SERUM CALCIUM, PHOSPHATE AND PARATHYROID HORMONE LEVELS IN KIDNEY TRANSPLANT RECIPIENTS

THIS DISSERTATION HAS BEEN SUBMITTED IN PART FULFILLMENT FOR THE DEGREE OF MASTER OF MEDICINE IN INTERNAL MEDICINE, UNIVERSITY OF NAIROBI

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DECLARATION

I Dr Fredrick Kalokola hereby certify that this work is my original work and that this has not been submitted to any other university.

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Dr Fredrick M. Kalokola

Dedication

I dedicate this to my parents Dr Festus M. Kalokola and Mrs. Evangelina Kalokola for their amazing love, encouragement and support.

Acknowledgements

I give thanks to God almighty for giving abundant life and good health.

I would like to appreciate my supervisors, Prof J. Kayima, Prof S. Mc'ligeyo and Prof C. Kigondu for their guidance, contributions and great supervision.

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ABSTRACT

Background

Successful renal transplantation corrects the abnormalities of mineral metabolism that lead to mineral bone disease. This includes correction of uremia, normalization of serum calcium and phosphorus levels, and restoration of calcitriol production. However, the degree of renal function recovery is usually incomplete, and persistence of hyperparathyroidism is common. In addition, the immunosuppressive drugs used to prevent graft rejection exert profound effects on bone metabolism. Disturbances of mineral metabolism and skeletal problems are common causes of morbidity and have been associated with cardiovascular risk after kidney transplantation.

Objective of the study

To determine the levels of serum calcium, phosphate and parathyroid hormone levels among kidney transplant recipients attending clinic at Kenyatta National Hospital.

Methodology

A cross-sectional study was carried out among 85 renal transplant recipients who were at leas t 6 months post transplant consecutively recruited for over a period of 5 months. At the time of recruitment demographic data, duration of dialysis, time since transplantation and etiology of kidney disease were entered into study performa. Blood was drawn and measurements of serum creatinine, calcium, phosphorus and albumin levels were determined using automated clinical chemistry analyzer. Serum intact parathyroid hormone (iPTH) assays were performed using electro-chemiluminiscence immunoassay on the fully automated analyzer (Cobas 601). Patients had their glomerular filtration rate estimated (eGFR) using Modified Diet in Renal Disease (MDRD) formula and staged as per kidney disease outcome quality initiative (K/DOQI) criteria

Data management and analysis

Data analysis was performed using SPSS version 17.0 software. Characteristics of participants were summarized into means/medians and proportions for continuous and categorical variables respectively. Correlation between serum calcium, phosphate and parathyroid with estimated glomerular filtration rate was measured using Pearson correlation coefficient. All statistical tests

were performed at 5% level of significance (95% confidence interval). The findings were presented using tables and graphs.

Results

A total of 85 renal transplant recipients who were at least 6 months post transplant attending transplant clinic at Kenyatta National Hospital were studied.

The study population was categorized into 5 groups as per K/DOQI staging of chronic kidney disease. CKD stage 1T had 8 patients, stages 2T, 3T, 4T and 5T had 45, 28, 2 and 2 patients respectively.

These 85 patients were on dialysis for an average of 12 months with a mean estimated glomerular filtration rate of 64 ml/min/1.73m2.

The mean parathyroid hormone level was 38.1pg/ml with 20% of patients having hyperparathyroidism and 16.5% having hypoparathyroidism. There was no significant correlation between serum PTH and eGFR.

Hypocalcemia was found in 49.4% of patients and was associated with relatively younger patients [age 37.5 (±11.5) years] compared to those with normal serum calcium levels [age 43.8 (±12.4) years] ($p=0.017$). No significant correlation was found between hypocalcemia and eGFR. Majority of patients had normal serum phosphorus levels (92.9%).

Conclusion

This study demonstrated that hyperparathyroidism is still present even after successful kidney transplantation. It appeared not be associated with duration of dialysis or graft function as it was found in other populations.

Hypocalcemia as well as hypoparathyroidism are also common in the transplant population and there seem to be no significant correlation with graft function though hypocalcemia was significantly associated with younger patients. Majority of our patients had normal phosphorus levels.

LIST OF ABBREVIATIONS

1. ALP Alkaline phosphatase
2. BMD Bone Mineral Density
3. BMI Body Mass Index
4. CKD Chronic Kidney Disease
5. CNI Calcineurin Inhibitors
6. CsA Cyclosporine A
7. DEXA Dual energy X ray Absorptiometry
8. eGFR Estimated Glomerular Filtration Rate
9. ESRD End Stage Renal Disease
10. FGF-23 Fibroblast Growth Factor- 23
11. GFR Glomerular Filtration Rate
12. HPT Hyperparathyroidism
13. iPTH Intact Parathyroid Hormone
14. K/DOQI Kidney Disease Outcomes Quality Initiative
15. KDIGO Kidney Disease Improving Global Outcome
16. KNH Kenyatta National Hospital
17. KTx Kidney Transplant
18. MMF Mycophenolate Mofetil
19. MDRD Modification of Diet in Renal Disease
20. PTH Parathyroid Hormone
21. RANKL Receptor Activator of Nuclear Kappa B Ligand
22. RRT Renal Replacement Therapy

23. SHPT Secondary Hyperparathyroidism
24. SSA Sub- Saharan Africa
25. WHO World Health Organization

1. LITERATURE REVIEW

1.1 Introduction

Kidney transplantation restores renal function, thereby improving renal bone disease. Various studies have demonstrated an improvement in secondary hyperparathyroidism (SHPT) after kidney transplantation; although parathyroid hormone (PTH) concentrations remained elevated even in the presence of excellent graft function. [1-5]. Hypercalcemia and hypophosphatemia are frequently encountered post transplant mineral bone disease. [6-9].

Hypophosphatemia can negatively impact on either skeletal or muscular systems, contributing to the increased incidence of bone fractures in kidney transplant patients. The putative causal factors for this metabolic alteration are persistent HPT and increased levels of FGF-23. [6]

Hypercalcemia can negatively impact on both the graft and patient outcome, increasing the incidence of nephrocalcinosis, which can induce a worse graft outcome, inducing vascular calcifications and increasing the incidence of pancreatitis.

Factors that have been associated with persistent hyperparathyroidism are graft dysfunction, longer time on dialysis and low concentration of 25OH. [8]

Bone mineral density (BMD) as assessed by dual X-ray absorptiometry has been used as a noninvasive method to assess bone mass loss. Several studies have documented that early bone mass loss occurs after renal transplantation. [9]

Numerous studies have confirmed the increased fracture risk after renal transplantation as compared with patients who have CKD stage 5 and are on dialysis. [9]. Ball et al. found that after 3 years after kidney transplantation, the risk of hip fracture increased by 34% when compared with dialysis patients.

Fractures occur during the early and late post transplantation periods, with a wide range of incidence depending on, among other factors, time after transplantation, severity of previous bone disease, diabetics, and immunosuppressive regimens used. Although a low BMD is a potent fracture risk factor, many transplant patients with low BMD do not have fractures. [9]

Immunosuppressive therapies have also been implicated as one of the major cause of bone disease after renal transplantation. Glucocorticoids are implicated as the main cause of post transplant bone loss. [10, 11]

Both cyclosporine and tacrolimus have been associated with post transplantation bone loss, although it is not always possible to distinguish clearly between the effects of CNI and those of glucocorticoids. Preventive strategies include minimizing glucocorticoid exposure and implementing therapies to counter the increase in bone resorption and decrease in bone formation that follows transplantation. Antiresorptive agents, especially biphosphonates, appear capable of retarding or halting the early bone loss and possibly reduce fracture rates also. [12]

1.2 CHRONIC KIDNEY DISEASE- MINERAL AND BONE DISORDER (CKD-MBD)

The incidence of CKD and ESRD has been increasing steadily for decades. The CKD epidemic is a worldwide public health problem, associated with premature death, increased morbidity and last but not least, enormous costs related to renal replacement therapy (RRT). [13-14]

The abnormalities in bone in the setting of CKD include the effects of high levels of PTH on bone, which results in the high turnover bone disease osteitis fibrosa. In addition, in the setting of CKD, a different skeletal abnormality known as adynamic bone, which is characterized by an

extremely low bone turnover, may occur. Some cases may demonstrate mineralization defects and show frank osteomalacia. A wide variety of disturbances of bone metabolism may occur in the setting of CKD. [15]

Parathyroid hormone has been identified as an important cardiotoxin in ESRD. The circulating level of parathyroid hormone is partly regulated by plasma concentration of calcium. A falling plasma calcium level triggers synthesis and release of PTH whereas an increase in plasma calcium has an inhibitory effect. In vitro, PTH has been shown to induce hypertrophy of cardiomyocytes and from clinical studies there are also indications that PTH may contribute to the development of left ventricular hypertrophy [16].

In patients undergoing dialysis, elevated serum levels of calcium, phosphorus, PTH, alkaline phosphatase, and FGF23 have been associated with death and cardiovascular events [17]. Disorders of mineral metabolism are thought to contribute to arterial calcification and diminished vascular compliance, [100] contributing to myocardial ischemia, heart failure and sudden death.

