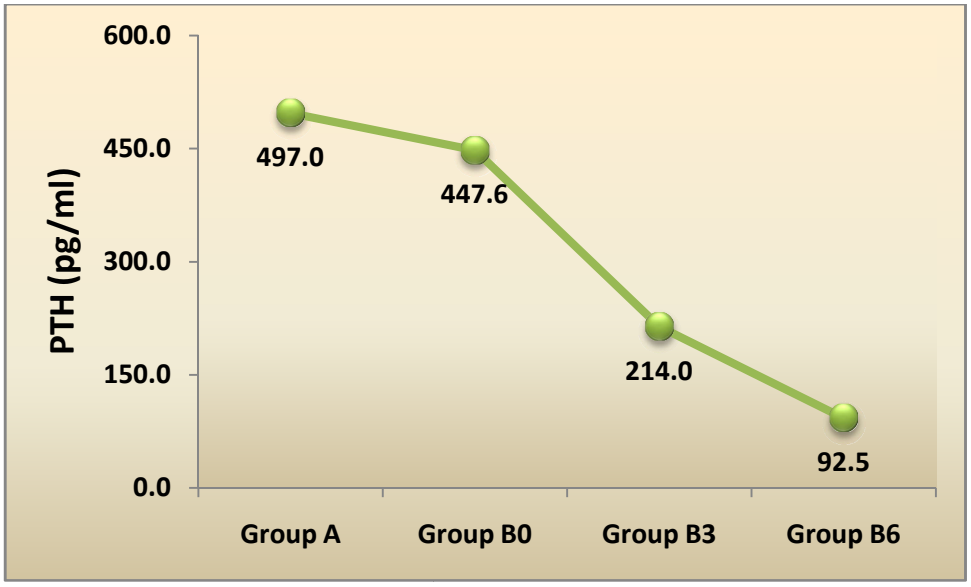


Fig 6.7 : Scatter plot showing the correlation between duration of dialysis and FGF-23 level before transplantation

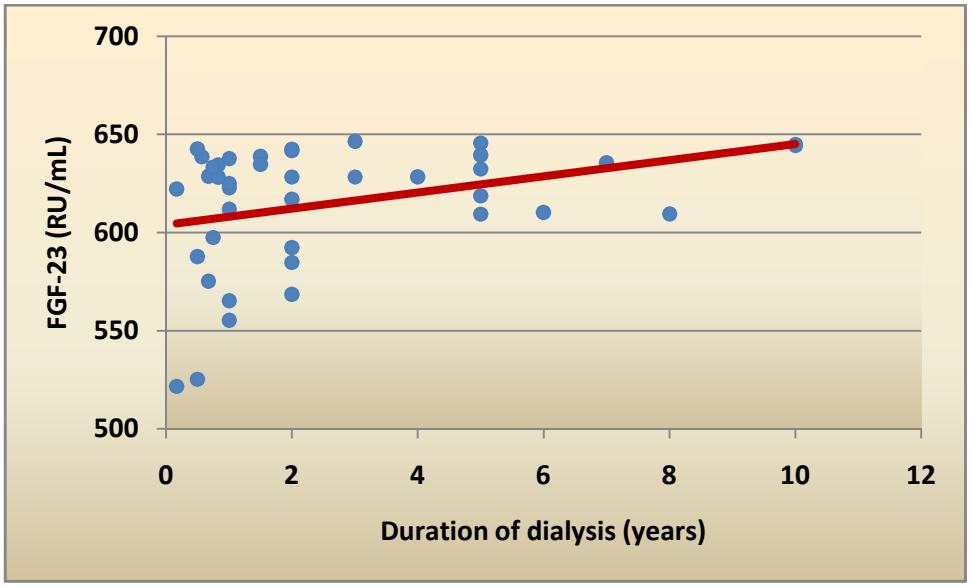


Fig 6.8: Scatter plot showing the correlation between serum calcium and FGF-23 level before transplantation

