INTRODUCTION

There are several types of bone disease that are commonly seen in kidney transplant recipients. These include pre-existing uremic osteodystrophy, osteopenia, osteoporosis, bone fracture, osteonecrosis and bone pain syndrome**(Zisman and Sprague, 2006).**

Kidney transplant recipients are now living longer than ever, and thus, proper prevention and management of bone disease has become an increasingly important part of their long-term care. Complications from posttransplant bone disease not only cause significant morbidity, but also increase the cost of care, hospitalization, and mortality **(Vatour, et al., 2004).**

Bone disease after kidney transplant is a multifactorial process that includes continuing bone loss superimposed on pre-existing renal osteodystrophy **(Zhang et al., 2008).**

During the first 6 to 12 months after kidney transplant, there is a rapid bone loss. After this time period, patients may either continue to lose bone at a slower rate, stabilize, or improve BMD depending on numerous factors including medication usage, overall health, and renal function **(Sprague, et al., 2006).**

There can be several different bone histologies and no single clinical biomarker can distinguish between the various bone disorders **(Rolla, et al., 2006).**

The 2009 Kidney Disease Improving Global Outcome (KDIGO) clinical practice guideline provides recommendations for the evaluation, prevention, and treatment of bone disorder in renal transplant patients. The same measures that are used to prevent osteoporosis in the general population also apply to transplant recipients. General recommendations should include the following: All patients should receive counseling regarding smoking cessation, early mobilization after transplantation and fall prevention**(KDOQI, 2009).**

AIM OF THE WORK

This study aims to:

A) Clarify the prevalence of bone mineral disease among renal transplant patient.

B) Determine the relationship between the duration of renal transplantation, renal function & prevalence of bone mineral disease.

Chapter *1*

PRE-EXISTING UREMIC OSTEODYSTROPHY

There are several different types of pre-existing renal osteodystrophy that may be encountered in kidney transplant patients including osteitis fibrosa cystica, adynamic bone disease, osteomalacia, osteopenia or osteoporosis.

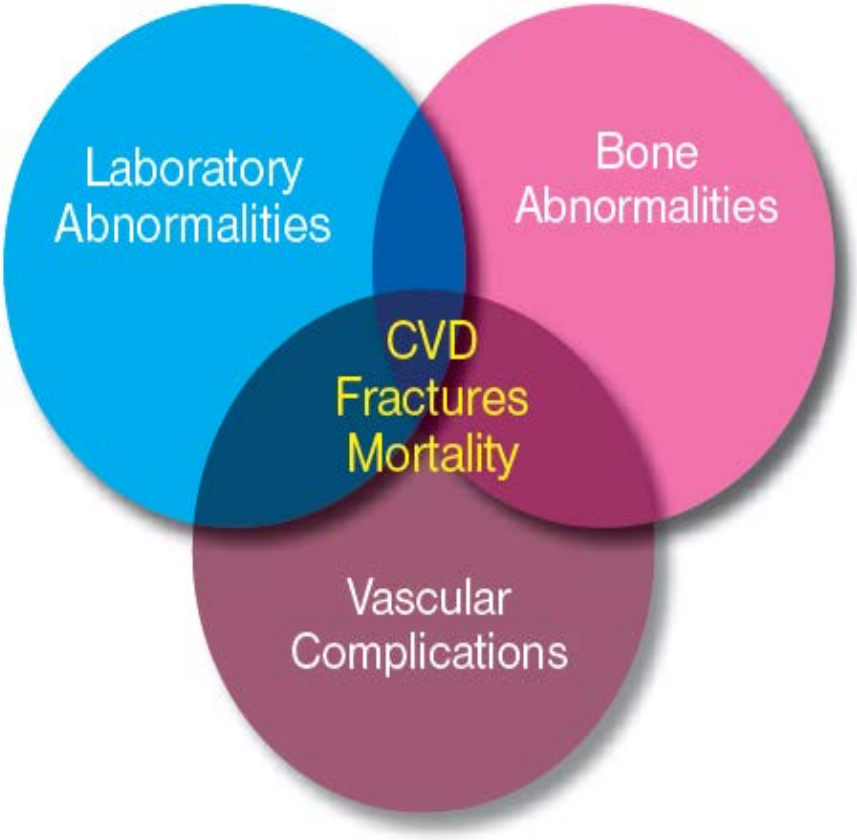

Fig. (1): Chronic kidney disease-mineral and bone disorder (CKD-MBD) ***(Moe et al., 2006).***

a. Osteitis fibrosa cystica:

Persistent secondary or tertiary hyperparathyroidism (HPT), reported in up to 30-50% of renal transplant patients, can lead to osteitis fibrosa cystica, a form of high turnover bone disease**(Heaf et al., 2003)**.

