Controversies surrounding the use of Parathyroid hormone as a marker of bone metabolism in chronic kidney disease(CKD) patients on haemodialysis in Zimbabwe: The way forward

BY

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THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE DEGREE OF MASTER OF SCIENCE IN CLINICAL BIOCHEMISTRY.

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DEPARTMENT OF CHEMICAL PATHOLOGY

FACULTY OF MEDICINE

SEPTEMBER 2013

ACKNOWLEDGEMENTS

This thesis could not have been completed without the advice, encouragement and supervision of Mr T.Nyamayaro. Thank you for your support, encouragement and guidance. Your enthusiasm is what kept me going.

Secondly, I would like to thank all my friends for all the help and contributions to my project. You made it all worthwhile. To Dr L. Muchemwa I say thank you for your guidance.

My acknowledgements go to the members of staff at Parirenyatwa hospital renal unit, Chitungwiza hospital renal unit and PSMI renal and laboratory units for all the assistance you gave me towards this project. Beckman coulter and Medtech I salute you for helping me with the procurement of my reagents.

Last and by no means least, I would like to thank my family at home and abroad who supported me throughout, to cite in particular my wonderful sister Mrs C. Chapwanya and DR A. Chapwanya my brother-in-law who tirelessly helped me to bring this thesis into shape. Finally a big thank you to my parents for everything you have given me.

I give glory to my God the Almighty for his faithfulness and dedicate this work to my parents.

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LIST OF ABBREVATIONS

CKD	Chronic Kidney Disease
KDOQI	Kidney Disease Outcomes Initiative
GF R	Glomerular Filtration Rate
ROD	Renal Osteodystrophy
PTH	Parathyroid Hormone
ADB	Adynamic Bone Disease
ALP	Alkaline Phosphatase
bALP	bone Alkaline Phosphatase
OC	Osteocalcin
PIPC	Procollagen Type 1 Carboxy -Terminal Peptide
TRAP	Tartarite Resistant Acid Phosphatase 5b
ICTP	Procallagen Type 1 Cross-linked Terminal Telopeptide
CTx	C-Linked Terminal Telopeptides
NTx	N-Linked Terminal Telopeptides
HTBD	High Turnover Bone Disease
LTBD	Low Turnover Bone Disease
Mg	Magnesium

Phos	Phosphate
Cal	Calcium
ALB	Albumin
Creat	Creatinine
RBS	Random Blood Sugar
Na^+	Sodium
k^+	Potassium
CAMP	Calcium, Albumin, Magnesium, Phosphate
CV	Coefficient of Variance
GEDTA	Glycoletherdiaame-N,N,N,N Tetracetic Acid
NADH	Nicotinamide Adenosine Dinucleotide Hydrogenase……
NAD	Nicotinamide Adenosine Dinucleotide ….
nm	nano moles
µg/L	Micrograms per litre
mmol/l	Millimoles per litre
pg/mL	Picograms per millilitre
µmol/L	Micromoles per litre
GLM	General Linear Modules

T1 -T4 Time 1-Time 4

% Percentage

ABSTRACT

Background

The progressive loss of renal function in chronic kidney disease (CKD) necessitates the eventual use of dialysis. Renal Osteodystrophy (ROD) is a serious complication of CKD with diverse and non-specific manifestations. Serum biochemical markers such as Parathyroid hormone (PTH) are used worldwide to monitor bone changes in patients on haemodialysis although bone biopsy has remained the gold standard. The use of PTH as a marker for bone changes has however been associated with a lot of controversies linked to its measurement and interpretation. Recommendations from several studies have been proposed on how to improve its clinical robustness in monitoring bone changes of patients on haemodialysis. Evaluation of these recommendations has however not been done extensively especially in Zimbabwe.

Objectives

To determine which serum marker(s) of bone offers the best diagnostic and monitoring sensitivity in CKD patients on haemodialysis before and during three months on haemodialysis.

Materials and methods

A short prospective study was carried out on CKD patients admitted on haemodialysis at three selected Renal Units (Parirenyatwa, Chitungwiza, PSMI). A total of 32 patients aged 22-75 years (Mean 50.5 ± 16.5 years) were randomly selected. Study group was divided into

those who had been on dialysis for more than three months (Group 1) and those who had been enrolled to start haemodialysis (Group 2). Serum levels of calcium, phosphate, PTH and bALP were determined over a three month period.

Results

There were significant increases in bALP (P=0.02) in group 1. A 69.6% increase in mean phosphate was observed in participants using low calcium concentration dialysate fluid.PTH and bALP showed a positive correlation at both 0.05 and 0.01 level of confidence interval using Pearson correlation 2-tailed test. Significant gender differences (P<0.05) in PTH were observed in both groups. Statistically insignificant decreases in PTH were noted for both groups.

Conclusion

The study findings have shown that PTH levels in an individual patient on haemodialysis fluctuate greatly and therefore diagnosis and monitoring of bone changes in these patients ought to be based on trends rather than a single value of PTH. The study concludes that bALP is indeed a potentially useful bone marker if we are to make headways in resolving some of the issues associated with the use of PTH alone.

CHAPTER ONE

1. INTRODUCTION

Chronic kidney disease (CKD) is a progressive loss in renal function over time. It is an emerging public health problem associated with serious complications such as cardiovascular pathology and renal osteodystrophy (1). The onset of the disease can be sudden or over time and is not limited to any particular gender or age group, although the aged are over represented in sufferers (2). Renal function, and in particular glomerular filtration rate (GFR) is finely regulated by angiotensin and corticosteroid hormones among other factors (3). The Kidney Disease Outcome Quality Initiative 2002 (KDOQI) concluded that the risk for CKD increases as GFR falls below 60ml/min/1.73m^2 for a period of more than three months (4). GFR is the index used to measure the severity of kidney disease. The KDOQI distinguishes five grades of CKD based on severity and recommends classification into CKD stages 1-5. Stage 1 is characterised by a normal kidney function with some evidence of kidney disease such as haematuria and protenuria or structurally abnormal kidneys like renal dysgenesis.Patients with CKD stage 2 present with the same renal problems as in stage 1 in addition to a slightly reduced kidney function with GFR of between 60-90ml/min/1.73m^2. In stage 3 and 4 renal function is moderately to severely reduced with GFRs of 30-49 and 15-29ml/min/1.73m^2 respectively. The last stage of CKD, end stage renal failure is when the patient now survives on dialysis with GFR of less than 15ml/min/1.73m^2 and severely disturbed bone metabolism. Recent research has shown that 1 in 10 people have some form of CKD at any given age (2).

This is especially so among women and those aged above 75 years, the latter being due to aging kidneys. Aging kidneys as the cause of CKD rarely progress beyond stage 3 unless if complicated by diabetic mellitus and hypertension (2).The burden of CKD on health and social services lies in the cost of renal replacement therapy and the emerging complications of CKD. According to a study by Moeller 2002, it is estimated that the number of people with end stage renal disease now on renal replacement therapy is approximately 1.4 million in the 122 countries studied worldwide (5). Other studies report that many who suffer with stage 5 CKD in developing countries die due to lack of funds to access regular maintenance dialysis as well as due to limited dialysis units .It is thought that less than 5% of all dialysis units in North Africa are distributed predominantly in urban centres (6). Cardiovascular accident is one of the most prevalent complication of CKD, accounting for about 40% deaths among patients on dialysis in Australia in 2003 (6). Other complications include cancer, respiratory infections and bone and muscle problems. Nearly all patients with CKD experience some form of renal osteodystrophy which has now an overall incidence of between 90-100% in patients with advanced renal disease and those treated with maintenance haemodialysis worldwide (7).This study has therefore focused on the issues in the monitoring of renal osteodystrophy owing to its high prevalence, and its impact on the lives of the sufferers. Furthermore in Zimbabwe, as in many developing countries renal osteodystrophy is sometimes overlooked, and information about renal osteodystrophy is sparse among our CKD patients..

1.1 Overview of Renal Osteodystrophy

Renal osteodystrophy (ROD) describes a severe bone disease common among CKD patients. It occurs when kidneys fail to maintain the proper levels of calcium and phosphorus in blood. In the early stages of CKD (stages 2 and 3) this manifests as disorders of calcium and phosphorus metabolism, and their regulating hormones parathyroid hormone and calcitriol. (8). Factors implicated in the pathogenesis of renal osteodystrophy include the inability of affected kidneys to produce adequate calcitriol,reabsorb calcium and excrete Phosphate thus causing hypocalcaemia and secondary hyperparathyroidism. Recently studies by Harris 2006 and Goodman 1994 have reported other causes associated with dialysis being the use of calcium containing phosphate binders orally or in dialysate fluids (9, 10). Some of these factors may dominate throughout the course of the disease and as a result ROD manifests differently in individual CKD patients. The disease is therefore classified into three main categories; *high turnover bone disease* (osteitis fibrosa) more common with haemodialysis, *low turnover bone disease* (adynamic bone disease and osteomalacia) more common with peritoneal dialysis and *mixed uremic osteodystrophy* (osteitis fibrosa and osteomalacia) (11).

Osteitis fibrosa is a high turnover bone disease characterised by high bone formation and resorption rates. It occurs in secondary hyperparathyrodism patients when excess production of PTH results in the proliferation of osteocytes in long bones. Unfortunately these cells are unable to produce normal bone, instead woven unstructured bone often with evidence of erosion cavities and marrow fibrosis are formed due to the high rates of cell proliferation (11). Initially it manifests as hyperphosphotaemia which becomes significant as the GFR fall below 40 ml/min/1.73m^2 and when about 50% of kidney function is lost (8, 12).

Hyperphosphotaemia enhances formation of calcium-phosphate complexes which in turn lower serum calcium levels.

For many years it was believed that the Barkers 'new steady state' is developed following hyperphosphotaemia (12). According to his 'Trade of hypothesis' hyperphosphotaemia by causing hypocalcaemia will stimulate PTH production which has the effect of increasing calcium reabsorption and phosphate excretion resulting in normalisation of both minerals at a higher set point of PTH (12). However it was demonstrated that this pattern was not shown by every patient (13). Hyperphosphotaemia has been shown to directly stimulate parathyroid hyperplasia and to lower arachidonic acid production by the parathyroid gland both of which have the effect of increasing PTH production (13).

Mixed uremic osteodystrophy exhibit both features of high and low bone turnover i.e. high rates of bone formation with evidence of marrow fibrosis, and abnormal bone mineralisation with osteoids. Osteomalacia is another manifestation of ROD which shows low bone formation with a high volume of unmineralised bone, the osteoids (8). This occurs due to inadequate production of calcitriol, the active form of vitamin D. Calcitriol is synthesised by the kidney via the hydroxylation of vitamin D in the presence of α – hydroxylase an enzyme located in the kidney epithelial tubular cells. In diseased kidneys this enzyme synthesis is reduced resulting in less calcitriol production. Calcitriol is important for calcium absorption in the gastrointestinal and for bone mineralisation. In the absence of adequate calcitriol weak and unstable bone structures are therefore formed (14).

