Renal replacement therapy and bone mineral metabolism

Vishal Dey
MBBS MRCP (UK) SCE (Nephrology)

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Institute of Cardiovascular and Medical Sciences
University of Glasgow ©

Abstract

Mineral bone disturbances are common in chronic kidney disease (CKD), and associated with significant risk of mortality and morbidity in patients on renal replacement therapy (RRT). Surrogate biomarkers of bone turnover such as parathyroid hormone (PTH), phosphate, calcium and Vitamin D are used to diagnose, evaluate, and guide treatment.

This thesis examines the effect of RRT on mineral bone disturbances, it's association with bone morbidity, and management strategies for phosphate control.

Initially the incidence of radiologically proven bone fracture by site, in prevalent RRT groups is quantified and the relationship to associated risk factors studied. In this multicentre observational study of 2096 patients over a 3-year period the risk of fracture is higher in haemodialysis (HD) patients than in transplant patients even when controlling for other risk factors. Exposure to lanthanum and Vitamin D is apparently a protective factor in the HD group.

I then examine a thrice-weekly nocturnal in-centre dialysis model in which we attain normal phosphate levels without dietary restriction or supplementation by altering the dialysis prescription and time. This observational trial over a 2-year period with over 2000 sessions of dialysis in 14 patients is associated with reduction of blood pressure medications.

Subsequently I investigate the relationship of phosphate to FGF23 in a group of peritoneal dialysis patients. Finally, I study the effect of dialysis on clearances of FGF23 and expand on the knowledge of FGF-23 during a session of dialysis.

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Papers and publications arising from this work:

Original manuscripts

Dey V, Farrah TE, Traynor JP, Spalding EM, Robertson SE, Geddes CC. Symptomatic fracture risk in the renal replacement therapy population. Nephrol Dial Transplant 2017 Jul 1;32(7):1211-1216.

Dey V, Hair M, So B, Spalding EM. Thrice-Weekly Nocturnal In-Centre Haemodiafiltration: A 2-Year Experience. Nephron Extra 2015 Aug 29;5(2):50-57.

Poster presentations

Dey V, Jones A, Hillyard D, Hair M, Stevens K, Spalding EM, et al. Mp539 FGF23 Clearance in Peritoneal Dialysis. Nephrology Dialysis Transplantation 2017;32(suppl_3):iii627-iii627. 54th ERA-EDTA, Madrid 2017

Dey V, Stevens K, Hillyard D, Jardine A, Spalding EM. Mp684 Effect of a Single Dialysis Session on Fgf23 Levels. Nephrology Dialysis Transplantation 2017;32(suppl_3):iii682-iii682. 54th ERA-EDTA, Madrid 2017

Dey V, Farrah T, Traynor J, Spalding EM, Robertson S, Geddes CC. Fp410 Multi-Centre Analysis of Fracture Risk in Renal Replacement Therapy Patients. Nephrology Dialysis Transplantation 2015;30(suppl_3):iii207-iii208. 52nd ERA-EDTA, London 2015

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So B, **Dey V**, Spalding EM, Control of plasma phosphate on thrice weekly in-centre haemodiafiltration, 50th ERA-EDTA, Istanbul 2013

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Author's Declaration

The work presented in this thesis was that of the author and his supervisors, Dr Elaine Spalding and Professor Alan Jardine. All clinical research work was carried out by the author.

All statistical analyses were carried out by author with input from Dr Colin Geddes (Chapter 2) and Mario Hair (Chapter 3).

I declare that this thesis has been composed by myself and is a record of the work I have performed. It has not been previously submitted for a higher degree although individual components have been presented at scientific meetings or have been submitted as papers to peer-reviewed journals.

Vishal Dey

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Definitions/Abbreviations

$1,25(OH)_2$ D	1,25 dihydroxy Vitamin D
aa	amino acid
aFGF	acidic fibroblast growth factor
bALP	bone alkaline phosphatase
bFGF	basic fibroblast growth factor
A&A	Ayrshire & Arran
ABD	adynamic bone disease
ArMORR	Accelerated Mortality on Renal Replacement
AVF	Arterio-venous fistula
BCM	Body composition monitor
CaMos	Canadian Multicentre Osteoporosis Study
CAC	Coronary artery calcification
CARI	Caring for Australians with Renal Impairment
CHI	Unique patient Identifier Number
CHO	Chinese hamster ovary
COPD	Chronic obstructive pulmonary disease
CKD	Chronic kidney disease

CKD-MBD	Chronic Kidney Disease – Mineral bone disorder
CT	Computerised Tomography
CV	Cardiovascular
ELISA	Enzyme-linked immunosorbent assay
ERA-EDTA	European renal association – European dialysis and transplant association
ESRD	End stage renal disease
EVOLVE	Evaluation of Cinacalcet Therapy to Lower Cardiovascular Events
FGF-19	Fibroblast growth factor 19
FGF-21	Fibroblast growth factor 21
FGF-23	Fibroblast growth factor 23
FGFR	Fibroblast growth factor receptor
FREEDOM	Fracture Reduction Evaluation of Denosumab in Osteoporosis Every 6 Months
FRAX®	Fracture Risk Assessment Tool
GFR	Glomerular filtration rate
GGC	Greater Glasgow and Clyde
GN	Glomerulonephritis

GRADE	Grading of Recommendations, Assessment, Development and Evaluation
GRI	Glasgow Royal Infirmary
HMG-CoA	3-hydroxy-3-methyl-glutaryl-coenzyme A
HD	Haemodialysis
HDF	Haemodiafiltration
HR	Hazard ratio
ICD	International classification of diseases
IHD	Ischaemic heart disease
Kd	measured dialyzer clearance
kDa	k-Daltons
KDIGO	Kidney Disease Improving Global Outcomes
K/DOQI	Kidney Disease Outcomes Quality Initiative
LVH	Left ventricular hypertrophy
MI	Myocardial infarction
mls	millilitres
MDRD	Modification of diet in renal disease study equation
MICS	Malnutrition-inflammation complex syndrome

NKF	National Kidney Foundation
NHS	National Health Service
NHANES	National Health and Nutrition Examination Surveys
PD	Peritoneal dialysis
PEM	Protein energy malnutrition
PIS	Participant information sheet
PMS	Patient management system
PO_4	Phosphate
PRD	Primary renal disease
PRIMO	Paricalcitol capsules benefits in Renal failure Induced cardia Morbidity
PTH	Parathyroid hormone
Qb	Dialysis blood flow
Qd	Dialysate flow
RR	Relative risk
RRT	Renal replacement therapy
RT	Renal transplant
SD	Standard deviation
SERPR	Strathclyde Electronic Renal Patient Record

SPSS	Statistical analysis software
t½	Half-life
TBW	Total body water
Td	Dialysis time
VisSim™	Visual Solutions Inc.
WHI	Women's Health Initiative
$UFE_{phosphate}$	Urinary fractional excretion of phosphate
UK	United Kingdom
XH	Crosshouse hospital

Reference Keys

Conversion factors of metric units to SI units

	Metric Unit	Conversion	SI Unit
Albumin	g/dl	10	g/L
Calcium, total	mg/dl	0.249	mmol/l
Calcium ionised	mg/dl	0.25	mmol/l
Creatinine	mg/dl	88.4	μmol/l
Parathyroid hormone	pg/ml	0.106	pmol/L
Phosphorus	mg/dl	0.322	mmol/L
Vitamin D, 1,25 -dihydroxyvitamin D	pg/ml	2.6	pmol/l
Vitamin D, 25-hydroxyvitamin D	ng/ml	2.496	nmmol/l

Chapter 1

Introduction & Background

1.1 Introduction

Chronic Kidney Disease (CKD) is a major public health problem affecting 8-16% of the population worldwide. (1) The estimated lifetime risk of CKD stage 3a is greater than 50%; higher than that of diabetes and increases dramatically with age. (2) It causes progressive loss of renal function, decreased quality of life and increased mortality and morbidity. The increased deaths from cardiovascular disease in CKD are not well understood, though vascular calcification from disturbed mineral metabolism is believed to play a major role. These metabolic derangements and bony abnormalities in renal failure were traditionally described as 'Renal osteodystrophy'.

1.2 History of Renal Osteodystrophy

Bone disease in renal failure, was first seen in the mid-nineteenth century by Virchow. By 1950s the roles of Vitamin D and parathyroid hormone to bone abnormalities were being increasingly recognised. Some authors believed Vitamin D deficiency caused bone symptoms and showed good response to calciferol in biopsy proven osteomalacia. Others demonstrated resolution of metastatic calcification and bone healing following parathyroidectomy. (3) Not all cases of uremic osteomalacia responded to Vitamin D, possibly due to resistance. In 1960s complications of vascular calcification arose from improved survival by modern dialysis techniques. During this time the roles of calcium and phosphate became prominent with Scribner noting his patients 'turning to stone'. (3)

Bone disease by then was being recognised histologically as three different forms

1. Hyperparathyroid form, associated with an enlarged parathyroid gland, high levels of PTH, increased bone turnover and osteitis fibrosa

2. Osteomalacia with unmineralised osteoid and low bone turnover

3. Mixed form, a combination of the above patterns.

Though histological diagnosis provided a picture of the bone morphology, its relationship to abnormal mineral metabolites continued to remain a mystery.

To understand the homeostasis of calcium, phosphate and vitamin D in the context of renal failure various hypotheses were proposed. An initial simplistic overview was as kidneys lost nephrons, its excretory function declined resulting in hyperphosphatemia. This in turn caused hypocalcemia and stepwise rise in PTH. Thus, secondary hyperparathyroidism occurred at the cost of calcium and phosphate homeostasis known as the 'trade off hypotheses'. (4)

1.3 Initial management

Initial management of renal osteodystrophy was aimed at correcting calcium and phosphate levels with Vitamin D supplementation. Dialysate calcium-controlled calcium levels while aluminium salts as aluminium hydroxide and aluminium carbonate served as phosphate binders. Aluminium being insoluble and poorly absorbed via the intestine was considered safe and controlled secondary hyperparathyroidism effectively.

1.4 Aluminium based phosphate binders

In 1977 Platts *et.al* found an association of encephalopathy and spontaneous fractures in home dialysis patients that were supplied with water that had high concentrations of aluminium and manganese. Aluminium levels measured using optical emission spectrography and Neutron activation was high in water and tissues (75% of the samples from bones though only traces were seen in the brain) of affected patients. Values were averaged and difference of means calculated using Student's t tests. The findings in the brain tissue was in contrast to work done by Alfrey (5) who found high concentrations in the grey matter of the cerebral cortex of patients who died of encephalopathy. The analysis of brain by Platts *et al*. without separation of grey and white matter probably explains this paradox. (6)

Toxicity from oral aluminium hydroxide used as phosphate binders was considered but couldn't be proven, as 5 of the 11 patients had not been taking

their medications. Contamination from plumbing was unlikely given the similarity of results obtained from water source and patients home. (6)

During this period there was an improvement in the water purification systems with introduction of ion exchangers, but the use of oral aluminium containing phosphate binders continued. The prevalence of aluminium related osteomalacia decreased and shifted the epidemiology from an epidemic problem to an endemic form. By now it was widely believed that oral aluminium contributed to toxicity by prolonged slow exposure in the context of decreased renal function and inability to excrete metabolites effectively. Despite poor understanding of the mechanism by which aluminium caused osteodystrophy there was increasing evidence that aluminium had effects on osteoblasts, osteoclasts and bone mineralisation. (7) It altered 1,25$(OH)_2$D metabolism (8) and decreased secretion of PTH. (9)

In the absence of an alternative, effective binder, aluminium continued to be used in lower doses along with calcium salts to control hyperphosphatemia. Serum levels were closely monitored, and patients advised to avoid high phosphate containing diets. In severe cases desferoxamine was used as an aluminium chelator. Though these measures proved effective, desferoxamine had its own problems. Apart from minor side effects of headaches, flushing and rash, which were managed with decreasing infusion, there were grave concerns of some patients developing systemic mucormycosis. (10)

In the absence of consensus and lack of therapeutic guidelines the management of bone disease was fragmented and suboptimal.

1.5 Management of renal osteodystrophy

The first attempt to an international consensus on the management of mineral bone disturbances in CKD was described by Cannata-Andia JB *et.al* in 2000 in a document, 'Clinical Algorithms on renal osteodystrophy'. (11)These were followed by the, Caring for Australians with Renal Impairment (CARI) guidelines in March 2000 and Kidney Disease Outcomes Quality Initiative (K/DOQI) by the National Kidney Foundation (NKF) in 2003. (12)

The work of the NKF began at a conference, 'Controversies in Mineral Metabolism and Bone Disease in CKD' on March 14-16, 2003. Individuals with expertise on basic sciences, vascular pathology, parathyroid, Vitamin D, and diagnostics participated. Three sub-groups discussed, bone turnover, osteoporosis in CKD and vascular calcification. This resulted in a comprehensive set of guidelines for nephrologists to integrate latest evidence into clinical practice. A new definition of renal osteodystrophy was proposed: 'A constellation of bone disorders present or exacerbated by chronic kidney disease that lead to bone fragility and fractures, abnormal mineral metabolism, and extra skeletal manifestations'. This however failed to gain acceptance across the globe resulting in the formation of Kidney Disease: Improving Global Outcome (KDIGO). (12-15)

1.6 Kidney Disease: Improving Global Outcome (KDIGO)

In 2003, Kidney Disease: Improving Global Outcome (KDIGO) an independent, non-profit foundation governed by an international board of directors was formed. Its mission was to 'improve the care and outcomes of kidney disease patients worldwide through promoting coordination, collaboration, and integration of initiatives to develop and implement clinical practice guidelines'. Following the successes of the initial conference in 2004 on 'Definition and classification of CKD' a second consensus conference was held in September 15-17, 2005, Madrid, Spain. The objectives were to develop a clinically clear, definition and classification, reach a consensus on bone evaluation and assess the utility of serum markers and imaging procedures of bone disease in CKD. The meeting consisted of more than 70 physicians from 21 countries across six continents. The conference proceedings consisted of plenary and breakout sessions of three separate working sub-groups addressing – biomarkers, histomorphometry and imaging. Specific recommendations were approved resulting in a statement 'Definition, evaluation, and classification of renal osteodystrophy: A position statement from KDIGO'.(13)

The principal recommendations and conclusions of the conference were the use of the term '*renal osteodystrophy*' exclusively to define bone pathology and '*Chronic Kidney Disease –Mineral Bone Disease*' incorporated a syndrome of clinical, biochemical and imaging abnormalities.

To develop a standardised approach based on the highest quality evidence KDIGO in 2009 published specific guidelines for diagnosis, evaluation, prevention and treatment of chronic kidney disease – mineral bone disorder (CKD-MBD). While providing guidance it identified gaps and lack of high-quality evidence of recommendations. Further work at a controversies conference, in 2013 'CKD-MBD: Back to the Future', identified 12 recommendations for re-evaluation. In June 2015, a working group convened where decisions considered important including those on outcomes were made. Multiple randomised control trials (RCTs) and prospective cohort studies were examined from December 2006 to September 2015, supplemented till February 2017.

A selective clinical practice update using the GRADE system (16), to define the strength and levels of evidence were published in July 2017. (17) The recommendations are graded as Grade 1 (strong or we recommend) or Grade 2 (weak or we suggest) and quality designated as Grade A (high), Grade B (moderate), Grade C (low) or Grade D (very low).

1.7 UK Renal Association – UK RA

The UK Renal Association is a leading professional body of UK Renal Community that has provided guidance on best practice management of kidney disease since 1995. Established in 1950 and NICE (National Institute of Health and Clinical Excellence) accredited in 2010, the latest UK RA guidelines on management of CKD-MBD was published in 2015, with further commentaries in June 2018. (18,19) It grades most of the evidence as Grade 2C or lower whereas only two of the original recommendations by KDIGO guidelines were graded as 1A, both within the paediatric nephrology. 20 of these were graded as 2C and 12 ungraded. (17)

1.8 Definitions

1.8.1 Renal osteodystrophy

The definition of renal osteodystrophy is summarised in Table 1.1. It involves evaluation using bone biopsy and reporting using the nomenclature recommended by the American Society for Bone and Mineral research. Its

classification is based on the TMV system – bone turnover, mineralisation and volume. This was mainly established as a research tool and for use in select group of patients with diagnostic uncertainty. It did not form a part of routine assessment of CKD-MBD.

Table 1-1 Definition of Renal Osteodystrophy

Renal osteodystrophy is an alteration of bone morphology in patients with CKD
It is one measure of the skeletal component of the systemic disorder of CKD-MBD that is quantifiable by histomorphometry of bone biopsy.

CKD, chronic kidney disease; CKD-MBD, chronic kidney disease-mineral and bone disorder.

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1.8.2 CKD-MBD

CKD- MDB was defined as a syndrome with definition summarised in table 1.2. A framework for classification of CKD-MBD into four types based on laboratory abnormalities (L), bone disease (B) and calcification of vascular or soft tissues (C) was proposed. The purpose was to improve communication and facilitate research.

Table 1-2: Definition of CKD-MBD

A systemic disorder of mineral and bone metabolism due to CKD manifested by either one or a combination of the following:
Abnormalities of calcium, phosphorus, PTH, or vitamin D metabolism
Abnormalities in bone turnover, mineralization, volume, linear growth, or strength
Vascular or other soft tissue calcification

CKD, chronic kidney disease; CKD-MBD, chronic kidney disease-mineral and bone disorder; PTH, parathyroid hormone.

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1.9 Definition and Classification of CKD

Chronic kidney disease or '*CKD is defined as abnormalities of kidney structure or function, present for >3 months, with implications for health and CKD is classified based on cause, GFR category, and albuminuria category (CGA)'*. (20)

Its classification based on GFR and albuminuria was categorised in 2012 by KDIGO and represented below in Figure 1-1.

Figure 1-1: CKD Nomenclature used by KDIGO(20)

Prognosis of CKD by GFR and albuminuria category

Prognosis of CKD by GFR and Albuminuria Categories: KDIGO 2012				Persistent albuminuria categories Description and range		
				A1	A2	A3
				Normal to mildly increased	Moderately increased	Severely increased
				<30 mg/g <3 mg/mmol	30-300 mg/g 3-30 mg/mmol	>300 mg/g >30 mg/mmol
GFR categories (ml/min/ 1.73 m^2) Description and range	G1	Normal or high	≥90			
	G2	Mildly decreased	60-89			
	G3a	Mildly to moderately decreased	45-59			
	G3b	Moderately to severely decreased	30-44			
	G4	Severely decreased	15-29			
	G5	Kidney failure	<15			

Green: low risk (if no other markers of kidney disease, no CKD); Yellow: moderately increased risk; Orange: high risk; Red, very high risk.