High bone turnover is usually associated with cortical bone loss and weakening its mechanical function **(Malluche et al., 2010)**.

Bone biopsy characteristically shows increased bone resorption, extensive osteoclastic activity and endosteal fibrosis **(Malluche & Monier-Faugere 1994)**.

High serum calcium (Ca), high phosphorus (Phos), low active vitamin D, high parathyroid hormone (PTH), and elevated alkaline phosphatase (ALP) and osteocalcin are common. Alkaline phosphatase and osteocalcin are secreted by osteoblasts and can serve as useful clinical markers of high bone turnover. The cornerstone of treatment aims to suppress PTH secretion by a variety of methods including dietary phosphate restriction and use of phosphate binders, the use of the calcimimetic agent, cinacalcet, or surgical parathyroidectomy **(Block et al., 2004)**.

b. Adynamic bone disease:

This condition is usually caused by over-suppression of PTH and other growth factors, including gonadal

hormones,growth hormone, and insulin-like growth hormone-1 **(Zisman & Sprague, 2006)**.

Bone biopsy findings include a low bone formation rate as assessed by tetracycline fluorescence-labeling, little or no evidence of cellular activity, a paucity of osteoblasts and osteoclasts, and thin osteoid seams **(Malluche & Monier-Faugere 1994).**

Low bone turnover is frequently associated with loss of cancellous bone and abnormal mineral metabolic activity. Inability to maintain mineral homeostasis may contribute to cardiovascular and soft tissue calcifications, which may explain the high mortality rate in patients with low bone turnover **(Malluche et al., 2010).**

Patients may have a high serum Ca, a relatively low PTH and low AP levels. Groups at highest risk include the elderly, diabetics, patients previously on peritoneal dialysis, those on calcium-containing phosphate binders, and those with over-suppressed PTH by vitaminD analogues. The prevention and treatment of adynamic bone disease is avoidance of over suppression of PTH secretion **(Eknoyan et al., 2003).**

Historically, excessive aluminum accumulation was a major cause of adynamic bone disease in ESRD patients before the strict water purification and the avoidance of

aluminum-containing phosphate binders were adopted **(Zhang et al., 2008).**

c. Osteomalacia:

Osteomalacia in post-transplant patients has numerous causes including a deficit in bone mineralization due to hypophosphatemia, malnutrition, vitamin D deficiency, or aluminum toxicity **(Zisman & Sprague, 2006).**

Characteristic findings on bone biopsy include wide unmineralized osteoid seams, low bone formation, absence of osteoblasts and osteoclasts and endosteal fibrosis **(Malluche et al., 1994).**

Patients may have low serum Ca and Phos levels but PTH and AP levels are frequently within normal limits or slightly high. The gold standard for the diagnosis of osteomalacia from aluminum toxicity is aluminum staining of the bone biopsy **(Eknoyan et al., 2003).**

However, a useful, noninvasive clinical test in patients suspected to have chronic aluminum toxicity is desferoxamine stimulation of aluminum release. Treatments are targeted toward the underlying causes and include Ca and vitamin D supplementations. The treatment of osteomalacia from aluminum toxicity is desferoxamine administration or kidney transplantation **(Zhang et al., 2008).**

d. Osteopenia and osteoporosis:

These conditions are usually diagnosed by bone mineral density (BMD) measurement with dual energy X-ray absorptiometry. Many patients undergoing transplant already have low bone mineral density. Thus, it is not surprising that low BMD (osteopenia and osteoporosis) is very common in kidney transplant recipients **(Gallego et al., 2006).**

Common risk factors include older age, female gender, Caucasian race, chronic disease, immobility and malnutrition. In addition, hypogonadism is very common, but not routinely screened for or treated among the ESRD population. Chronic metabolic acidosis and uremic osteodystrophy can also contribute to bone loss **(Zisman & Sprague, 2006).**

e. Other bone disease:

Dialysis-related amyloidosis is caused by β2 - microglobulin deposition as amyloid fibrils, leading to chronic inflammatory response, destructive arthropathy and lytic bone lesions. The articular symptoms associated with this disorder rapidly improve after renal transplantation. Although new cystic lesions are unusual, resolution of existing cysts is unusual **(Zhang et al., 2008).**

f. Clinical course:

Patients often have a combination of the different type of bone diseases as described above, commonly termed mixed bone diseases. Due to the dynamic nature of renal osteodystrophy, it is not uncommon for one type of bone disease to evolve into another type of bone disease, depending on the clinical setting and management **(Zisman & Sprague, 2006).**