The last type of renal osteodystrophy which has been found increasingly of late amongst CKD patients on dialysis is the adynamic bone disease (ADB). This is characterised by low bone formation with normal to low bone mineralisation (15). Previously, the use of aluminium in dialysate as phosphate binders was the predominant cause of adynamic bone disease in dialysis patients. Recent studies have shown occurrences of adynamic bone disease in the absence of aluminium (16) and its prevalence has been shown to be increasing amongst CKD sufferers being treated with maintenance dialysis. It is postulated that aggressive use of calcium in diet, dialysates and calcium containing phosphate binders and administration of vitamin D analogs over suppress PTH production below the recommended target range of 150-300 pg/ml resulting in low bone formation (1,17,18,19,20). In USA in 2007, 25% of patients on dialysis had PTH levels above target range (>300pg/ml) (high bone turnover), 25% had PTH within the target range and 50% had PTH levels below 150pg/ml (adynamic bone disease) (13). The relative prevalence of high or low bone turnover disease differs with communities and the dialysis modalities used (21). In Zimbabwe, CKD patients attending renal clinic at the selected hospitals Parirenyatwa and Chitungwiza are using high calcium containing dialysis fluid (1.75 mmol/l) and are therefore at risk of developing the ADB with time. Furthermore studies done elsewhere have shown that use of low dialysate calcium in haemodialysis (1.25-1.3 mmol/l) has the effect of reversing bone ADB. The same study also showed that low dialysate calcium allows the use of vitamin D supplements and calcium containing phosphate binders without a risk of developing hypercalcaemia, calcification and low bone turnover disease (9, 22).

In advanced CKD stages, the loss of renal mass accompanied by reduced synthesis of calcitriol and the increasing resistance to parathyroid hormone (PTH) actions by the kidney

and skeletal tissue all necessitate higher levels of PTH to maintain normal bone metabolism, resulting in secondary hyperparathyroidism. The biochemical picture shown is that of high PTH levels, hypocalcaemia, hyperphosphotaemia and low calcitriol. This forms the basis of renal treatment of using phosphate binders such as calcium carbonate to reverse hyperphosphotaemia, calcitriol and other vitamin D analogs to correct hypocalcaemia. It is postulated that the aggressive use of these treatment modalities has resulted in the shift from the above biochemical picture to that shown in low turnover bone disease of a low serum PTH and hypercalcaemia (14,15) as discussed above.

Renal osteodystrophy is most serious in children because their bones are still growing (23). The condition slows bone growth and causes deformities. Legs may bend inward toward each other or outward away from each other; this deformity is referred to as "renal rickets." Another important consequence is short bone stature. In adults if left untreated, the bones gradually become thin and weak with increased risk of bone fractures. According to Edgar and Lerma, patients with ROD usually tend to be clinically asymptomatic and for those with symptoms they tend to be generally vague and nonspecific (23). Patients may complain of joint and bone pain mainly of lower back, hips and lower extremities which is a common symptom. General pruritus is another presentation especially in patients already on maintenance renal replacement therapy owing to deposition of calcium phosphate in the skin (12).

1.2 CLINICAL UTILITY OF BIOCHEMICAL SERUM MARKERS OF RENAL OSTEODYSTROPHY (ROD)

Patients with renal osteodystrophy are usually asymptomatic, so diagnosis is based on laboratory tests. At present, circulating biomarkers such as PTH, Alkaline phosphatase (ALP), phosphate and calcium, as well as bone biopsies are used to evaluate bone turnover. However the gold standard for a definitive diagnosis of CKD related bone disease is through bone biopsy. The disadvantage of this method is that it is invasive, expensive and an anaesthetic risk (24). Routinely, biochemical markers are measured to monitor treatment in dialysis.

Measurement of PTH has been widely used since PTH is the major regulator of bone turnover and skeletal cellular activity.PTH is a single chain polypeptide (84 amino acids) hormone produced by the parathyroid gland in responses to hypocalcaemia. It functions to restore serum calcium by increasing osteoclastic resorption and renal calcium reabsorption and phosphate excretion and calcitriol synthesis (8, 25). However its use of late has been associated with a lot of controversies about its measurement and interpretation of the findings (26). The use of iPTH immunometric assays suffer from cross reactivity with large PTH fragment (fragment 7-84) and the tendency to overestimate PTH levels in dialysis patients (26). Second generation immunometric assays (bioPTH) that measure the entire PTH molecule (1-84) were thought initially to be more helpful in differentiating between high and low bone turn over, but studies have failed to prove this in dialysis patients and children (17,27). Determination of PTH levels may establish the diagnosis and define the severity of secondary hyperparathyrodism, but it can't predict the type of bone disease especially where PTH levels are only slightly elevated (24). PTH levels <100 pg/ml are associated with

presence of ADB, whereas that of >450 pg/ml are associated with high turnover bone disease or mixed disease.PTH in the range between 100-450 pg/ml are associated with normal bone or any of the above patterns (24).

The interpretation of PTH results has always been based on the recommendation of target PTH by the K/DOQI (2005) of 150-300 pg/ml for patients on CKD stage 5 and on dialysis (1). However a randomized trial by Barreto and co-workers in 2008 proved that the K/DOQI recommended intact PTH levels of 150-300 pg/ml did not prevent development of adynamic bone disease owing to the different assay methods and dialysis treatment modality used (28). Furthermore with a half life of only a few minutes, single values of PTH may be misleading being unable to distinguish ROD disease, and recommendations made by other researchers are that it is preferable to evaluate the trend of PTH levels in diagnosis and subsequent treatment decisions (24, 26, 29.). Optimal target PTH values at all stages of CKD remain controversial and hence the need for more studies. It is on the basis of these PTH issues and recommendations that this study is done to test this approach prospectively by measuring PTH levels in CKD patients before they commence dialysis treatment and thereafter for three months. The pre-dialysis values will form the baseline upon which other values will be compared thereby overcoming the problem of interpretation of PTH values. This will also standardise the variation in PTH measurement due to assay method and individual variations. Furthermore establishment of different target PTH ranges for patients on different stages of CKD will allow individualised therapy based on the PTH trend shown by the patient. Renal replacement therapies associated with less risk of ROD are now available elsewhere in the world (29). One of these is a non-calcium containing phosphate binder Sevelamer hydrochloride which has been found to be extremely useful in patients on dialysis being able

to control phosphate levels and at the same time maintaining calcium levels within homeostatic levels (30, 31). In USA the use of vitamins analogs (such as neither 19-Nor 1.25dihydroxyvitamin D_2) which have been structurally altered in such a way that there is avoidance of over suppression of PTH has been introduced. Paricalcitrol is another analog which has proved to have a survival benefit for those who have been using it (32). Considering such developments results of an individual becomes more useful in choosing the most appropriate therapy.

Given the limitations and uncertainty of PTH estimations for accurate non invasive diagnosis of bone turnover and management of therapy in CKD patients, it is clear that other new markers of bone remodelling are needed. Several studies have shown that a combination with other new markers improves PTH utility as a marker of bone metabolism (33). The newer markers are divided into those of bone formation and bone resorption. Bone specific Alkaline phosphatase (b ALP), osteocalcin (OC), procollagen type 1 carboxy-terminal peptide (PIPC) are indicative of formation while pyridinole, deoxypyridinole, tartarite-resistant acid phosphatase 5b (TRAP), procollagen type 1 cross linked c-terminal telopeptide (ICTP) and C and N terminal telopeptides (CTx and NTx) signify resorption (34,35). OC is the most abundant non-collagenous protein of the matrix produced by osteoblasts in response to calcitriol hormone. It has a high sensitivity in distinguishing between high turnover bone formation (HTBD) and normal or low turnover bone disease (LTBD) but not between normal and LTBD. PIPC is a by product of collagen synthesis and increases with high bone turnover. It is not affected by renal clearance thus reflect bone metabolism in pre-dialysis but not useful in haemodialysis. Pyridinole and deoxypyridinole are products of degradation of collagen normally excreted in urine. In renal disease serum levels increase 50-100-fold especially in

HTBD and correlates well with PTH in LTBD and useful for evaluating ROD especially deoxypyridinole (34,36). However studies by Ferreira and co-workers showed that the high levels of PICP measured in CKD do not correlate with the parameters measured in biopsies nor with other serum markers of bone turnover (37,38). The clinical value has not been established in the diagnosis of bone disease in haemodialysis (37). TRAP isoforms 5b is a lysosomal enzyme produced by osteoclasts during bone resorption. Its concentration reflects the number of osteoclasts and percentage of bone surface resorption. It rises in end-stage renal disease and correlates well with PTH and ALP. Its value still remains to be established and more sensitive and simple methods to be evaluated (36). ICTP is a component of type 1 collagen that is released during bone resorption with levels rising not only in CKD but also in bone metabolism disorders hence reducing its sensitivity as a marker of bone metabolism in uraemia (39). CTX is another type I collagen released during bone resorption and is excreted by the kidney. In CKD there is an increase in CTX and effect of renal replacement therapy on its concentration is only noted after six months of treatment (40).

This study will focus on measurement of bALP and PTH because several studies have shown that the combination of PTH and bALP improves the specificity and sensitivity of diagnosing ROD in suffers (41, 42).

Bone alkaline phosphatase (bALP) is an 80kda glycoprotein anchored in cellular membrane of osteoblasts by the hydrophobic glycosylphospatidylinositol anchor where it catalyses the hydrolysis of pyrophosphate an inhibitor of bone mineralization. bALP serum levels reflect specifically the rates of bone formation as its rate is not affected by renal clearance or dialysis. Owing to the coupling effect of bone formation and resorption bALP measurement reflect overall bone metabolism (43). Development of specific antibodies for bALP provided

a more specific index of bone formation. Studies by Urena 1996 showed that bALP by the Ostase method for diagnosis of ADB has a specificity, sensitivity and positive predictive value of 100% as compared to that of iPTH of 72%, 80% and 47% respectively (39,41). The same study has also demonstrated that using a combination of iPTH and bALP increases the sensitivity and specificity in diagnosis of ROD by 80% and 100% respectively using cut off points of PTH of 200pg/ml and bALP of 20 mg/ml (42,43). Coutenye 2007, using agarose gel electrophoresis demonstrated that bALP<27u/l and PTH<150pg/ml are gold markers of ADB (44,45).

1.3 RATIONALE

CKD related bone diseases have clinically relevant repercussions such as bone pain, stunted growth, high risk of bone fracture and cardiac calcification (1). It has been shown that low levels of bALP are associated with high cardiovascular morbidity in patients on haemodialysis (46). Currently circulating biological markers such as PTH and calcium, as well as bone biopsies are both used to evaluate bone turnover. However the gold standard for a definitive diagnosis of CKD related bone disease such as ADB is through bone biopsy. The disadvantage of this method is that it is invasive, expensive and is an anaesthetic risk. Therefore this study is focusing on serum markers which are non-invasive, simpler to obtain but highly diagnostic. In Zimbabwe at present bone biopsies are not readily available and limited to a few hospital sites. Thus in areas where bone biopsies are not possible the use of a combination of bALP, PTH and the routinely used markers such as phosphate, magnesium and calcium as serum markers for ROD may offer acceptable realistic alternatives for diagnosis and monitoring treatment of CKD patients. Given the limitations of measurement and interpretation of PTH a combination of bALP and PTH can be used to monitor bone changes in dialysis in CKD patients and appropriate therapy adjustments can be made. In Zimbabwe, CKD patients attending renal clinic at the selected hospitals are using high calcium containing dialysis fluid and are therefore of at risk of developing the above mentioned ADB with time. Considering the progress made in development of renal replacement therapies that are ameliorating the effect of dialysis on the bone establishment of individualised optimal PTH target ranges will help to reduce the risk of ROD. Accordingly renal replacement therapy must be carefully monitored and adjusted to maintain optimal

serum biochemical parameters of bALP, PTH and calcium within the homeostatic levels according to the stage of CKD (1, 19, 45).