1.10 Prevalence of CKD

The prevalence of CKD varies across nations with 6% of men and 7% of women affected with CKD 3-5D in the Health Survey (HS) report of 2010 in England. Using the HS report the Office for National statistics estimated that 2.71 million had CKD stage 3-5 in 2009. The highest numbers were in those >75 years (29% in men, 35% women). (21) A National Health and Nutrition Examination Survey (NHANES) in the US from 1999 – 2004 of 13233 patients aged >20 years estimated this to be 13.1%. (22) In a cross-sectional study of 47204 patients in China, from January 2007 to October 2010 the prevalence of CKD was 10.8% (8.7% men, 12.9% women) equating to 119.5 million adults > 18 years with CKD. (23) Estimation of early asymptomatic CKD is however difficult due to heterogeneity of population, different methodologies to estimate GFR, single timed measurements and asymptomatic nature of the disease.(1)

The prevalence of adult patients on renal replacement therapy (RRT) in UK on 31st December 2013 was 56,940 patients, an absolute increase of 4.0 % from 2012. This was an increase of 1.2% for haemodialysis (HD), 7.1% for renal transplant (RT) and a decrease of 3.3% for peritoneal dialysis (PD). (24) In the US 615,899 patients had end stage renal disease (ESRD) with 430,273 on dialysis and 185,626 with a functioning transplant, a one-year growth rate of 3.4% in 31st December 2011. (25) On a global scale it's estimated that more than 80% of patients receiving RRT are from affluent nations. (1) Numbers from developing countries are small but these are largely due to incomplete data collection, absence of universal health care systems and lack of resources to accept patients into RRT programs. These numbers are expected to rise with expanding age, improved socio-economic status and population explosion in developing countries(1).

1.11 Prevalence of CKD-MBD

CKD-MBD is a systemic disorder that manifests with abnormalities affecting bone biochemical parameters and vascular calcification. Its prevalence is best defined by characterising each component individually.

1.11.1 Bone abnormalities in CKD-MBD

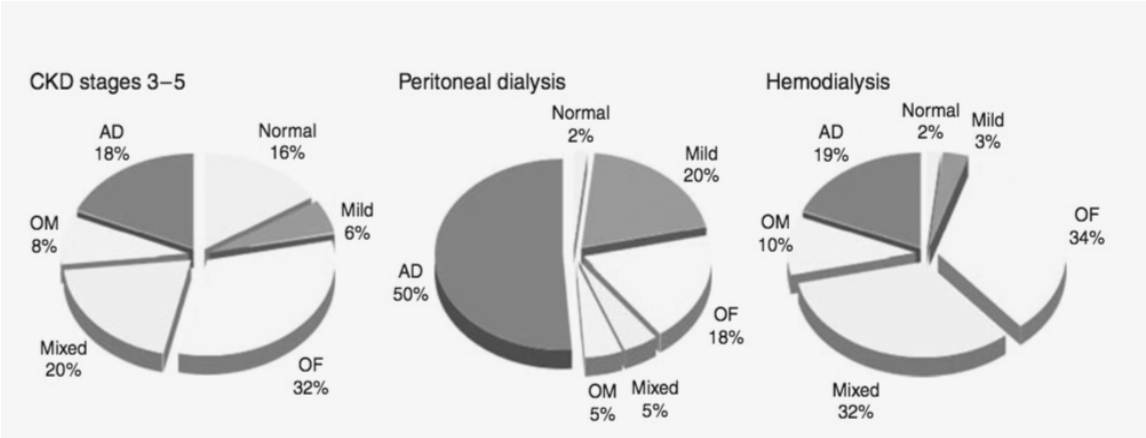
Histological classification, the gold standard for diagnosing renal osteodystrophy based on turnover and mineralisation is described in table 1-3. (17)

Table 1-3: Histological classification on turnover and mineralization

Histology	Turnover	Mineralisation
Mild	Slightly increased	Normal
Osteotitis fibrosa	Increased	Normal
Osteomalacia	Decreased	Abnormal
Adynamic bone disease	Decreased & acellular	
Mixed	Increased	Abnormal

An analysis of bone disease by histological types from 1983 to 2006 in systematic literature based on RRT modality is shown in figure 1-2. There were wide differences in reported numbers due to classification methods, treatment modalities and genetic background. (17)

Figure 1-2: Prevalence of types of bone disease by bone biopsy in CKD-MBD



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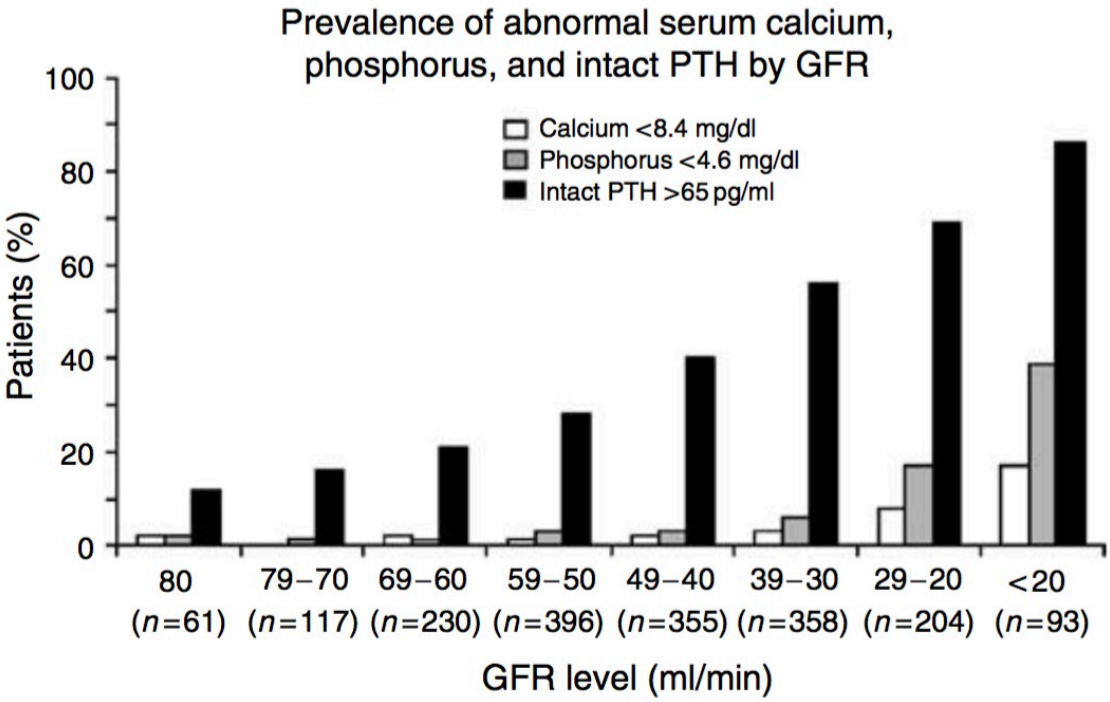
More recent data studying the prevalence of renal osteodystrophy using TMV classification found 58% patients with low bone turnover, 18% normal turnover and 24% high turnover. This study evaluated 630 bone biopsies from 2003-2008 across 2 continents (US 316, Europe 314; 87 black and 543 white patients). All patients were on routine dialysis (600 HD and 30 PD) for at least 6 months and not taking any treatment known to affect bone metabolism apart from Vitamin D compounds or calcimimetics. High turnover was associated with higher phosphorus and PTH and more common in the younger age group. Mineralisation defect was rare (only 3%) with normal and low trabecular thickness in 40% and 37% patients respectively. There were significant racial differences with low turnover common in whites (62%) and high turnover in blacks (45%). Approximately same number of patients had low, normal or high cancellous bone volumes in whites while two-thirds of the black patients had high cancellous bone volume. Most black patients had high cortical porosity while almost equal numbers of white patients had low or normal porosity. No racial differences were seen in trabecular thickness. (26)

Interactions between TMV and architecture showed high bone turnover had increased cortical porosity, and patients with increased cortical porosity had higher erosion depth. Low bone turnover was associated with low cancellous bone volume or thin cortices in most white patients. No relationship was present between bone turnover, defective mineralisation, cancellous bone volume, or cortical thickness. (26)

1.11.2 Biochemical abnormalities in CKD-MBD

Prevalence of biochemical abnormalities in relation to various stages of CKD was studied by Levin *et al*. In a cross-sectional analysis of 1814 subjects, using non-referred populations in the community PTH levels greater than 65pg/ml and 1,25 OH_2 D_3 levels below 22pg/ml (deficiency defined as levels <15ng/ml by KDOQI; < 22pg/ml by Levin based on lowest tertile data) were identified early at eGFR >80ml/min per 1.73 m^2. Normal calcium and phosphate levels are maintained until eGFR fell below 40 ml/min per 1.73 m^2. (27)(Figure 1-3)

Figure 1-3: Prevalence of secondary hyperparathyroidism, hypocalcemia and hyperphosphatemia by eGFR at levels 10mls/min intervals.



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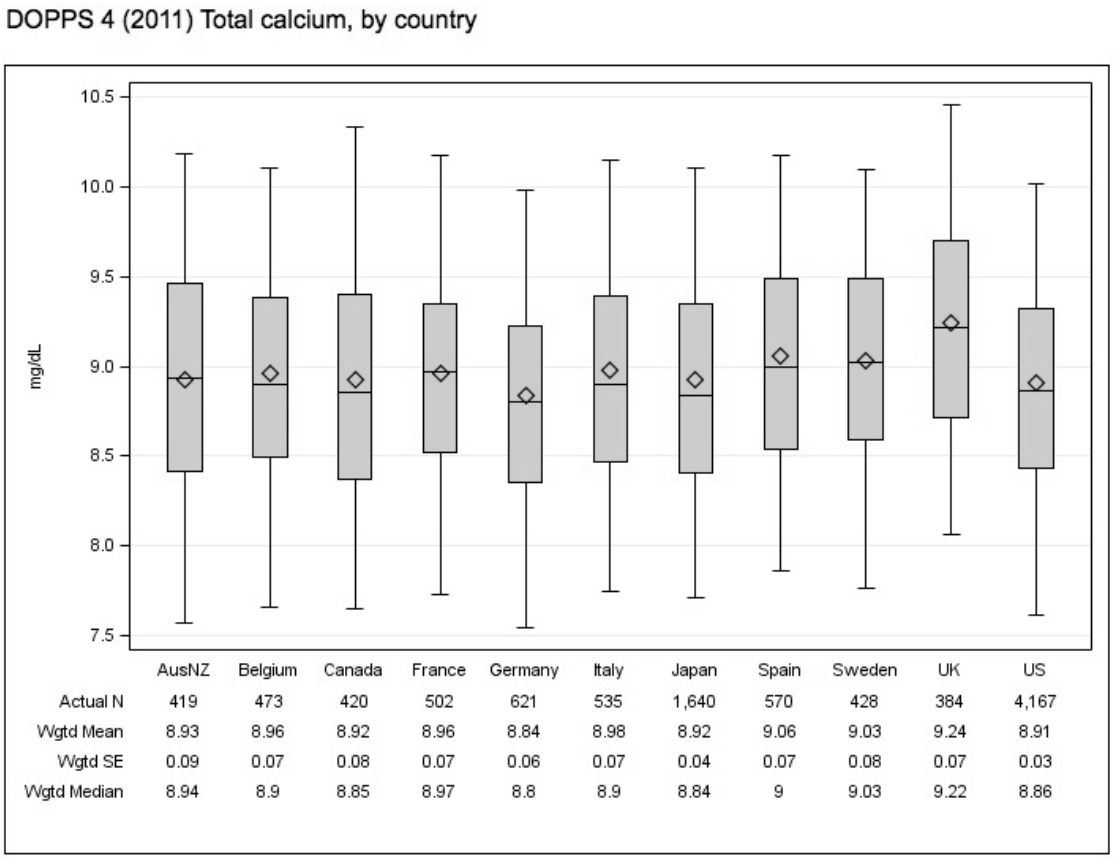
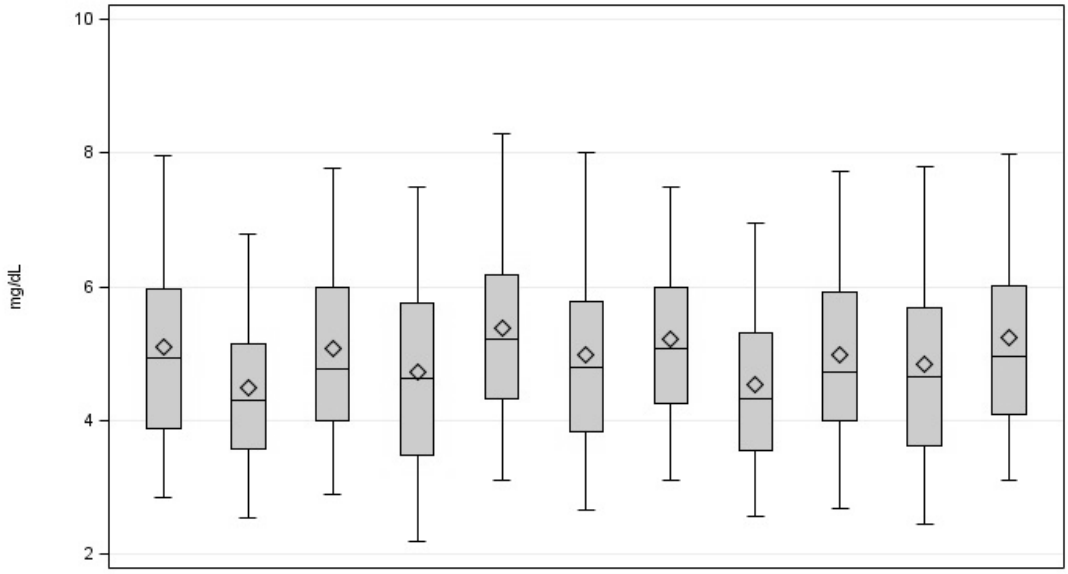
The prevalence in abnormalities of calcium, phosphorus and PTH in CKD 5D patients on haemodialysis across the globe are illustrated in the longitudinal DOPPS 4 (2011) data set. This represents results from a collection of samples from random facilities of country-specific cross-sectional cohorts. (29) (Figure 1-4)

Figure 1-4: Serum Calcium, phosphorus & iPTH in HD patients of DOPPS 4 (2011) countries



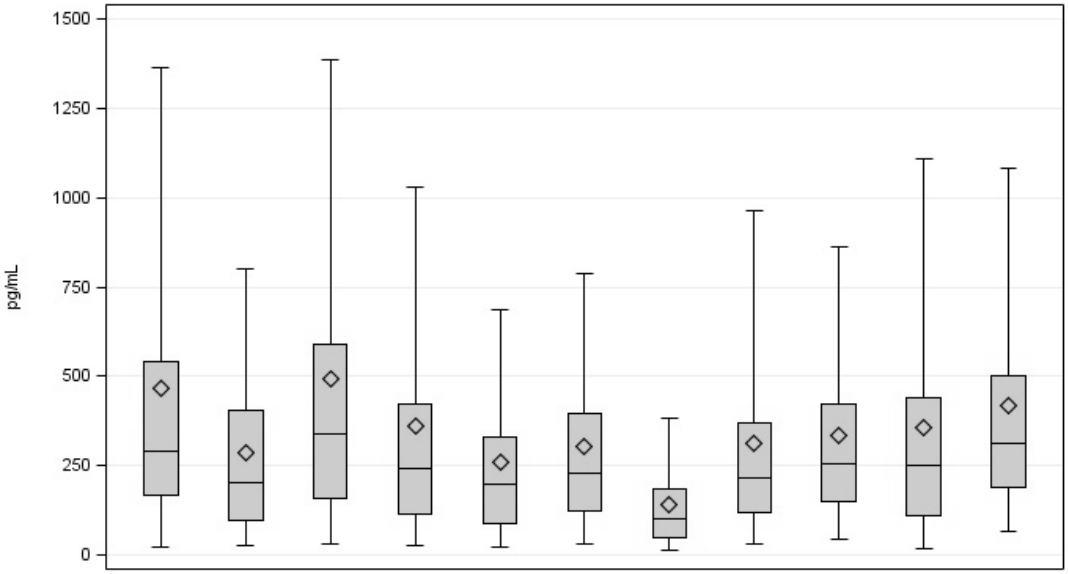
Top & bottom boxes indicate 25th & 75th percentiles. Horizontal line within box indicates median (50th percentile) & diamond mean. Vertical lines extend to 5th and 95th percentile. Reproduced with permission (29)

DOPPS 4 (2011) Serum phosphorus, by country



	AusNZ	Belgium	Canada	France	Germany	Italy	Japan	Spain	Sweden	UK	US
Actual N	453	483	484	503	669	607	1,702	582	538	418	4,234
Wgtd Mean	5.10	4.48	5.06	4.73	5.39	4.97	5.21	4.54	4.98	4.83	5.23
Wgtd SE	0.10	0.07	0.12	0.13	0.12	0.14	0.06	0.07	0.10	0.09	0.04
Wgtd Median	4.93	4.31	4.77	4.64	5.22	4.79	5.07	4.31	4.73	4.66	4.97

DOPPS 4 (2011) Serum PTH, by country



	AusNZ	Belgium	Canada	France	Germany	Italy	Japan	Spain	Sweden	UK	US
Actual N	396	421	434	447	568	537	1,364	571	491	350	4,042
Wgtd Mean	467.58	286.37	493.84	361.03	257.64	303.66	140.75	310.48	336.77	354.74	418.71
Wgtd SE	39.20	38.03	68.14	32.03	13.77	19.12	8.19	23.94	26.77	28.12	14.93
Wgtd Median	292.57	204.23	339.57	240.96	198.63	228.76	102.98	216.55	253.58	252.55	312.94

Top & bottom boxes indicate 25th & 75th percentiles. Horizontal line within box indicates median (50th percentile) & diamond mean. Vertical lines extend to 5th and 95th percentile. Reproduced with permission (29)

1.12 Mortality & morbidity

1.12.1 Introduction

Mortality and morbidity in chronic kidney disease (CKD) remain high with cardiovascular (CV) events being the major cause of death. This is 10-30 times higher in ESRD than the general population when matched for age, sex and ethnicity. (1) CKD patients on dialysis, unlike the general population exhibit reverse epidemiology of cardiovascular risk factors. Large multi-center trials targeting traditional risk factors as hypercholesterolemia, showed no significant effect on death reduction from cardiovascular disease, myocardial infarction (MI) or stroke in HD patients. (30,31) Similarly an inverse relationship has been seen between hypertension and increased body mass index (BMI) with cardiovascular mortality in CKD. (32)

The aetiology of this phenomenon is not clear and various hypotheses have been proposed. The survival bias, postulates that CKD patients that survive to reach RRT have undergone a specific selection process, as many do not reach ESRD due to high mortality and are thus distinct from the general population. The time discrepancy among competitive risk factors theory assumes that short-term survival advantages of obesity, hypercholesterolemia or hypertension outweighs the long-term cardiovascular risk. The malnutrition-inflammation complex syndrome (MICS) in dialysis patients is believed to offer the best explanation. It proposes that dialysis patients have a higher proportion of malnutrition, hypoalbuminemia and inflammation that are stronger risk factor for cardiovascular disease than the traditional risk factors seen in the general population. (32)

In recent years novel surrogate biomarkers such as Fibroblast Growth Factor – 23 (FGF-23), and traditional markers such as phosphate have emerged as powerful predictors of adverse effects. These abnormal markers from defective bone metabolism are strongly associated with vascular calcification and fractures, common causes of morbidity in the CKD population.