The nature and evolution of pre-existing renal osteodystrophy after kidney transplant has yet to be fully established, largely due to a lack of serial histological studies by bone biopsy in this population. Several small studies do provide some insight into this issue. In a histological study of 20 patients who had bone biopsies before and 6 months after kidney transplant were compared **(Cruz et al., 2004).**

Five of the 12 patients with adynamic bone disease recovered completely and the remaining cases had some improvement. Five of 8 patients with high turnover bone disease developed low-turnover bone disease (4 with adynamic bone disease, 1 with osteomalacia). In a long-term study of 57 patients followed for a mean of 5.6 years after kidney transplant, 56% of patients were demonstrated to

have decreased cancellous bone volume, 46% of patients had low bone turnover, and 59.7% of patients had reduced bone formation indices. High bone turnover was rarely seen, despite the fact that 63% of patients had elevated serum creatinine levels **(Monier-Faugere et al., 2000).**

In another report of 25 patients at least 5 years after transplant with good renal allograft function, bone biopsy revealed mixed bone disease in 10 patients, adynamic bone in 7 patients, high turnover bone in 4 patients, and normal bone in 3 patients **(Cueto-Manzano et al., 2003).**

These studies suggest that pre-transplant renal osteodystrophy may not resolve completely, but often persists or evolves into a different disease process, depending on the allograft function, PTH level, immune-suppressive medications, and clinical management.

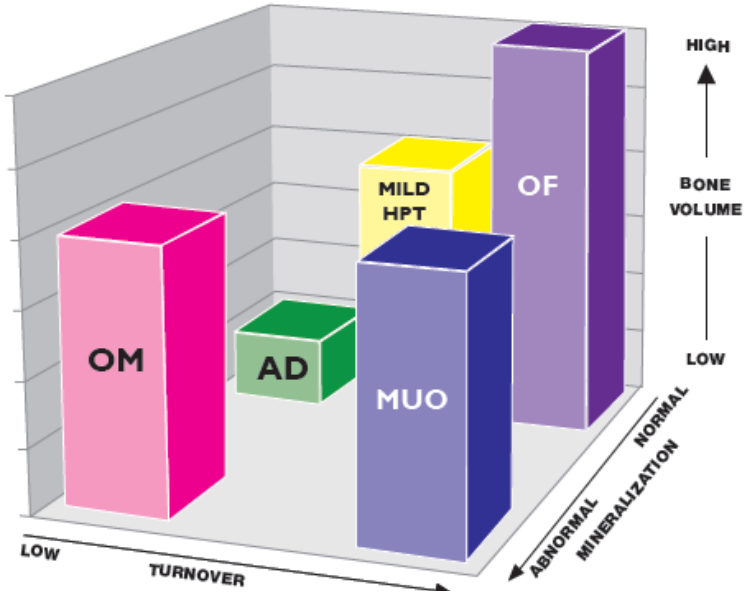

Fig. (2): Renal osteodystrophy ***(Moeet al., 2006).***

Turnover mineralization volume (TMV) classification system for bonehistomorphometry. The TMV system provides more information than the previously used classification scheme. Each axis represents one of the descriptors in the TMV classification: turnover (from low to high), mineralization (from normal to abnormal), and bone volume (from low to high). Individual patient parameters can be plotted on the graph, or mean values and ranges of grouped data can be shown. For example, many patients with renal osteodystrophy cluster in areas shown by the bars. The red bar (osteomalacia [OM]) previously was described as low-turnover bone with abnormal mineralization. Bone volume may be low to medium, depending on the severity and duration of the process and other factors that affect bone. The green bar (a dynamic bone disease [AD]) previously was described as low-turnover bone with normal mineralization, and bone volume in this example is at the lower end of the spectrum, but other patients with normal mineralization and low turnover will have normal bone volume. The yellow bar (mild hyperparathyroid-related bone disease [HPT]) and purple bar (osteitis fibrosa [OF], or advanced hyperparathyroid-related bone disease) previously were considered distinct categories, but in actuality represent a range of abnormalities along a continuum of medium to high turnover and any bone volume depending on the duration of the disease process. Finally, the blue bar (mixed uremic osteodystrophy [MUO]) is variably defined internationally. In the present graph, it is shown as high-turnover, normal bone volume, with abnormal mineralization. In summary, the TMV classification system more precisely describes the range of pathologic abnormalities that can occur in patients with chronic kidney disease.