1.4 RESEARCH QUESTIONS

Does a single Parathyroid hormone (PTH) value reflect the whole picture of changes in bone metabolism in CKD patients on maintenance renal replacement therapy at selected hospitals in Zimbabwe?

1.5 HYPOTHESIS

Parathyroid hormone levels decrease in patients with chronic kidney disease undergoing renal replacement therapy.

1.6 OBJECTIVES

To determine the serum marker(s) of bone metabolism (bALP and PTH) individually or in combination that offers the best diagnostic sensitivity in CKD patients before and during three months of renal replacement therapy.

1.7 SPECIFIC AIMS

1.To determine the levels of serum markers of bone metabolism PTH, bALP in CKD patients undergoing renal replacement therapy before and three months on dialysis.

2. To evaluate the clinical utility of serial measurements of combined biochemical markers bALP and PTH in the diagnosis and monitoring of therapy in ROD on CKD patients on renal replacement.

3. To determine the relationship between new serum markers of bone metabolism (bALP and PTH) with the old markers (calcium, phosphate) in CKD patients undergoing renal replacement therapy at selected hospitals.

4. To evaluate the effect of dialysates with different calcium concentration on markers of bone metabolism and ROD.

CHAPTER TWO

2 METHODOLOGY

2.1 Participants

A short prospective study was carried out on 32 CKD patients who were admitted to start renal replacement therapy at selected renal clinics. Participants were recruited from two Central hospitals (Chitungwiza, Parirenyatwa) and one private hospital (PSMI Renal Clinic) within the Greater Harare area. Parirenyatwa is one of the main referral hospital in the country serving patients referred from all the different medical centres within the country .Chitungwiza hospital serve mainly this satellite area and other patients from nearby rural areas. Both Parirenyatwa and Chitungwiza central hospitals offer high dialysate concentration haemodialysis and also peritoneal dialysis. The former because of its large catchment area has more haemodialysis patients (more than 100 at any given time) than Chitungwiza. PSMI Renal centre serve mainly those from within the catchment area of Harare offering both haemodialysis and peritoneal dialysis. In addition it is one of the few centres in the country offering use of a dirty haemodialysis machine hence also attract patients from all over the country requiring this service. Only those patients attending renal clinic with CKD and requiring haemodialysis were recruited voluntarily for this study from the three centers.CKD patients newly admitted on haemodialysis were recruited daily during the study period as they require the service. Ethical approval for the study was sought and granted. All the participants were aged above 18 years (Range 20-79, Average age 50.5 years). No selection restrictions regarding gender, dialysis modalities or cause of CKD was used in recruiting

participants for the study. The participants were selected based on not suffering from diabetes mellitus or undergone para-thyroidectomy or renal transplant.

The participants were then divided into two groups. The first group of participants was composed of CKD patients who had been on haemodialysis for three or more months. The second group was of CKD Patients who had just been admitted for renal replacement therapy and were on calcium carbonate and starting haemodialysis. The two groups were further stratified into those using high calcium concentration dialysate fluid (1.75 mmol/l) and those on lower calcium concentration dialysate (1.50 mmol/l).

For both groups, serum markers and other biochemical tests were measured at the beginning of the project and once every month thereafter for three months as shown in the schedule in Figure 1.

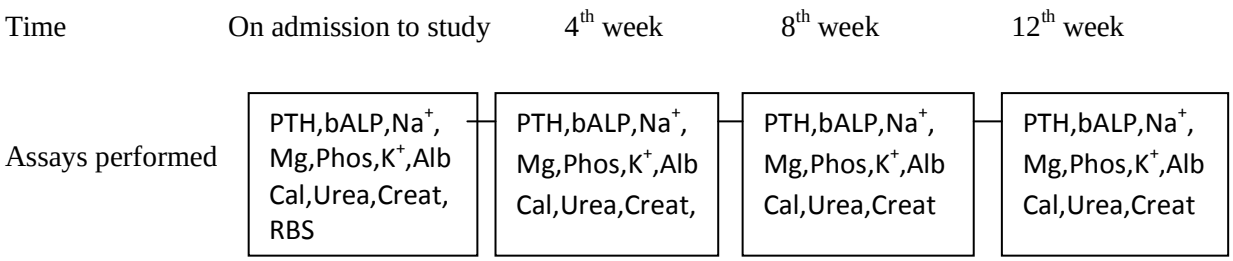

Figure 1. Summary of the schedule showing the times at which serum markers and other biochemical molecules were measured during the study period.

The serum markers and biochemical tests measured were: PTH, bALP, Calcium (Cal),Magnesium (Mg), Phosphate (Phos), Albumin (Alb), Sodium (Na^+), Potassium (K^+), Urea, Creatinine (Creat) .In addition random blood sugar (RBS) was also measured to rule out diabetic mellitus.

Left over venous blood samples collected for routine testing at the beginning of each month at the renal clinic was used to assay the above tests in the two groups. The Cockcroft and Gault method was used to calculate the GFR to monitor the changes in stages of CKD of patients (47). Patients' demographic details were obtained from their records.

2.2 LABORATORY PROCEDURES

2.2.1 Blood collection

Blood was collected from all participants over a three month period at the beginning of each month when patients come for their routine monthly bleeds. For those who were starting haemodialysis, the first blood sample was collected before their very first dialysis session and thereafter monthly as a pre-dialysis sample. Blood was collected via the patient catheter just before dialysis commence, by the renal clinic nurses into plain tubes with clot activators. The samples were immediately collected by the researcher in batches to the laboratory for analysis.

2.2.2 Laboratory analysis of blood samples

Laboratory assays were carried out at Parirenyatwa Hospital Public Laboratory. Left over blood samples collected from participants in plain tubes were centrifuged for 5 minutes at 3000 rpm and serum aliquoted into serum tubes within four hours and frozen at -70^{0}c until analysis. To ensure confidentiality, the serum pots were labelled with a unique identifying number for each patient. This number could not be linked to the patient details by any other person except the researcher. Samples were thawed only once for the assaying of Intact PTH and bALP. The rest of the analytes were analysed immediately prior to freezing. Calcium, Phosphorus, Magnesium, Albumin (CAMP), Urea, Creatinine and glucose were measured on the Beckman AU680 Olympus analyser (Beckman Coulter Instruments, Inc. Fullerton, CA, USA). Intact PTH and bALP were measured on the Beckman Immunoassay Access 2

analyser (Beckman Coulter Instruments, Inc. Fullerton, CA, USA). All reagents used were supplied by Beckman Coulter Instruments and calibrations and use of quality control were performed as per manufacturer‘s instructions.

2.2.3 Measurement procedure for access 2 Immunoassay

PTH and bALP were measured by the immunoassay method. Samples, calibrators and controls were thawed prior to assaying. Calibrations and controls were run according to the manufacturer’s instructions. 0.5ml serum was added to serum cups and assayed on the ACCESS 2 Auto analyser.

2.2.4 Measurement procedure for the AU680 Olympus

Serum calcium, phosphate, magnesium, albumin, urea, Creatinine, sodium and potassium were all measured on the Beckman AU680 Olympus auto analyser .The samples were analysed soon after centrifugation .First calibrations and controls were run and when they passed according to the manufacturer’s instructions the samples were then processed. 0.5 ml 0f the patient serum was poured in the Hitachi cups and the test was programmed on the machine using the machine unique sectors.

2.3 PRINCIPLES OF TESTS

2.3.1 PARATHYROID HORMONE

PTH is measured by the Access intact PTH assay method that uses paramagnetic particles as solid phase and an enzyme mediated chemiluminescent reaction in the detection phase for the determination of PTH in serum and plasma (Ref 48). In the reaction, serum iPTH binds to the monoclonal Anti –PTH on solid phase while goat Anti-PTH-ALP conjugate reacts with a different antigenic site on serum iPTH. After incubation unbound material are washed and a chemiuminescent substrate (Lumi-phase 530) is added which will be catalysed by ALP to produce light. The light is then measured by a luminometer and is directly proportional to iPTH concentration of the sample. The amount is determined from a stored calibration determined from a stored calibration curve. According to the manufacturers claims the assay has a sensitivity of 1 pg/ml and inter assay CVs of 2.6.

2.3.2 BONE ALKALINE PHOSPHATASE

bALP is measured by the Access Ostase immunoassay method. This is a one step immuno-enzymatic assay method that measures serum bALP by use of a mouse monoclonal specific for bALP and solid phase paramagnetic particles coated with goat Anti-mouse polyclonal antibodies. In the reaction vessel the bALP-antibody complex become bound to the solid phase and unbound material is the washed. A chemiluminescent substrate (Lumi-Phos*530) is added and light generated by the reaction is measured with a Luminometer. The light production is directly proportional to the concentration of bALP in the sample. The amount of analyte in the sample is determined from a stored multi-point calibration curve (49).

According to the manufacturer the assay has a sensitivity of 0.1µg/L and inter assay CVs of 4.2.

2.3.3 CALCIUM

Calcium in serum is measured using an automated dye binding calorimetric method on the Beckman AU680 Olympus analyser. In this method calcium ions in the sample react with Arsenazo 111 to form an intense purple coloured Complex. The absorbance of the complex is measured bichromatically at 660 and 700 nm. The resulting increase in the absorbance of the reaction mixture is directly proportional to calcium concentration in the sample. According to the manufacturers claims the assay has a sensitivity of 0.01 mmol/l and inter assay CVs of 0.57% at 1.61 mmol/l and 0.48% at4.19 mmol/l.

2.3.4 ALBUMIN

On the Beckman AU680 Olympus analyser, Albumin is measured by the Bromocresol Green method. A coloured complex is formed when albumin in sample reacts with Bromocresol Green at acidic pH of 4.2. The absorbance of the complex measured at 600/800 nm is proportional to the concentration of Albumin. The assay sensitivity is0.07 g/L and the inter assay CVs of 1.80% and 0.92% at 24.04 g/L and 57.77 g/L respectively.

2.3.5 MAGNESIUM

Magnesium ions in the serum react with Xylidly blue to form a coloured complex in a strongly basic solution of pH 11.4. The absorbance of the coloured complex measured bichromatically at 520/820 nm is directly proportional to the concentration of the magnesium ions in the sample. Calcium interference with this method is eliminated by addition of Glycoletherdiamine-N, N, N, N tetracetic acid (GEDTA). According to the manufacturers claims the assay has a sensitivity of 0.01mmol/L and inter assay CVs of 1.2% and 0.90% at 0.80 mmol/L and 3.02 mmol/L respectively.

2.3.6 PHOSHORUS

An automated dye binding calorimetric method is used to measure serum phosphate on the Beckman AU680 Olympus analyser. Inorganic phosphorus reacts with Molybdate to form a heteropolyacid complex. A surfactant is added to eliminate the need to prepare protein free filtrate. The absorbance of the complex is measured at 340/380nm and is proportional to the concentration on phosphorus. According to the manufacturers claims the assay has a sensitivity of 0.10mmol/L and inter assay CVs of 1.03% and 0.61% at 0.96 mmo/L and

3.36 mmol/L respectively.

2.3.7 UREA

Bun reagent is used to measure blood urea nitrogen by an enzymatic rate method using the Beckman AU680 Olympus analyser. In the reaction urea is hydrolysed by Urease to produce ammonia and carbondioxide.The ammonia produced combines with 2-oxoglurate and NADH in the presence of Glutamate dehydrogenase to yield glutamate and $NAD^{+.}$ The decrease in the NADH absorbance per unit time is proportional to the concentration of urea in the sample. According to the manufacturers claims the assay has a sensitivity of 0.23 mmol/L and inter assay CVs of 1.80% at both 4.90 and 25.64 mmol/L.