A study done by Mugera on prevalence and patterns of hyperparathyroidism (HPT) and mineral bone disease in patients with CKD at Kenyatta National Hospital (KNH) reported 22.4% of the study population to have elevated levels of serum iPTH. The mean corrected calcium levels were all below the lower limit of normal while mean levels of serum phosphorus progressively increased with worsening kidney function. [18]

1.3 KIDNEY TRANSPLANTATION

Solid organ or stem cell transplantation is a well established procedure in the treatment of end-stage organ diseases (renal disease, chronic liver failure, end stage pulmonary disease, heart failure). Improved outcome for these patients is accompanied with complications. One of these is metabolic bone disease, which can hinder their long term survival and quality of life. [19]

There are a number of risk factors contributing to bone loss in these patients: hypogonadism, vitamin D deficiency, malabsorption, low body weight, physical inactivity, excessive use of tobacco or alcohol and immunosuppressive therapy. Management of pretransplant risk factors has improved resulting in better mineral density (BMD) levels before transplantation [19]

After transplantation rapid and marked bone loss is observed in the first 3-6 months. The speed of the bone loss suggests that corticosteroids are heavily involved. Greater bone losses at vertebral and hip sites and high rates of incident of fragility fractures have been reported. [20]

Organ transplantation is an effective therapy for end-stage organ failure and is widely practiced around the world. According to WHO, kidney transplants are carried out in 91 countries. Around 66,000 kidney transplants, 21,000 liver transplants and 6000 heart transplants were performed globally in 2005. (WHO). The first successful living-donor kidney transplant was performed in 1954 in the United States. [21]

More than 10% of people or more than 20 million, ages 20 years and older in the United States had CKD (2010). In 2008, 17,413 people underwent kidney transplantation [22].

Sub- Saharan Africa accounts for more than 80% of the landmass of the African continent with an estimated population of 800 million. Unlike in high-income countries, the incidence of ESRD in Sub Saharan Africa (SSA) is high in young patients who are 20-50 years old. While 30% of patients with ESRD in the United States, Europe and Middle East undergo renal transplant (RTx), due to the excessive costs less than 1% of ESRD patients in SSA receive a transplant. [23]

1.4 RENAL REPLACEMENT THERAPY IN KENYA

Renal replacement therapy in Kenya has come a long way. Between the years of 1984-1986, 77 patients with ESRD had been dialyzed and 65% died while on dialysis and 2 had successful renal transplantation [24].

In the mid 1990's, fifteen living donor recipients were followed up for at least 24 months post transplantation. The majority of the donors and recipients were males. Within this time, one graft has been lost at one year and patients restarted on hemodialysis. Three patients died, two within the first year, the third at 23 months after transplantation, all with functioning grafts. The one year graft and patient survival rates were 93% and 86% respectively. The second year graft survival rates remained at 93% and the patients' survival rates 80% [25].

1.5 PREVALENCE OF BONE AND MINERAL DISORDERS IN KIDNEY TRANSPLANT RECIPIENTS

The renal osteodystrophy present in the dialysis stage continues after transplantation, due to the treatment with corticosteroids and calceneurin inhibitors and persistent hyperparathyroidism. In nearly a third of all patients, the HPT is not resolved and persist even for several years after kidney transplantation, occasionally requiring surgery [26, 27].

Lobo et al observed that 55% of renal transplant recipients have PTH concentrations greater than normal [28].

Although PTH levels tend to decrease after kidney transplant surgery, PTH levels remain elevated in a subset of patients. Some studies report more 50% of patients still having elevated PTH levels more than two years after transplant [39]. A study done in Norway among 360 renal transplant recipients with normal transplant function, one hundred and eighty eight patients (52%) had elevated iPTH levels more than 1 year after transplantation. Twenty-six patients (7%) had iPTH levels >2.5 times the upper limit of normal [30].

During the first 6 to 12 months after kidney transplant, there is rapid bone loss. After this time period, patients may continue to lose bone at a slower rate, stabilize or improve BMD depending on numerous factors [31].

About 30% of patients may still have elevated PTH levels beyond 1 year despite the presence of normal renal function and vitamin D metabolism. [32].

Renal phosphate wasting, and hypophosphatemia are very common (up to 90%) in the early post transplant period, though they tend to resolve overtime. [33-34]. Despite of the successful kidney transplant, the active 1, 25 hydroxyvitamin D levels are lower than the expected [35].

Reinhardt et al report hypercalcemia after renal transplant in 52% of 129 patients 3 months after grafting [36]. However, in about 5 to 10% of recipients, hypercalcemia persists beyond the first year but resolves gradually within 2 to 5 additional years [37, 38]. In a prospective study looking at calcium metabolism in the early post transplantation period among renal transplant recipients,

Evenepoel et al showed a serum calcium levels following a biphasic pattern, a significant decline during the first postoperative week, followed by a significant increase [39].

1.6 NATURAL HISTORY OF BONE AND MINERAL DISORDERS AFTER TRANSPLANTATION

Ideally, successful kidney transplantation corrects the abnormalities in mineral metabolism that, during uremia, lead to secondary HPT and renal osteodystrophy. This includes reversal of uremia, abolition of hypocalcaemia, hyperphosphotemia and acidosis, and restoration of calcitriol production and reversal of skeletal resistance to PTH and vitamin D. [40]

A study done by Pieter et al showed that after an initial fall, intact parathyroid hormone (iPTH) levels showed a slow but steady decline towards the upper normal limit. The prevalence of persistent HPT, defined as an iPTH level more than or equal to 2.5 times the upper normal limit or the need for parathyroidectomy following transplantation, remained stable at 17% up to 4 years after transplantation. Patients with persistent HPT had significantly elevated serum levels of iPTH, calcium and phosphorous at the time of renal transplantation, and has spent a larger time on dialysis. [41]

A study done at Tokyo Women's Medical University among living-donor kidney transplant recipients (N=34) revealed, increased serum calcium levels until the fourth week post transplantation, after which it reached a plateau. Serum phosphate decreased substantially at one week post kidney transplantation but recovered to the reference level at two months. HPT persisted for 12 months after transplantation. [42]

Post transplant HPT may be associated with many complications and morbidities, including bone loss and vascular calcification [43, 44], and the related hypercalcemia has been linked to allograft dysfunction [40-45] and graft loss. [47]

1.7 RISK FACTORS OF BONE AND MINERAL DISORDERS POST RENAL TRANSPLANTATION

Although some early reports have linked post transplantation bone disease mainly to glucocorticoid excess, it has become clear that it rather comprises a spectrum of metabolic alterations of bone metabolism during dialysis (secondary HPT, adynamic bone disease, osteomalacia and mixed bone disease), as well as factors that occur after transplantation (Table1). [9]

Table1: Contributing factors of post transplantation bone disease.

○ **PRE TRANSPLANTATION**	○ **POST TRANSPLANATION**
○ Osteitis fibrosa	○ Immunosuppressive drugs
○ Mixed bone disease	○ PTH status
○ Adynamic bone disease	○ Hypophosphatemia
○ Osteomalacia	○ Renal function

In a study done to assess predictive factors for persistent HPT after kidney transplantation, 7.1% of patients had elevated iPTH levels. There was a statistically significant relationship between

increased age of the patients as well as duration of dialysis and a post kidney transplant high PTH level ($p<0.001$). [43]

The magnitude of pretransplant HPT and renal function determine the long-term post transplant parathyroid function. Torres et al showed that in 62 renal transplant recipients with good renal function (creatinine <177umol/l), iPTH decreased from 214±29 pre transplantation to 116±70pg/ml post transplantation ($p<0.01$). Of the many variables analyzed, creatinine ($p=0.001$) and pre transplant PTH ($p=0.02$) significantly correlated with post transplant PTH [27].

Heaf et al reported that high prednisone dose (>9mg/day), high cyclosporine trough concentration, hyperparathyroidism and high alkaline phosphatase levels were factors associated with long-term bone loss after transplantation. [31]

A study done in Turkey among renal transplant recipients, low BMI values and decreased 25OH vitamin D levels were found to be major risk factors for loss of bone mineral density. [48]

Osteopenia and osteoporosis are frequent among kidney transplant recipients (66%). Those with low bone density tend to have elevated iPTH levels and low creatinine crealence. [49]. Priyanka et al reported a positive correlation between BMI and bone mineral density in spine and femur. Longer CKD duration prior to transplant showed greater loss of BMD in the femur. [50]

In a study done to determine prevalence and patterns of bone loss in the first year after renal transplant, about 45% had low BMD at baseline. Factors that were correlated with low BMD

were older age, post menopausal status, and tertiary HPT. There was a linear decrease in total hip and lumbar spine BMD from baseline to12 months [51].

Female gender has also been linked as an independent risk factor. This was negatively associated with BMD, suggesting that the female skeleton is more vulnerable to transplant-related hormonal changes than that of the male. [52]. Regular weight-bearing, physical activity also had a beneficial effect on bone mass, while prolonged bed rest had a powerful negative impact on skeletal health. [53]

1.8 PATHOGENESIS OF BONE AND MINERAL DISORDERS AFTER TRANSPLANTATION

The possible causes of bone loss after kidney transplant are numerous and usually multiple factors are present in each patient. These factors include pre existing continued uremic osteodystrophy, immunosuppressive drugs, persistent HPT, hypophosphatemia, poor allograft function, loop diuretic, acidosis, smoking, alcohol abuse, hypogonadism, aging, chronic disease, physical inactivity/immobilization, and poor nutrition. [48, 54, 55]

It is evident that post transplantation bone disease is a complex problem that ranges from low to high turnover, indicating that the pathogenesis of post transplantation bone disease is multifactorial [55, 57- 58].