Fibroblast Growth Factors(FGFs):

Physiology of FGFs23:

Fibroblast growth factors (FGFs) comprise a family of 22 molecules, which can be grouped into seven subfamilies **(Itoh & Ornitz 2004)**which have in common the ability to bind to one of the four FGF receptors (FGFR), typically in a paracrine manner**(Mohammadi et al.,2005).**

The paracrine FGFs require the presence of heparin sulfate glycoseaminoglycans to allow signal transduction, resulting in a variety of effects including embryonic development, tumor growth, angiogenesis and wound healing **(Powers et al.,2000).**

Secreted FGFs belong to the FGF19 family and include FGF19, FGF21 and FGF23. Secreted FGFs appear unique in that the topology of their heparin-binding region diverges from the typical structure seen in the canonical FGFs, which reduces their affinity for heparin sulfate **(Harmer et al.,2004)**and enables them to avoid capturing in the extracellular matrices and hence allows them to function as endocrine factors **(Goetz et al., 2012).**

The weak heparin-binding ability of the FGF19 subfamily also reduces their affinity for FGFR**(Mohammadi et al., 2005)** which has been shown to be weak even at high concentrations **(Zhang et al., 2006).**

Rather, the activation of FGFRs by FGF19 family members requires the presence of a different co-factor, namely members of the Klotho family of membrane-boundglucosidases. Expression of α-klotho is limited to certain tissues, most notably to the distal convoluted tubule, and to a lower extent to the parathyroid and pituitary glands, the sinoatrial cells of the heart, placenta, skeletal muscle, urinary bladder, aorta, pancreas, testis, ovary and colon **(Ben-Dov et al.,2007).**

The limited distribution of the full-length, trans-membrane α-klotho molecule explains the tissue-restricted physiologicactions of FGF23 (vide infra). FGF23 is a 32 kDa protein expressed in the osteocytes and osteoblasts, which primarily targets the FGF receptor-α-klotho complex in the kidney. Excess FGF23 production results in hypophosphatemia, suppressed 1, $25(OH)^2$ vitamin D levels and elevated PTH levels and impaired bone and cartilage mineralization. FGF23 deficiency, on the other hand, results in hyperphosphatemia, elevated 1, $25(OH)^2$ vitamin D level, suppressed PTH and soft tissue calcification **(Liu& Quarles 2007).**

The regulation of FGF23 is complex and incompletely understood. PTH, 1,25(OH)2 vitamin D, secreted klotho, glucocorticoids, calcium and phosphate appear to regulate FGF23 production but the response is context dependent and

the molecular mechanism underlying the transcriptional regulation of FGF23 remain unclear. The principal regulator of FGF23 appears to be 1, 25(OH)2 vitamin D, which stimulates FGF23 production in the bone (**Liu et al.,2006).**

The role of other regulators of FGF23 remains controversial. Stimulation of FGF23 production by PTH was shown in some**(Rhee et al., 2011)**but not all, studies **(Lavi-Moshayoff et al., 2010).**

Recent studies also suggest that bone mineralization and remodeling may have a direct effect on FGF23 production, and mutations in genes that regulate bone mineralization, such as Phex, Dmp1, Enpp1, as well as FGFR1 and HMW-FGF2 increase FGF23 gene transcription **(Quarles.2012).**

These local regulators may allow FGF23 to control renal phosphate metabolism according to the actual influx/efflux of calcium and phosphate to and from the bone. In addition, leptin, estrogen and glucocorticoids also regulate FGF23 (**Tsuji, et al 2010).**

Interestingly, although phosphate correlates with FGF23 levels in some settings, such as ESRD, changes in serum phosphorus level do not appear to have an immediate or consistent effect on FGF23 production. Studies that examined the effects of oral phosphate intake on FGF23

levels have detected either no effect **(Isakova et al.,2011)**or described changes in FGF23 production in response to alterations in dietary phosphate intake after a lag time of up to 1 week **(Moe et al.,2011).**

This suggests that phosphate may affect FGF23 indirectly, either through vitamin D and/or through bone mineralization (**Liu et al., 2006).**

FGF23 undergoes cleavage into N- and C-terminal fragments that do not activate FGFR/Klotho complexes. The enzymes responsible for FGF23 cleavages have not been identified (**Liu et al., 2003).**

Cleavage of the intact FGF23 abrogates its effects not only by removing the binding site to the FGFR-klotho complex, but also by a direct inhibitory effect of the C-terminal peptide fragments**(Goetz et al., 2010).**

Cleavage of the intact FGF23 molecule may occur in blood samples too and could affect the accuracy of laboratory measurements, depending on the length of time elapsed from obtaining the sample **(Smith et al.,2011)** and on the type of laboratory assay (intact, which measures the whole molecule, or C-terminal, which measures whole molecule and C-terminal fragments) The primary physiologic actions of FGF23 involve regulation of bone and mineral metabolism through bone–kidney endocrine