2.3.8 CREATININE

Creatinine is measured by the Creatinine Enzymatic method using the Beckman AU680 Olympus analyser. In this method Creatinine is hydrolysed in the presence of creatininase to form Creatine which in turn is hydrolysed to sarcosine and urea by the enzyme creatinase. Sarcosine is oxidatively demethylated by sarcosine oxidase to form glycine, formaldehyde and hydrogen peroxide. The latter then reacts with 4-amino antipyrine and N (3suloproply)-3-methoxy -5- methlyaniline (HMMPS) in the presence of peroxidise to form a blue colour whose absorbance is measured at 600/700 nm. The concentration of Creatinine is directly proportional to the absorbance of the blue pigment formed. According to the manufacturers claims the assay has a sensitivity of 0.88 µmol/L and inter assay CVs of 1.2% and 0.7% at 62.1 and 908.3 µmol/L respectively.

2.4 STATISTICAL ANALYSIS

The participants were firstly placed into two groups, those who have been on dialysis for a period of more than a year (n=18) and those starting dialysis (n=14). The two groups were further stratified into those using high calcium concentration dialysate fluid (calcium = 1.75 mmo/L) and those using a lower calcium concentration dialysate fluid (calcium = 1.50 mmol/L).

Individual plots were drawn to show the changes in levels of calcium, phosphate, PTH and bALP for the two main groups. One way analysis variance was used to check for significance of difference for the measured analytes between gender, age and dialysate concentration for the two groups. Pearson Correlation test was performed to measure the relationship between PTH and each of the three analytes measured, calcium, phosphate and bALP.

Lastly the General Linear Models (GLM) that employs a number of statistical analyses was used to analyse the data firstly using the Multivariate analysis to detect any repeated measures effects (time effect) on calcium, phosphate, PTH and bALP levels by group, age, gender and dialysate concentration used. Secondly by performing the Mauchly Test of Sphericity that test for both effect of homogeneity and correlations between the variances.

The averages of each of the analytes (cal, phos, PTH and bALP) for the four different time periods were calculated using the Bonferroni analysis to show any significant difference between the measurements. These estimated marginal means were then used to draw profile plots showing trends in cal, phos, PTH and bALP for the two groups.

CHAPTER 3

RESULTS

Two groups of participants were involved in this study. Group 1 had participants who had been on haemodialysis for a period of more than three months and Group 2 those who had just started dialysis. Both groups were followed up for three months.

3.0 STUDY PARTICIPANTS CHARACTERISTICS

Table 1. The distribution of the study participants by group, age, and gender and dialysate concentration used.

	Group 1		**Group 2**	
	Frequency	**Percentage**	**Frequency**	**Percentage**
	18	**56.3**	**14**	**46.8**
Gender –Males	**15**	**83.3**	**9**	**64.3**
-Females	**3**	**16.7**	**5**	**35.7**
-Total	**18**	**100**	**14**	**100**
Age -Below 50 years	**8**	**44.4**	**5**	**35.7**
-Above 50 years	**10**	**55.6**	**9**	**64.3**
-Total	**18**	**100**	**14**	**100**
Dialysate concentration				
- High	**10**	**55.6**	**9**	**64.3**
-Low	**8**	**44.4**	**5**	**35.7**
-Total	**18**	**100.0**	**14**	**100**

The summary of sex,age and dialysate concentration characteristics is shown in table 1.There is no significant difference between the two groups , between males and females and betweenthe low and high dialysate concentration used($p>0.05$).The age range of the participants was 20-79 years and the mean age 50.50 years.

3.1 CHANGES IN THE LEVELS OF THE ANALYSED MARKERS PER GROUP WITH TIME

Mean changes in calcium, phosphate, PTH and bALP were calculated per group with the study participants stratified into two groups of gender and concentration of dialysate fluid used. The figures below show these mean changes per analyte.

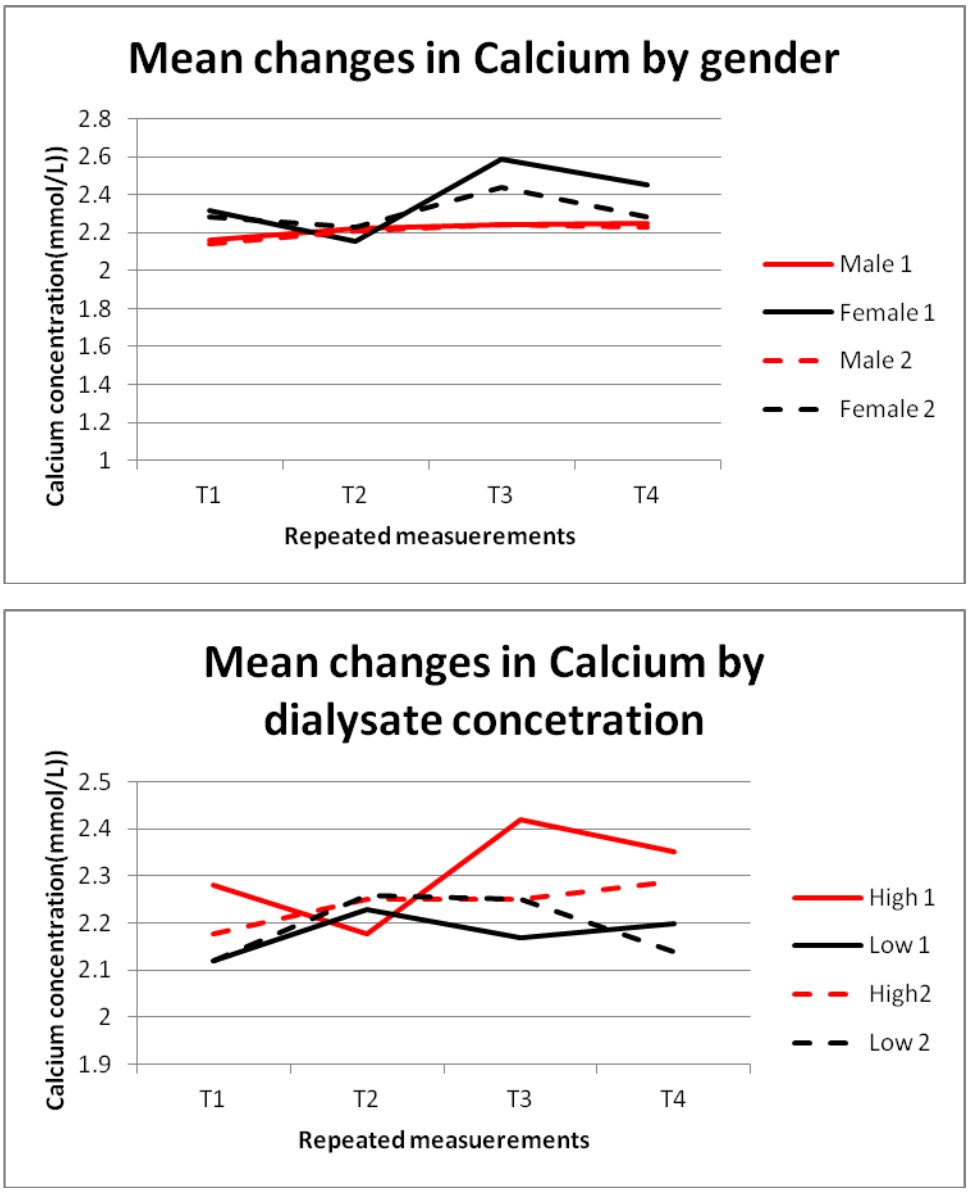

FIGURE 2. Changes in mean calcium levels by gender and dialysate concentration used.

During the period of study mean calcium levels in both males and females participants fluctuated between 2.1 with mean levels being slightly higher in females than males. No group differences are observed with gender (P>0 05). Those using high calcium concentration dialysate fluid show slightly higher mean calcium levels although the difference is not statistically significant

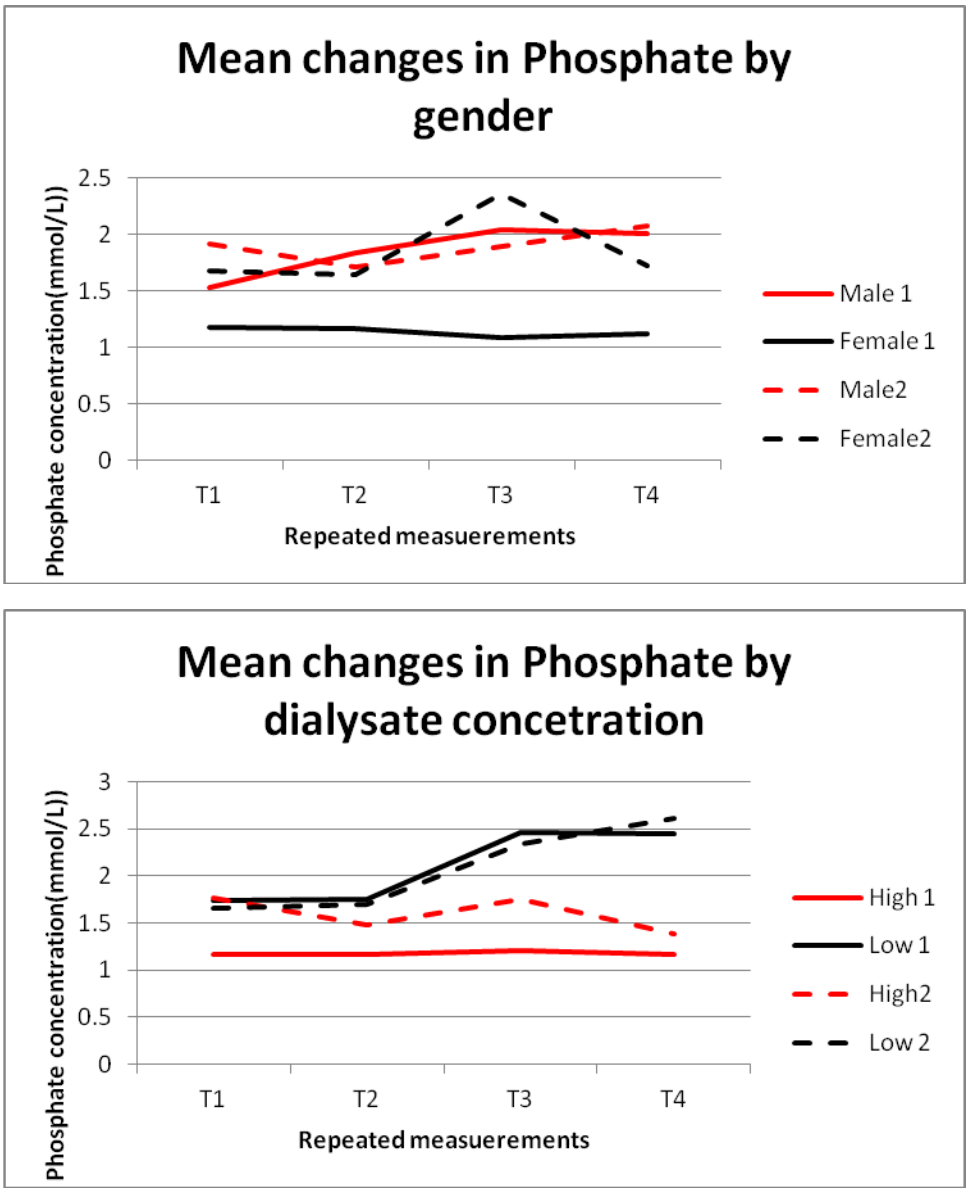

FIGURE 3. Mean changes in phosphate levels by gender and dialysate concentration used.