1.8.1 PRE TRANSPLANTATION MINERAL AND BONE DISORDERS

There seems to be no doubt that pre transplant mineral and bone disorders play an important role in the maintenance or development of post transplant alterations of bone remodeling. Indeed,

most transplant patients suffer different forms of pre existing bone disease that may persist after transplantation. [59]

Studies by Julian et al showed that, 6 months after transplantation patients exhibited a low bone mass, decreased mineral apposition rate, and delayed mineralization consistent with a pattern of adynamic bone disease [59]. The condition is usually caused by over suppression of PTH and other growth factors, including gonadol hormones, growth hormone, and insulin like growth hormone-1. [31, 33]

1.8.2 **PERSISTENT HYPERPARATHYROIDISM AND HYPERCALCEMIA**

In patients with non suppressible 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. In addition, some patients may develop de novo secondary hyperparathyroidism resulting from progressive functional alterations of the transplanted kidney. However, in many studies the bone histopathologic findings are heterogeneous, ranging from high bone turnover to low bone turnover without apparent correlation to post transplant serum PTH levels, suggesting that other factors that begin to operate after transplantation play a central role in the development of the bone alterations observed in these patients. [27]

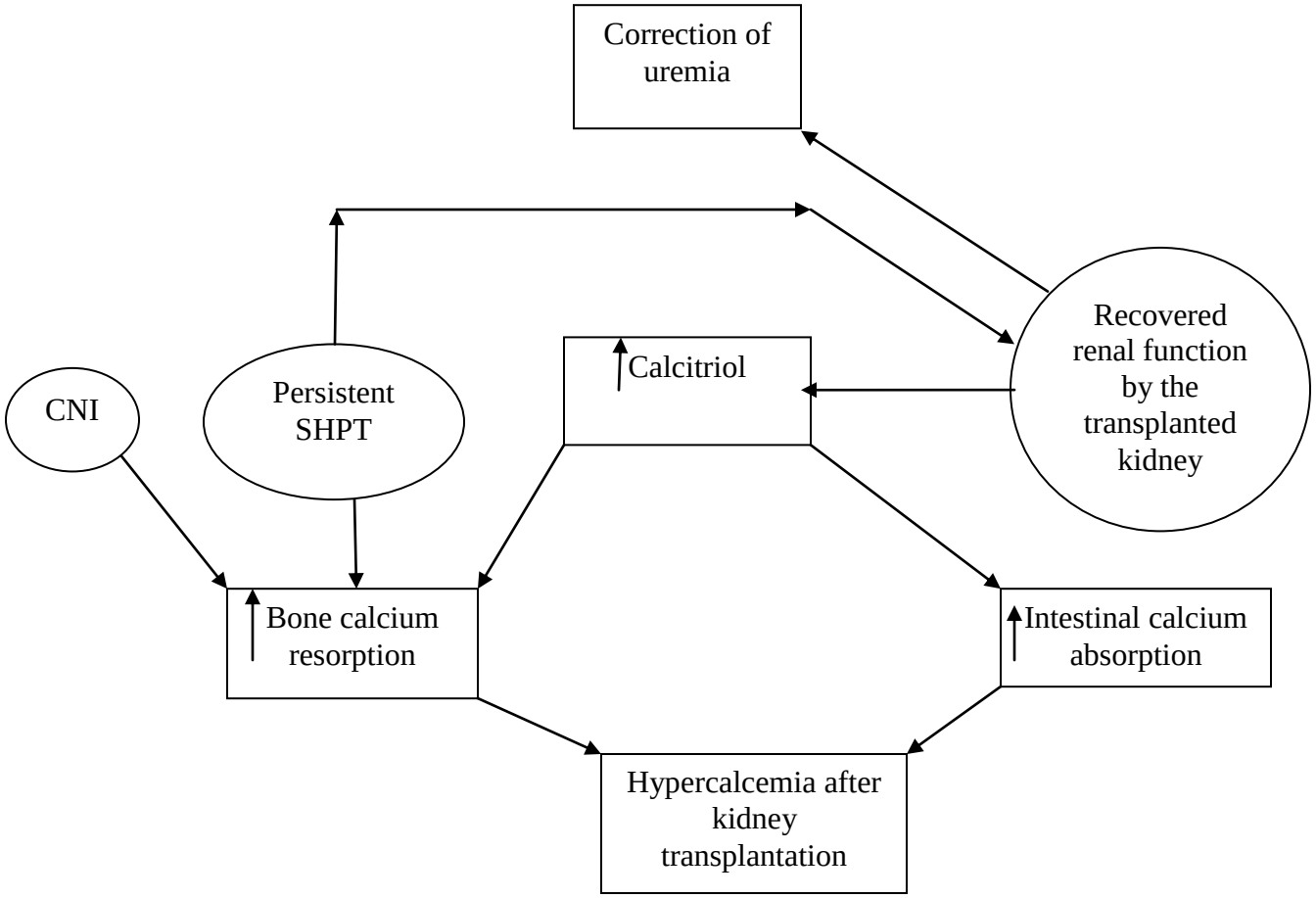
Serum PTH concentrations decrease progressively during the first 3 to 6 months after grafting. However, 1 year after transplantation resolution of hyperparathyroidism is incomplete in 50% of recipients. Duration of dialysis, parathyroid gland size and development of nodular/ or monoclonal hyperplasia of parathyroid gland are the most important factors responsible for HPT. [44]

Post transplantation hypercalcemia is a common problem that results from the effect of increased PTH concentration on different target tissues. High PTH concentrations stimulate the renal production of calcitriol that, in turn, increases intestinal calcium absorption and improves the skeletal mobilization of calcium. Correction of uremia and normalization of serum phosphorus levels are additional factors contributing to the resolution of the skeletal resistance to PTH, thus facilitating the release of calcium due to osteoclastic bone resorption. [44]

Another potential factor causing hypercalcemia after kidney transplant is the recovered circulating levels of calcitriol, secondary to its increased renal tubular synthesis, further stimulated by the inappropriately high PTH and low phosphorus levels. The recovered calcitriol levels might concur in inducing hypercalcemia by both its intestinal and bone effects. [1]

Figure 1 summarizes the main hypothesized pathogenic mechanisms through which hypercalcemia might develop after KTx.

Figure 1: The main supposed mechanisms responsible for the occurrence of hypercalcemia after kidney transplant [60]



1.8.3 HYPOPHOSPHATEMIA

Renal phosphate wasting and hypophosphatemia are very common (up to 90%) in the early post transplant period, though they tend to resolve over time [33-34]. Persistent HPT and elevated phosphatonin fibroblast growth factor 23 (FGF23) are the main causes of hyperphophaturia. Other possible causes include steroid therapy, reduced intestinal phosphorus absorption, reduced

proximal tubular Na/Pi co- transporter expression or increased tubular sensitivity to PTH. [32, 34, 61]

1.8.4 IMMUNOSUPPRESSIVE THERAPY

Several studies suggest that post transplantation immunosuppressive therapy constitutes a major factor in the pathogenesis of post transplantation bone disease. [12, 62-63]

The major cause of post transplantation bone loss is corticosteroid treatment. This has been clearly shown in several reports comparing the evolution of BMD in patients on cyclosporine A (CsA) monotherapy versus those receiving CsA plus prednisone with or without azathioprine [64-65]. Twelve to 18 months after grafting BMD remained unchanged or even increased both at the lumbar spine [64-65] and femoral neck [64] in the group without corticosteroids and significantly decreased in the corticosteroid treated group.

The pathogenesis of corticosteroids- induced bone loss is multifactorial and has been reviewed extensively. The main deleterious effect of corticosteroid is direct and profound inhibition of bone formation. They inhibit osteoblasts differentiation and induce apoptosis in mature osteoblasts and osteocytes. They also decrease gastrointestinal calcium absorption, resulting in a negative calcium balance and SHPT. In addition, corticosteroids directly suppress gonadotropins and may cause hypogonadism [66-67].

The possible role of calceneurin inhibitors cyclosporine remains controversial. Studies in animals and humans have shown that cyclosporine causes high bone turn over [12, 62-63]. In rats, cyclosporine causes bone loss that is associated with increases bone resorption and formation.

[63]; however other studies have failed to demonstrate an effect of the drug on mineral and bone metabolism in renal transplant recipients [58, 11].

Tacrolimus is a calceneurin inhibitor that suppresses T cell activation and the production and release of IL-2 and other cytokines. It induces severe trabecular bone loss in rats, but this effect appears to be less severe in humans [12].

In humans, CsA and tacrolimus may contribute to post transplant bone loss by decreasing osteoprotogerin mRNA expression and increasing RANKL gene expression in osteoblasts [68-69]. The effect of other drugs such as sirolimus, MMF and azathioprine has been poorly investigated but they appear to have little or no effect on bone [70].

1.8.5 ROLE OF DETERIORATING RENAL FUNCTION

The level of renal function achieved as a result of transplantation is a critical determinant of whether SHPT will be present. Patients that do not achieve a GFR greater than 70ml/min/1.73m2 are at a greater risk for progression of renal bone disease [71].

Bone loss after transplantation is related to adverse effects of immunosuppressive drugs (glucocorticoids and calceneurin inhibitors) as illustrated in figure 2.