1.12.2 Fractures in CKD-MBD

Abnormal bone formation in common in CKD and contributes to bone pain, postural instability, weakness and increased risks of fractures. Data studying the relationship between bone histology, and risks of fractures are scanty. A review of 2507 bone biopsies in symptomatic patients over 16 years (1985 - 2001 over three time periods 1985-1990, 1991-1996 and 1997-2001) from Brazil (2340 patients, 93.1 % HD; 6.9% PD) and Uruguay (167 patients on HD) noted an increased prevalence of hyperparathyroid bone disease, mixed bone disease and adynamic bone disease and decreased low turnover osteomalacia over the three time periods. (33)

A review of the role of patient characteristics, RRT modality, biochemical parameters and medications in relationship to fractures is discussed further in chapter 2.

1.12.3 Vascular calcification in CKD-MBD

Vascular calcification is a common complication (34) and an independent predictor of mortality in CKD. (35) Various authors have studied the association of different histomorphometric characteristics and vascular calcification. London *et al.* reported an increased association of adynamic bone disease (ABD) and aortic calcification in HD patients. (36) In contrast Barreto *et al.,* found no association between coronary artery calcification and different types of bone disease. (37)

A prospective study of 64 stable HD patients with 1 year follow up by 16-slice coronary tomography (CT) and bone biopsy concluded that coronary artery calcification development was associated with lower trabecular bone volume while an improvement in bone turnover was associated with lower coronary artery calcification progression in patients with high- and low-turnover bone disorders. (38) An evaluation of 150 patients >40 years old at various stages of CKD (47 HD and 103 CKD) using pulse wave velocity showed a significant increase in arterial stiffness compared to healthy population. This remained significant after adjustment for age, sex, mean arterial pressure, heart rate and body mass index (BMI). No differences were noted between pulse wave velocity and

glomerular filtration rate (GFR) in the predialysis group, but vascular calcification assessed by X-ray and CT showed a gradual and significant increase in aortic calcification. (39)

1.13 Phosphate

1.13.1 Introduction

Inorganic phosphate or phosphorus is an important part of cellular components used for energy storage, oxygen transport, acid-base regulation, membrane transport, signal transduction and bone mineralisation. Serum phosphate is regulated between 0.8-1.5mmol/L in healthy individuals by hormones (PTH, Vitamin D, FGF-23, growth hormone amongst others) that modulate intestinal uptake, renal excretion and mobilisation from bone. Bone is the largest single source of phosphate present in the form of hydroxyapatite complexed with calcium. The daily phosphorous balance is the sum of the total intake and the amount excreted via the urinary and faecal route.

1.13.2 Phosphate, cardiovascular disease and CKD

High phosphate levels are associated with vascular calcification and increased cardiovascular mortality. This has been seen both in the CKD (40,41) and general population. (42) Goodman *et al.* noted coronary-artery calcification is common (88%) in dialysis patients as young as 20-30 years age. In this group cholesterol concentrations were lower and phosphate levels higher in those with calcification, despite showing no association with blood pressure, male gender and diabetes mellitus. (40) A similar association has been seen with elevated phosphate being an independent risk factor for increased intima-medial thickness of the carotid artery of haemodialysis patients both, with and without diabetes. (41)

Post hoc data analysis of patients with ischaemic heart disease (IHD) and normal renal function, demonstrated a graded independent relationship between high serum phosphate within the normal reference, to increased cardiovascular events and deaths. (43) An evaluation of 3368 participants from the Framingham Offspring study over a period of 16.1 years found that higher serum phosphate was associated with increased mortality from cardiovascular events in patients

with no CKD (defined as MDRD GFR of ≥60 ml/min per 1.73 m^2) or cardiovascular disease (defined as history of coronary heart disease, cerebrovascular disease, peripheral vascular disease or heart failure). On further scrutiny in a subgroup with eGFR of >90ml/min per 1.73m^2 and no proteinuria, it emerged that the association remained robust. (42)

Hyperphosphatemia is associated with progression of kidney disease in the CKD population. The composite end point determined by doubling of serum creatinine or reaching ESRD found a 29% higher risk with each mg/dl of rise in serum phosphate despite correction for multiple confounders. The study was limited by its retrospective nature, being confined to male US veterans and inability to measure the confounding effect of PTH. (44)

A systematic review of 35 studies from 1980 to 2007 studying the relationship of mineral metabolism disturbances to all-cause mortality (29 studies) and CV outcomes (11 for CV mortality & 4 CV events) in CKD found a strong association of increased mortality with phosphorus concentration. Despite the heterogeneity of the studies this association was present in both HD and PD patients though data on risk was less conclusive in pre-dialysis patients. (45) These findings are similar to a recent study examining early CKD patient population (n=10672) from a community-based screening program over a 2.3-year period, which found no significant association between quartiles of serum phosphate and all-cause mortality. Though the association for progression to ESRD was present between higher quartiles of phosphate, this was non-significant following adjustment for cofounders. (46)

1.14 Vitamin D

1.14.1 Introduction

Vitamin D is a fat-soluble compound that plays an essential role in CKD-MBD. It consists of two main parent forms D_2 (ergocalciferol) and D_3 (cholecalciferol) that are metabolised in the liver to 25(OH) D_2 or 25(OH) D_3 known as ercalcidiol or calcidiol respectively. This undergoes further hydroxylation in kidneys into 1, 25(OH) $_2$ D_2 or 1, 25(OH) $_2$ D_3 (ercalcitriol and calcitriol). Calcitriol is the most active naturally occurring Vitamin D derivative. The primary source of Vitamin D

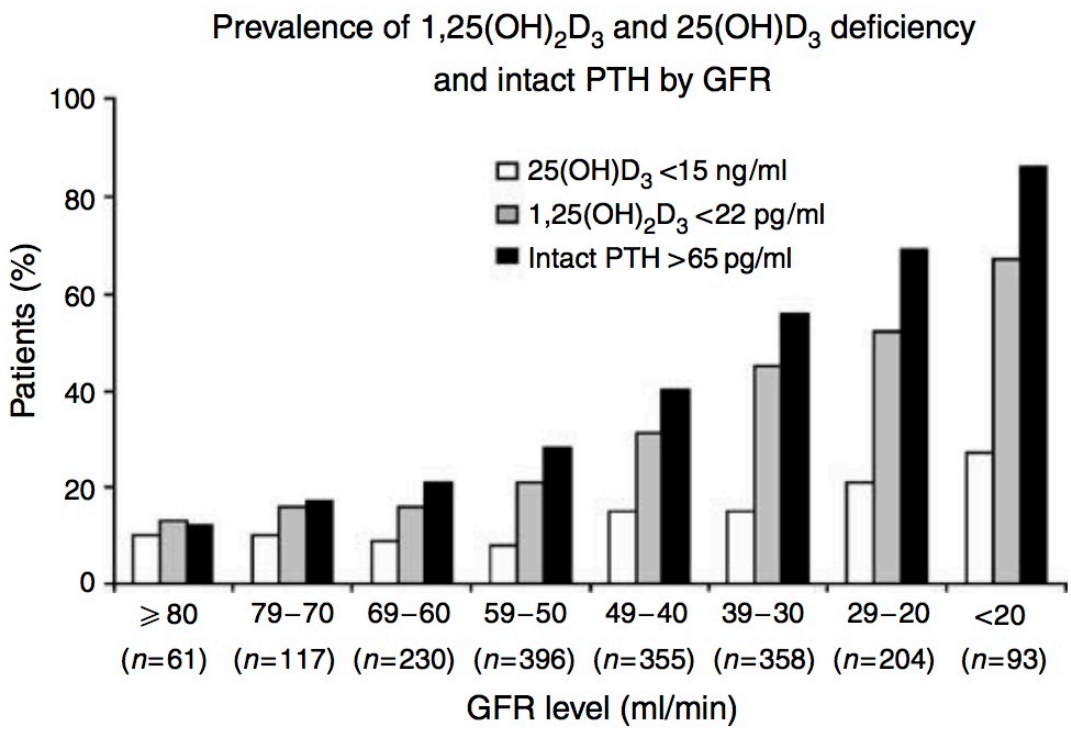
is sunlight from exposure of the skin to ultraviolet B (UVB) solar radiation that converts 7-dehydrocholesterol to previtamin D3 that undergoes rapid conversion to Vitamin D3. Vitamin D deficiency occurs as a result of decreased endogenous synthesis production from insufficient exposure to sunlight or dietary deficiency.

Various assays for measurement of Vitamin D exist and the definition of deficiency is not standardised. The current recommendations suggest using the same laboratory for measurement of levels.

1.14.2 Vitamin D, mortality and CKD

The prevalence of Vitamin D deficiency in CKD is common though its relationship to stages of CKD is unclear. Some studies reported lower levels with advanced disease (47,48) while others found no such relationship. (28) Observational studies in the general (49) and CKD population(50) with low Vitamin D levels have been associated with adverse clinical events.

Figure 1-5: The prevalence of deficiency of 1,25 OH_2 D_3, 25(OH) D_3, and secondary hyperparathyroidism by GFR



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A secondary analysis of a prospective double blinded, randomised placebo-controlled trial in healthy postmenopausal women >55 years, from the community found a lower incidence of cancer in the Vitamin D treated group compared to the placebo arm. 1179 subjects were followed for 4 years and randomly assigned to receive placebo, calcium (1400-1500mg) or calcium & Vitamin D3 (1000IU, 25µg). The unadjusted relative risk (RR) of incident cancer in the calcium and Vitamin D group was 0.402 (CI: 0.20 – 0.82, p=0.013) and calcium group 0.532 (CI: 0.27 – 1.03, p = 0.063). The effect remained when cancers after first 12 months were excluded in the calcium and Vitamin D group (RR – 0.232, CI: 0.09 – 0.60, p <0.005). The change in Vitamin D levels was most in the Vitamin D and calcium group (23.9±17.8 nmol/L). (51)

A similar randomised study in the Women's Health Initiative (WHI) showed an inverse relationship between 25(OH)D and incident risk for all cancers though there was no significant effect of Vitamin D on colorectal cancers. The baseline Vitamin D, dose of Vitamin D used (400 IU versus 1100 IU) and changes of serum Vitamin D levels achieved were lower in the WHI study. (51)

Data from 825 incident patients in the US on haemodialysis suggests increased mortality with lower serum levels of Vitamin D within 90 days of initiating RRT. This was independent of residual renal function, biomarkers of mineral bone disease, nutritional factors and co-morbidities. (50)

A meta-analysis of Randomized Controlled Trials of 57,311 participants with a mean follow up of 5.7 years (range 6months – 7years), adjusted for study size, suggested Vitamin D supplementation reduces all-cause mortality in adults and older individuals. The mean daily dose of Vitamin D, adjusted for trial size was 528 IU (range 300IU – 2000IU) with most patients receiving a dose between 400IU to 833 IU/day. Patients with chronic renal disease, ESRD, those on dialysis and advanced prostate cancer were excluded. It however did not examine the relationship with baseline Vitamin D status and the dose of Vitamin D supplementation. (49,52)

1.15 Parathyroid hormone - PTH

1.15.1 Introduction

Parathyroid hormone is an 84 amino acid protein composed of N-terminal, C-terminal and mid-region fragment. It is secreted in response to low ionised calcium - the most important determinant, hyperphosphatemia and Vitamin D deficiency. Levels are suppressed in response to calcitonin, hypercalcemia and high FGF-23 levels.

Conventional assays for PTH measured C terminal or N-terminal while 2nd generation tests detected the full-length molecule i.e. both the N & C terminus ('intact PTH' or 'active PTH') using two-site radioimmunoassay. 3rd generation assays that truly determine the full length molecule of 1-84 amino acid residues ('whole'/'bioactive' PTH) have been developed, but not widely available, and do not show better predictive value. Further methodological issues differ - based on collection mode (serum or plasma), sitting temperature (ice or room) and use of multiple commercial kits that make standardisation difficult.

1.15.2 Diagnostic role as bone marker & mortality

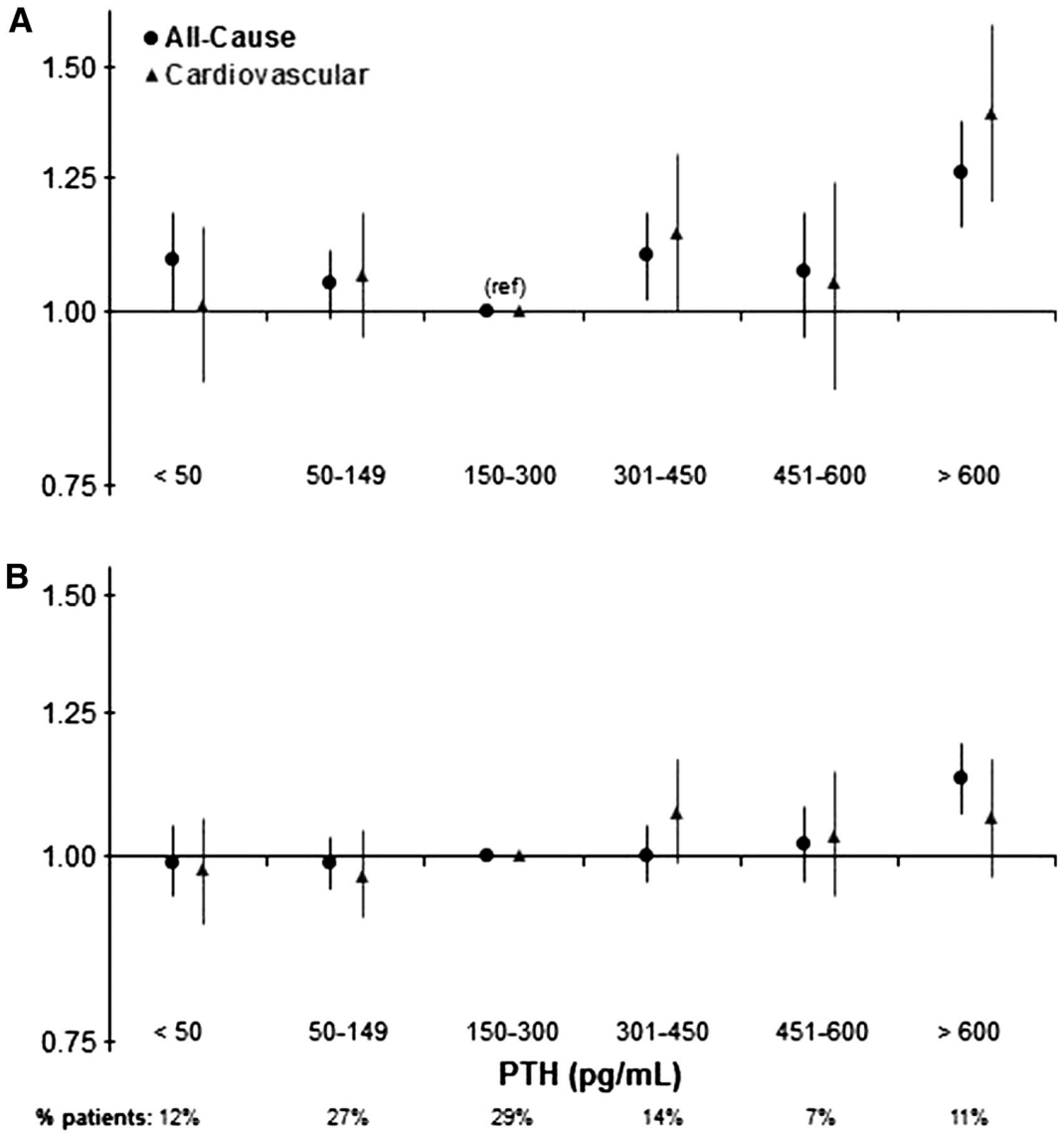
KDIGO recently assessed the diagnostic value of PTH as a marker of bone turnover in a cross-sectional retrospective study of 492 dialysis patients with bone biopsies. PTH was able to differentiate high turnover (iPTH >9 times upper limit of normal; sensitivity, 37.0%, specificity, 85.8%) from nonhigh and low (iPTH <2 times upper limit of normal; sensitivity, 65.0%, specificity, 67.3%) Addition of bone alkaline phosphatase improved this marginally. Low, normal, and high bone turnover could not be diagnosed with a single or combination of biomarkers. (53) This is further discussed in Section 1.18.2.

Raised PTH a consequence of secondary hyperparathyroidism is common in CKD stages 3-5D and associated with increased mortality and morbidity. Optimal levels of PTH in stages CKD 3-5D is unknown, though it is the most commonly used surrogate markers of bone turnover to guide treatment.

Analysis of the DOPPS phases 1to 4 data over the last 15 years (1996-2011) suggests increased risk of all cause mortality with very high or low levels of PTH.

Cardiovascular and all cause mortality was high for PTH = 300-450 pg/ml (HR, 1.09) and PTH > 600pg/ml (HR, 1.23) while hospitalisation rates were highest in the group with PTH levels >600pg/ml in the adjusted models. A further subgroup analysis of patients with no exposure to treatment for secondary hyperparathyroidism showed a similar association of increased mortality with very low (PTH <50 pg/ml; HR, 1.25; 95% CI, 1.04 to 1.51) and high levels of PTH (PTH >600 pg/ml; HR 1.15; 95% CI, 0.86 to 1.53), Figure 1-6. (54)

Figure 1-6: Associations of (PTH) levels with mortality and hospitalizations in DOPPS participants.



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This is consistent with other data (55,56) that suggests a U or J shaped relationship between adverse outcomes and PTH thresholds. However, a recent meta-analysis did not find an association between PTH and mortality.(57)This is possibly due to analysis of single cohort studies and evaluation of PTH as a continuous variable with linear association. The use of multiple analytical

methods and different assays adds to the complexity of being able to interpret the data accurately.