Both groups showed high mean phosphate levels (above 1mmol/L) within normal but near the upper limit or above the normal upper limit (0.6-1.7).This is regardless of gender or dialysate fluid used. Patients who have been on dialysis for more than 3months show mean phosphate levels which have stabilised above 1mmol/l especially women. A 69.6 % increase in mean phosphate levels was shown in participants using low calcium concentration dialysate fluid in both groups and this difference has been proved to be significant ($P<0.05$).

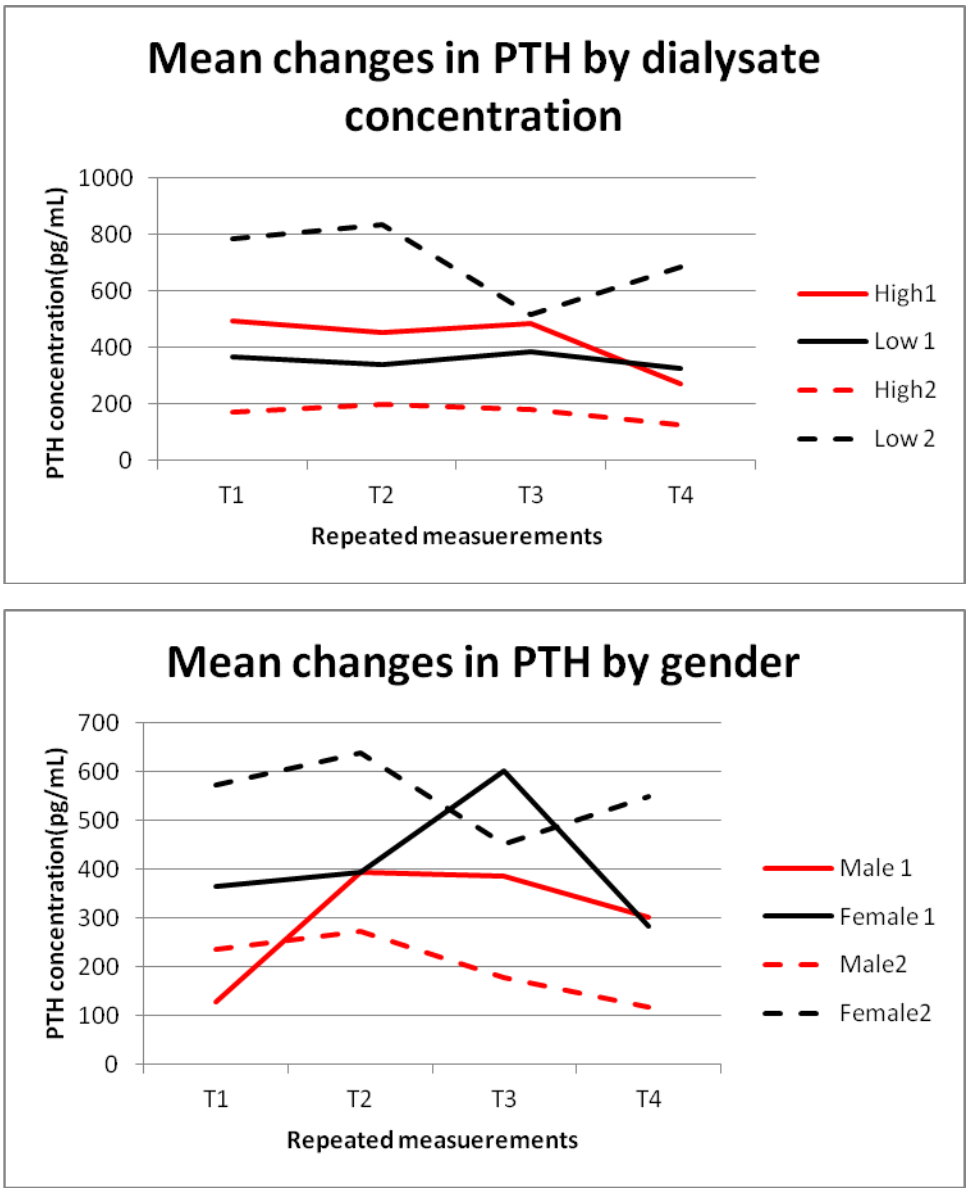

FIGURE 4. Mean changes in PTH by gender and dialysate concentration used.

Higher PTH values obtained with the participants using the lower calcium concentration than those using a higher concentrate dialysate fluid especially so with group 2 (Mean PTH > 500 pg/mL). Females have higher mean PTH (> 300 pg/mL) than males (between 118- 300 pg/mL ie females show slower decrease in PTH than males, (P=0 .044).Initial mean values of PTH in both males and females were higher in group 2 than 1(at T1:235 and573 pg/mL) and (T1 1.29 and 364 pg/mL) respectively.

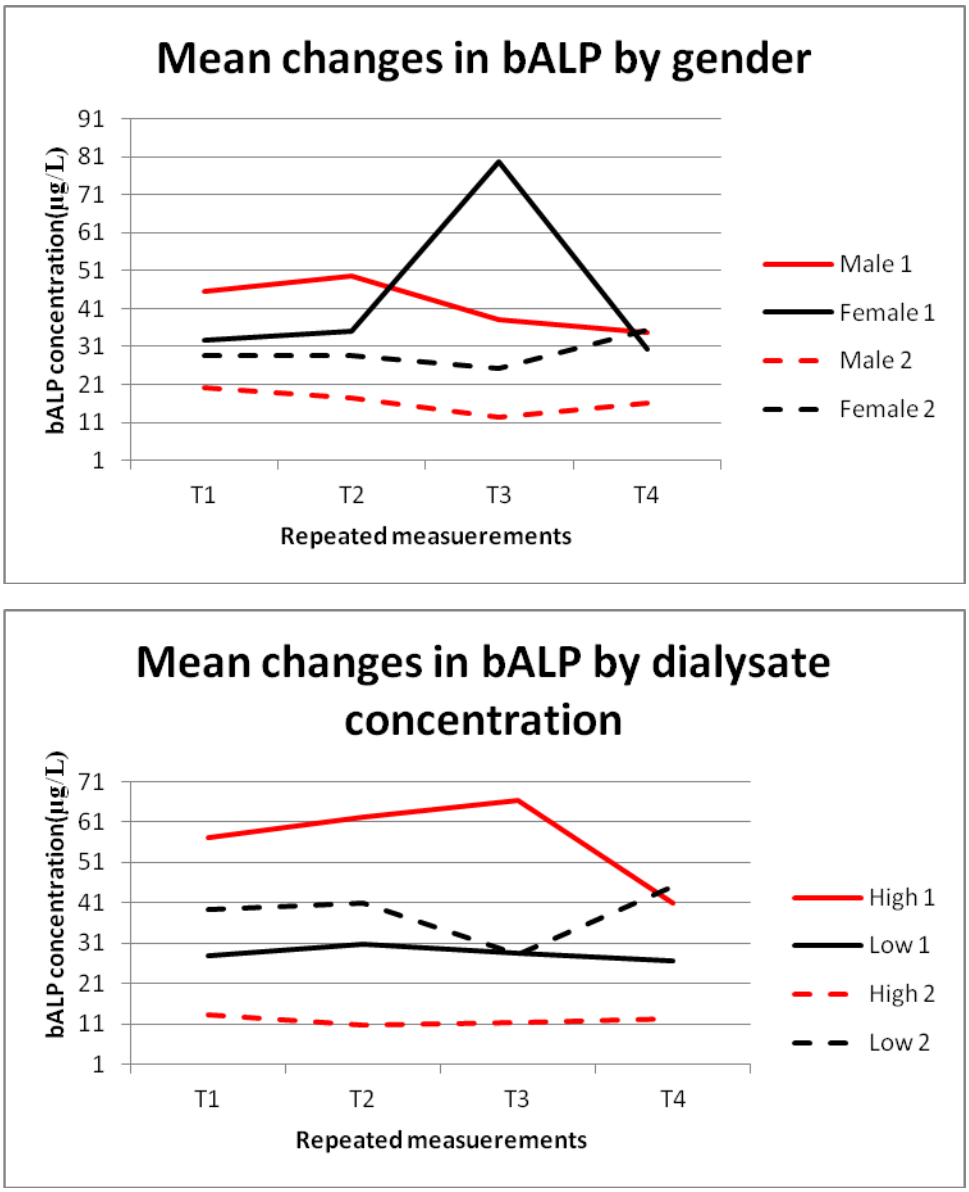

FIGURE 5. Mean changes in bALP by gender and haemodialysate concentration used.

No significant differences noted between males and females (P>0.05). bALP levels stable at 11ug/L in those who had just started dialysis in both males and females receiving high calcium concentrate fluid unlike their counterparts where bALP increases were noted (>41µg/L). These differences in bALP between group was found to significant (P<0.05).

3.2 RELATIONSHIP BETWEEN PTH AND OTHER MARKERS OF BONE CHANGE ANALYSED

Table 2. Correlations between PTH and other markers of bone change.

Correlations

		Mean PTH	Mean CAL	Mean PHOS	Mean bALP
Mean PTH	Pearson Correlation	1	.122	-.156	.649(**)
	Sig. (2-tailed)		.505	.395	.000
	N	32	32	32	32
Mean CAL	Pearson Correlation	.122	1	-.126	.125
	Sig. (2-tailed)	.505		.491	.495
	N	32	32	32	32
Mean PHOS	Pearson Correlation	-.156	-.126	1	-.360(*)
	Sig. (2-tailed)	.395	.491		.043
	N	32	32	32	32
Mean bALP	Pearson Correlation	.649(**)	.125	-.360(*)	1
	Sig. (2-tailed)	.000	.495	.043	
	N	32	32	32	32

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

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

There is positive relationship between PTH and bALP (0.649) which is significant at both 0.05 and 0.01 levels of confidence interval. A negative significant correlation exists between bALP and phosphate (0.05 level of confidence interval). Calcium and phosphate show weak insignificant relationships with PTH.

3.3 COMPARISONS OF MEAN VALUES TO SHOW DIFFERENCES BETWEEN REPEATED MEASUREMENT BY ANOVA AND THE TEST OF HOMOGENEITY

Table 3. P values generated by two statistical analysis summarising significance of differences between the groups, gender and dialysate concentration.

		Group(time)	Gender	Dialysate concentration
Calcium	**1**	0.364	0.392	0.771
	2	0.810	0.901	0.668
	3	0.551	0.928	0.842
	4	0.801	0.779	0.485
bALP	**1**	0.047	0.075	0.565
	2	0.161	0.843	0.271
	3	0.007	0.246	0.150
	4	0.169	0.734	0.746
Phosphate	**1**	0.987	0.506	0.193
	2	0.977	0.054	0.136
	3	0.237	0.584	0.119
	4	0.563	0.438	0 036
PTH	**1**	0.189	0.044	0.494
	2	0.762	0.005	0.420
	3	0.099	0.002	0.546
	4	0.179	0.100	0.197

P< 0.05 shows significant changes. The Mean and F values not shown here

There is no significant change in calcium with regard to time ,gender and type of dialysate concentration fluid used($P > 0.05$).whilst there is significant change in bALP ($P=0.007$) between the two groups with time ,the change in bALP remains non significant with gender and dialysate concentration used. There is significant change ($P=0.036$) in phosphate with type of dialysate fluid used .However the change in phosphate with regard to gender and group(time) is not significant($P>0.05$).Although a statistically significant change in PTH is noted between males and females ($P<0.05$) this is not so with regard to time and dialysate concentration used.