Figure 2: Mechanisms of bone loss in the early phase of post transplantation period. (72)

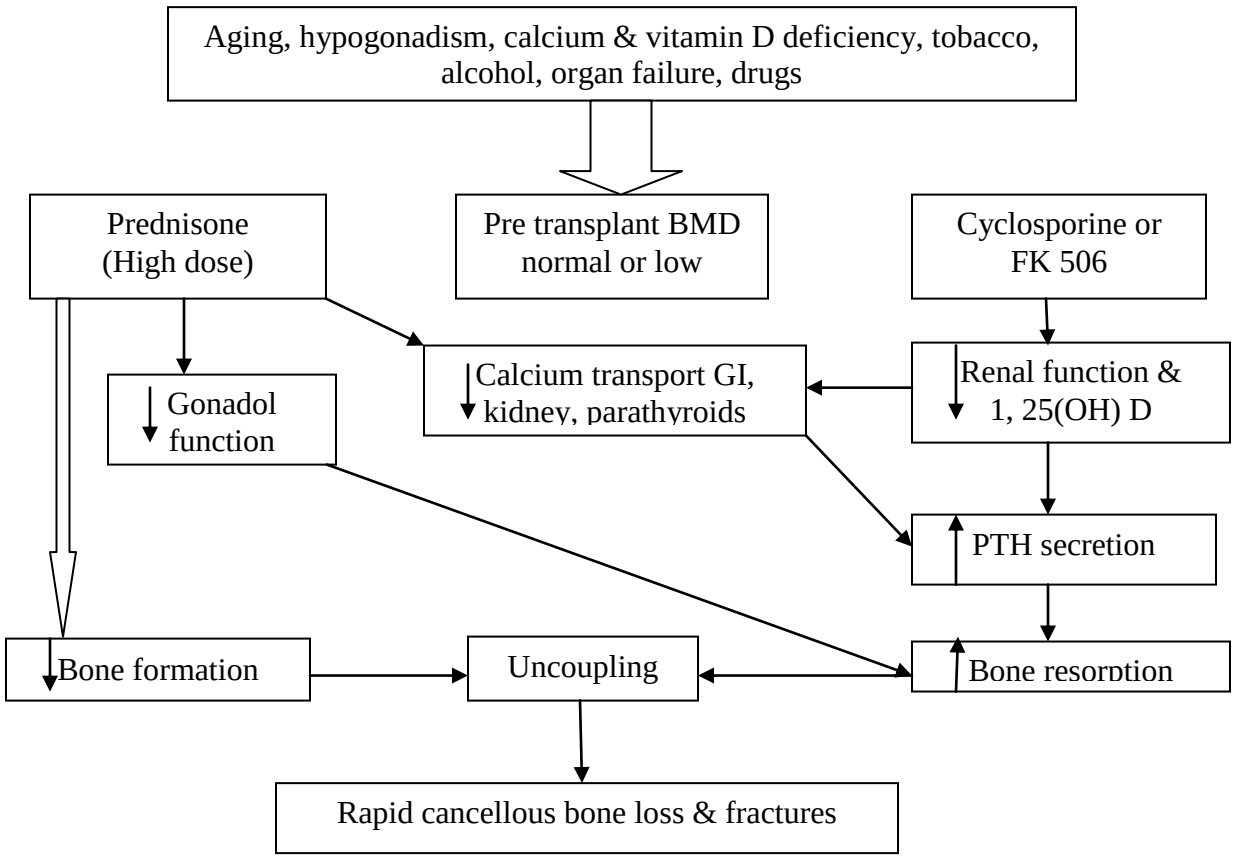
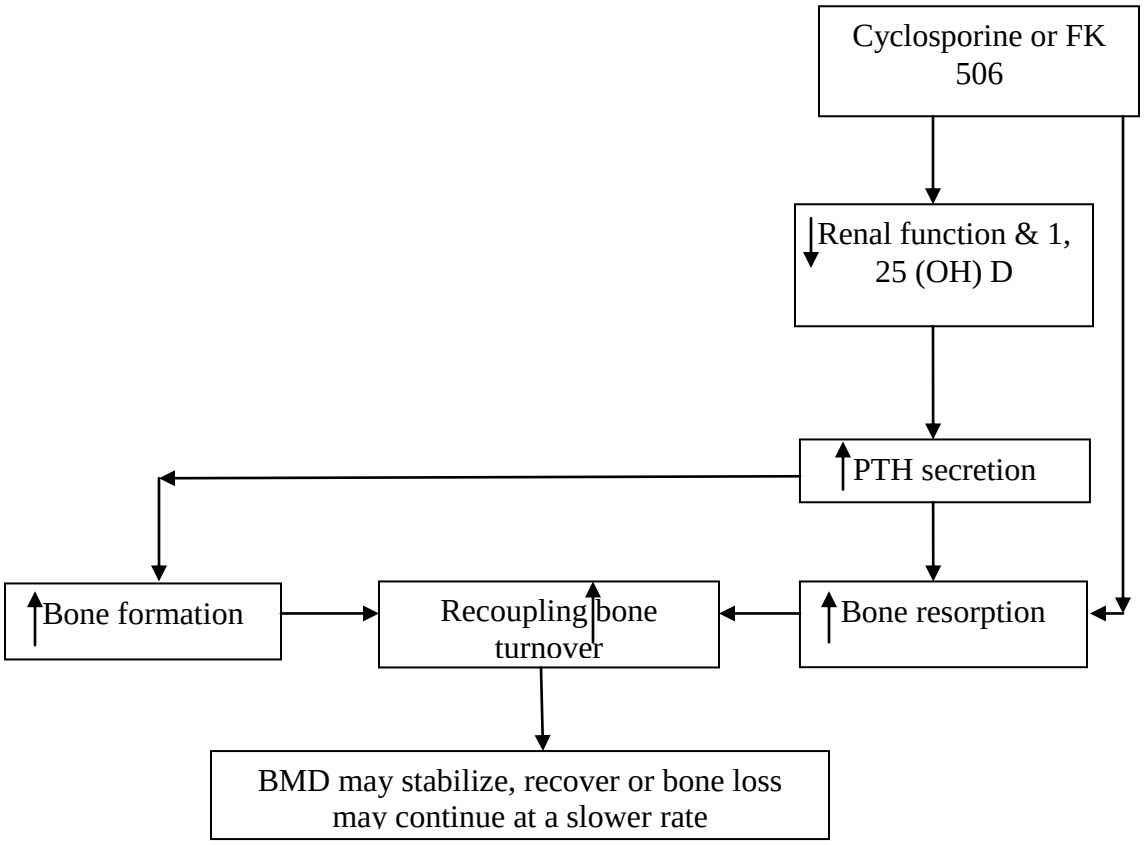


Figure 3: Mechanisms of bone loss in the later phase of post transplantation period. (72)



1.9 BIOCHEMICAL PARAMETERS

A. Parathyroid hormone

PTH levels are consistently below pre- transplant levels 6 months after surgery with reported decreases between 19 and71% [73-76]. PTH levels immediately (<2weeks) after surgery, however, were variable with 2 studies reporting decreases [70, 77] and 2 reporting increases [78, 2].

Although PTH levels tend to decrease after kidney transplant surgery [79, 80], PTH levels remains elevated in a subset of patients. At time points greater than 2 years after transplant, >50% of patients still have elevated PTH levels [81-82] and this trend continued more than 5 years after surgery [81]. Elevated PTH levels negatively impact BMD related outcomes in kidney transplant patients. Patients with higher post transplant PTH levels tend to have significantly greater BMD loss than patients with lower post transplant PTH levels.

The percent of patients with high calcium tend to decrease after kidney transplant although elevated calcium levels pesist in a subset of patients. At time points greater than 2 years depending on the study, between 1.4 and 47% of patients have elevated calcium levels [36, 81-83]. The normal calcium range varies among studies.

B. Phosphate

Phosphate levels which are elevated prior to surgery, decreases rapidly after transplantation falling to within or below range for patients with normal kidney function (0.78-1.32mmol/l) in the first postoperative month [70, 74, 75].

Although mean phosphorus levels are within normal limits by the end of the 2nd month post transplant hypophosphatemia is still observed in between 1.6 and 39% of patients 6 months after transplant [1, 80, 83]. Definitions of a normal phophorous range also varies across studies.

C. Vitamin D

Baseline 1,25 (OH) vitamin D tend to fall outside and below the normal range and then increases slowly after surgery to within normal limits by month 12 [36, 73, 80].

Justification of the study

Despite significant improvement in renal function, recipients of kidney transplantation fail to recover from CKD- related mineral and bone disorders (MBD). Hypercalcemia and hypophosphatemia are frequently encountered post- transplant mineral bone disorders [41].

Hypercalcemia can adversly affect either the graft (nephrocalcinosis) and other organs or systems (vascular calcifications, pancreatitis). Though many factors have been claimed to induce hypercalcemia after kidney transplant, the persistence of post transplant HPT of moderate-severe degree is universally considered the first causal factor [85].

Renal transplant recipients with calcification have been shown to have significantly higher serum PTH and calcium levels. [86]. Coronary artery calcification has been shown to be a strong independent predictor of cardiovascular events in transplant recipients [87].

The restoration of vitamin D synthesis, clearance of phosphate and reduction of PTH levels are all beneficial to the bone after transplantation. However, bone loss still remains an important problem due to posttransplantation factors such as steroid use, persisitent HPT, and other metabolic and acid-base disturbances [51].

The therapeutic stratergies for alleviation of post transplantation bone disease include low dose steroid, steroid elimination, calcium, vitamin D, calcimimetic agents, biphosphonates and, to a lesser extent, calcitonin [9].

Despite the knowledge of existence of mineral disorders after renal transplantation, there are no studies describing the pattern of the disorder among renal transplant recipients in Kenya.

Knowing the levels of serum calcium, phosphorus and PTH in the posttransplant population physicians maybe in the position to correct any abnormalities in the levels and possibly prevent complications known to arise from bone related mineral disorders. This study was done to

determine the levels of serum calcium, phosphorus and parathyroid hormone levels among renal transplant recipients attending clinic at Kenyatta National Hospital.

Research question:

What are the patterns of bone related mineral disorders among kidney transplant recipients?

OBJECTIVES

Broad objective

To determine the levels of bone mineral metabolism parameters among kidney transplant recipients attending renal clinics at KNH.