1.15.3 Cinacalcet & effect on PTH

Cinacalcet a calcimimetic agent lowers PTH along with calcium and phosphate levels. It acts on calcium-sensing receptors on the parathyroid gland and used to treat refractory secondary hyperparathyroidism in patients on HD who do not respond to conventional therapy or cannot have a parathyroidectomy. An analysis of 4 randomized, placebo controlled clinical trials of >1100 patients showed a significant reduction in fracture rates, surgical parathyroidectomy and cardiovascular hospitalisation with improvement in the physical component summary and bodily pain of the short form (SF-36) health survey in the Cinacalcet group. (58) The Evaluation of Cinacalcet Therapy to Lower Cardiovascular Events (EVOLVE) Trial reported a non-significant reduction (HR 0.93; P = 0.112) in risk of deaths or cardiovascular events in patients with secondary hyperparathyroidism treated with cinacalcet use versus placebo. Total follow up was for 64 months (median 21.1 months, on Cinacalcet) with median iPTH levels of 692 pg/mL. The number of surgical parathyroidectomies fell by >50%. (59)

1.16 Calcium

1.16.1 Introduction

Calcium in the bone accounts for 99% of the total calcium distribution. The remainder (1%) is present in the extracellular fluid, measurable as serum calcium with the rest being in intracellular spaces. 40-50% of the serum ionised calcium (Ca^{2+}) is physiologically active while the rest is bound to albumin or anions as phosphate. (17) Levels vary as per protein levels with laboratories reporting 'corrected calcium' in addition to ionised Ca^{2+}. PTH, Vitamin D, various other hormones and pH tightly regulate serum calcium levels in a narrow reference range of 2.1-2.6 mmol/L in healthy individuals. The amount absorbed by the intestines, the exchanges across the intracellular, extracellular and bone compartments and those excreted via the kidneys maintains calcium homeostasis. The net balance is neutral in healthy adults, positive in children of

growing age, and negative in the elderly. The regulation of Calcium in relation to phosphate, Vitamin D and FGF-23 is described in Section 1.20.

1.16.2 Calcium in CKD

The kidneys play a vital role in calcium regulation. While no data exists on the association of high serum calcium on mortality and morbidity in CKD stage 3a- 5, high serum calcium in CKD Stage 5D increases mortality, independent of age, gender, race, diabetes, vintage, phosphorus and PTH. (60,61) A prospective cohort study of 25,588 patients with end stage renal disease on HD for >180 days across 925 dialysis facilities found increased risk of all-cause mortality (HR, 1.10) and cardiovascular mortality (HR, 1.17) with calcium (or corrected calcium) levels of > 10 mg/dL (2.5 mmol/l) or calcium levels of ≤ 1.88 mmol/L. (60)Data from North America is conflicting with increased risk of mortality with calcium levels of <8.5 mg/dL (2.1 mmol/l) in univariable models which reversed with multivariable adjustment. (61)

Calcium used in dialysate in HD patients is an important determinate of the total calcium balance. Mass assessment on calcium is difficult and levels during HD are affected by duration of HD, ultrafiltration rate and intradialytic interval. (62) Two studies in the early 1990's (63,64) evaluated the effects of varying dialysate calcium on plasma concentration. Acute (63,64) and long-term changes (64) in calcium were measured with different concentrations of dialysate (0.75, 1.25, & 1.75 mmol, Hou SH. *et al.*; 1.25, 1.5 & 1.75 mmol; Argiles A. *et al.*). Dialysate calcium of 1.25 mmol/l was considered to be near neutral for most patients. The optimal dialysate calcium should induce a positive balance in patients with deficiency and reduce flux in those with calcification. Since then 2 RCTs have been conducted, the results of which are discussed in Section 1.19.4.

1.17 Management of CKD-MBD

Management of mineral bone disease in CKD is complex. It involves education, dietary restriction, medications, increased duration of dialysis and parathyroidectomy in severe cases. Therapies are focussed on correcting surrogate markers of bone turnover, the benefits of which remain uncertain.

1.18 KIDIGO 2017 & UK RA June 2018 Updates

The summary of the original 2009 KDIGO guidance on Diagnosis, Evaluation, Prevention and Treatment of CKD-MBD and 2017 guidelines is compared and enlisted in Appendix 1.1. The guidance has not been reproduced verbatim to allow discussion of the recommendations. Here we consider the relevant KDIGO 2017 updates, which include some changes in the order of recommendations. (65)

The update has provided some clarity but raises further questions of implementation, investigations and treatment. The major shift in management is towards an individualised, multi-professional approach and deviation from specific targets. The evidence and rationale behind the latest guidance is included and the recommendations briefly examined.

UK RA published an Update in June 2018 a 'Commentary on the KDIGO Guideline on the Diagnosis, Evaluation, Prevention and Treatment on CKD-MBD in June 2018' to guide UK clinical practice. (19) This is covered with each section to summarise UK recommendations.

1.18.1 Diagnosis of CKD-MBD using DEXA

KDIGO Guideline 3.2.1, 2017 Update, Grade 2B

A substantial advance in the new guidelines is the consideration for use of DEXA BMD in patients with CKD G3a- G5D to assess fracture risk if this information impacts treatment decisions in patients at risk.

KDIGO Guidance 3.2.2, 2009, Grade 2B

Dual energy X-ray absorptiometry (DEXA) BMD assessment was not routinely recommended in patients with CKD G3a – G5D due to lack of evidence and its inability to predict fracture risk. Besides, BMD did not predict histological form of renal osteodystrophy.

Rational & Evidence

Fractures in CKD are higher than the general population. (66,67)Previous guidance on the use of DEXA, was based on cross sectional studies. Latest data from 4 prospective cohort studies (68-71) have consistently demonstrated hip BMD predicted fractures across the spectrum of CKD G3a – G5D, despite not making distinction between different histological forms i.e. adynamic bone disease, osteodystrophy, osteoporosis or high bone turnover. (71)

The latest evidence comes from Naylor *et al.,* who examined data from the Canadian Multicentre Osteoporosis Study (CaMos) using The Fracture Risk Assessment Tool (FRAX®) in the CKD cohort. FRAX® assesses the risk of fractures over a 10- year period using clinical risk factors and BMD at the femoral neck in the general population. The CaMos cohort, stratified by eGFR had 320 patients all ≥ 40 years age with CKD 3a – 5 (72.2 % Stage 3a, 23.8 % stage 3b). Individuals were followed up for a mean of 4.8 years for major incident fractures. FRAX® predicted risk was 6.4% with BMD and 8.2% without BMD. This was similar to the major observed fracture risk of 5.3% in eGFR <60ml/min/1.73 m^2 (3.3% - 8.6%, 95% CI). The major limitations were relatively small number of fractures and inability to further stratify prognostication by stages of eGFR that reduced statistical power.(71)

West *et al*. studied 131 patients from three tertiary care hospitals across Toronto ≥ 18 years with CKD stage 3a- 5 over a 2-year period. DEXA BMD was measured at baseline and 24 months. Baseline and 2 years DEXA BMD were significantly lower in the total hip, lumbar spine, ultradistal and ultradistal 1/3 radius in the fracture group compared to those without fractures. The risk of incident fractures increased by 1.98 (95% CI, 1.53 – 2.43) for hip fractures and 1.89 (95% CI, 1.44 – 2.34) for lumbar spine for every 1 standard deviation (SD) decrease in DEXA BMD. The association did not change in the adjusted models. (72)

Yenchek *et al*. measured hip BMD in 2754 participants and reported its association to non-spine fragility fractures. DEXA BMD identified osteoporosis at

baseline and recorded fractures using self-reported surveys (contacted every 6 months with annual review). Median observation period was 11.3 years and 587 participants (21%) had a GFR of < 60 ml/min/ 1.73 $m^{2.}$ 98 fractures were recorded in the CKD group and 286 in the non – CKD group. Time to first fracture was analysed with BMD being the main predictor. Greater fractures were associated with lower femoral neck BMD (each lower SD BMD, HR 2.14, 95% CI 1.80 – 2.55, no CKD; HR 2.69, 95% CI 1.96-3.69, CKD) independent of CKD. The model was adjusted for age, sex, race and BMI.

Iimori *et al*. assessed usefulness of PTH, bone alkaline phosphatase (b-ALP) and BMD in predicting fracture risks in patients with CKD stage 5D. This single centred study included 485 patients in Japan over a 5-year period. PTH levels <150 pg/ml (HR – 3.47, p <0.01, n = 148) or > 300 pg/ml (HR – 5.88, p <0.0001, n = 141) were associated with increased risk of all type of fractures. High b-ALP was a significant predictor of all fractures. Low baseline BMD at every site except lateral lumbar spine predicted fracture risk. (HR 0.96, P = 0.01, femoral neck; HR = 0.95, P = 0.003, trochanter; HR = 0.97, P = 0.005, total hip) The risk persisted in both unadjusted and adjusted demographic parameters. (68)

What is not clear from the 2017 updates is what defines, risk factors for osteoporosis, how these patients should be monitored, with what imaging, and when treatment should be commenced.

The effectiveness of Denosumab and safety in CKD stage G1 – G4 was evaluated in the FREEDOM trial (Fracture Reduction Evaluation of Denosumab in Osteoporosis Every 6 Months). 3902 subjects (2817 women with CKD stage 3a – G3b and 73 with G4) with an average age of 72.3 ±5.2 years received Denosumab and were followed up for 36 months. (73) Denosumab reduced incident fractures, with increases in BMD at all sites. Adverse effects were similar in both groups. There was no robust evidence to support a theoretical fear of adynamic bone disease though a major limitation was the lack of data on CKD 5- 5D.

UK RA Update, June 2018

The UK RA recommends a multi-professional approach between rheumatologist/osteoporosis expert and renal physicians to aid decision on management following DEXA.

1.18.2 Bone biopsy

Use of bone biopsy, the 'gold standard' in diagnosis of renal osteodystropy is limited due to lack of physician expertise and patient choice.

KDIGO Guideline 3.2.2, 2017, Not Graded

Bone biopsy continues to be recommended for CKD G3a-G5D, if the knowledge will impact treatment decisions. This is no longer necessary for initiation of treatment.

KDIGO Guidance 3.2.1, 2009, not graded

KDIGO suggested bone biopsy in certain clinical settings in CKD G3a- 5D (unexplained fractures, persistent bone pain, unexplained hypercalcemia, unexplained hypophosphatemia, possible aluminium toxicity, and prior therapy with bisphosphonates, though not limited to these) and prior to the use of antiresorptive therapy.

Rational & Evidence

DEXA BMD or laboratory parameters do not provide an accurate assessment of bone histology in CKD. To predict diagnostic accuracy of bone turnover to bone histology a retrospective analysis of bone biopsies and biomarkers (iPTH, whole PTH, b- ALP and amino –terminal propeptide of type 1-collagen) were undertaken across 4 nations from 492 dialysis patients. Levels of biomarkers singly or in combination did not reach acceptable levels of discrimination for a single time point diagnosis of different forms of BMD. iPTH, whole PTH and b-ALP levels were associated with bone turnover. (53)

PTH to help diagnostic decision-making is illustrated in Table 1-4.

Table 1-4: Utility of PTH thresholds for diagnosis decision-making (KDOQI & KDIGO)(53)

	NKF-KDOQI[a]				KDIGO[b]			
	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
Differentiating low from nonlow turnover bone disease, or "When do I stop therapy?"	68.5%	61.2%	71.6%	57.7%	65.7%	65.3%	73%	57%
Differentiating high from nonhigh turnover bone disease, or "When do I start therapy?"	58.0%	77.7%	34.8%	90%	37.0%	85.8%	34.9%	86.9%

Abbreviations: iPTH, intact parathyroid hormone; KDIGO, Kidney Disease: Improving Global Outcomes; NKF-KDOQI, National Kidney Foundation–Kidney Disease Outcomes Quality Initiative; NPV, negative predictive value; PPV, positive predictive value.

[a]Using serum iPTH < 150 pg/mL for lower and >300 pg/mL for upper threshold.

[b]Using serum iPTH < 130 pg/mL for lower and >585 pg/mL for upper threshold (2× and 9× upper limit of normal for assay).

Reprinted from AJKD, Sprague SM et al., Diagnostic Accuracy of Bone Turnover Markers and Bone Histology in Patients With CKD Treated by Dialysis, 2016; 67:559-66 with permission from Elsevier

UK RA Guidance June 2018

The UK RA acknowledges that bone biopsy may be an appropriate test and that implementation of an ungraded guidance may be limited by local expertise.

1.18.3 Treatment targets

KDIGO Guidance 4.1.1, 2017, not graded

The new guideline suggests treatment to be focussed on serial assessments of phosphate, calcium, and PTH together in CKD G3a-G5D.

Rational & Evidence

Bone markers undergo diurnal changes and levels are affected by various other factors i.e. type of food intake, mediations, RRT treatment etc. amongst others. (74) This section is further covered in KDIGO Guidance 4.1.5

1.18.4 Serum phosphate levels

KDIGO Guideline 4.1.2, 2017, Grade 2C

Elevated phosphate levels should be lowered toward normal in CKD G3a-G5D.

KDIGO Guideline 4.1.1, 2009, Grade 2C

Previous guidance was to maintain serum phosphate in the normal range in CKD G3a – G5 and lowering this towards normal range in CKD G5D.

Rational & Evidence

The association of high phosphate to mortality has been proven in multiple trials. (60,75,76) However, there is no data supporting benefit from maintaining phosphate in the normal range with treatment, in CKD G3a – G4.

A study on 148 patients with GFR between 20-45ml/min/1.73m^2 treated with phosphate binders (calcium acetate, lanthanum, sevelamer or placebo) showed a fall in serum phosphate from 4.2 mg/dl (1.34 mmol/l) to 3.9 mg/dl (1.26

mmol/l) in the treatment arm compared to 4.1 mg/dl in the placebo arm. iPTH increased in the placebo group and was stable in the treated group. There was however an increase in coronary artery and abdominal aorta calcification in the treated group. (77)

UK RA Guidance June 2018

The RA suggests an individualised approach towards achieving the normal range. Previously NICE and UK RA Update of 2015 recommended target serum phosphate level of 0.9 – 1.5 mmol (Guideline 3.1, 2015, Grade 2C) for CKD 3-5 and 1.1 – 1.7 mmol for CKD 5D (Guideline, 3.2, 2015, Grade 2C). (18)

1.18.5 Serum calcium levels

High calcium levels are associated with increased mortality and morbidity.

KDIGO Guideline 4.1.3, 2017, Grade 2C

An individualised approach to managing mild asymptomatic hypocalcemia and avoidance of hypercalcemia is suggested rather than correction.

KDIGO Guideline 4.1.2, 2009, Grade 2D

The maintenance of normal calcium was suggested in CKD Stages 3- 5D.

Rational & Evidence

In the EVOLE (Evaluation of Cinacalcet Therapy to Lower Cardiovascular Events) trial, subjects with hypocalcemia from cinacalcet did not come to harm, and correction of hypocalcemia had the risk of positive calcium balance. The findings of the EVOLVE trial is discussed further in later sections of the thesis. (78)

UK RA Guidance June 2018

RA recommends avoidance of hypercalcemia and maintenance of levels below the upper range of normal for the reference laboratory.

1.18.6 Dialysate calcium

KDIGO, Guideline 4.1.4, 2017, Grade 2C

In patients with CKD G5D a dialysate calcium concentration of 1.25 to 1.50 mmol/l has been suggested.

KDIGO, Guideline 4.1.3, 2009, Grade 2D

Guidance 4.1.3, 2009, Grade 2D has been upgraded to Guidance 4.1.4, 2017, Grade 2C with no change in the advice.

Rationale & Evidence

2 RCTs (79,80) have been published since 2009 of better quality though they do not discriminate between harm and benefit.

Ok *et al*. examined the effect of dialysate calcium on coronary artery calcification (CAC) score and bone histomorphometry. Patients with PTH ≤ 300pg/ml were randomised to high calcium dialysate of 1.75 mmol/l (HCD, n=213) or low calcium dialysate 1.25 mmol/l (LCD, n = 212). Multislice computerised tomography (CT) and bone biopsy was performed at baseline and 24 months. Overall there was an increase in CAC scores in both groups (452 ± 869 to 616± 1086, LCD; 500± 909 to 803± 1412, HCD) but the differences were not significant (p = 0.25). However, the mean absolute difference i.e. the progression of CAC scores (-138, p=0.03) by Agatston method, a semi-automated tool to assess CAC score and the mean absolute difference in progression of volume (-118, p = 0.001) were significantly different. In 108 patients that had a bone biopsy an increase in bone turnover (bone formation rate and activation frequency) was seen in the LCD compared to the HCD. (low turnover decreased from 85.0 % to 41.8%, P = 0.001, LCD; 79.2 % to 64.2 %, HCD). (79)

Another RCT in 52 patients with iPTH <100 pg/ml randomised to 1.25 mmol or 1.75 mmol/l calcium dialysate over a 6-month period demonstrated an improvement in the bone and mineral parameters in the LCD group. Pre-dialysis mean total calcium was not significantly different between the two groups but

post dialysis total calcium was significantly higher in the HCD group (2.59± 0.18 HCD; 2.44± 0.19, LCD; p<0.01). Ionised calcium was significantly higher in the HCD group both pre (1.08± 0.05 mmol/l, HCD; 1.04± 0.06, LCD; p = 0.02) and post dialysis (1.8 ± 0.04, HCD; 1.04 ± 0.04, LCD p <0.01), while this was not noted in the total or ionised calcium levels in the LCD group. Mean total and ionised calcium increased in the HCD compared to the LCD. iPTH and total alkaline phosphatase increased in the LCD group at 3 and 6 months (p < 0.001) At 3 months the pre-dialysis total and ionised calcium in the LCD decreased and then levelled off. (80) The study did not look at bone biopsies but based the likely diagnosis of adynamic bone disease on the iPTH levels.

UK RA Guidance June 2018

RA recommendations with CKD G5D are a dialysate of 1.25 to 1.5 mmol/l with a degree of flexibility to meet patients' individual requirements.

1.18.7 Treatment of hyperphosphatemia

KDIGO Guidance 4.1.5, 2017, Not Graded

The decision on phosphate lowering treatment should be based on progressive or persistent changes in phosphate levels.

KDIGO Guidance 4.1.4, 2009, CKD 3a-G5, Grade 2D; CKD G5D, Grade 2B; Choice of binder, Not Graded

Phosphate binders were recommended in CKD G3a- G5D for the treatment of hyperphosphatemia. The choice of binder was based on concomitant treatment and presence of other components of CKD.

Rational & Evidence

The study by Block et al. has been discussed in Section 1.18.4, KDIGO Guidance 4.1.2, 2017. It highlights the risk of the associated arterial calcification with phosphate binding therapy. (77)

Hill *et al.* studied 8 patients with CKD Stage 3-4 during two 3-week periods in a placebo-controlled crossover study. Samples from blood, urine and faeces were collected at baseline and on a weekly basis. Patients on calcium carbonate had a greater calcium balance compared to placebo while phosphorus balance did not differ significantly. Fasting serum phosphate and PTH were unaffected by calcium carbonate despite a modest increase in urinary phosphate. (81)

Both these studies challenge the rational of using calcium-based phosphate binders in the prevention of hypocalcemia and treatment of hyperphosphatemia.

UK RA Guidance June 2018

In CKD G3a-G5D the UK RA advises against the use of phosphate binders 'pre-emptively' but reserving this for those with progressively rising or persistent hyperphosphatemia. A multi-professional approach to its management was suggested.

KDIGO Guidance 4.1.6, 2017, Grade 2B

The use of calcium-based phosphate binders in CKD G3a- 5D should be restricted in patients receiving phosphate-lowering treatment.