3.4 TRENDS IN BONE MARKERS BY GENERAL LINEAR MODULE

There were no significant changes in PTH, bALP, calcium and phosphate with time for the two groups (p>0 05). The marginal changes observed showed a general decrease in PTH with time for both groups (**Fig 6**). Mean calcium levels remain within normal range (2.05-2.55 mmol/L) with time during the study period for the two groups. Mean phosphate levels for both groups remained high throughout the study period (>1.5 mmol/L) with levels higher in the group who had just started dialysis (mean at time 1=1.73 mmol/L) as compared to 1.46 mmol/L at time 1 for group with more than a year on dialysis (**Table 4).**

TABLE 4. Mean at Time 1 for the serum markers of bone change

	Group(time)		**Gender**		**Dialysate concentration**	
	1 N=18	2 N=10	Male N=20	Female N=8	High N=17	Low N=11
CALCIUM	2.20 SD-0.264	2.60 SD-0.122	2.15 SD-0.27	2.28 SD=0.144	2.23 SD-0.273	2.12 SD-0.193
PHOSPHATE	1.46 SD-0.79	1.73 SD-0.67	1.60 SD-0.83	1.43 SD-0.51	1.57 SD-0.89	1.52 SD-0.51
PTH	429.45 SD-419.27	342.15 SD-494.08	376.35 SD-341.00	452.75 SD-654.37	324.69 SD-418.31	511.98 SD-469.68
bALP	42.64 SD-74.49	21.42 SD-23.71	36.67 SD74.37	31.05 SD-38.01	36.46 SD-79.20	32.92 SD-38.63

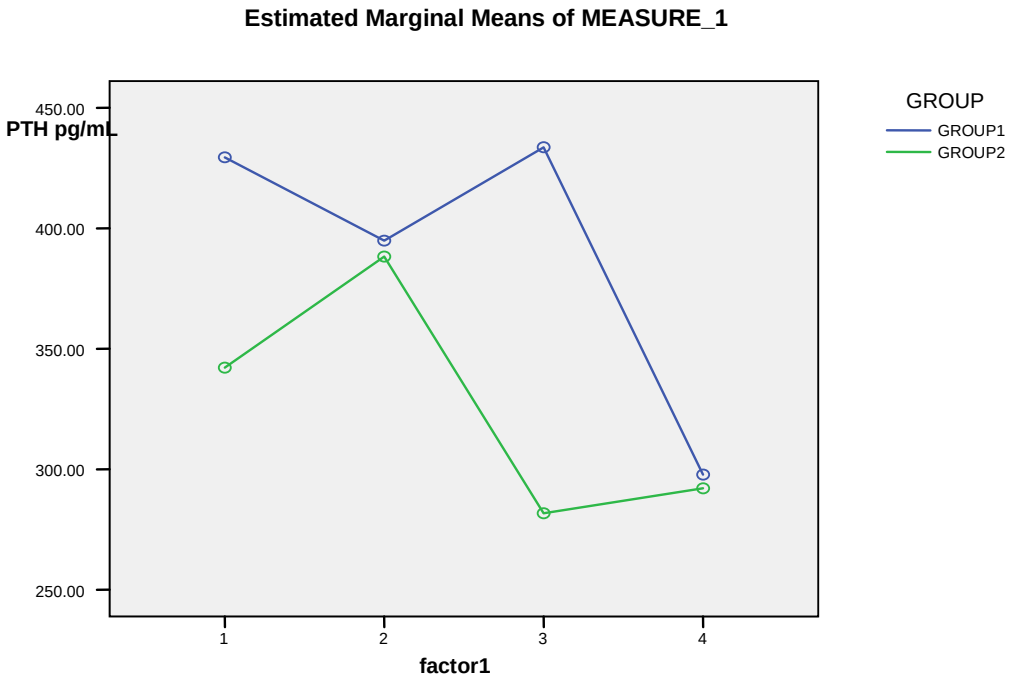

Figure 6 Minimal changes in PTH by group

The marginal changes observed showed a general decrease in PTH with time for both groups .However the change is not significant (P>0.05).

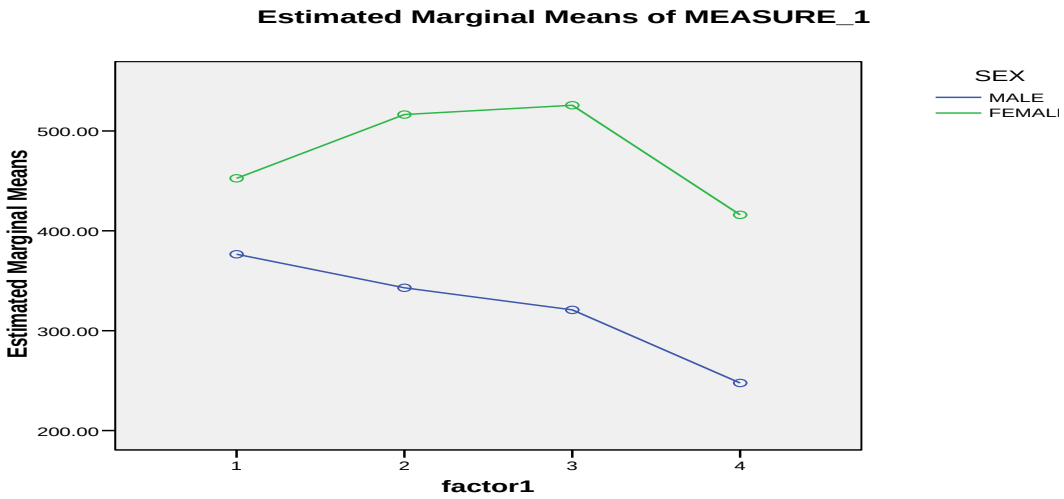

FIGURE 7, Variation in PTH levels by gender.

There were significant changes in the PTH with time when data was analysed by gender showing decreases in PTH in both males females (P<0.05).The decrease in PTH in females was less as compared to than in males (P=0.044).

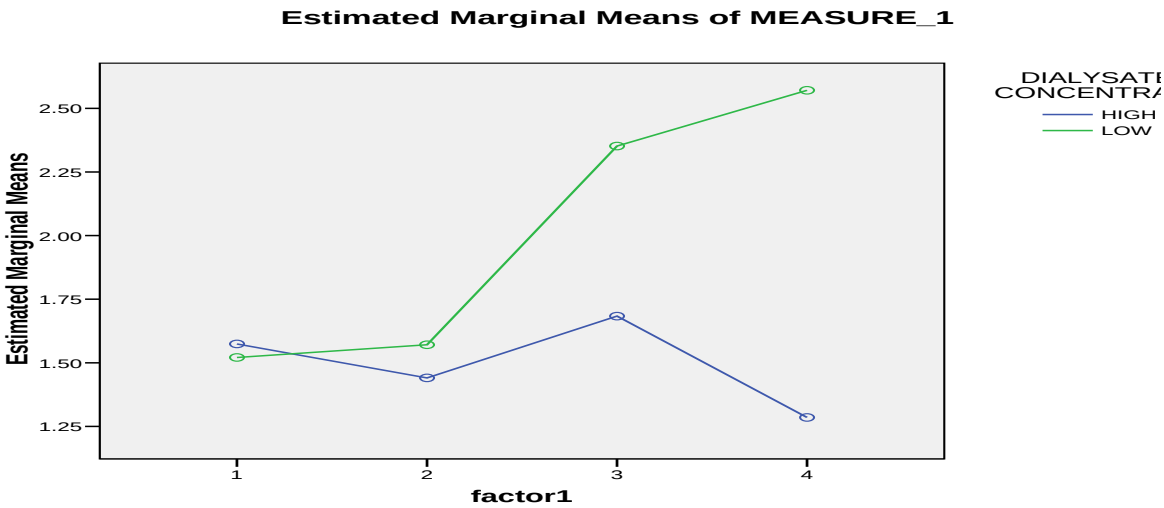

FIGURE 8. Variation in phosphate with time in participants using different dialysate concentration.

When the data was analysed stratified into those using high and low calcium concentration dialysate fluid statistically significant changes were observed for phosphate with time ($P<0.05$) using all the four multivariate tests of GLM and Mauchly test of sphericity (**Tables 5 and 6**). The profile plot showed increasing phosphate levels in low calcium concentration users and lower levels in those using the high concentration dialysate fluid (**Figure 8**).

TABLE 5. Significant changes in phosphate levels of participants on different dialysates concentration (P<0.05).

Multivariate Tests(c)

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power(a)
Factor 1 (TIME)	Pillai's Trace	0.382	4.949(b)	3.000	24.000	0.008	0.382	14.848	0.859
	Wilks' Lambda	0.618	4.949(b)	3.000	24.000	0.008	0.382	14.848	0.859
	Hotelling's Trace	0.619	4.949(b)	3.000	24.000	0.008	0.382	14.848	0.859
	Roy's Largest Root	0.619	4.949(b)	3.000	24.000	0.008	0.382	14.848	0.859
factor1 * DIALY	Pillai's Trace	0.397	5.266(b)	3.000	24.000	0.006	0.397	15.799	0.881
	Wilks' Lambda	0.603	5.266(b)	3.000	24.000	0.006	0.397	15.799	0.881
	Hotelling's Trace	0.658	5.266(b)	3.000	24.000	0.006	0.397	15.799	0.881
	Roy's Largest Root	0.658	5.266(b)	3.000	24.000	0.006	0.397	15.799	0.881

(a) Computed using alpha = .05 **P (<0 05)**

(c)Design: Intercept +DIALY (b) Exact statistics

TABLE 6. Significant changes in phosphate as shown by Mauchly Sphericity Test (P<0.05).

Test of within-subject effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power(a)
factor1	Sphericity Assumed	5.450	3	1.817	3.308	0.024	0.113	9.925	0.734
	Greenhouse-Geisser	5.450	2.108	2.586	3.308	0.042	0.113	6.973	0.619
	Huynh-Feldt	5.450	2.386	2.284	3.308	0.035	0.113	7.895	0.659
	Lower-bound	5.450	1.000	5.450	3.308	0.080	0.113	3.308	0.418
factor1 * DIALY	Sphericity Assumed	7.266	3	2.422	4.411	0.006	0.145	13.232	0.859
	Greenhouse-Geisser	7.266	2.108	3.447	4.411	0.015	0.145	9.296	0.753
	Huynh-Feldt	7.266	2.386	3.045	4.411	0.012	0.145	10.525	0.792
	Lower-bound	7.266	1.000	7.266	4.411	0.046	0.145	4.411	0.525
Error(factor 1)	Sphericity Assumed	42.834	78	0.549					
	Greenhouse-Geisser	42.834	54.801	0.782					
	Huynh-Feldt	42.834	62.046	0.690					
	Lower-bound	42.834	26.000	1.647					

P (<0.05)

(a) Computed using alpha =0 .05

Table 7. Distribution of PTH and bALP in study Group 1**.**

.