Specific objectives

1. To determine the serum levels of calcium, phosphate and intact parathyroid hormone among kidney transplant recipients
2. To determine the relationship between bone biochemical parameters and eGFR

Secondary objective

1. To determine the relationship between age, gender, duration of dialysis prior to transplantation, duration after transplantation and biochemocal parameters (calcium, phosphate and iPTH)

MATERIALS AND METHODS

Study Design

It will be a cross-sectional descriptive study

Study Area

Renal transplant clinic at the Renal Unit of the Kenyatta National Hospital including the biochemistry laboratory of Kenyatta National Hospital

Study Population

Renal transplant recipients of 18 years and above who have signed an informed consent

Study Duration

Enrollment was done from march 2013 to august 2013

Sample Size

A total number of 85 renal transplant recipients were studied. The following formula was used to obtain the sample size:

An estimated 110 kidney transplants patients are currently seen in KNH. Based on these numbers, the accessible population is described as finite (less than 10,000) hence the appropriate formula used to calculate the sample size will be as follows [88]:

$$n = \frac{NZ^2 \times p(1-p)}{d^2(N-1)+Z^2 p(1-p)}$$

Description:

n = required sample size

N= the expected number of the accessible population – total kidney transplant patients in KNH-110

z = confidence level at 95% (standard value of 1.96)

p = estimated prevalence of mineral disorders (42%)

d = margin of error (5%)

PATIENT SELECTION

Inclusion criteria

1. Kidney transplant recipients who are at least 6 months post transplant and are on follow up at renal transplant clinic in KNH
2. Patients 18 years of age and above who have signed an informed consent

Exclusion criteria

1. Patients who have returned to dialysis
2. Patients who have declined to participate in the study

Case definitions:

Staging severity of chronic kidney disease with transplant (2009 KDIGO Clinical Practice Guideline For the Care of Kidney Transplant Recipients)

Stage 1T: Kidney damage with normal or increased kidney function, glomerular filtration rate ≥90 mL/min/1.73 m2

Stage 2T: Kidney damage with mildly decreased kidney function: glomerular filtration rate 60-89 mL/min/1.73 m2

Stage 3T: Moderately decreased kidney function: glomerular filtartion rate 30-59 mL/min/1.73 m2

Stage 4T: Severerly decreased kidney function: glomerular filtration rate 15-29 mL/min/1.73 m2

Stage 5T: Kidney failure: glomerular filtration rate <15 mL/min/1.73 m2 or on dialysis.

DEFINITIONS

Hypoparathyroidism; Serum iPTH <15 pg/ml

Hyperparathyroidism; Serum iPTH >65 pg/ml

Hypocalcemia; Serum calcium <2.2 mmol/l

Hypercalcemia; Serum calcium >.2.6 mmol/l

Hypophosphatemia; Serum Pi <0.8 mmol/l

Hyperphosphatemia; Serum Pi>1.6 mmol/l

Sampling method

Consecutive sampling was used to recruit patients at visit to the KNH renal transplant clinic over a period of 5 months, till the sample size of 85 was attained. The clinic runs every Tuesday morning except for public holidays.

METHODS

Screening, Consenting and Recruitment

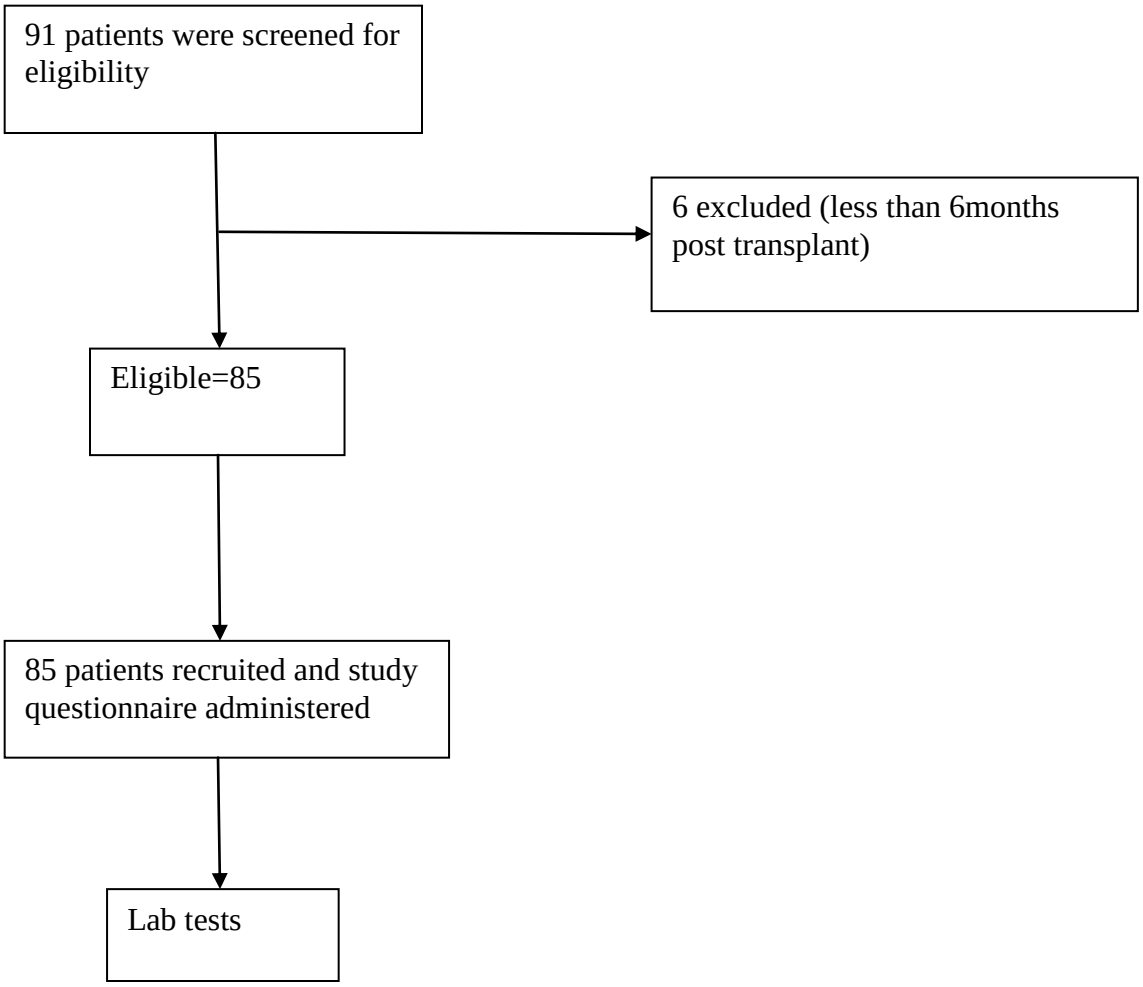
All patients who had satisfied the inclusion criteria and signed the informed consent were recruited into the study. The study principle investigator administered the consent forms to the study participants.

Clinical methods

An investigator administered questionnaire was used to collect data from recruited patients. Data consisted of socio demographic data including age, gender and marital status. Information on possible etiology of kidney disease, time on dialysis, time since transplantation and current medications were recorded.

PATIENT RECRUITMENT FLOW CHART

85 patients were recruited between March 2013 and August 2013.



LABORATORY METHODS

About 5ml of blood was drawn from the cubital vein in sterile plain vacutainer for creatinine, calcium, phosphate, albumin and intact PTH.

The blood samples were immediately processed for determination of biochemical markers and if they were not immidiately processed they were then stored at -20 degree Celsius until time of analysis.

The measurements of serum creatinine, albumin, calcium and phosphate were determined using automated clinical chemistry analyzer Mindray Clinical Chemistry Analyzer in the KNH Renal laboratory while serum iPTH assays were performed in KNH main laboratory using electrochemiluminesce immunoassay (ECLIA) (Cobas®)…..(Appendix 1)

Parathyroid hormone (PTH) is formed in the parathyroid glands and secreted into blood stream. Intact PTH consists of a single polypeptide chain containing 84 amino acids. The biologically active N-terminal fragment has a half-life of only a few minutes. Selective measurement of the (mainly) intact parathyroid hormone permits direct ascertainment of the secretory activity of the parathyroid gland. [89-90]

The results were analyzed after daily calibration using standard calibration methods and materials and tests assayed against controls.

The glomerular filtration rate (GFR) was estimated using the MDRD formula [105]:

$eGFR = 32788 \times \text{Serum Creatinine}^{-1.154} \times \text{Age}^{-0.203} \times [1.212 \text{ if Black}] \times [0.742 \text{ if Female}]$

The formula was calculated using a free internet-based clinical equation calculator [94]

Total serum calcium was corrected for serum albumin using the equation: Corrected Calcium= Ca [40-measured serum albumin] x 0.02 + measured serum calcium. (A typical correction is that

for every 1g/l that the albumin concentration is below 40g/l, the calcium concentration is 0.02mmol/l below what it would be if the albumin concentration was normal).

Laboratory reference ranges were as follows:

Serum intact PTH 15-65pg/ml

Serum calcium 2.2-2.6mmol/l

Serum phosphate 0.8-1.6mmol/l

Serum albumin 35-54g/l

Serum creatinine

Male……60-130µmol/l

Female…….40-110µmol/l

DATA MANAGEMENT AND ANALYSIS

At the end of data collection, questionnaires were coded, entered and managed in Microsoft Excel. Data cleaning was performed at the end of data entry and analysis performed using SPSS version 17.0 software.

The characteristics of the participants were summarized into means/medians and proportions for continuous and categorical variables respectively. eGFR, creatinine, serum calcium, phosphate and iPTH were analyzed and presented as means and medians where appropriate. Furthermore, calcium, phosphate and iPTH were categorized according to patients with low, normal and high levels and presented as proportions. The relationship between bone biochemical parameters and eGFR was measured using Pearson correlation coefficient (r) and linear regression coefficient (β) and illustrated using scatter plots. The levels of iPTH, calcium and phosphate were associated with sex of the patient using chi-square test. Age of the patients was compared between groups using Student's t test while duration of dialysis and length of time since transplant was compared using Mann Whitney U test. All statistical tests were performed at 5% level of significance (95% confidence interval). The findings were presented using tables and graphs.

ETHICAL CONSIDERATIONS

The study was undertaken after approval by the Department of Internal Medicine, University of Nairobi and the Kenyatta National Hospital Scientific and Ethical Review Committee.