KDIGO Guidance 4.1.5, 2009, Grade 1B

Restriction on the use of calcium containing phosphate binders and/or dose of Vitamin D analogs in the presence of persistent or recurrent hypercalcemia

Rational & Evidence

Three new RCTs were published since the last recommendation; 2 published by the INDEPENDENT (Reduce Cardiovascular Calcifications to Reduce QT Intervals in Dialysis) investigators from the *Di Iorio* group. (82,83) In the first cohort 466 incident haemodialysis patients across 18 Italian centres were randomly assigned to receive Sevelamer (4300 ± 1400 mg/day, median 4800 mg/day) or calcium containing phosphate binder (2200 ± 1000 mg/day, median 2000 mg/day) for 24 months and followed up for 36 months. It excluded patients > 75 years of age

and those with a history of cardiac arrhythmia. Phosphate levels at baseline were higher (mean 5.6 ± 1.7 mg/dl versus 4.8 ± 1.4 mg/dl) and CAC scores lower (median 19, IQR 0-30 versus 30, IQR 7-180) in the Sevelamer group compared to the calcium group. There were no significant changes in the use of Vitamin D analogs, Cinacalcet, beta-blockers or HMG (3-hydroxy-3-methyl-glutaryl) coenzyme reductase inhibitors. There was a decrease in serum phosphate (-0.65 ±0.12mg/dl, P <0.001), calcium (-137 ± 0.09 mg/dl, P <0.001) and iPTH (-173.7 ± 15.85 pg/ml, P< 0.001) in the Sevelamer group compared to the calcium group at 24 months from baseline. Cardiovascular mortality due to cardiac arrhythmias and all cause cardiovascular deaths was lower in the sevelamer group compared to the calcium treated group (P<0.001). This association did not change after adjusting for CAC scores, time varying phosphate levels and C- reactive protein. Mortality from non-cardiovascular events were however not significant in the 2 groups. (83)

Mortality in kidney disease patients treated with phosphate binders (INDEPENDENT Study) was a randomised, multicentre, non-blinded pilot trial that looked at the use of phosphate binders on mortality. Patients with CKD Stage 3-4 were administered Sevelamer (n=107, mean dose 2184 ± 592 mg/day) or calcium carbonate (n = 105, mean dose 2950± 703 mg/day). CAC sores were assessed at 6 monthly intervals for a 2-year follow up period. Baseline median CAC scores (122 AU, IQR 0-180, Sevelamer group; 0 AU, IQR 0-215, Calcium group) and percentage of patients with calcification (62.6 % versus 47. 6%, P = 0.02) were higher in the Sevelamer group. Average phosphate concentration (4.37 ± 1.33 mg/dl versus 4.85 ± 0.79 mg/dl; p<0.01) was lower and average serum calcium (8.6 ± 0.7 mg/dl versus 9.4 ± 0.6 mg/dl) decreased in the sevelamer group. Average concentrations were defined as mean of all values up to the event or closing of the study. Patients randomised to the Sevelamer group had a lower rate of all-cause mortality at dialysis inception. These associations did not change following adjustment with baseline covariates. The major limitation of this study was the sample size. (82)

The study by *Block et.al*, (77) previously discussed in Section 1.18.4 highlights the potential harm from phosphate binders.

The working group acknowledged that calcium in any form might be harmful in all stages of CKD irrespective of presence of other markers (low PTH, hypercalcemia, arterial calcification or adynamic bone disease). Its use in certain high-risk scenarios may however be valid, thus the previous qualifier from 2009 recommending restricted use of calcium-based binders in persistent hypercalcemia was deleted.

Further evidence has since emerged from meta-analysis though this did not have a bearing on the 2017 guidelines.

Jamal *et al.* analysed data from 11 randomised trials (August 2008 – October 2012) with 4622 participants as a part of systematic review and showed a 22% reduction in all-cause mortality in patients assigned to non-calcium binders (Sevelamer and Lanthanum) compared to those on calcium binders (RR 0.78, 95% CI (0.61 – 0.98). The reduction in mortality was independent of serum phosphate and significant at 24 months but not 36-42 months, possible due to insufficient power. (84)

Patel *et al.* reviewed further data of 25 studies until March 2015 and showed a 46% reduction in all-cause mortality in patients receiving Sevelamer compared to those on calcium containing binders (RR 0.54, 95% CI 0.32 – 0.93). 88 % of the 4770 participants were on haemodialysis. Patients in the Sevelamer group had lower serum calcium, higher iPTH and lower total and LDL cholesterol. The phosphate levels in the two groups however did not differ significantly. (85)

Palmer *et al.* reviewed data from 77 trials with 12562 patients (11009 dialysis participants) and found no evidence that phosphate binder treatment reduced all call mortality compared to placebo. Sevelamer was better than calcium containing binders in reducing all-cause mortality (odds ratio, OR 0.39, 95% CI 0.2- 0.74) but for all other groups (lanthanum, iron, colestilan) the effects were non-significant. All binders reduced phosphate more than placebo. (86)

UK RA Guidance June 2018

The RA suggests limiting calcium-based binders in CKD G3a-5D. Calcium-based binders is no longer recommended as the first line of treatment specially as more generic version of non-calcium containing binders are available.

1.18.8 Dietary phosphate restriction

KDIGO Guidance 4.1.8, 2017, not graded

The 2017 guideline added a qualifier for better education about food choices in addition to dietary phosphate restriction.

KDIGO Guidance 4.1.7, 2017, not graded

Dietary phosphate restriction is recommended in both the 2009 and 2017 update.

Rational & Evidence

From a patient perspective and on a practical basis dietary phosphate restriction is difficult and could potentially restrict protein intake. Additives in food are high in phosphate and phosphate from plants is less absorbable (20 – 50%) than phosphate from animals (40-60%) due to presence of phytates in plants. Besides vegetable based diet had a lower phosphate absorption compared to meat based diet. (74) A better understanding and knowledge is suggested to help patients make better, informed choices.

UK RA Guidance June 2018

RA recommends personalised, evidence-based advice from Specialist Renal dietician in patients with CKD 3a- 5D for the management of hyperphosphatemia with our increased knowledge of bioavailability of phosphate from different food sources.

1.18.9 PTH in CKD-MBD

Modest rises in PTH in early stages of CKD is an adaptive response to declining eGFR.

KDIGO Guidance 4.2.1, 2017, Grade 2C

The Work Group updated guidance to reflect treatment should not be based on single elevated PTH. Modifiable risk factors i.e. hyperphosphatemia, hypocalcemia, high phosphate intake and Vitamin D deficiency should be evaluated

KDIGO Guidance 4.2.1, 2009, Grade 2C

Rational & Evidence

There was no data from RCTs for recommendation of an optimal PTH. Despite high PTH being associated with higher mortality, there is no data that's shows improvement in outcomes with lower PTH.

An RCT on 87 patients with CKD stage G2 to G4 randomised to treatment with 5,000 or 20,000 IU of cholecalciferol reported no difference in the PTH levels between the two groups at completion of study. There was PTH reduction in both groups and Vitamin D levels increased in both arms with serum levels being significantly higher in the high dose cohort. (87)

Thus recommendation 3.1.3 of the 2009 KDIGO guideline, which suggest 25 (OH) OD (calcidiol) level may be measured, and repeat testing determined by baseline values and therapeutic interventions remain. Replacement of Vitamin D deficiency and correction should continue as per strategies for the general population (Grade 2C).

UK RA Guidance June 2018

RA suggests evaluation of modifiable risk factors i.e. hyperphosphatemia and Vitamin D deficiency in patients with persistently elevated PTH and that decisions on treatment should not be based in single values.

1.18.10 Vitamin D analogs and calcitriol

KDIGO Guidance 4.2.2, 2017, not graded

The 2017 guidelines for CKD G3a - G5 no longer recommends routine use of calcitriol or Vitamin D analogs in non-dialysis patients, and that it should be reserved for severe and progressive secondary hyperparathyroidism.

KDIGO Guidance 4.2.2, 2009, Grade 2C

The use of calcitriol or Vitamin D analogs were suggested in CKD G3a - G5 in patients with rising PTH that remained persistently above the upper limit of normal despite correction of modifiable factors.

Rational & Evidence

Information from 2 RCTs, the PRIMO (88) and OPERA (89) showed no benefit of cardiac end points but increased risks of hypercalcemia with Vitamin D.

In the OPERA trial, a prospective, double blind RCT, 60 patients with left ventricular (LV) hypertrophy and CKD Stage 3-5 were administered a placebo (n=30) or 1µg of paricalcitol (n=30), daily for 52 weeks. LV mass measured with cardiac magnetic resonance imaging (MRI) at baseline and end of study showed no significant change (median – 2.59, IQR -6.13 to 0.32 g/m^2, placebo; median -4.85, IQR -9.89 to 1.10 g/m^2, paricalcitol) in the 2 groups despite significant reduction in the iPTH levels. The incidence of hypercalcemia (calcium >2.55 mmol/L) occurred in 43.3 % (n=13) in the treated group compared to 1 patient in the placebo group. (89)

The PRIMO (Paricalcitol capsules benefits in Renal failure Induced cardia Morbidity) study used a larger dose of paricalcitol (2 µg) and compared its effects to placebo in 112 patients with CKD 3- 5 over a 48-week period. Levels of PTH were reduced within 1 month and maintained in the normal range throughout the study. LV mass not indexed to height, assessed by cardiac MRI showed no changes (1.29 g, 95% CI, −0.72 to 3.29 g, paricalcitol; −0.20 g, 95% CI, −2.19 to 1.80 g, placebo; P = 0.12). For a subgroup of patients with LV

hypertrophy the LV mass index increased at week 48 in the paricalcitol group compared to placebo (0.46 g/m2.7, 95% CI, −0.15 to 1.08 g/m2.7, paricalcitol; −0.23 g/m2.7, 95% CI, −0.87 to 0.41 g/m2.7, placebo; P = .05) though the overall LV mass index did not differ (0.34 g/m 2.7, 95% CI, -0.14 to 0.83 g/m 2.7; paricalcitol; -0.07 g/m 2.7, 95% CI, -0.55 to 0.42 g/m 2.7; placebo; P= 0.06). The incidence of hypercalcemia defined as 2 consecutive measurements of greater than 10.5 mg/dL, was higher in the paricalcitol group compared to placebo (22.6% versus 0.9%). (88)

UK RA Guidance June 2018

RA no longer recommends calcitriol or its analogues in CKD 3a – G5 with reservation of these treatments to severe, progressive secondary hyperparathyroidism. Where treatment is commenced it should be started at the lowest dose, avoiding hypercalcemia.

1.18.11 PTH lowering therapy

KDIGO Guidance 4.2.4, 2017, Grade 2B

The use of calcimimetics, calcitriol, Vitamin D analogs or a combination of these medications has been suggested in patients with CKD G5D

KDIGO Guidance 4.2.4, 2009

This guidance suggested the use of calcitriol, Vitamin D analogs or calcimimetics to lower PTH (Grade 2B) and that the choice of the initial drug for treatment of elevated PTH be made on the serum calcium and phosphate levels (non graded). The binder used to adjust PTH should not compromise the serum phosphate and calcium levels (non graded) and that in patients with hypercalcemia Vitamin D analogue can be stopped (Grade 1B).

Rational & Evidence

The EVOLVE study (59)in 3883 HD patients, recruited across the globe showed a non-significant reduction of all-cause mortality and non-fatal cardiovascular events with cinacalcet use versus placebo (HR 0.93; P = 0.112). Though this did

not meet its primary unadjusted composite end point there seemed to be benefit of cinacalcet use when adjusting for baseline imbalances. The working group could not reach a consensus on the interpretation of data (use of primary analysis versus the suggestion of benefit from post hoc analysis) and recommendations for use of cinacalcet as the first line in CKD 5D. The new guidance thus recommends all concomitant treatment in CKD 5D in alphabetical order i.e. calcimimetics, calcitriol, or vitamin D analogs, or a combination of calcimimetics with calcitriol or Vitamin D analogs where PTH lowering therapy is needed.

UK RA Guidance June 2018

RA suggests that for dialysis patients requiring PTH lowering therapy calcimimetics, calcitriol and Vitamin D are all acceptable therapies. Parathyroidectomy has been suggested for patients that fail to respond to medical therapy in severe hyperparathyroidism.

1.18.12 Role of bisphosphonates

Guidance on the role of bone biopsy has been updated and covered in Section 1.18.2.

KDIGO Guidance 4.3.3, 2017, Grade 2D

Treatment with bisphosphonates in patients with CKD G3a – 5D with low BMD, fragility fractures or biochemical abnormalities should take into account magnitude and reversibility of biochemical abnormalities with consideration of bone biopsy.

KDIGO Guidance 4.3.3, 2009, Grade 2D

The suggestion was treatment choices take into account biochemical abnormalities, progression of CKD and consider a biopsy in patients with CKD Stage 3 with low BMD or fragility fractures.

Rational & Evidence

This has been covered in guidance 3.2.2 and the findings of the FREEDOM trial discussed. Recommendation 4.3.4 from 2009 has been removed with broadening of treatment choices.

UK RA Guidance June 2018

RA recommends treatment choices take specific side effects and bone phenotype into account in patients with CKD G3a-G5D. This is all the more relevant from a UK perspective as antiresorptive are not authorised for those with eGFR <30ml/min/$m^{2.}$

1.18.13 Assessment of BMD in transplant recipients

KDIGO Guidance 5.5, 2017, Grade 2C

Bone mineral density testing to assess fracture risks in CKD G1T – G5T in patients with risk factors for osteoporosis has been suggested.

KDIGO Guidance 5.5, 2009, Grade 2D

Assessment of BMD in patients with eGFR of >30ml/min/1.83 m^2 was suggested in the first three months after transplantation if they received steroids or had risk factors for osteoporosis.

Rational & Evidence

This has been broadly covered in recommendation 3.2.1. Currently there's no prospective data that looks into the association of DEXA to fractures in transplant patients.

A retrospective analysis of 238 transplant recipients from 1995 – 2007 examined the association of DEXA with fracture events. In the 670 examinations performed the incidence of osteopenia was 32.5% in the lumbar region and 46.0 % in the hip region, osteoporosis 14.1% in the lumbar and 13.9% in the hip region. There were 53 fractures in 46 patients. The relative risk of fractures with osteopenia and

osteoporosis was significantly higher than those with normal BMD. This was independent of age, female gender and diabetes (HR 2.7, 95% CI 1.6 – 4.6, P = 0.0003, Osteopenia; HR 3.5, 95% CI 1.8 – 6.4, P = 0.0001, Osteoporosis). (90) This study however did not form part of the review process due to its retrospective design.

UK RA Guidance June 2018

RA suggests that DEXA may be a useful tool to assess fracture risk in transplant recipients while acknowledging that evidence for intervention to prevent fracture is limited.

1.18.14 Role of Vitamin D post transplantation

KDIGO Guidance 5.6, 2017, Grade 2D/2C

The use of Vitamin D analogs and or antiresorptive agents is to be considered in patients with low BMD and eGFR ≥ 30 ml/min/m^2 in the first 12 months after kidney transplant. The guidance however acknowledges that there's insufficient data to guide treatment.

KDIGO Guidance 5.6, 2009, Grade 2D/2C

The guidance was similar to the updated 2017 guideline except that it recommended, consideration of bone biopsy prior to commencement of antiresorptive in the first 12 months with eGFR of > 30mil/min/m^2

Rational & Evidence

This has been covered in section 4.3.3.

UK RA Guidance June 2018

The guidance is largely similar to the 2017 KDIGO guidance. On the use of antiresorptive agents it suggests guidance as per local practice in the absence of optimal treatment strategy.

1.18.15 KDIGO & RA Updates on children

Guidelines specifically related to children 4.1.3, 4.1.6, 4.2.2 have not been considered for the purposes of this thesis.

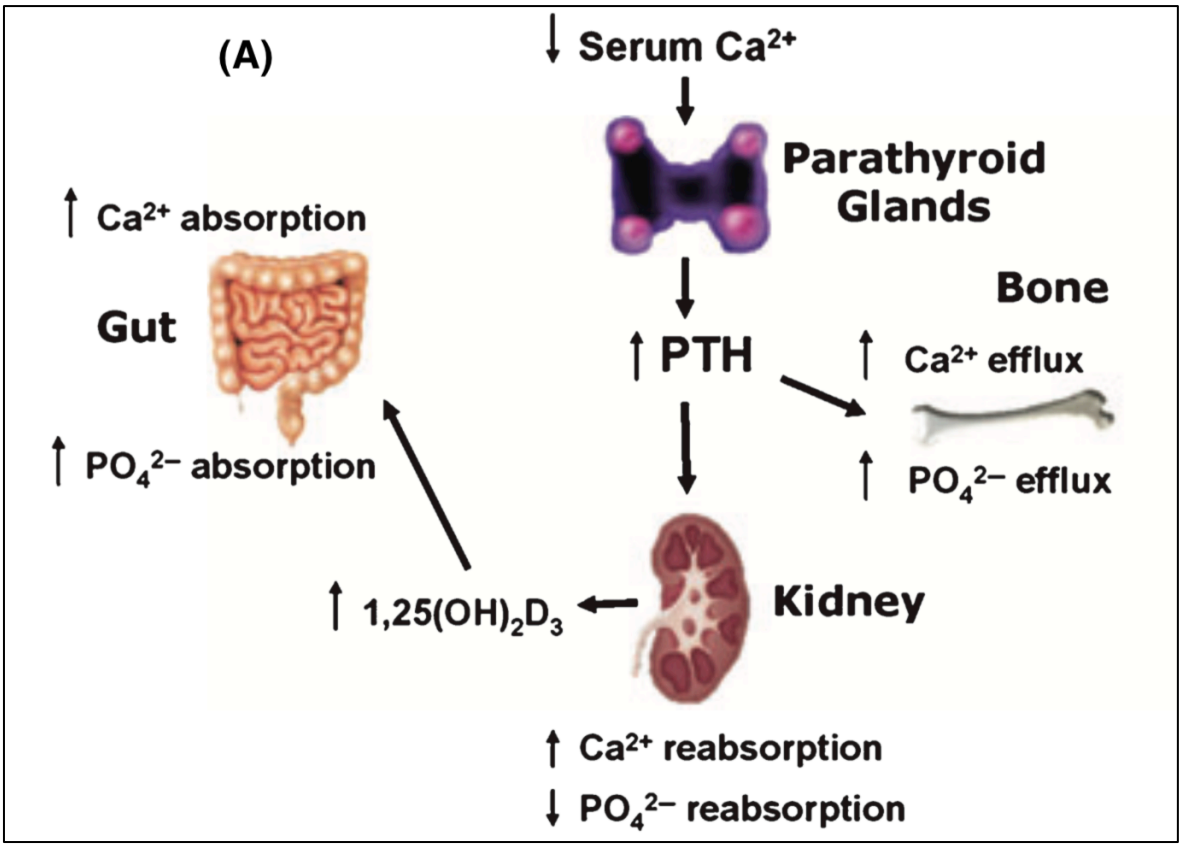
1.19 Novel biomarkers

1.20 Fibroblast growth factors (FGF-23)

Fibroblast growth factor 23, a novel molecule has emerged as an important regulator of phosphate metabolism. It maintains phosphate and Vitamin D homeostasis, independent of Calcium - PTH – Vitamin D axis by inducing phosphaturia and decreasing plasma $1,25(OH)_2D_3$. Its structure, assays and biological properties are described further in Chapter 4.