Type of bone change	**PTH(pg/mL)**	**bALP(µg/L)**	**% distribution**
High bone turnover	450-1367	29-359	38.9
Low bone turnover	<100	5.06-9.28	22.2
Normal bone turnover	150-300	5.34-12.13	38.9

The grouping is based on classification of ROD by K/DOQI (2005) that classifies all patients with PTH of >100 pg/mL and above 300 pg/mL as having low and high bone turnover respectively.

Normal ranges of bALP according to the manufacturer:

Males -3.7-20.9µg/L

Pre menopause females -2.9-14.5µg/L

Post menopause female - 3-8-22.6µg/L

Group 2 had PTH within 150-300 pg/ml range except one female (55years) with PTH levels above 1500 pg/mL and high bALP reaching up to 92.40 µg/mL.

CHAPTER 4

DISCUSSION

This study was undertaken to test prospectively the utility of PTH as a marker of bone changes in monitoring CKD patients on haemodialysis using trends rather than single values of PTH (18, 50, 51, 52, 53, 54). The findings of this study showed an overall non significant decrease in PTH with time which is however consistent with literature that says there is a general decline in PTH with dialysis especially where high calcium containing dialysate fluids are used (55, 56). Other factors have however been cited to influence PTH changes. In a study by Claire et al (2010) the effect of biological variation on PTH was demonstrated. PTH in patients dialysed in the morning were lower than of those dialysed in the afternoon (57). In the present study, the time of sample collection was not standardised since samples were collected at different times of the day as the patients came for their dialysis sessions. Different drug regimens used by the participants from the three different centres and the duration of this study which was short could have contributed to the difference in findings.

Females who had been newly enrolled for dialysis showed higher PTH mean levels than males in the same which was found to be statistically significant . This is in agreement with findings by Olafur et al (1995) indicating that PTH levels in females decrease at a slower rate than in males upon renal replacement intervention (50). The actual cause of this difference is still not fully established but literature points this to oestrogens influencing the secretion of PTH via its interaction with Osteoprotegerin (57).

In this study it was also observed that mean PTH values were higher in patients who were using a lower dialysate concentration in both group 1 and 2. Although the differences were not significant the PTH change was consistent with the significant increasing phosphate levels found in each of the same group. This is consistent with other studies that used even lower calcium concentration dialysate of 1.25mmo/l (55, 56) leading to a conclusion that use of even lower concentration dialysates worsens hyperparathyroidism (54). Literature also shows that use of low calcium containing dialysate fluid reduces the suppressing effect of calcium on PTH (44). A study by Harris et al (2006) showed significant changes in PTH in those who were using an even lower calcium concentration dialysate of 1mmol/L while in those using 1.62 mmol/l no significant changes were observed after a study period of 16 months (9). With the use of new vitamins regimens now available, the suppressing effect has been reduced. Conflicting views however still remains as to which dialysate concentration to use (58).

Calcium showed no significant increases in participants using high calcium containing dialysate fluid and is consistent with Zhao et al (2012) who also obtained non significant changes in calcium after 6 months of dialysis (55,56). Other studies however have shown hypercalcaemia and consequently hypoparathyrodism and development of adynamic disease (9, 59).

Significant increases in phosphate (69.6%) were seen in patients using low calcium dialysate fluid. Calcium remained within the lower limits of normal with PTH levels non significantly elevated. Considering that there were no significant differences in calcium between the two groups it is becoming increasingly possible that phosphate has a direct effect on PTH levels in patients on dialysis. Other studies have alluded to the same effect of phosphate directly

stimulating PTH (1, 50, 55, 60, 61, 62). However a study by Kilpatrick et al (2010) has shown that PTH directly induces hyperphosphotaemia via bone resorption (63). There remains a question as to which of the two is responsible for the initial rise. The same study by Kilpatrick concluded that it is more that that there is a vicious pathological cycle of PTH and phosphate in patient on dialysis (63.64)

bALP levels were found to be higher in participants with a longer history of dialysis of more than a year. bALP production increases with increased bone activity and for those who have just started haemodialysis there are initially no bone changes observed as these take a year to develop (51). The bALP differences between these two groups were significant. The findings of the study also showed that there was a significant positive correlation (0.649) at both 0.05 and 0.001 confidence intervals between bALP and PTH. This finding is supported by findings of other researchers showing the same positive relationship between the two (45, 49). This therefore indicates that the use of bALP improves the clinical utility of PTH in monitoring bone changes in patients on haemodialysis. bALP showed a negative relationship with phosphate . There was however a non significant relationship between PTH and both calcium and phosphate in this study diverging from the study by Olafur et all where calcium ingestion in the participants was standardised. Changes in calcium in the Olafur et all study were significantly linked to changes in PTH (50).

In this study the relationship between clinical presentations of patients with the biochemical findings was no ascertained due to the vagueness of bone manifestations in patients on haemodialysis. Biochemical findings in this study has however shown that 38.9% of patients had PTH and bALP levels above 450 pg/mL and 29 µg/L (ranging from 450-1367 pg/mL and 29-359 µg/L) respectively indicating high bone turnover changes; 22.2% showed PTH levels

below 100pg/mL and bALP within the lower limits of the normal ranges (2.9-22.6 according to the manufacture) indicating manifestations of adynamic bone disease .Another 38.9 % had PTH values ranging between 150 -300 pg/mL and bALP within normal limits indicating normal bone changes. This is according to the classification by Wang et al (1995) and the Kidney disease Outcomes Quality Initiative of 2003 (1, 34). The widely used classification by the KDOQI which recommends PTH levels in patients on haemodialysis to be 150-300 pg/mL proved not to be effective in preventing the low turnover bone disease by Barreto et al (28). This study therefore recommends the use of these PTH target values only when the PTH value has been obtained after an established trend. Claire et al (2010) recommended 26 serial measurements of PTH for one to establish any individual optimum target PTH value and that there should be at least a 70% increase change in these PTH values. This was based on the study findings which show biological variations of PTH even in healthy people (57).

RECOMMENDATIONS AND LIMITATIONS

It is recommended that renal units ought to record serial measurements of PTH, calcium and phosphate at least monthly until they establish the trends in individual patients and attain targets values for calcium, phosphate and PTH in order to avoid over-correcting one marker at the expense of the other. Furthermore, renal centres should offer PTH assays because this study and several others show that monitoring calcium and phosphate levels alone does not reflect all bone changes.

The limitation of this study was having a small sample size and financial constraints to buy adequate reagent kits for many PTH and bALP assays. It was a challenge to recruit the new members for group two as some would defer midway through the study due to financial problems or death. Therefore given enough finances and resources this study will ascertain the bone changes and manifestation occurring in patients with renal failure requiring haemodialysis.

CONCLUSION

The study findings have shown that PTH levels in an individual patient on haemodialysis fluctuates greatly and therefore diagnosis and monitoring of bone changes in these patients ought to be based on trends rather a single value of PTH. Gender and Phosphate have been shown to be strong factors that influence PTH levels in patients on haemodialysis. The study has also confirmed that bALP is indeed a reliable bone marker to consider using if we are to make headways in solving some of the issues associated with PTH as a bone marker when used alone.

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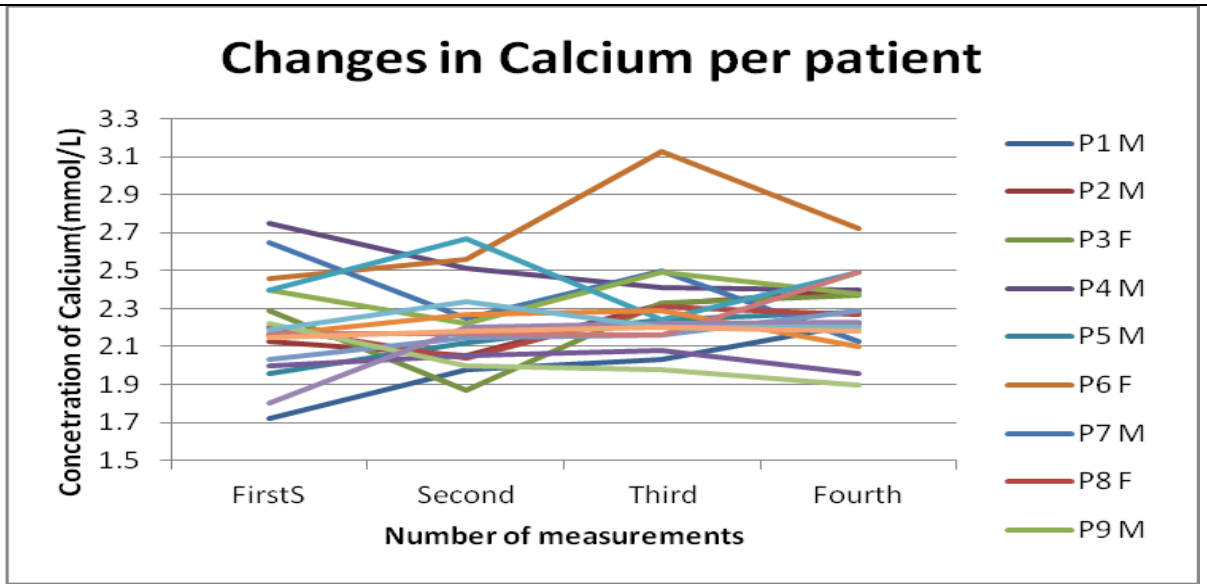
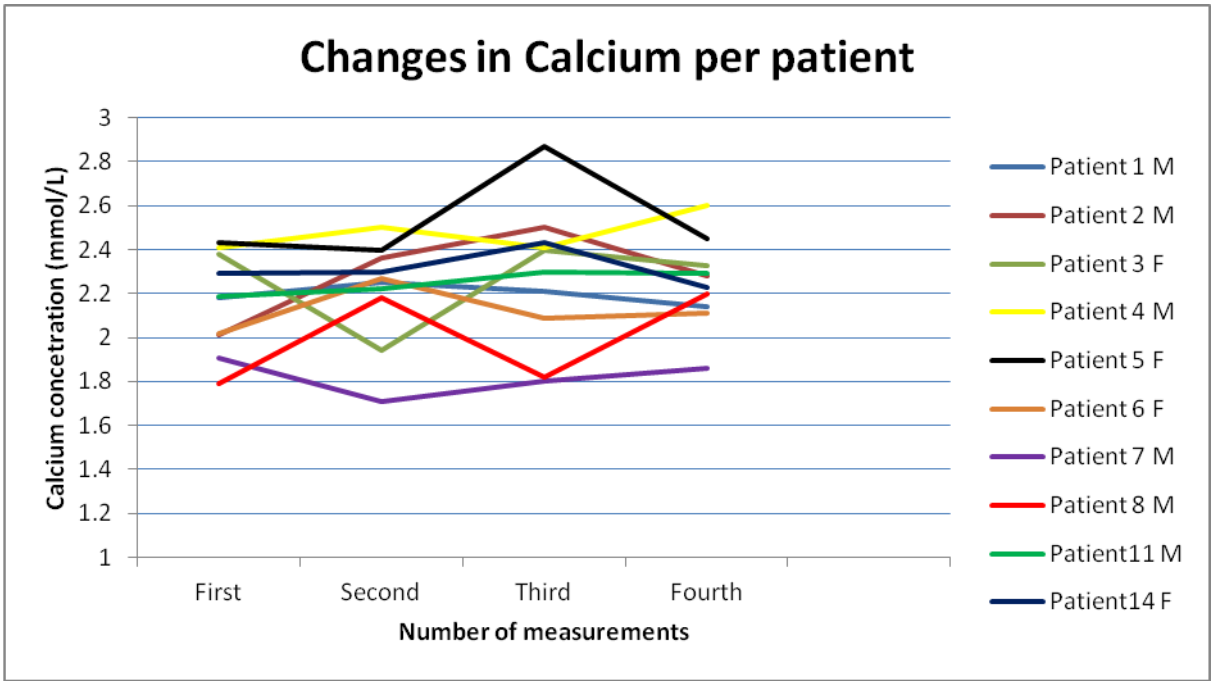
APPENDICES