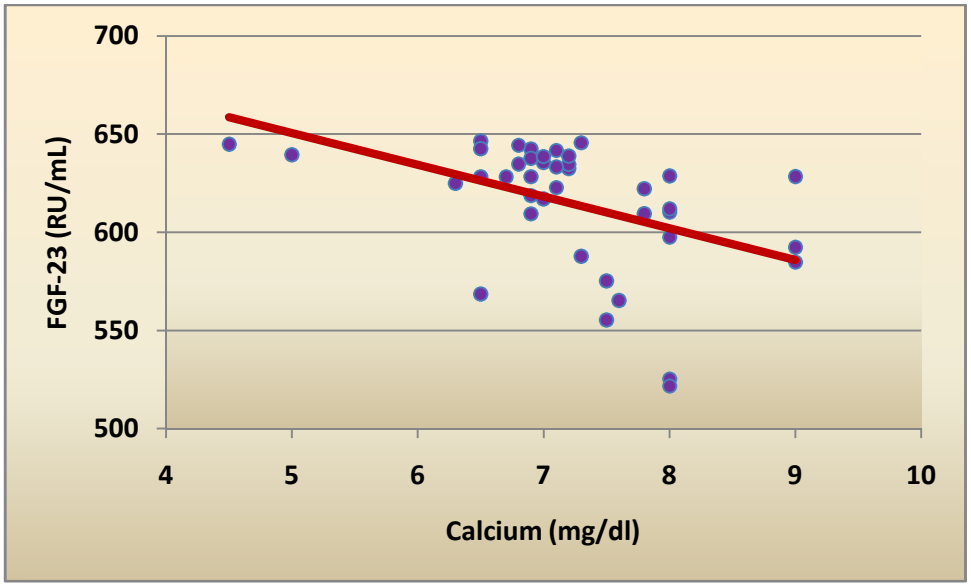
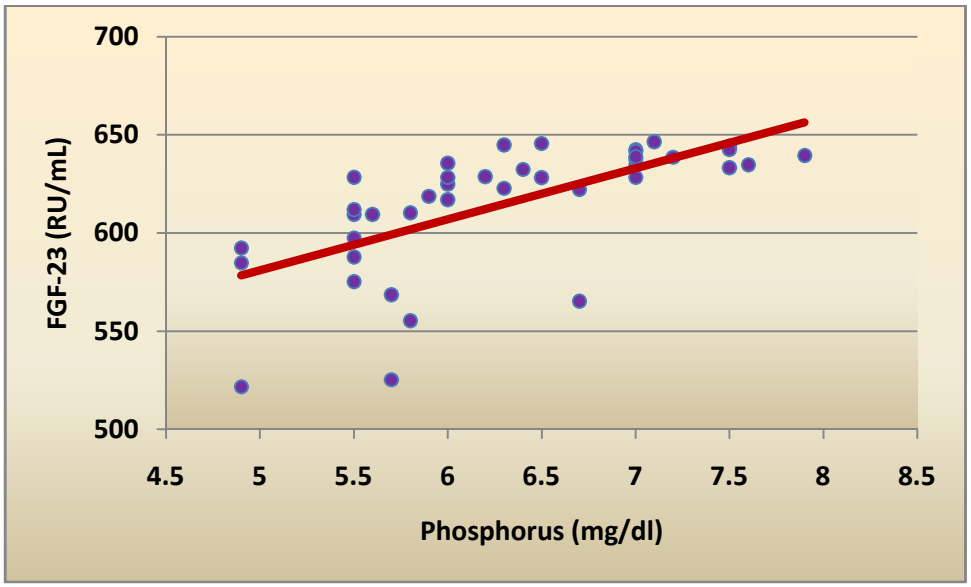
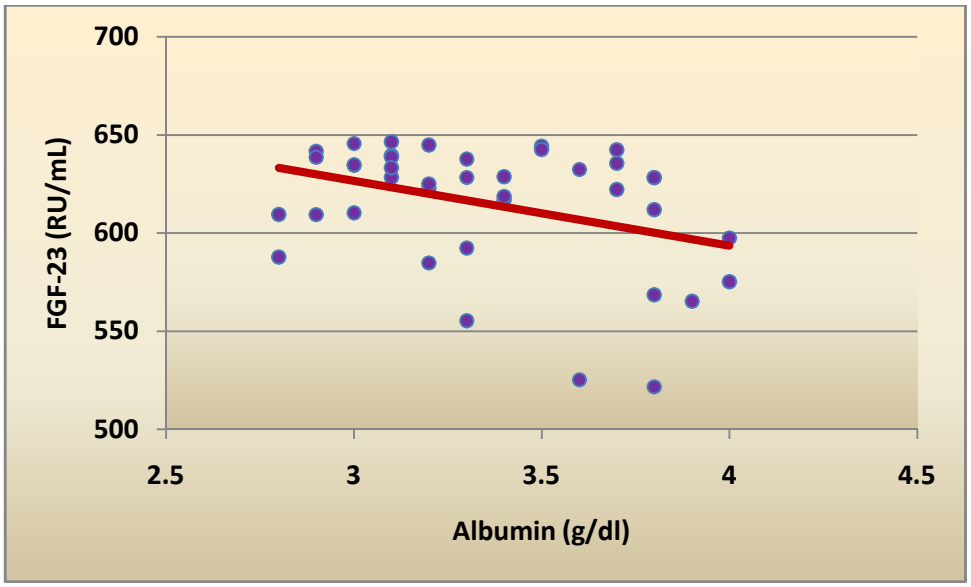