Traditionally our understanding of bone metabolism was limited to the Vitamin D- PTH- Calcium axis. Low calcium is the primary stimulant of PTH from the chief cells in the parathyroid gland. PTH increases 1-alpha-hydroxylase activity from the kidneys that promotes circulating $1,25(OH)_2D_3$ levels. $1,25(OH)_2D_3$ increases calcium reabsorption by the kidneys. Calcium and phosphate efflux from bone by PTH and increased absorption of calcium from the intestine in response to increasing levels of $1,25(OH)_2D_3$ levels helps restore calcium levels. The secondary effects of increased phosphate are offset by PTH, which promotes phosphaturia. With loss of renal function Vitamin D levels are reduced and PTH levels increased to help maintain normal calcium – phosphate levels. These mechanisms fail with progressive renal failure. (91) Figure 1-7

Figure 1-7 : Traditional Calcium- PTH- Vitamin D axis



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FGF-23, first isolated from the ventrolateral thalamic nucleus of mouse brain (92) is mainly secreted from osteocytes and osteoblasts of bone, thymus and lymph nodes. High FGF-23 levels is associated with deficiency of $1,25(OH)_2D_3$ and seen in X-linked hypophosphatemic rickets, autosomal dominant/recessive hypophosphatemic rickets and tumour induced osteomalacia.

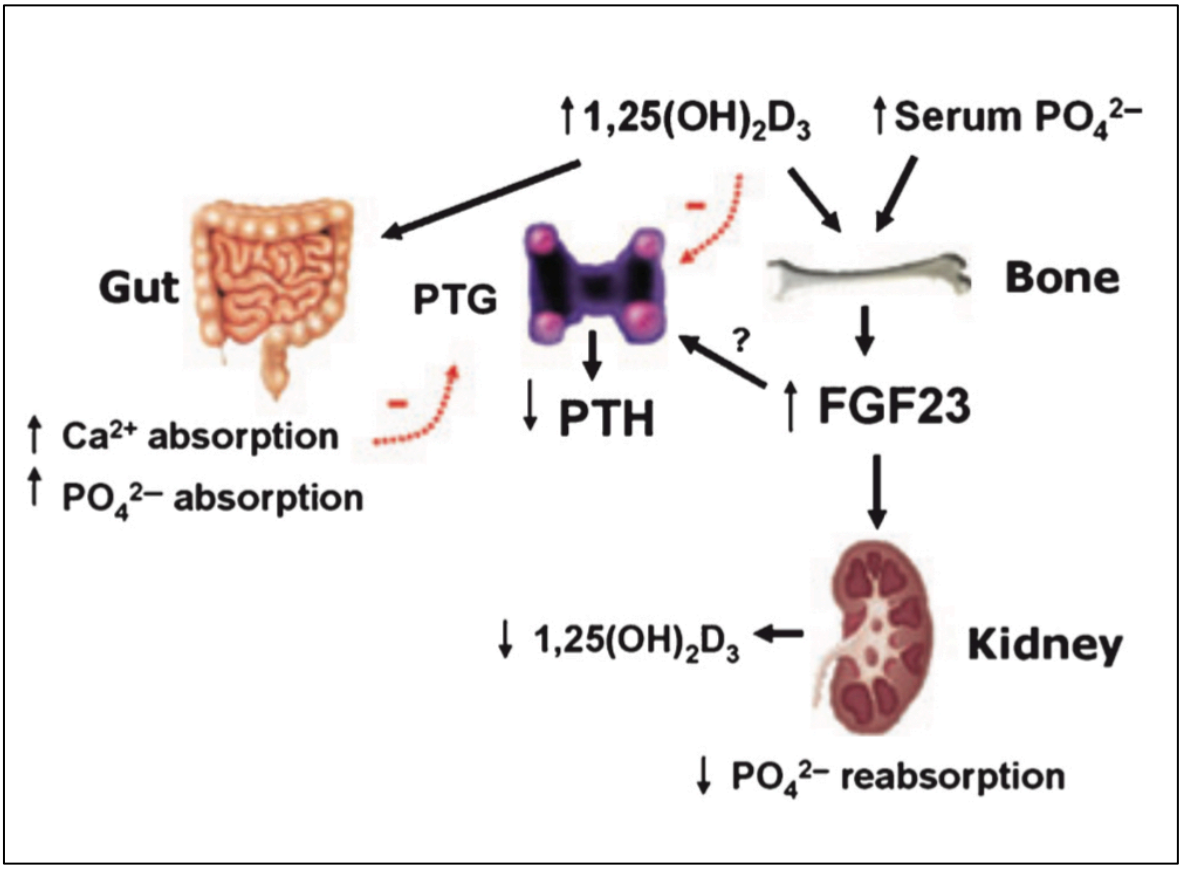
FGF-23 inhibition by administration of antibodies in animal models leads to hyperphosphatemia and normal $1,25(OH)_2D_3$ levels by decreasing urinary fractional excretion of phosphate ($UFE_{phosphate}$). Rats with mild CKD and normal serum phosphate had high $UFE_{phosphate}$ and low $1,25(OH)_2D_3$ levels prior to administration of FGF-23 antibodies. This suggests the changes in Vitamin D and phosphate are FGF-23 dependant. The rise in FGF-23 in early CKD before any increases in PTH and phosphate abnormalities further supports the role of FGF-23 in maintenance of normal phosphate levels. (93)

The data on dietary phosphate acting as a stimulant of FGF-23 production is conflicting. Larsson *et al*. administered six healthy males with normal renal function a phosphate-supplemented diet. Blood samples were collected 4 times a day with measurement of phosphate and FGF-23 levels. FGF-23 levels were within the normal range and no circadian variation seen. Serum phosphate, PTH and $1,25(OH)_2D_3$ levels did not change. Phosphate excretion was increased but no changes in FGF-23 levels were noted during the phosphate loading in the 24-hour urine samples. (94) In contradiction to the previous study phosphate restricted diet in 13 healthy individuals produced a significant reduction of FGF-23 concentrations and increase in $1,25(OH)_2D_3$ levels. There was an inverse relationship of FGF-23 to Vitamin D concentrations. (95)

These suggest that FGF-23 is a counter regulatory hormone for Vitamin D. It increases phosphaturia and maintains serum Vitamin D levels. With failing nephrons phosphate excretion decreases and the effects of FGF-23 to maintain phosphate haemostasis becomes less effective. FGF-23 levels gradually rise, often 1000- fold in ESRD to maintain phosphate homeostasis.

There may be a direct role of FGF-23 on PTH but the exact mechanism surrounding this is not fully understood. A simple representation of the role of FGF-23 in the regulation of phosphate, Vitamin D, calcium and PTH is shown in Figure 1-8.

Figure 1-8 FGF-23 & bone metabolism axis



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1.20.1 FGF-23 and mortality

The ArMORR (Accelerated Mortality on Renal Replacement) study examined the association of FGF-23 and mortality in haemodialysis patients from a cohort of 1056 US dialysis centres. Data was analysed prospectively from 10,044 subjects beginning haemodialysis treatment and followed up for 1 year. cFGF-23 levels (iFGF 23 and cFGF-23 had a linear correlation) were measured after excluding patients exposed to Vitamin D at entry point and multivariable models were adjusted for confounding factors. Baseline demographics, primary renal diagnosis, associated comorbidities, coexisting conditions, dialysis dose and facility-specific standardized mortality rates were included in the models. High cFGF-23 levels were strongly associated to mortality, independent of phosphate and other known risk factors. The relationship of FGF-23 and mortality was dose-responsive and seen on a continuous scale (odds ratio 1.8; 95% CI, 1.4 - 2.4 per unit increase in log cFGF-23) and for each quartile (Quartile 1 served as

reference and odds ratio for quartile 2, 3 and 4 were 1.6 [95% CI, 0.8 - 3.3]; 4.5 [95% CI, 2.2 - 9.4]; and 5.7 [95% CI, 2.6 to 12.6] respectively). (96)

Secondary analysis from The EVOLVE Trial a randomised, prospective, multicentre placebo-controlled trial, showed treatment with cinacalcet significantly lowers FGF-23. FGF-23 levels were recorded at baseline and 20 weeks, when 91% of patients had blood samples and were still on the original treatment. 2985 (77%), of the 3883 randomised patients had baseline FGF-23 and 2602(67%) had both baseline and week 20 results available. Samples were analysed using Luminex- based microbead assay which uses polyclonal capture and detection antibodies. Median FGF-23 levels at baseline (Cinacalcet group, 5555pg/ml; Placebo, 5600 pg/ml; P= 0.86) were similar. At week 20 weeks FGF-23 levels were significantly lower in the cinacalcet group (2255 pg/ml) compared to the placebo group (5580 pg/ml), P <0.001. Events defined in the primary end points were recorded up to 5 years and associations with FGF-23 levels recorded. Reductions in FGF-23 were associated with lower cardiovascular events and deaths. (97)

While one cannot conclude that lower FGF-23 levels contributed to the lower cardiovascular events the association of high FGF-23 with LVH and the ability of Cinacalcet to lower calcium is relevant.

1.21 Further avenues in management of CKD-MBD

A systematic review and meta- analysis is underway to assess the use of cinacalcet versus standard treatment in CKD. All data sources from MEDLINE, EMBASE, Cochrane register and Web of Science from 1996 to June 2015 are being reviewed and will be reported using the GRADE summary results. (98)

1.22 Aims of this project

This research project evaluates end points that are of direct importance to patients (fractures & quality of life) and investigates the effect of different dialysis strategies on biochemical markers such as phosphate, FGF-23 and others.

1.22.1 Primary aims

To investigate the association between bone biochemistry and hard end points such as fractures in a prevalent RRT population

To assess the impact of a novel tele-health technology on clinical outcomes and quality of life in PD patients

To study the effect of a nocturnal HDF program on bone biochemistry and fluid management

1.22.2 Secondary aims:

To expand on the knowledge of FGF-23 during a session of dialysis

To study the association of phosphate and FGF-23 in a group of dialysis (PD & HD) patients

1.23 Hypothesis

Accepting that the management of CKD-MBD in patients with ESRD is challenging, it is hypothesised that better control of biochemical parameters is achievable by using different RRT modalities, innovative information technology and self-management techniques. This in turn may impact on morbidity, mortality and Quality of life (QOL).

1.24 Limitations

Phosphate levels fluctuate within individuals and have interassay, post prandial, diurnal and seasonal variability. No study conducted during the routine delivery of dialysis can fully take these variations into account.

Chapter 2

Symptomatic Fractures in RRT population

2.1 Introduction

Fractures are an important cause of morbidity and mortality in patients on renal replacement therapy (RRT) (99). Histological changes of bone disease begin early in chronic kidney disease (CKD) but the clinical consequences of bone pain and fracture occur mainly in CKD stage 5d. Features specific to chronic kidney disease – mineral bone disease (CKD-MBD) (hyperphosphatemia, diminished activation of vitamin D, secondary hyperparathyroidism, elevated fibroblast growth factor 23 [FGF-23]) and exposure to medications altering bone metabolism in patients with CKD are likely to contribute to fracture risk. Markers of chronic kidney disease - mineral bone disease (CKD-MBD) improve substantially with successful renal transplant but transplant patients have the additional fracture risk associated with steroid induced osteoporosis. The true fracture risk in RRT patients and its association with surrogate markers of CKD-MBD is poorly defined.

In a recent report of the temporal trends in fracture risk in a large cohort of dialysis patients the incidence of pelvic/hip fractures fell from 29.6/1000 patient years in year 2000 to 20.6/1000 patient-years in 2009 (100). A systematic review looking into fracture risk in kidney transplant recipients, with follow up from 1.7 to 5.3 years found a variable incidence rate of 3.3 to 99.6 fractures per 1000-person years (101). The methods of detecting fracture events, difference in fracture definitions used and the fracture sites studied make it difficult precisely to define the true incidence and risk factors for fractures in the RRT population.

2.2 Aims

The aim of this multicentre observational study was to quantify the incidence of radiologically proven bone fracture by anatomical site in prevalent renal replacement therapy groups, and study its relationship to potential risk factors.

2.3 Material and Methods

All adults (>18 years of age) prevalent on RRT on 07/07/2010 were identified from the West of Scotland electronic patient record. This prospectively maintained database includes all patients managed by the 4 adult renal units (National Health Services (NHS) Ayrshire & Arran (n=287), NHS Dumfries & Galloway (n=123), NHS Greater Glasgow & Clyde (n=1376) and NHS Lanakshire (n=310) at inception) serving the 2.5 million population of the West of Scotland and has interfaces that receive all radiology reports from all of the 20 hospital radiology departments in the region. Initial electronic search was performed on all reported fractures from 07/07/2010 until 01/08/2013. New fractures were identified by reviewing all radiology reports manually (X-ray, computerised tomography (CT), magnetic resonance imaging (MRI), ultrasound (USG), nuclear medicine scans, angiographic reports and others) and included all radiology investigations from date of inception. The clinical indication for the radiological examination was used to determine if the fracture was likely new. Where it was unclear from the report if the fracture was new or old a previous radiological investigation was viewed if available. Fractures reported in this study were either symptomatic or incidental findings reported as a part of other radiological investigation for a different purpose. From the clinical indication included in the radiological reports, the majority of fractures identified were symptomatic.

This meant that all fractures in the study patients would be identified with the exception of fractures occurring outside the region (for example if the patient was on vacation elsewhere). All of these reports were read to identify new fracture events. If multiple anatomically distant sites were involved in one incident then they were recorded as separate fracture events. One of the 4 renal units joined the West of Scotland renal electronic patient record in 2011 and so 01/08/2011 was selected as the inception date for RRT patients from this centre. Median follow up period was 3.0 years.

2.3.1 Definition of fractures:

We based the grouping of anatomical fracture sites on International Classification of Disease tenth version (ICD10) (102) (Table 2-1).

Table 2-1 Definitions of fractures in RRT population

Definition	Includes fractures of
Foot	Toe, metatarsals, calcaneus, os calcis, cuboid, cunieforms
Tibia	Fibula, ankle, malleolus, malleolar, bimalleolar and trimalleolar
Femur	Shaft of femur, diaphysis and condyles
Hip	Neck of femur, pertronchanteric, intertrochanteric, trochanteric
Pelvis	Sacrum, ilium, acetabulum and pubis
Shoulder	Clavicle, acromion, glenoid process and scapula
Humerus	Elbow, epicondyles and greater tuberosity
Radius	Ulna, wrist and scaphoid
Hand	Metacarpals etc.
Skull	Face - maxilla, orbit, scaphoid
Thorax	Ribs and sternum
Others	Patella

RRT – renal replacement therapy

2.3.2 Covariates:

Covariates identified as potential risk factors for fractures were age, sex, primary renal diagnosis (PRD), RRT modality, RRT duration, biochemical parameters (serum albumin, phosphate, adjusted calcium, alkaline phosphatase, intact parathyroid hormone (PTH)), haemoglobin, and medications affecting bone metabolism (corticosteroids, cinacalcet, alfacalcidol, calcium and non-calcium containing phosphate binders).

For calculation of biochemical parameters, the average of the last three results prior to inception was calculated. The pre-dialysis values performed routinely each month were used for patients on haemodialysis (HD). The ERA-EDTA Coding system for Primary Renal Disease and the related web-based PRD search tools were used in recording the diagnosis of PRD. (103) They were grouped as familial/hereditary nephropathies, glomerular disease, systemic disease, tubulointerstitial disease and diabetes.

2.3.3 Statistical analysis:

Follow up continued until the date of death or date of last recorded serum laboratory result in the electronic record before 4th September 2013. The incidence of fracture was expressed as the total number of fractures per 1000 patient years of follow up. Patient baseline characteristics and fracture risk ratio were compared between RRT modalities. Risk for sub-groups was the total number of fractures divided by the total follow up time in that group and risk ratios with 95% confidence intervals calculated to compare groups.

Actuarial time to first fracture was calculated by Kaplan Meier survival method and log rank test applied to determine statistical differences between RRT groups. Censor date was time to first fracture, death or lost to follow up. Survival analyses were studied for different fracture groups: 'all fractures', hip fractures, pelvic fractures and major fractures (femur, hip and pelvic fractures combined).

Cox- proportional hazard models were constructed with time to first fracture as the dependent outcome. The hazard ratio (HR) for fractures was estimated for each covariate in the univariable model. All covariates in the univariable model were selected for the multivariable model. Further multivariable models were created in the same way for the renal transplant (RT), HD and PD groups separately.

Statistical analysis was performed using SPSS version 21 (IBM Corp., New York, USA). P values <0.05 were regarded as statistically significant.

2.3.4 Ethics review:

This study involved anonymous, observational, electronic data collection and thus no ethics approval or informed consent was sought.

2.4 Results

2.4.1 Patient characteristics:

2096 patients on RRT at the start of the study. 907 were on HD, 108 on peritoneal dialysis (PD) and 1081 had a functioning RT. Mean age at inception was 55.7 years (50.4 years RT and 61.8 years HD group). Median duration of subsequent follow up was RT 1112 days (range 8-1155), HD 1086 days (range 1 – 1155) and PD 1126 days (range 13-1155). Patient characteristics at study inception are shown in table 2-2. As expected, mean serum phosphate, PTH and alkaline phosphatase were lowest in the RT group and this group also had the highest haemoglobin concentration. Prevalent corticosteroid use was highest in the RT group, and prevalent phosphate binder and activated vitamin D use highest in the HD group.