Appendix 1. Variation in calcium per patient with time for Group 1 and 2

GROUP1

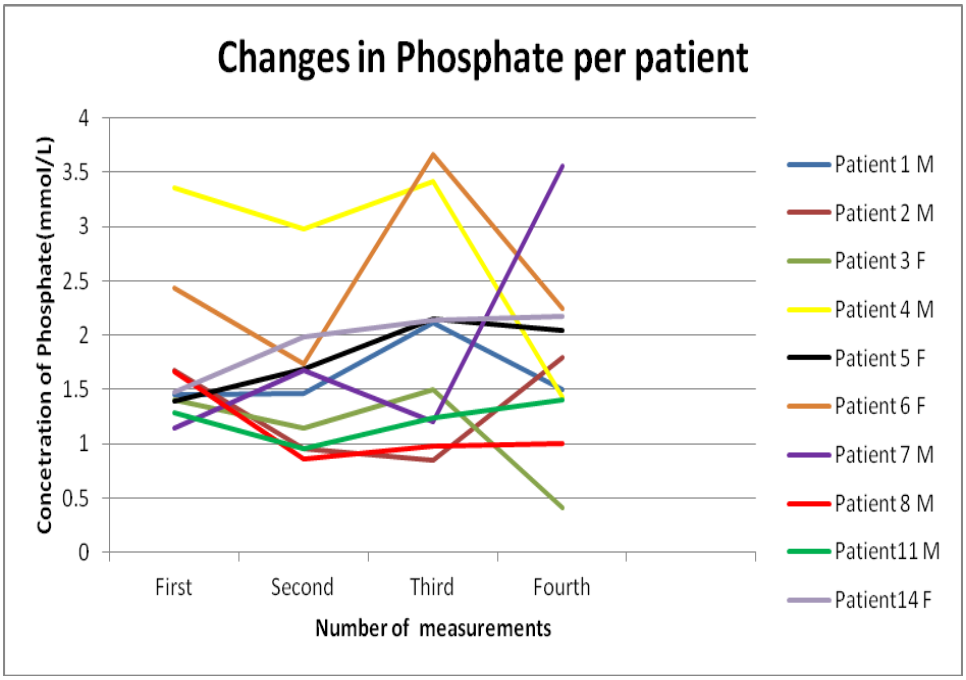


GROUP2

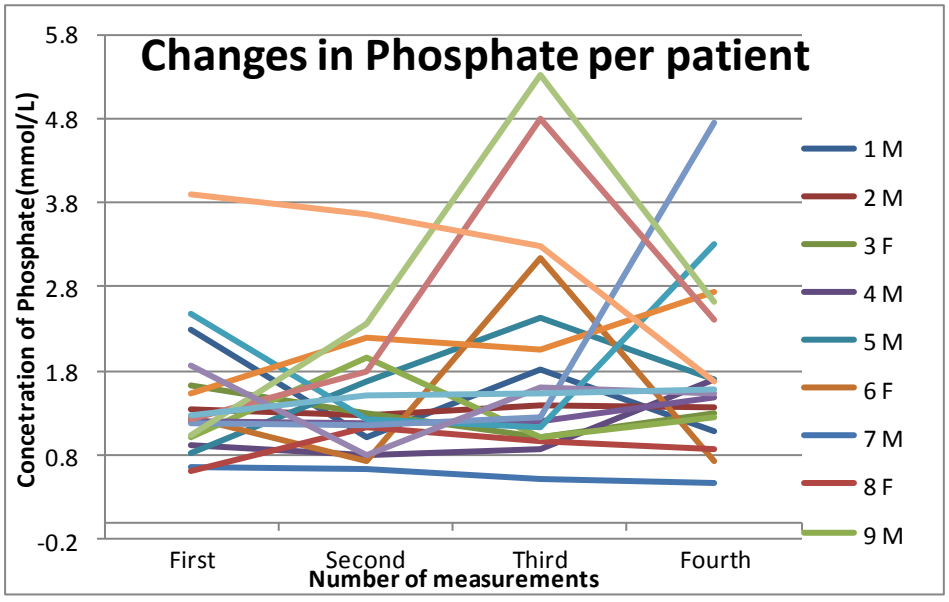


Appendix 2. Variation in phosphate per patient with time for Group 1 and 2.

GROUP2

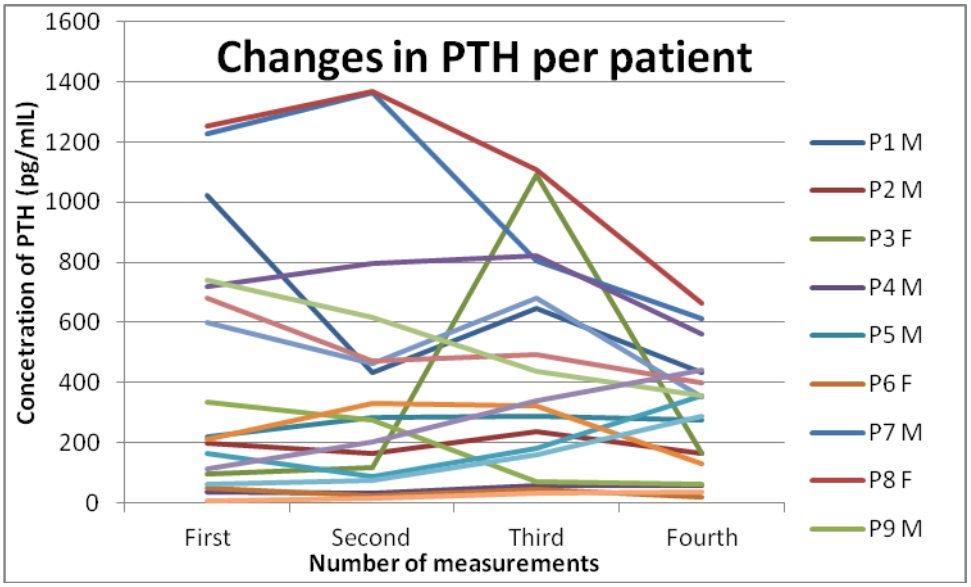


GROUP 1

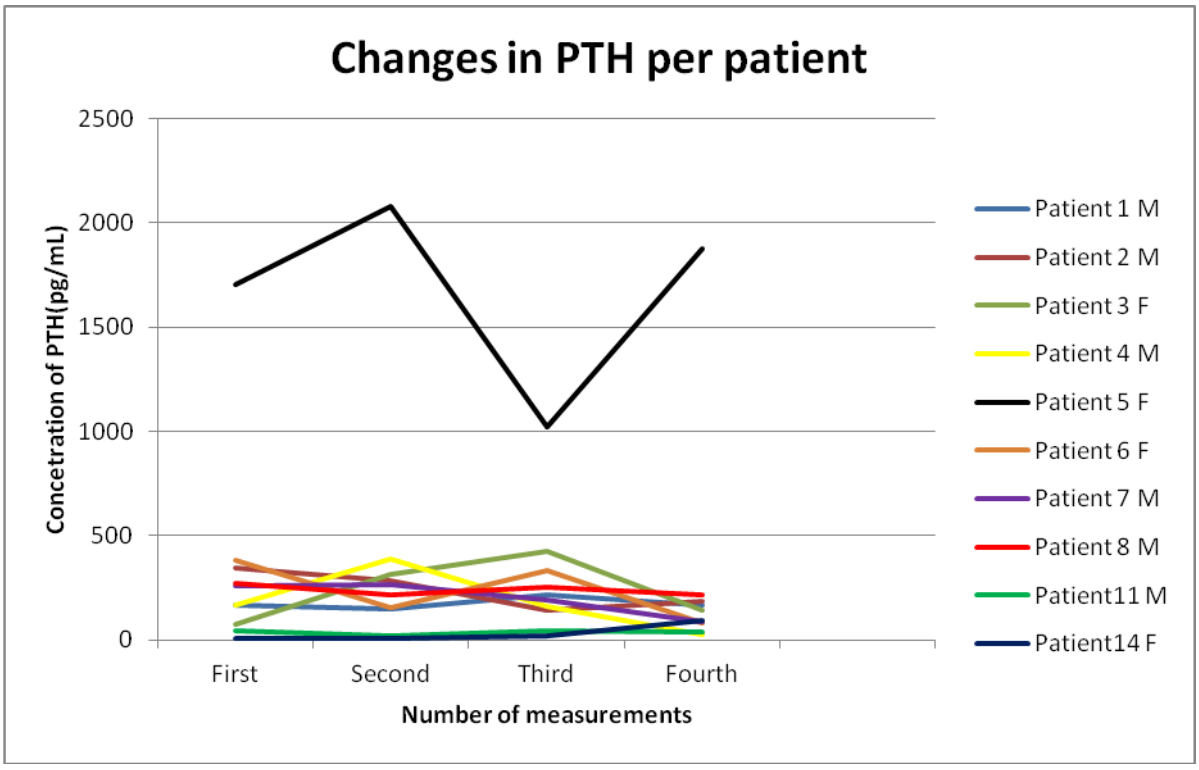


Appendix 3. Variation in PTH per patient with time for Group 1and 2.

GROUP1

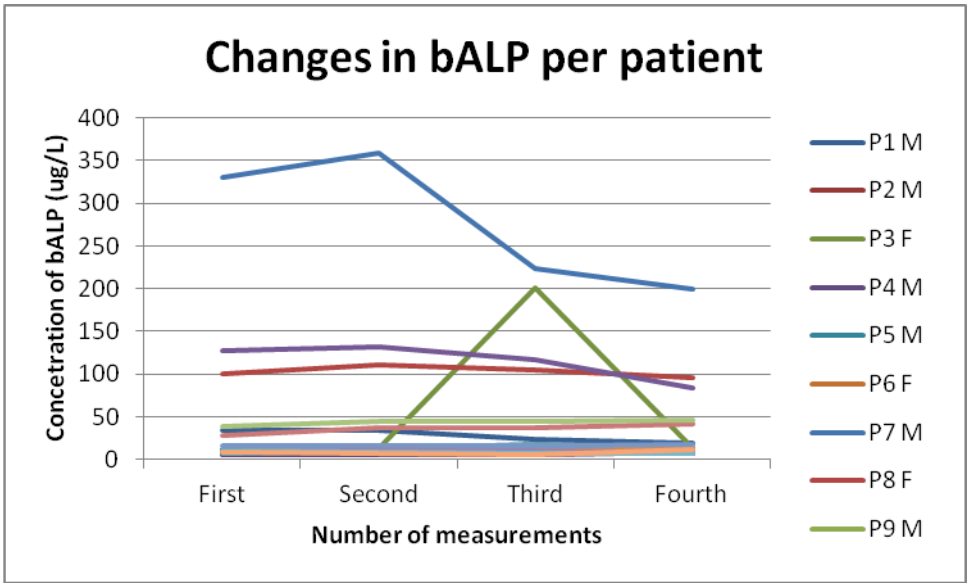


GROUP2



Appendix 4. Variation in bALP per patient with time for Group 1and 2.

GROUP1



GROUP2