Cases eligible to participate in the study were included only after providing consent following the process as outlined below:

1. The cases were informed that the project involves local research
2. They were told the purpose of the study
3. The procedures of the study were explained clearly with full details of all the test to be done
4. They were assured that participation is voluntary and that no medical attention would be denied should they decline to participate
5. The participants were informed of the medical benefits and also physical and psychological harms to their satisfaction prior to being included in this study
6. The subjects were assured of full and free access to their results and that the therapeutic interventions would be recommended where the need arises, according to accepted standard of practice
7. It was asserted that confidentiality would be strictly maintained and all data would be securely stored and only revealed upon a need-to-know basis and that all costs regarding investigations in this study will be borne by the principle investigator

- Following the full explanation and acceptance by the patient of the above, the subject was requested to sign the consent form (Appendix 2)

RESULTS

Patient clinical and socio-demographic characteristics

Table 2: Socio-demographic characteristics of the study population

Variable	Frequency (%)
Mean age in years (±SD)	40.7 (12.3)
Range	18-64
Gender	
Male	60 (70.6)
Female	25 (29.4)
Marital Status	
Married	60 (70.6)
Single	25 (29.4)
Level of Education	
Primary	4 (4.7)
High school	33 (38.8)
College/ University	48 (56.5)

A total of 91 renal transplant recipients who were attending the KNH renal transplant clinic between March 2013 and august 2013 were screened for eligibility into the study. Six of these patients were excluded as they were less than six months since transplantation. The 85 patients who gave consent were interviewed and blood was drawn for laboratory investigations.

Our study population was composed of 60 (70.6%) males and 25 (29.4%) females, with a mean ± SD age of 40.7 ± 12.3 years (range: 18-64). Majority of the patients were married 60 (70.6%) and had a college/university education 48 (56.5%). The socio-demographic characteristics of the study population are presented in Table 2.

The median duration of dialysis prior to transplantation was 12 months (IQR: 9- 19). The median time since transplantation was 21 months (IQR: 10-29). The etiology of the kidney disease as documented in the file of the study subjects included hypertension in 62, diabetes mellitus in 11, diabetes mellitus and hypertension in 6, SLE in 3, polycystic kidney disease in one, renal tuberculosis in 1, and chemotherapy in one patient. The study population was categorized into 5 groups as per KDIGO Clinical Practice Guidelines for the Care of Kidney Transplant Recipients.

Majority of patients were in CKD stage 2T (52.9%) with 95.2% being in stage 3T and above. The maintenance immunosuppression therapy was comprised of prednisone in 85 (100%), mycophenolate mofetil in 79 (92.9%), cyclosporine in 55 (64.7%) and tacrolimus in 28 (32.9%) subjects. Only 5 patients were on azathioprine and one patient on everolimus. Four (4) patients had been calcium supplements. Other medications included antihypertensives, oral hypoglycemic or insulin as illustrated in Table 3.

Table3: **Patient clinical characteristics**

Gender (male)	**60 (70.6%)**
Median duration in months of dialysis (IQR)	12 (9-19)
Median duration in months since transplantation (IQR)	21 (10-29)
Etiology of the Kidney Disease (%)	
Hypertension	62 (72.9)
Diabetes	11 (12.9)
Diabetes/Hypertension	6 (7.1)
Systemic Lupus Erythromatosus	3 (3.5)
Polycystic Kidney Disease	1 (1.2)
Renal-Tuberculosis	1 (1.2)
Chemotherapy	1 (1.2)
CKD Stage (%)	
1T	8 (9.4)
2T	45 (52.9)
3T	28 (32.9)
4T	2 (2.4)
5T	2 (2.4)
Immunosuppressants (%)- Prednisone	85 (100)
Mycophenolate mofetil	79 (92.9)
Cyclosporine	55 (64.7)
Tacrolimus	28 (32.9)
Azathioprine	5 (5.9)
Everolimus	1 (1.2)
Other medications (%)- Antihypertensive	70 (82.4)
Oral hypoglycemic/insulin	11 (12.9)
Calcium supplements	4 (4.7)

Biochemical characteristics of the study population

The median serum creatinine was at 119 µmol/l with calculated mean GFR ± SD at 64 ± 19 ml/min/1.73m2. The mean corrected serum calcium was 2.2mmol/l. Normal serum calcium levels were found in 43 (50.6%) patients while 42 (49.4%) patients had hypocalcemia. The median iPTH was 38.1 pg/ml with majority of patients (63.5%) having normal serum levels. Hyperparathyroidism was found in 17 patients (20%) and hypoparathyroidism was seen in 14 patients (16.5%). The Mean serum phosphorus level was 1.2±0.2 mmol/l with majority of patients having normal levels 79 (92.9%). Hyperphosphatemia was seen in 3 patients and hypophosphatemia in another 3. Table 4 illustrates biochemical parameters of the study population.

Table 4: Biochemical characteristics of the study population

Variable	**N=85**
Mean eGFR, ml/min/1.73m2, (±SD)	64 (±19)
Median serum creatinine (µmol/l) (IQR)	119.0 (105.0-140.0)
Mean serum calcium (mmol/l) (±SD)	2.2 (±0.2)
Mean corrected serum calcium (mmol/l) (±SD)	2.2 (±0.2)
Categories, n (%)	
Hypocalcemia	42 (49.4)
Normal	43 (50.6)
Mean serum calcium in hypocalcemic patient in mmol/l (SD)	2.0 (0.1)
Mean serum albumin, g/L (SD)	40.7 (±4.0)
Median iPTH (pgl/ml) (IQR)	38.1 (25.0-58.6)
Categories, n (%)	
Low <15 pg/ml	14 (16.5)
Normal 15-65 pg/ml	54 (63.5)
Hyperparathyroidism >65 pg/ml	17 (20.0)
Mean serum phosphorus (mmol/l) (SD)	1.2 (±0.2)
Categories, n (%)	
Hypophosphatemia	3 (3.5)
Normal	79 (92.9)
Hyperphosphatemia	3 (3.5)

Correlation between bone biochemical parameters and transplant kidney function (eGFR)

The correlation between bone biochemical parameters and eGFR is shown in table 5. No significant correlation was found between eGFR and bone biochemical parameters (serum calcium, phosphate and iPTH).

Table 5: Pearson correlation between bone biochemical parameters and eGFR

Variable	Pearson correlation coefficient (r)	Beta coefficient, β (95% CI)	P value
Corrected serum calcium (mmol/l)	0.082	0.001 (-0.002-0.003)	0.455
iPTH (pg/ml)	-0.079	-0.155 (-0.583-0.272)	0.472
Serum phosphorus (mmol/l)	-0.024	0.000 (-0.003-.003)	0.829

Table 6: Distribution of calcium among different study variables

variable	Hypocalcemia	Normal	P value
Age	37.5 (11.5)	43.8 (12.4)	0.017
Sex F M	 11 (26.2%) 31 (73.8%)	 14 (32.6%) 29 (67.4%)	0.519
Median duration of dialysis (IQR)	12.0 (9.0-18.0)	13.0 (10.0-24.0)	0.163
Median duration since transplantation (IQR)	22.0 (14.0-29.0)	18.0 (8.0-29.0)	0.408

Hypocalcemia was associated with relatively younger patients with mean age of 37.5(±11.5) years compared to those with normal serum calcium levels with a mean age of 43.8 years (p=0.017). There was no significant difference between males and females, time on dialysis and time since transplantation in those with hypocalcemia (Table 6). No factors were found to be associated with serum iPTH levels (Table 7).

Table 7: Distribution of iPTH among different study variables

Variable	Hypo-parathyroidism	Normal	P value	Hyper-parathyroidism	P value
Mean age (SD)	36.9 (13.6)	42.9 (11.7)	0.103	36.8 (12.2)	0.982
Sex F M	 2 (14.3%) 12 (85.7%)	 18 (33.3%) 36 (66.7%)	 0.204	 5 (29.4%) 12 (70.6%)	 0.763
Median duration in months of dialysis (IQR)	10.0 (6.0-12.0)	13.0 (10.0-18.0)	0.067	16.0 (12.0-25.0)	0.306
Median duration in months since transplantation (IQR)	22.5 (13.0-31.0)	21.5 (10.0-33.0)	0.879	18.0 (9.0-26.0)	0.491

Likewise for serum phosphate no factors were associated with either hypophosphatemia or hyperphosphatemia (Table 8)

Table 8: Distribution of serum phosphate among different study variables

Variable	Hypo-phosphatemia	Normal	P value	Hyper-phosphatemia	P value
Age (SD)	50.3 (0.6)	40.6 (12.2)	0.175	33.0 (17.3)	0.297
Sex F M	 1 (33.3%) 2 (66.7%)	 24 (30.4%) 55 (69.6%)	 1.000	 0 (0.0%) 3 (100.0%)	 0.552
Median duration of dialysis (IQR)	12.0 (1.0-26.0)	12.0 (9.0-18.0)	0.716	16.0 (12.0-27.0)	0.394
Median duration since transplantation (IQR)	19.0 (10.0-151.0)	22.0 (10.0-29.0)	0.702	18.0 (6.0-31.0)	0.630

Distribution of patients with hypocalcemia and normal calcium levels among each of CKD stage is as illustrated in figure 9. Thirty-eight patients (95.3%) out of 42 with hypocalcemia belonged to CKD stages 1T- 3T. There was no difference in CKD stages among patients with hypocalcemia and those with normal serum calcium levels (p=0.950).

Table 9: Distribution of patients with low and normal calcium levels among different CKD stages

	Hypocalcemia	Normal	P value
CKD Stage			
1	5 (11.9%)	3 (7.0%)	0.950
2	22 (52.4%)	23 (53.5%)	
3	13 (31.0%)	15 (34.9%)	
4	1 (2.4%)	1 (2.3%)	
5	1 (2.4%)	1 (2.3%)	

Twenty-eight patients out of 42 with hypocalcemia had normal iPTH levels. Only 6 patients with hypocalcemia had low iPTH levels and 8 patients with hypocalcemia had elevated iPTH levels.

Twenty-eight (51.9%) patients with hypocalcemia had normal serum iPTH levels while 6 of hypocalcemic patients had low serum iPTH. Eight of 42 patients with hypocalcemia had elevated serum iPTH levels (>65pg/ml).