Fig 6.9: Scatter plot showing the correlation between Serum phosphorus and FGF-23 level before transplantation



6.10 : Scatter plot showing the correlation between serum albumin and FGF-23 level before transplantation



DISCUSSION

DISCUSSION

The chronic kidney disease-bone and mineral disorders (CKD-MBD) represents a dynamic area of research. ***(Mejía N et al,2011)***

Disturbances of mineral metabolism are common if not ubiquitous during the course of chronic kidney disease (CKD) and lead to serious and debilitating complications unless these abnormalities are addressed and treated.

Recently, the collection of biochemical abnormalities such as calcium, phosphorus, vitamin D and PTH disorders, changes in bone morphology such as variation in turnover, volume and bone mineralisation, and vascular or other soft-tissue calcifications, have been included under the definition of "chronic kidney disease mineral and bone disorders" (CKD-MBD). ***(Moe SM,2008)***

Recently, new factors such as FGF-23 have been added to the classic list of regulators of bone metabolism, which include calcium, phosphorus, PTH and calcitriol. ***(Mejía N et al,2011)***

Patients with chronic kidney disease have seriously compromised homeostatic mechanisms, giving rise to different adaptive changes in calcium (Ca), phosphorus (P), parathyroid hormone (PTH), vitamin D and fibroblastic growth factor (FGF-23) levels. ***(Torregrosa JV et al ,2011)***

Fibroblast Growth Factor 23 (FGF23) is identified as a "phosphatonin" that is thought to be implicated in the systemic balance of phosphate maintained by the interaction of kidneys, intestine and bone. ***(Fugakawa M et al, 2005).***

The discovery of FGF23, a novel bone-derived hormone that inhibits phosphate reabsorption and calcitriol production by the kidney, has uncovered primary regulatory pathways and new systems biology governing bone mineralization, vitamin D metabolism, parathyroid gland function and renal phosphate handling. ***(Evenepoel P et al,2007)***

This phosphaturic hormone, which is made predominately by osteocytes in bone, appears to have a physiologic role as a counter-regulatory hormone for vitamin D. ***(Stubbs J et al, 2007)***

Pathologically, high circulating levels of FGF23 result in hypophosphatemia, decreased production of 1,25(OH)2D, elevated parathyroid hormone and rickets / osteomalacia in patients with functioning kidneys, where as low levels are associated with tumoral calcinosis, hyperphosphatemia and elevated 1,25(OH)2D. ***(Stubbs J et al, 2007).***