Table 2-2 Baseline characteristics at inception in RRT population

	RT	HD	PD	Total
Number (%)	1081(51.6)	907(43.3)	108(5.2)	2096
Females, n (%)	442 (40.9)	375 (41.3)	54 (6.2)	871 (41.6)
Age, years±2SD	50.4±13.3	61.8±15.8	57.9±15.3	55.7±15.6
Age Groups, n (%)				
16-44 years	360 (33.3)	151 (16.6)	21(19.4)	532
45-64 years	568 (52.5)	338 (37.3)	51(47.2)	957 (45.7)
65-74 years	124 (11.5)	201 (22.2)	21(19.4)	346 (16.5)
>75 years	29 (2.7)	217 (23.9)	15 (13.9)	261 (12.5)
Total	1081	907	108	2096
RRT Vintage, n (%)				
0-1 year	28(2.6)	324(35.7)	53 (49.1)	405(19.3)
2-5 years	180 (16.7)	333 (36.7)	37(34.3)	550(26.2)
6-10 years	250 (23.1)	121(13.3)	10 (9.3)	381(18.2)
11-20 years	369 (34.1)	71(7.8)	5 (4.6)	445(21.2)
>20 years	254 (23.5)	58(6.4)	3(2.8)	315(15)
Primary Renal Diagnosis, n (%)*				
Tubulointerstitial disease	181(27.2)	104(18.2)	8(16.3)	293(22.8)
Systemic disease	35(5.3)	68(11.9)	3(6.1)	106(8.2)
Miscellaneous disease	105 (15.8)	113(19.8)	9(18.4)	227(17.7)
Glomerular disease	205(30.8)	131(23.0)	10(20.4)	346(26.9)
Familial/hereditary	113(17.0)	56(9.8)	8(16.3)	177(13.8)
Diabetes	27(4.1)	98(17.2)	11(22.4)	136(10.6)
Blood lab, Average (SD)				
Phosphate (mmol/L)	0.98 (0.25)	1.42 (0.44)	1.53 (0.44)	1.2 (0.42)
Adjusted Calcium (mmol/L)	2.45 (0.16)	2.37 (0.17)	2.42 (0.18)	2.41(0.17)
Alkaline Phosphatase (U/L)	96.7 (60.48)	131.12	135.87	113.64
Albumin (g/L)	38.85 (4.91)	34.26 (5.33)	36.92(5.1)	36.65 (5.59)
Parathyroid hormone (pmol/L)	28.38	41.11(28.27)	39.86	37.3 (28.32)
Hemoglobin (g/L)	117.48	98.97	108.32	108.57
Medication exposure at baseline,				
Corticosteroid	996 (92.14)	70 (7.72)	6 (5.56)	1072
Cinacalcet	28 (2.59)	110 (12.13)	5(4.63)	143 (6.82)
Alfacalcidol	220 (20.35)	663 (73.10)	29(26.85)	912 (43.51)
Alucaps	14 (1.30)	59 (6.50)	7 (6.48)	80(3.82)
CCPB	48 (4.44)	250 (27.56)	12 (11.11)	310 (14.79)
Lanthanum	6 (0.56)	156 (17.20)	7 (6.48)	169 (8.06)
Sevelamer	27 (2.50)	283 (31.20)	22 (20.37)	332 (15.84)

CCPB, calcium containing phosphate binder; HD, haemodialysis; n, number of patients; PD, peritoneal dialysis; RRT, renal replacement therapy; RT, renal transplant; SD, standard deviation * Data for Primary renal diagnosis available for 1285 (61.3%)

2.4.2 Incidence of fractures

There were 340 fractures in the three-year study period with an overall incidence of 62.8 per 1000 patient years. The incidences were 37.6, 99.2, and 57.6 per 1000 patient years in the RT, HD and PD groups respectively ($p<0.05$). Radial, foot and hip fractures were the 3 commonest sites (n=53, 47 and 46 respectively). Fractures at other sites are illustrated in Table 2-3.

The risk ratio (RR) of fracture in HD group compared to RT was 2.60, (95% CI: 2.59- 2.61), and in HD compared to PD was 1.70 (95% CI: 1.68-1.70) for all fracture types.

Table 2-3 Absolute number of radiological fractures in RRT population

RRT patients	**RT**	**HD**	**PD**	**Total**
Number	1081	907	108	2096
Fractures n (%)				
Foot	24 (2.2)	21 (2.3)	2 (1.9)	47
Tibia	14 (1.3)	23 (2.5)	3 (2.8)	40
Femur	7 (0.6)	6 (0.7)	1 (0.9)	14
Hip	12 (1.1)	32 (3.5)	2 (1.9)	46
Pelvis	3 (0.3)	12 (1.3)	2 (1.9)	17
Vertebrae	9 (0.8)	29 (3.2)	0	38
Shoulder	2 (0.2)	11 (1.2)	0	13
Humerus	5 (0.5)	10 (1.1)	0	15
Radius	19 (1.8)	32 (3.5)	2 (1.9)	53
Hand	12 (1.1)	12 (1.3)	2 (1.9)	26
Skull	3 (0.3)	1 (0.1)	0	4
Thorax	2 (0.2)	20 (2.2)	1 (0.9)	23
Others	1 (0.1)	2 (0.2)	1 (0.9)	4
Total	**113**	**211**	**16**	**340**

HD, haemodialysis; n, number of patients; PD, peritoneal dialysis; RRT, renal replacement therapy; RT, renal transplant

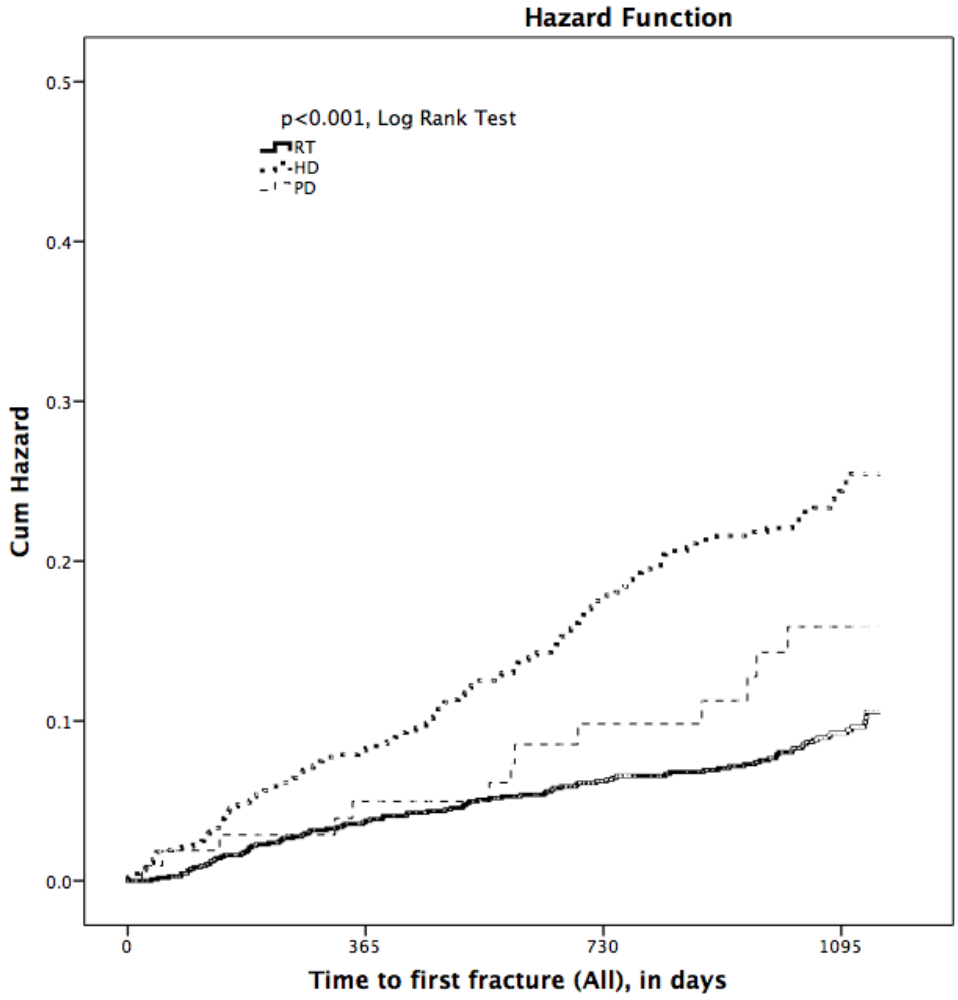
2.4.3 Univariable analysis

Figure 2-1 shows Kaplan Meier cumulative hazard plots for first fracture based on RRT modality. Patients with RT had lower risks than HD and PD for all fractures (median time 492, 464 and 588 days for RT, HD and PD respectively).

Sub-analysis of time to first hip fracture, pelvic fracture, and 'all major fractures' (hip, pelvis and femoral studied cumulatively as a group) showed similar results (figure 2-2 to 2-4).

This risk difference was not seen for femoral fractures (p=0.77) (figure 2-5).

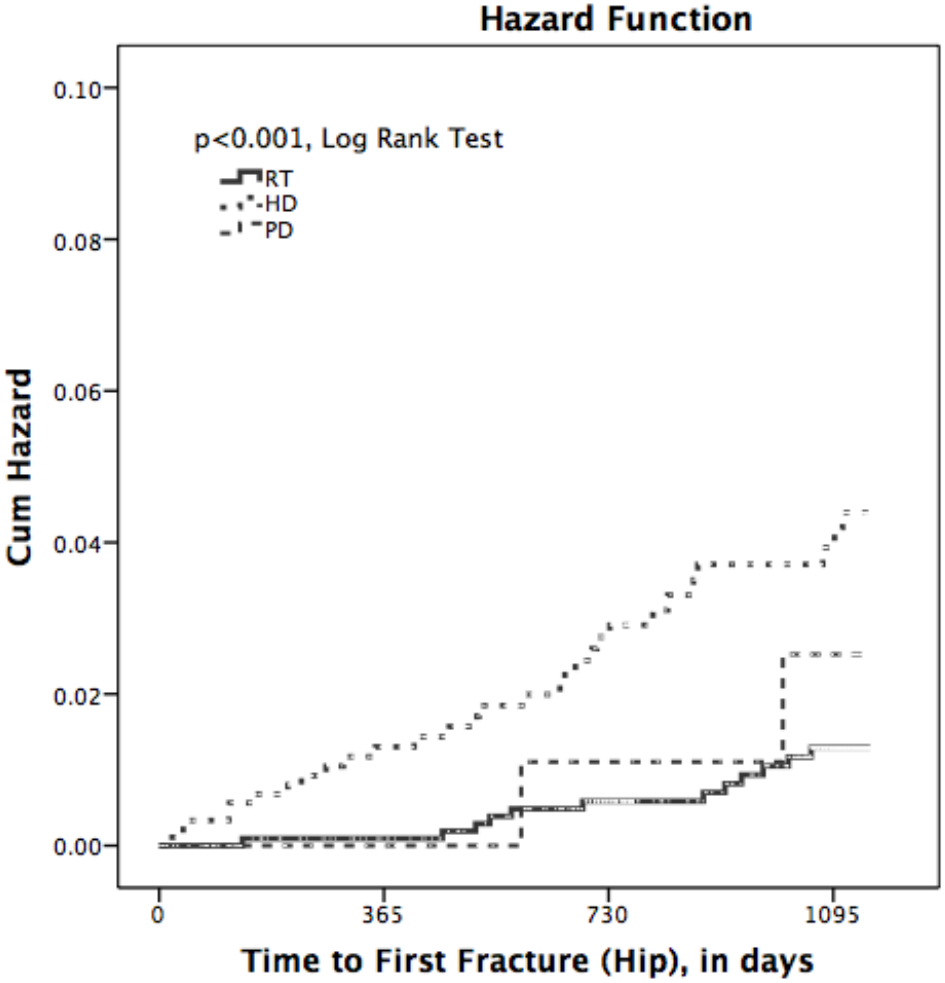
Figure 2-1 Kaplan Mier curves for time to 1st fracture (includes 'all fractures') based on RRT modality (p<0.001, log rank test)



RT	1080	1002	904	602
HD	906	724	566	373
PD	107	94	77	53

HD, haemodialysis; PD, peritoneal dialysis; RT, renal transplant

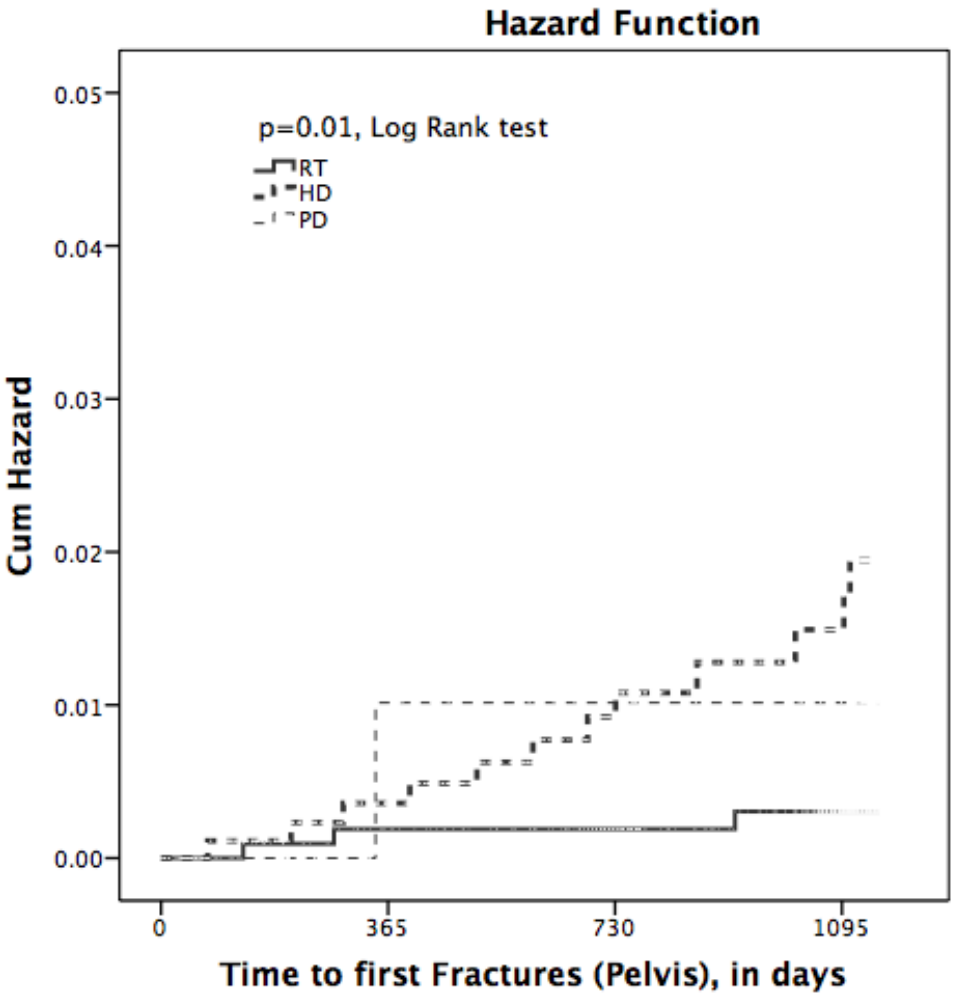
Figure 2-2 Kaplan Mier curves for time to 1st fracture (Hip) based on RRT modality (p<0.001, log rank test)



RT	1080	1038	953	653
HD	906	772	634	440
PD	107	99	85	61

HD, haemodialysis; PD, peritoneal dialysis; RT, renal transplant

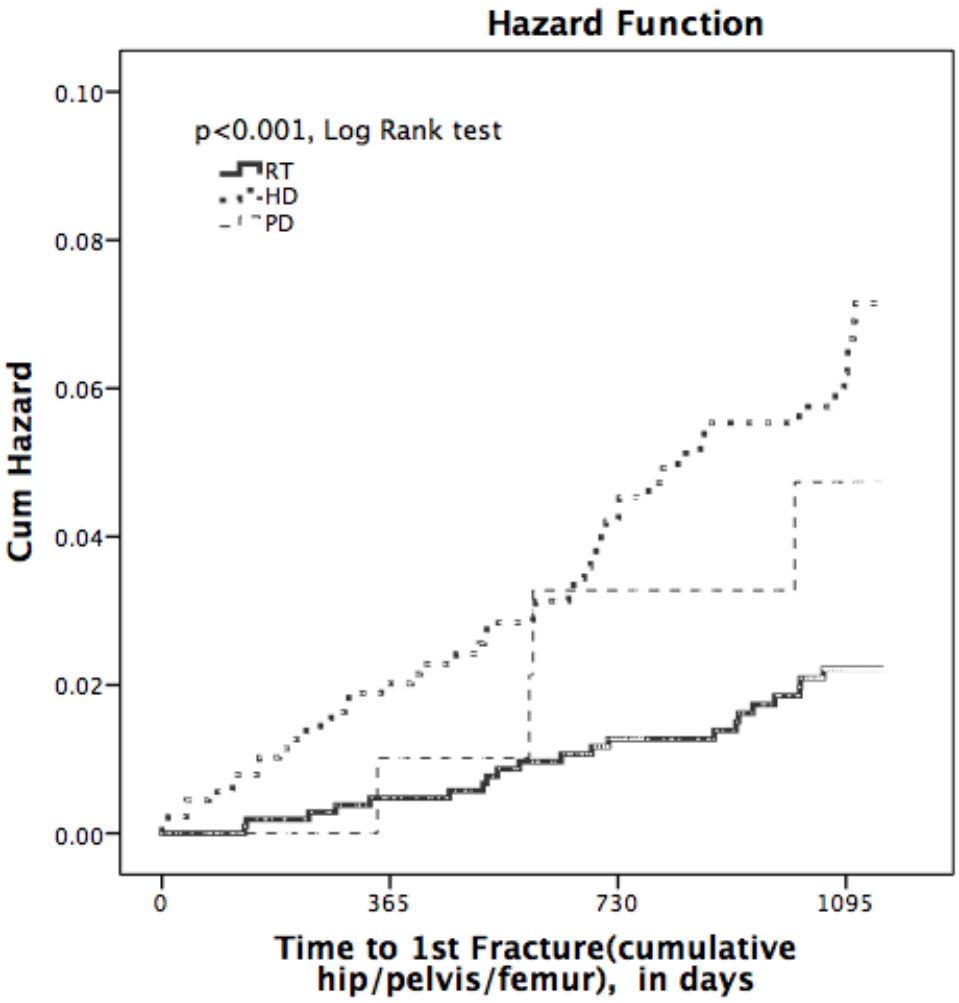
Figure 2-3 Kaplan Mier curves for time to 1st fracture (Pelvis) based on RRT modality (p=0.01, log rank test)