There was no significant association between hypocalcemia and low or elevated iPTH levels with p=0.549 and p=0.730 respectively as shown in table 10.

Table 10: The association between serum calcium and serum iPTH

	Normal 15-65 pg/ml	**Low <15 pg/ml**	**P value**	**Hyper-parathyroidism >65 pg/ml**	**P value**
Corrected serum calcium (mmol/l)	2.2 (0.2)	2.2 (0.3)	1.000	2.1 (0.2)	1.000
Calcium					
Hypocalcemia	28 (51.9%)	6 (42.9%)	0.549	8 (47.1%)	0.730
Normal	26 (48.1%)	8 (57.1%)		9 (52.9%)	

Discussion

The chronic kidney disease epidemic is a worldwide public health problem associated with high morbidity and mortality [10-11]. Metabolic bone disease is a common complication of CKD and is a part of broad spectrum of disorders of mineral metabolism which include hypocalcemia, hyperphosphatemia, low vitamin D and elevated PTH levels; abnormalities of bone turnover, mineralization, volume, linear growth and strength; and vascular or soft tissue calcification [15-18].

Kidney transplantation from either a live or a deceased donor is preferable to dialysis therapy because transplantation provide a better quality of life and improved survival [16]. However even after otherwise successful kidney transplantation, mineral bone disorders do not always resolve [26-30, 36, 38, 39].

Secondary hyperparathyroidism after kidney transplantation has been reported even in the presence of excellent graft function [1-5]. In this study, post transplant calcium, phosphorus, creatinine and iPTH considerably normalized in majority of patients.

The mean age (±SD) of our study population was 40.7 (±12.3) years. Patients were at least a decade younger than those in the studies by Evenepoel et al and Kawarazaki et al with mean ages of 54.5 and 50.0 years respectively [7, 43]. The average age of patients with ESRD in Africa has been reported to be much younger than that in the developed world with figures of less than 40 years [102-103].

Males made up the majority of the patients with 70.6% similar to what was reported in other studies [7, 43, 94]. In other parts of Africa (Nigeria, Senegal and Burkina Faso) studies have also reported a male preponderance among patients with ESRD [102].

Hypertension was recorded as the etiology of kidney disease in 62 (72.9%) patients followed by diabetes mellitus in 11 (12.9%) patients. This is in contrast to the studies done in Japan and Iran where diabetes was reported as etiology of kidney disease in 26.5% and 78.6% of the study subjects respectively while in Iran hypertension was reported in 23.6% [41, 42].

The etiology of End Stage Renal Disease in SSA differs from that reported in the western population [110]. In Europe and United States, diabetic nephropathy constitutes about 50% of the causes of ESRD while in Africa the predominant causes are hypertension and CGN. Presentation of ESRD in Africa is often late and diagnosis prior to presentation is often inaccurate without the recognition of renal failure by the referring unit. In a ten-year study of 368 patients with CKD in Nigeria, the etiology of renal failure was undetermined in 62% and of the remaining patients whose etiology was ascertained, hypertension accounted for 61%, diabetes mellitus for 11% and CGN for 5.9% [102].

Majority of patients had relatively good graft function with a mean estimated GFR of 64ml/min/1.73m2. Eighty one (95.2%) out of 85 patients were between CKD stage 1T and 3T. The mean eGFR was comparable to that in a study done in Boston (United States) among 106 renal transplant recipients where 12% were African American and the mean was 62.9ml/min/m2 [91].

In this study which comprised of 85 renal transplant recipients with 62.3% having CKD stage between 1T and 2T, hyperparathyroidism was found in 17 (20%) patients, at a median (IQR) duration of 21 (10-29) months post transplant. The prevalence of HPT in a cross-sectional study done by Madhumati et al (91) among 106 renal transplant recipients who were at least 6 months post transplant with 55% belonging to CKD stage 1T and 2T was 66%. Post transplant median (IQR) duration was 12.8 (7.5-30.9) months. Similar cutoff levels for defining hyperparathyroidism were used i.e. iPTH> 65pg/ml. The difference in the prevalence of HPT could be explained by the fact that high levels of FGF-23 were associated with HPT in the study by Mathumati et al though the levels of FGF-23 were not assessed in this study.

We found no correlation between HPT and duration since transplant, time on dialysis and eGFR similar to what was reported by Mathumati et al. Gholamhossein reported hyperparathyroidism (iPTH>60pg/ml) in 7.1% of the study population 3 months post transplantation and was associated with duration of dialysis prior to transplantation with a mean ±SD of 54 ± 18.68 months compared to a mean ±SD of 14.26 ± 8.34 months ($p< 0.001$) in those with post transplant iPTH of ≤60pg/ml. Though our patients with elevated PTH had been on dialysis for a longer time

(median duration 16 months) prior to transplantation compared to those with normal PTH (median duration 13 months) or low PTH (median duration 10 months), the difference was not statistically significant.

The prevalence of hyperparathyroidism in this study was similar to that among CKD patients who were not yet on dialysis attending clinic at KNH where 21.6% of the study population had HPT. These results suggest a persistence of hyperparathyroidism post kidney transplantation. Pre transplant hyperparathyroidism has been associated with persistent hyperparathyroidism post renal transplantation [27, 94]. A shorter time on dialysis prior to transplantation or preemptive transplantation may reduce the risk of persistence hyperparathyroidism after kidney transplantation.

Secondary hyperparathyroidism has been associated with metabolic bone disease which manifests as bone pain and skeletal fractures [37, 104, 81]. PTH receptors have been demonstrated in the heart, and in vitro, PTH induces hypertrophy of cardiomyocytes. Severe left ventricular hypertrophy was found in 70% of patients with ESRD, who exhibit elevated plasma PTH levels [95]. However, an analysis of published literature found that the evidence for links between PTH and cardiovascular disease or mortality was poor [96]. In the EVOLVE study, the use of calcimimetic agent cinacalcet in patients with moderate- to – severe secondary HPT who were undergoing hemodialysis, did not significantly reduce the risk of death or major cardiovascular events [97].

Almost half (49.4%) of our study population had hypocalcemia with mean calcium levels of 2.2 mmol/l. Hypocalcemia was found to be associated with patients with relatively younger age with mean age of 37.5 years (p= 0.017). We found no significant correlation between hypocalcemia and estimated glomerular filtration rate (eGFR). None of our patients had hypercalcemia as it has been reported in other studies with hypercalcemia ranging from 3%- 21% [41, 39, 43, 91, 92]. In the early post transplant period serum calcium has been reported to follow a biphasic pattern with an immediate post op decline followed by a significant increase. A low pre transplant PTH has been found to be a predictor of severe hypocalcemia post renal transplantation [41]. In this

study pretransplant iPTH was not done thus we were not able to assess its correlation with hypocalcemia.

Mugera et al [13] reported a higher prevalence of low turnover bone disease (50.9%) of the patients with biochemical evidence of mineral bone disease, than a higher turn-over disease among CKD pre-dialysis patients attending clinic at KNH. This might help explaining presence of hypocalcemia in our population. The effect of corticosteroids on serum calcium levels among renal transplant recipients can never be ignored. They decrease gastrointestinal calcium absorption resulting in negative calcium balance and secondary hyperparathyroidism [66- 67].

In studies where hypercalcemia was reported among renal transplant recipients, factors that were reported to be associated with post transplant hypercalcemia included low eGFR, male sex, post transplant HPT and longer duration of dialysis [7, 46, 93, 95]. Another factor found to cause hypercalcemia after kidney transplantation is recovered circulating levels of calcitriol, secondary to its increased renal tubular synthesis, further stimulated by inappropriately high PTH and low phosphate levels. Calcitriol increases serum calcium levels through its effect in the intestine [1]. In this study calcitriol levels were not assessed because of financial constraints thus could not be correlated against other biochemical parameters.

Although almost half of our patients had hypocalcemia this was not associated with graft function as estimated by GFR. Only 2 (4.8%) of the 42 patients with hypocalcemia were between CKD stage 4T and 5T with over ninety five percent of patients with hypocalcemia being between CKD stage 1T and 3T.

Calcium is the major regulator of the parathyroid at the levels of secretion, gene expression and cell proliferation. Studies have shown hypocalcemia to cause a profound increase in parathyroid cell proliferation, PTH gene expression and subsequent PTH synthesis [98]. Hypocalcemia among CKD patients undergoing hemodialysis has been associated with increased mortality especially in the presence of hyperphosphatemia and elevated PTH levels [99].

Recently, concerns have also grown that calcium supply in excess of the requirements may increase cardiovascular risk [100]. The use of calcium containing phosphate binders has been associated in more rapid progression of coronary calcification than the use of sevelamer hydrochloride [101].

Only four patients were on calcium supplements despite half of transplant recipients having hypocalcemia. In this study vitamin D levels were not determined and thus any correlation with low serum calcium levels in this population could not be ascertained [89-90].

Majority (92.9%) of renal transplant recipients had normal serum phosphate levels. No correlation was found between serum phosphate and estimated GFR. Only 3 (3.5%) patients were found to have hypophosphatemia and another 3 (3.5%) patients with hyperphosphatemia. Age, sex, duration of dialysis, time since transplantation and PTH were not associated with either higher or lower levels of serum phosphate. Kawarazaki et al reported hypophosphatemia among 15% of renal transplant recipients 12 months post transplantation. Hypophosphatemia was associated with persistent HPT 12 months post transplantation. The difference in the prevalence of hypophosphatemia might be due to the fact that in the study by Kawarazaki et al the mean iPTH levels at 12 months post transplant was higher 107 (71.0-205.3)pg/ml compared to a mean of 38.1 (25.0-58.6)pg/ml at a median duration of 21 (10-29) months post transplantation in this study.