In addition, patients with chronic kidney disease (CKD) exhibit marked elevations of circulating FGF23. ***(Yamashita T, 2005)***

While the significance of increased FGF23 levels in CKD remains to be defined, it might contribute to phosphate excretion and suppression of 1,25(OH)2D levels in CKD stages 3 and 4, as well as potentially contribute to secondary hyperparathyroidism through direct actions on the parathyroid gland in more advanced renal failure.***(Kurosu H et al ,2006)***

As a result disorders of FGF-23 excess are characterized by hypophosphatemia with increased renal phosphate wasting and inappropriately low calcitriol levels for the degree of hypophosphatemia (**Fukagawa M,et al 2005).**

As our knowledge expands regarding the regulation and functions of FGF23, the assessment of FGF23 will become an important diagnostic marker as well as a therapeutic target for management of disordered mineral metabolism in a variety of acquired and hereditary disorders. ***(Stubbs J et al ,2007)***

Disordered mineral metabolism not only contributes to the pathogenesis of parathyroid and bone diseases (hyperparathyroidism and renal osteodystrophy), but also is associated with comorbidities, such as vascular calcifications, cardiovascular disease, growth retardation and cognitive dysfunction in patients with CKD. ***(Block GA et al ,2004)***

Recently, FGF-23 has been suggested to be responsible for the hypophosphatemia and inappropriately low calcitriol levels observed after renal transplantation .*(* ***Evenepoel P et al,2007)***

Still though, its role in the hypophosphatemia observed after renal transplantation remains largely unknown.

The aim of the present cohort prospective study was therefore to investigate FGF-23 levels in patients with end-stage renal disease before and after a successful renal transplantation and their probable association with markers of bone and mineral metabolism.

The key and novel findings in the present study are the following:

(i) intact FGF-23 levels decrease dramatically after successful renal transplantation when graft function is good.

(ii) Phosphorous levels correlate significantly with FGF-23 levels before transplantation.

In our prospective study, FGF-23 levels were determined in renal transplant recipients with stable renal function for 3 and 6 months posttransplant.

Intact FGF-23 was measured in order to avoid measurement of fragments that accumulate in end stage renal disease and do not probably reflect endogenous FGF-23 production.

In our study, patients with ESRD on regular hemodialysis had statistically significant correlation between level of FGF23 and Phosphorous, PTH, Calcium .

Patients with ESRD had high levels of FGF23, phosphorous , PTH and low levels of Calcium. This was in agreement with ***Yamashita T,2005*****,** patients with chronic kidney disease (CKD) exhibit marked elevations of circulating FGF23.

Kazuaki N, et al 2010, also agreed with our study , where serum FGF-23 levels positively correlated with serum phosphate and calcium levels, calcium–phosphate product and changes in serum phosphate in ESRD patients.

Fukagawa M ,et al 2005 , serum phosphate is one of the main regulators of FGF-23 levels in uremic patients on maintenance HD and suggest that production of FGF-23 in the bone is continuously stimulated by phosphate load, whereas the normal negative feedback loop is no longer active with respect to FGF-23.

These mechanisms may account for the markedly increased serum FGF-23 levels in uremic patients.

In our study we observed significant reduction of FGF23 level , after 3 months post transplantation(625.3 ± 18.2 versus 242.3 ± 9.5, P**<0.001**) and at 6 months there was a small further decline in FGF-23 levels (242.3 ± 9.5 versus 136.3 ± 9.8,P**<0.001).** Hypophosphatemia which is the major stimulant for FGF-23 secretion is resolved.

This was in agreement with **Economidou D et al,2009 where he found that** FGF-23 levels decreased by 89% 3months post transplantation (346 ± 146 versus 37 ± 9pg/mL,$P < .01$) and remained stable for 12 months .