Serum phosphorus levels are usually elevated prior to renal transplantation and decreases rapidly following transplantation to within or below range for patients with normal kidney function in the first post operative month [70, 74-75]. Post transplant hypophosphatemia has been reported in several studies in patients who were at least 6 months post transplant [1, 80, 83].

Conclusion

This study demonstrated that elevated serum iPTH levels are present even after renal transplantation and are not associated with graft function. It also revealed presence of low PTH levels though the prevalence is lower than those reported in the CKD counterpart. This may suggest continuing presence of adynamic bone disease if PTH levels are used as surrogate markers for bone turnover.

Hypocalcemia was present in almost half of renal transplant recipients as opposed to hypercalcemia reported in other studies. This may be as a result of higher prevalence of adynamic bone disease in our CKD patients. Hypocalcemia was associated with relatively younger patients and was not correlated with graft function as estimated by GFR.

Majority of our patients had serum phosphorus levels within reference ranges.

Recommendations:

- Monitoring for levels of serum PTH in addition to calcium, phosphate and creatinine since elevated PTH has been associated with fractures, bone pain and may contribute to cardiovascular morbidity and mortality among renal transplant recipients.
- Monitoring of serum calcium in young post transplant patients who may have hypocalcemia which may lead to elevated serum PTH.
- Studies should be done to look into the role of serum levels of vitamin D in CKD-MBD among our renal transplant recipients.
- Longitudinal follow up studies (from pre pretransplant to post transplant period) on mineral bone parameters should be done to look for trends and clinical outcomes among renal transplant recipients.

Limitations of the study

This was a cross-sectional study thus it was difficult to show temporal relationship among mineral bone parameters.

We could not test for vitamin D status in this population because of financial constraints

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APPENDIX 1

Albumin estimation (Mindray Clinical Chemistry Analyzer)

Principle of the method:
At a controlled pH, bromocresol green forms a colored complex with albumin.
The intensity of color t 360 nm is directly proportional to albumin content.

Procedure:

1. 10µl of sample is mixed with 1000µl of reagent and mixed well
2. The mixture will be incubated at 37˚C for 2 minutes
3. Absorbance will be read at 630 nm
4. Concentration of albumin will be reported in g/l

Phosphate (Mindray Clinical Chemistry Analyzer)

Principle of the method:
Phosphorus reacts with ammonium molybdate in an acid medium to form a phosphomolybdate complex which absorbs light at 340nm. The absorbance at this wavelength is directly proportional to the amount of phosphorus in the sample.

Procedure:

1. 20µl of the sample is mixed with 1000µlof reagent and mixed well
2. The mixture will be incubated at 37˚C for 5 minutes
3. Absorbance will be read at 340nm
4. Concentration of phosphate will be reported in mmol/l

Calcium estimation (Mindray clinical Chemistry Analyzer)

Principle of the method:
Arsenazo III reagent reacts with calcium to form a bluish-purple colored complex. The amount of color formed is measured by an increase in absorbance of the reaction mixture at 630 nm. The intensity of color is directly proportional to calcium present in the sample.

Procedure:

1. 5µ of sample is mixed with 350µl of reagent and mixed well
2. The mixture will be incubated at 37˚C for 1 minute
3. Absorbance will be read at 630 nm
4. Concentration of calcium will be reported in mmol/l

Creatinine estimation (Mindray Clinical Chemistry Analyzer)

Principle of the method:

Creatinine reacts directly with picrate ion under alkaline conditions to form a red-orange compound, called a Janovski complex, with an absorbance peak at 520 nm whose color intensity is directly proportional to the creatinine concentration in the sample.

Procedure:

1. 10µl of sample will be mixed with 1500µl of working reagent and mixed well
2. The mixture will be incubated for 5 min at 37˚C
3. Absorbance will be read at 520 nm
4. Creatinine concentration will be expressed in µmol/l

Estimation of PTH (Cobas® 601)

The equipment uses immunoassay technique for the in vitro Quantitave determination intact parathyroid hormone in human serum and plasma for the differential diagnosis of hypocalcemia and hypercalcemia.

Principle of test

1st incubation: 50µl of sample, a biotinylated monoclonal PTH-specific antibody, and monoclonal PTH-specific antibody labeled with a ruthenium complex form a sandwich complex.
2nd incubation: after addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with procell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
Results are determined via a calibration curve which is instrument-specifically generated by a 2-point calibration and a master curve provided via the reagent barcode. The methodology is fully automated and will require the use of 30µl of serum and results generated after about 20 minutes in print-out.

APPENDIX 2

CONSENT FORM

I, ---------- consent to participate in the study on calcium, phosphate and parathyroid hormone levels in kidney transplanted patients on follow up at Kenyatta National Hospital. I do this with full understanding of the purposes of the study and the procedures involved which include filling out a study questionnaire and having laboratory tests, all of which have been explained to me by Dr. Kalokola.

Signature of patient----------
Signature of witness----------
Date----------

If you have questions during the course of the study, you may contact the following:

Dr. Fredrick Kalokola
Mobile phone: 0716- 405-846

OR

The Chairman of Ethical and Review Committee
Kenyatta National Hospital
Tel:254 020 2726300 ext 44355, 726300-9

IDHINI

Nambari ya hospitali……. Umri……..

Mimi……...
Natoa idhini mwenyewe bila aina yoyote ya kushurutishwa au kulazimishwa kushiriki katika utafiti uliotajwa hapa kuhusu utafiti wa viwango vya madini ya "calcium" na "phosphate" na hormoni ya "parathyroid" kwa wagonjwa waliopandikizwa figo. Nimeelezewa kikamilifu kuhusu madhumuni na hali yake na naelewa kuwa nitaulizwa maswali kadhaa na nipimwe damu. Pia naelewa kuwa naweza kujiondoa wakati wowote iwapo nitabadilisha mawazo.

Sahihi ya mshiriki……
Sahihi ya shahidi…….
Tarehe……..

Ukiwa na maswali au jambo lolote unahitaji kuelezewa zaidi tafadhali wasiliana na Dkt. Fredrick Kalokola kwa nambari ya simu ifuatayo: 0716 405 846.

Asante

INVESTIGATOR'S STATEMENT

I the investigator have educated the research participant on the purpose and applications of this study.

Signed…… Date…….

APPENDIX 3
Statement of information Form

SERUM CALCIUM, PHOSPHATE AND PARATHYROID HORMONE LEVELS IN KIDNEY RECIPIENTS

Statement of information for Patients Participating in the Study

Purpose of the Study
I, Dr Fredrick Kalokola, am undertaking a study to learn about the levels of calcium phosphate and parathyroid hormone in patients who are recipients of kidney transplant on follow up at Kenyatta National Hospital. The calcium and phosphate are minerals that are important in maintaining a normal bone function. The parathyroid hormone is a protein that helps in regulating the levels of calcium and phosphate in the body. The study will be conducted at this hospital with cooperation from the staff and permission from the hospital administration.

Procedures

You are being asked to participate in a survey that will take between 20 and 30 minutes. If you agree to participate, I will ask you the questions and note your responses in writing. I will then send you to the laboratory for blood tests. I will inform you of the test results. All the results will remain confidential. The purpose of this consent form is to ask your permission to do so. If you agree to participate, I shall ask you to sign the consent form. However, this form will not be linked to your answers. Your individual responses will be seen only by the researchers, and will be stored in a locked place under their control.

The risks to you as a participant in this study include:

- Pain in the cubital region on your arm upon venepuncture
- Swelling at the venepuncture site may appear, this is the collection of blood under the skin (hematoma)
- NB: should any of the above happen to you, feel free to contact Dr Fredrick Kalokola for examination and management.

The benefits to you as a participant in this study include:

- Free evaluation of your current kidney function tests
- Free estimation of your Glomerular Filtratation Rate
- Free evaluation of your blood calcium and phosphate levels
- Free evaluation of the level of your parathyroid hormone (a protein which helps with control of the calcium and phosphorus metabolism in the body)
- A free copy of your results will be availed to you upon request
- The findings of this study may identify ways of preventing some complications that may arise after kidney transplantation.

Right to Refuse or Withdrawal

Your participation I this research is voluntary. You do not have to participate. If you do choose to participate, but prefer not to answer certain questions, you are free to do so. You are also free to terminate the interview and withdraw from the study at any time.

You are free to ask questions before signing the consent form. If you agree to participate in the study, please sign on the consent form.

APPENDIX 4

STUDY QUESTIONNAIRE

SERUM CALCIUM, PHOSPHATE AND PARATHYROID HORMONE LEVELS IN KIDNEY TRANSPLANT RECIPIENTS

PATIENT RECORD FORM

I. GENERAL DATA

Study number

Hospital Number

Study Date

Date Month Year

NAME

Last Name First Name Middle Name

SEX

1= Male 2= Female

DATE OF BIRTH

Date Month Year

Physical address: (District / Estate / Village / Location)

_____

_____

Telephone Number: _____

Marital Status: [ ]

1= Married; 2= Single; 3= Widowed; 4= Separated; 5=Other

Highest Educational Attainment: ☐

1= No formal Education; 2= Primary; 3= High School; 4= College / University

II. MEDICAL HISTORY.

1. Etiology of the kidney disease_____
2. Duration of dialysis prior to kidney transplantation (months) ☐
3. Time since transplantation (months) ☐

Appendix 5

LABARATORY PARAMETERS

BIOCHEMICAL PARAMETERS (MEASURED / CALCULATED)

LAB EXAM	RESULT	Date:
Plasma intact PTH (pmol/L)		
Serum Creatinine (mmol/L)		
Calculated (GFR) creatinine clearance (ml /min /1.73m2)		
Serum Calcium (mmol/L)		
Serum albumin		
Corrected serum calcium (mmol/L)		
Serum Phosphorus (mmol/L)		
CKD(KDOQI) STAGE (2/3/4/5)		