This was also in agreement with **Evenepoel P et al,2007**. In the study by Evenepoel et al., the results also showed a significant reduction of FGF-23 levels.

Our results was in agreement with those of **Pande S et al ,2006** who also observed FGF-23 level reduction as early as the fifth posttransplant day.

This was disagreed with **Krocker D et al,2006** who reported that patients with chronic renal failure and secondary hyperparathyroidism that have undergone renal transplantation, he justifies as it is expected that there is a greater production in the first trimester after transplantation, until osteoblasts become inactive and bone metabolism is suppressed.

Krocker D et al,2006 reported that corticosteroids, calcineurin inhibitors, and mTOR inhibitors stimulate FGF-23 production, even though in the first months after transplantation, with the use of higher doses of these drugs. our study observed greater reduction in FGF23 level.

In the aforementioned study, not only FGF-23 was determined but also C-terminal fragments. For this reason, the excessive and rapid reduction could be due to increased urinary excretion.

In our study we observed significant reduction in FGF23 level after 3 and 6 moths post transplant but still did not reach normal level .

This was in agreement with **Levi M,2001,** who also recognized persistant elevation of FGF23 level post transplant and explained that by FGF23 secretion can persist for months following transplantation, even in the presence of hypophosphatemia.

It is possible that the use of immunosuppressive drugs contributes to this situation.

Indeed, it has recently been shown that FGF23 mRNA expression in human osteoblasts increases in response to dexamethasone .

Evenepoel P et al ,2007 also recognized persistant elevation of FGF23 level post transplant and explained that uremic bone may develop resistance to feedback inhibition of FGF23, perhaps caused by the preceding years of chronic phosphate retention that stimulates FGF23 secretion .

In our study we observed that before transplantation: FGF23 level significantly correlated with phosphorous and iPTH levels. Also we observed negative correlation with calcium levels.

After transplantation there is no significant correlation between FGF23 level and phosphorous, calcium and iPTH levels at 3 and 6 months posttransplant.

This was disagreed with **Economidou D et al,2009** where he found Phosphate levels 3 month after transplantation significantly correlated with FGF-23 levels after transplantation.

In our study, mean iPTH levels decreased at 3 months posttransplantation and remained low, but higher than normal by 6 months post transplantation. This was agreed with **Torres A et al, 2002** where he found iPTH concentrations decrease progressively after renal transplantation. However, resolution of secondary hyperparathyroidism (SHPT) is incomplete 1 year after transplantation in about 50% of the recipients (A. Torres et al, 2002)

In our study a significant correlation was observed between pre transplantation iPTH and Ca and P levels and their levels at 3 and 6 months post transplantation.

This was agreed with Heaf JG et al 2003, who justified that successful renal transplantation, by normalizing urinary phosphate and ß2-microglobulin excretion and renal calcitriol production, reverses many of these abnormalities in mineral and bone metabolism, including:

-A fall in the plasma phosphate concentration to normal

-A reduction in plasma PTH levels

-A decrease in plasma alkaline phosphatase levels, indicative of less bone resorption

-Mobilization of soft tissue calcifications, as correction of hyperphosphatemia markedly lowers the calcium-phosphate product

-Improvement in aluminum bone disease

-Prevention of progression of amyloid osteodystrophy

In our study , we had a statistically significant (P< 0.001) on comparing phosphate level before transplantation and 3 and 6 months post transplantation. We also had temporary mild hyposphosphatemia in 20% of our patients at 3 months and 6 months post transplantation.

This was in agreement with **Economidou D et al,2009,** who reported temporary mild hyposphosphatemia in 28% of his patients at 3 months post transplantation.Phosphate levels and TmPO4/GFR were strongly correlated indicating that low P levels after renal transplantation are the result of renal phosphate wasting.

Evenepoel P et al,2007 ,Patients that developed hypophosphatemia tended to have higher pre- and post transplantation iPTH levels as well as higher pre transplantation FGF-23 levels. These findings suggest that FGF-23 and iPTH

could act synergistically to cause phosphaturia, as has been previously suggested .

Ghanekar H et al ,2006 : Hypophosphatemia occurs early following renal transplantation and resolves almost completely at 1 year after transplantation.

In our study , we had a statistically significant relation (P< 0.001) on comparing calcium level before transplantation and 3 and 6 months post transplantation. We noticed increase in serum calcium post transplantation .

This was agreed with **Papagianni A et al ,2009.**

This was in agreement with Saji F et al, 2009 In those patients who develop increase in serum calcium , the plasma calcium concentration frequently begins to rise in the first 10 days after transplantation; however, this response can be delayed for six months or more.

Borchhardt K et al 2007, explained hypercalcemia developed post transplantation as persistent hyperparathyroidism is the most common cause .In addition to hyperparathyroidism, other factors can also contribute to an elevation in the plasma calcium concentration:

- Resorption of soft tissue calcium phosphate deposits, which is often associated with persistent hyperphosphatemia.
- Normalization of calcitriol production, which both increases the PTH effect on bone and directly enhances intestinal calcium absorption.
- Enhanced tubular calcium resorption.
- To a lesser degree, a rise in the plasma albumin concentration (due to better nutrition.

- Our study has some advantages. One is that FGF-23 levels were determined with an assay that does not detect C terminal fragments.

Another is that serial measurements of FGF-23 after renal transplantation were conducted.

Possible limitations, on the other hand, are the small number of patients and the use of activated vitamin D analogs and calcium salts, which may have confounded our results.

In conclusion, FGF-23 levels decrease dramatically after successful renal transplantation and remain within normal limits when graft function is good.

iPTH and P levels also decrease significantly after renal transplantation, while Caclium increase.

The recent identification of FGF23 as a physiological regulator of phosphate and vitamin D metabolism has greatly advanced our understanding of mineral and bone disorders in CKD.

FGF23 plays a central role in the pathogenesis of altered mineral metabolism and secondary hyperparathyroidism in CKD patients and post-transplant hypophosphatemia in kidney transplant recipients.

Further elucidation of FGF23 function and regulation will help to establish a more rational approach for the management of the mineral and bone disorders that are associated with high burden of morbidity and mortality in CKD patients and also in renal transplant patients.

Summary & Conclusion

Summary & Conclusion and Recommendation

The discovery of fibroblast growth factor 23 (FGF23), a novel bone-derived hormone that inhibits phosphate reabsorption and calcitriol production by the kidney, has uncovered primary regulatory pathways and new systems biology governing bone mineralization, vitamin D metabolism, parathyroid gland function and renal phosphate handling.

As our knowledge expands regarding the regulation and functions of FGF23, the assessment of FGF23 will become an important diagnostic marker as well as a therapeutic target for management of disordered mineral metabolism in a variety of acquired and hereditary disorders.

CKD is likely the most common cause of chronically elevated FGF23 levels, and the clinical condition in which levels are most markedly elevated.

Recently,FGF-23 has been suggested to be responsible for the hypophosphatemia and inappropriately low calcitriol levels observed after renal transplantation.

We performed a cohart prospective study to investigate FGF-23 levels in patients with end-stage renal disease before and after renal transplantation and their probable association with markers of bone and mineral metabolism.

Intact FGF-23 levels were determined before transplantation , 3, and 6 post transplantation in 20 renal transplant recipients.

Intact parathyroid hormone (iPTH), calcium (Ca), P, VitD levels were measured at the same time periods.

Conclusion

- FGF23 are markedly increased in patients with end stage renal disease associated with increase in phosphorous and i PTH levels .
- FGF-23 levels decrease dramatically after successful renal transplantation and remain within normal limits when graft function is good.
- i PTH and Phosphorous levels also decrease significantly after renal transplantation, while Calicum increase.

Recommendations :

- An increased circulating level of FGF23 is an independent risk factor for mortality, cardiovascular disease, and progression of chronic kidney disease (CKD), but its role in transplant allograft and patient survival is unknown .

- We recommend further studies for role of FGF23 post transplant on a larger number of patients and follow up of patients for longer periods .

- Further studies are recommended to study role of FGF23 post transplant and its impact on graft survival, post transplant bone mineral disorders ,cardiovascular disease , and mortality.

- FGF23 can be used not only as a biomarker for assessing phosphate retention but also as a predictor of mortality and future development of refractory hyperparathyroidism.

- It is promising that anti-FGF23 monoclonal antibodies are effective to treat Hyp mice, a homologue of human XLH . This insight may present new strategies to manage persistent hypophosphatemia in kidney transplant recipients.

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الملخص العربى

إن إكتشاف عامل نمو الخلايا الليفية (FGF23) ، هرمون العظام المستمدة الذي يمنع إعادة امتصاص الفوسفات و إنتاج الكالسيتريول عن طريق الكلى ، ساعد فى فهم المسارات التنظيمية الأولية والجديدة بيولوجيا التي تحكم تمعدن العظام ، والتمثيل الغذائي لفيتامين دال و وظيفة الغدة الجاردرقية و امتصاص الفوسفات .

إن تقييم مستوى عامل نمو الخلايا الليفية FGF23 و زيادة معرفتنا بشأن تنظيم وظائف عامل نمو الخلايا الليفية أصبح علامة تشخيصية هامة ، فضلا عن الهدف العلاجي لإدارة الإختلال فى المعادن في مجموعة متنوعة من الأمراض الوراثيه و المكتسبة .

الإضطراب المزمن فى وظائف الكلى يعتبر السبب الأكثر شيوعا لإرتفاع مستويات عامل نمو الخلايا الليفية بشكل مزمن .

و في الآونة الأخيرة ،تم دراسه مسئوليه عامل نمو الخلايا الليفية عن نقص مستوى الفوسفات فى الدم ومستوي الكالسيتريول بعد زرع الكلى .

وقد أجرينا دراسة استطلاعية لدراسه مستوي عامل نمو الخلايا الليفية FGF-23 في المرضى الذين يعانون من فشل الكلوي مزمن قبل وبعد زرع الكلى وارتباطها المحتمل مع علامات من العظام واستقلاب المعادن.

تمت الدراسه على عشرين مريض من مرضى الفشل الكلوى قاموا بعمل زراعه كلى بشريه و مقارنتهم بعشرن مريض يعانون من فشل كلوى مزمن يقومون بعمل جلسات إستصفاء دموى

تم تحديد مستوى عامل نمو الخلايا الليفية FGF-23 قبل زرع الكلى و متابعه مستوى الهرمون بعد زراعه الكلى بثلاث أشهر و سته أشهر .و تم قياس هرمون الغدة الجاردرقية (iPTH)، و مستوى الكالسيوم و الفوسفور فى الدم في نفس الفترات الزمنية.

- وجد ان مستوى عامل نمو الخلايا الليفية FGF23 يزداد بشكل ملحوظ في المرضى الذين يعانون من فشل لكلوي مزمن ويرتبط مع زيادة في مستويات الفوسفور وهرمون الغده الجار درقية .
- انخفاض مستوي عامل نمو الخلايا الليفية بشكل كبير بعد نجاح زرع الكلى .
- لوحظ انخفاضا كبيرا فى مستويات الفوسفور وهرمون الغده الجار درقية بعد زرع الكلى ، في حين لوحظ زيادة فى نسبه الكالسيوم .

التوصيات :- ننصح بعمل المزيد من الدراسات لدراسة دور عامل نمو الخلايا الليفية FGF23 فى مرضى زراعه الكلى و متابعه تأثيره على الأوعيه الدمويه و المعادن و العظام و تأثيره على وظيفة الكلى المزوعه وتأثيره و علاقته بنسب الوفاه بعد زراعه الكلى لفترات اطول.