RT	1080	1038	958	661
HD	906	775	638	445
PD	107	98	85	62

HD, haemodialysis; PD, peritoneal dialysis; RT, renal transplant

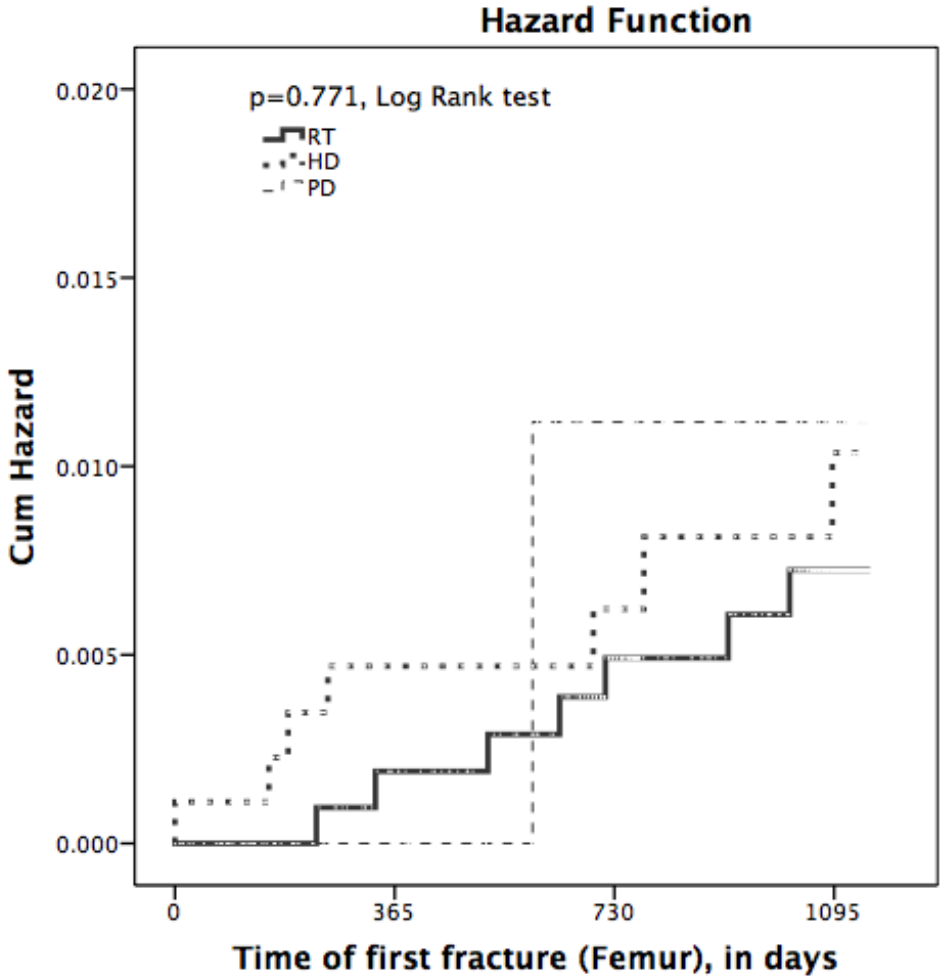
Figure 2-4 Kaplan Mier curves for time to 1st fracture (includes cumulative of Hip/Pelvis/Femur)) based on RRT modality (p<0.001, log rank test)



RT	1080	1033	949	647
HD	906	768	626	431
PD	107	98	83	59

HD, haemodialysis; PD, peritoneal dialysis; RT, renal transplant

Figure 2-5 Kaplan Mier curves for time to 1st fracture (Femur) based on RRT modality (p=0.771, log rank)



RT	1080	1036	954	657
HD	906	777	643	448
PD	107	99	85	62

HD, haemodialysis; PD, peritoneal dialysis; RT, renal transplant

In the univariable analysis age (HR 1.03 per year), female gender (HR 1.60), HD (HR 2.58), diabetes PRD (HR 2.38), serum phosphate (HR 1.73 per mmol/L), alkaline phosphatase (HR 1.0 per IU/mL), haemoglobin (HR 1.0 per g/L) and exposure to sevelamer (HR 1.59) were associated with significantly reduced time to first fracture. ($p<0.05$) (Table 2-4 below)

Table 2-4 Unadjusted risk of bone fractures among RRT population

	Unadjusted (All RRT Groups)			
	HR	**95.0% CI**		**p value**
		Lower	**Upper**	
Age (years)	1.03	1.02	1.04	<0.05
Female	1.60	1.26	2.04	<0.05
RRT Modality				
RT	**Reference**			
HD	2.58	1.99	3.33	<0.05
PD	1.57	0.88	2.80	0.13
RRT Vintage in years	0.97	0.95	0.98	<0.05
Primary Renal diagnosis				
Familial/hereditary nephropathy	**Reference**			
Tubulointerstitial disease	1.09	0.64	1.85	0.75
Systematic	1.04	0.51	2.10	0.92
Miscellaneous	1.16	0.67	2.00	0.60
Glomerular disease	0.71	0.41	1.23	0.22
Diabetes	2.38	1.38	4.11	<0.05
Biochemistry				
Phosphate (mmol/l)	1.73	1.33	2.24	<0.05
corrected Calcium (mmol/l)	0.89	0.43	1.84	0.76
Alkaline phosphatase (U/l)	1.00	1.00	1.00	<0.05
corrected albumin (g/l)	0.93	0.91	0.95	<0.05
Parathyroid hormone (pmol/l)	1.00	1.00	1.01	0.12
Haemoglobin (g/dl)	1.00	0.99	1.00	<0.05
Medication exposure				
Steroid	0.46	0.36	0.59	<0.05
Cinacalcet	1.24	0.79	1.93	0.35
Alfacalcidol	1.20	0.95	1.53	0.13
Alucaps	0.97	0.50	1.89	0.93
CCPB	1.22	0.88	1.68	0.24
Lanthanum	1.02	0.65	1.61	0.94
Sevelamer	1.59	1.19	2.12	<0.05

CCPB, calcium containing phosphate binders; Co., corrected; CI, confidence interval; HD, haemodialysis; HR, Hazard ratio; PD, peritoneal dialysis; PTH, parathyroid hormone; RRT, renal replacement therapy; RT, renal transplant

2.4.4 Multivariable model of all RRT groups:

In the multivariable model including all RRT groups (table 2-5), age (HR 1.02, p=0.002) and HD (HR 5.25, p<0.001) were independently associated with increased risk of fractures and glomerular disease PRD (HR 0.42, p=0.017), increasing serum albumin (HR 0.96 per g/L, p=0.06) and being on alfacalcidol (HR=0.51, p=0.001) or lanthanum (HR 0.41, p=0.002) at inception were associated with decreased risk.

Table 2-5 Adjusted risk of bone fractures among all RRT population

	Adjusted (All RRT Groups)			
	HR	**95.0% CI**		**p value**
		Lower	**Upper**	
Age (years)	1.02	1.01	1.04	<0.05
Female	1.16	0.80	1.68	0.42
RRT Modality				
RT				
HD	5.25	2.12	12.99	<0.05
PD	2.64	0.80	8.72	0.11
RRT Vintage in years	1.01	0.98	1.04	0.46
Primary Renal diagnosis				
Familial/hereditary nephropathy	**Reference**			
Tubulointerstitial disease	1.28	0.68	2.41	0.45
Systemic	0.58	0.25	1.32	0.19
Miscellaneous	0.95	0.50	1.81	0.87
Glomerular disease	0.42	0.21	0.86	<0.05
Diabetes	1.37	0.70	2.67	0.36
Biochemistry				
Phosphate (mmol/l)	1.47	0.92	2.35	0.11
corrected Calcium (mmol/l)	1.76	0.56	5.52	0.33
Alkaline phosphatase (U/l)	1.00	1.00	1.00	0.10
corrected albumin (g/l)	0.96	0.93	1.00	0.06
Parathyroid hormone (pmol/l)	1.00	0.99	1.00	0.30
Haemoglobin (g/dl)	1.01	1.00	1.02	0.30
Medication exposure				
Steroid	1.49	0.75	2.96	0.25
Cinacalcet	0.83	0.43	1.58	0.56
Alfacalcidol	0.51	0.34	0.76	<0.05
Alucaps	0.91	0.45	1.85	0.79
CCPB	0.78	0.49	1.23	0.28
Lanthanum	0.41	0.19	0.88	<0.05
Sevelamer	0.84	0.53	1.32	0.45

CCPB, calcium containing phosphate binders; Co., corrected; CI, confidence interval; HD, haemodialysis; HR, Hazard ratio; PD, peritoneal dialysis; PTH, parathyroid hormone; RRT, renal replacement therapy; RT, renal transplant

2.4.5 Multivariable model of HD group:

In a multivariable model of only HD patients age (HR 1.03, p=0.003) was independently associated with reduced time to first fracture and glomerular disease PRD (HR 0.36, p=0.026), increasing serum albumin (HR 0.95 per g/L, p =0.041) and being on alfacalcidol (HR =0.54, p=0.008) or lanthanum (HR 0.46, p=0.05) at inception were associated with decreased risk. (Table 2-6)

Table 2-6 Adjusted risk of bone fractures among HD population

	Adjusted (HD)			
	HR	**95.0% CI**		**p value**
		Upper	**Lower**	
Age (years)	1.03	1.01	1.04	<0.05
Female	1.10	0.73	1.68	0.64
RRT Vintage in years	1.01	0.98	1.05	0.53
Primary Renal diagnosis				
Familial/hereditary nephropathy	**Reference**			
Tubulointerstitial disease	1.46	0.67	3.14	0.34
Systemic	0.66	0.26	1.65	0.37
Miscellaneous	0.96	0.44	2.11	0.92
Glomerular disease	0.36	0.15	0.88	<0.05
Diabetes	1.38	0.62	3.07	0.43
Biochemistry				
Phosphate (mmol/l)	1.38	0.81	2.34	0.24
corrected Calcium (mmol/l)	0.79	0.22	2.79	0.71
Alkaline phosphatase (U/l)	1.00	1.00	1.00	0.08
corrected albumin (g/l)	0.95	0.91	1.00	<0.05
Parathyroid hormone (pmol/l)	1.00	0.99	1.01	0.59
Haemoglobin (g/dl)	1.01	1.00	1.02	0.18
Medication exposure				
Steroid	1.54	0.72	3.28	0.27
Cinacalcet	0.80	0.40	1.60	0.52
Alfacalcidol	0.54	0.34	0.85	<0.05
Alucaps	0.98	0.48	2.03	0.96
CCPB	0.76	0.47	1.25	0.28
Lanthanum	0.46	0.21	1.00	0.05
Sevelamer	0.88	0.54	1.42	0.59

CCPB, calcium containing phosphate binders; Co., corrected; CI, confidence interval; HD, haemodialysis; HR, Hazard ratio; PD, peritoneal dialysis; PTH, parathyroid hormone; RRT, renal replacement therapy; RT, renal transplant

2.4.6 Multivariable model of transplant group:

In a multivariable model in transplant patients there were no significant independent associations of baseline co-variates with time to first fracture. (Table 2-7 below)

Table 2-7 Adjusted risk of bone fractures among RT population

	Adjusted (RT) HR	95.0% CI Lower	Upper	p value
Age (years)	1.02	0.98	1.06	0.38
Female	1.77	0.59	5.25	0.31
RRT Vintage in years	1.01	0.95	1.07	0.77
Primary Renal diagnosis				
Familial/hereditary nephropathy	**Reference**			
Tubulointerstitial disease	1.24	0.27	5.77	0.79
Systematic	0.00	0.00	.	0.99
Miscellaneous	2.00	0.50	8.02	0.33
Glomerular disease	0.62	0.14	2.76	0.53
Diabetes	1.02	0.07	.	0.99
Biochemistry				
Phosphate (mmol/l)	2.90	0.37	.	0.31
corrected Calcium (mmol/l)	.	0.65	.	0.08
Alkaline phosphatase (U/l)	1.00	0.99	1.01	0.79
corrected albumin (g/l)	0.94	0.84	1.05	0.24
Parathyroid hormone (pmol/l)	0.98	0.95	1.02	0.36
Haemoglobin (g/dl)	0.99	0.97	1.02	0.59
Medication exposure				
Steroid	.	0.00	.	0.99
Cinacalcet	0.00	0.00	.	0.99
Alfacalcidol	0.18	0.02	1.48	0.11
Alucaps	0.00	0.00	.	0.99
CCPB	2.22	0.16	.	0.55
Lanthanum	0.00	0.00	.	1.00
Sevelamer	0.00	0.00	.	0.99

CCPB, calcium containing phosphate binders; Co., corrected; CI, confidence interval; HD, haemodialysis; HR, Hazard ratio; PD, peritoneal dialysis; PTH, parathyroid hormone; RRT, renal replacement therapy; RT, renal transplant

2.4.7 Multivariable model of PD group:

In a multivariable model in PD patients there were no significant independent associations of baseline co-variates with time to first fracture. (Table 2-8 below)

Table 2-8 Adjusted risk of bone fractures among PD population

	Adjusted (PD)			
	HR	**95.0% CI**		**p value**
		Lower	Upper	
Age (years)	0.54	0.00	1189.72	0.87
Female	9.70	0.00	.	0.98
RRT Vintage in years	1.00	0.97	1.03	0.89
Primary Renal diagnosis				
Tubulointerstitial disease				1.00
Familial/hereditary nephropathy	0.00	0.00	.	0.92
Glomerular disease	0.00	0.00	.	0.82
Miscellaneous	0.00	0.00	.	0.93
Systematic	0.00	0.00	.	0.93
Diabetes	.	0.00	.	0.88
Biochemistry				
Phosphate (mmol/l)	.	0.00	.	0.89
corrected Calcium (mmol/l)	.	0.00	.	0.85
Alkaline phosphatase (U/l)	0.95	0.30	3.04	0.94
corrected albumin (g/l)	0.49	0.00	.	0.96
Parathyroid hormone (pmol/l)	0.86	0.00	18542.73	0.98
Haemoglobin (g/dl)	1.01	0.02	46.81	1.00
Medication exposure				
Steroid	2401.68	0.00	.	0.94
Cinacalcet	.	0.00	.	0.79
Alfacalcidol	20.11	0.00	.	0.98
Alucaps	.	0.00	.	0.80
CCPB	891.69	0.00	.	0.89
Lanthanum	0.00	0.00	.	0.88
Sevelamer	.	0.00	.	0.91

CCPB, calcium containing phosphate binders; Co., corrected; CI, confidence interval; HD, haemodialysis; HR, Hazard ratio; PD, peritoneal dialysis; PTH, parathyroid hormone; RRT, renal replacement therapy; RT, renal transplant

2.5 Centre effect in univariable & multivariable analysis

We further analysed the data to see if there was a centre effect in the multivariable analysis but the results do not change. (Table 2-9 to 2-11)

Table 2-9 Unadjusted risk of bone fractures among RRT population with Centre effect

	Unadjusted (All RRT Groups)			
	HR	95.0% CI Lower	Upper	p value
Age (years)	1.02	1.01	1.04	0.00
Female	1.16	0.80	1.67	0.44
RRT Modality				
RT	**Reference**			
HD	5.00	2.01	12.44	<0.001
PD	2.46	0.74	8.17	0.14
RRT Vintage in years	1.00	1.00	1.00	0.61
Primary Renal diagnosis				
Familial/hereditary nephropathy	**Reference**			0.00
Tubulointerstitial disease	1.34	0.71	2.54	0.37
Systematic	0.61	0.27	1.41	0.25
Miscellaneous	1.01	0.53	1.96	0.97
Glomerular disease	0.44	0.22	0.90	0.02
Diabetes	1.45	0.74	2.84	0.28
Biochemistry				
Phosphate (mmol/l)	1.45	0.90	2.35	0.13
corrected Calcium (mmol/l)	1.95	0.60	6.32	0.27
Alkaline phosphatase (U/l)	1.00	1.00	1.00	0.08
corrected albumin (g/l)	0.95	0.91	1.00	0.03
Parathyroid hormone (pmol/l)	1.00	0.99	1.00	0.36
Haemoglobin (g/dl)	1.01	1.00	1.02	0.13
Medication exposure				
Steroid	1.51	0.76	3.00	0.24
Cinacalcet	0.79	0.41	1.53	0.49
Alfacalcidol	0.52	0.35	0.79	0.00
Alucaps	0.95	0.47	1.93	0.88
CCPB	0.81	0.51	1.31	0.40
Lanthanum	0.40	0.19	0.88	0.02
Sevelamer	0.90	0.56	1.45	0.66
RRT Centre				
A	**Reference**			
B	1.29	0.48	3.45	0.62
C	2.35	0.70	7.83	0.17
D	1.46	0.66	3.23	0.36

CCPB, calcium containing phosphate binders; Co., corrected; CI, confidence interval; HD, haemodialysis; HR, Hazard ratio; PD, peritoneal dialysis; PTH, parathyroid hormone; RRT, renal replacement therapy; RT, renal transplant

Table 2-10 Adjusted risk of bone fractures among RT population with centre effect

	Adjusted (RT)			
	HR	**95.0% CI**		**p value**
		Lower	Upper	
Age (years)	1.02	0.98	1.06	0.41
Female	1.92	0.63	5.81	0.25
RRT Vintage in years	1.00	1.00	1.00	0.74
Primary Renal diagnosis				
Familial/hereditary nephropathy		**Reference**		0.68
Tubulointerstitial disease	1.29	0.28	6.07	0.75
Systematic	0.00	0.00	.	0.93
Miscellaneous	2.27	0.55	9.44	0.26
Glomerular disease	0.68	0.15	3.00	0.61
Diabetes	1.10	0.07	16.81	0.94
Biochemistry				
Phosphate (mmol/l)	3.48	0.40	29.92	0.26
corrected Calcium (mmol/l)	25.78	0.46	1440.45	0.11
Alkaline phosphatase (U/l)	1.00	0.99	1.01	0.78
corrected albumin (g/l)	0.94	0.83	1.08	0.38
Parathyroid hormone (pmol/l)	0.98	0.95	1.02	0.35
Haemoglobin (g/dl)	0.99	0.96	1.03	0.69
Medication exposure				
Steroid	6371.88	0.00	.	0.93
Cinacalcet	0.00	0.00	.	0.93
Alfacalcidol	0.17	0.02	1.42	0.10
Alucaps	0.00	0.00	.	0.94
CCPB	1.87	0.13	27.28	0.65
Lanthanum	0.00	0.00	.	0.98
Sevelamer	0.00	0.00	.	0.93
RRT Centre				0.95
A	**Reference**			
B	24800.17	0.00	.	0.96
C	10715.33	0.00	.	0.96
D	14504.17	0.00	.	0.96

CCPB, calcium containing phosphate binders; Co., corrected; CI, confidence interval; HD, haemodialysis; HR, Hazard ratio; PD, peritoneal dialysis; PTH, parathyroid hormone; RRT, renal replacement therapy; RT, renal transplant

Table 2-11 Adjusted risk of bone fractures among HD population with centre effect

	Adjusted (HD)			
	HR	**95.0% CI**		**p value**
		Lower	Upper	
Age (years)	1.03	1.01	1.04	<0.001
Female	1.07	0.70	1.63	0.76
RRT Vintage in years	1.00	1.00	1.00	0.76
Primary Renal diagnosis				
Familial/hereditary nephropathy		**Reference**		0.01
Tubulointerstitial disease	1.53	0.70	3.32	0.28
Systematic	0.72	0.28	1.81	0.48
Miscellaneous	1.06	0.48	2.35	0.89
Glomerular disease	0.39	0.16	0.97	0.04
Diabetes	1.50	0.67	3.37	0.33
Biochemistry				
Phosphate (mmol/l)	1.34	0.78	2.31	0.29
corrected Calcium (mmol/l)	0.86	0.24	3.17	0.83
Alkaline phosphatase (U/l)	1.00	1.00	1.00	0.06
corrected albumin (g/l)	0.94	0.90	0.99	0.02
Parathyroid hormone (pmol/l)	1.00	0.99	1.01	0.70
Haemoglobin (g/dl)	1.01	1.00	1.03	0.07
Medication exposure				
Steroid	1.58	0.74	3.37	0.24
Cinacalcet	0.77	0.38	1.55	0.46
Alfacalcidol	0.56	0.35	0.90	0.02
Alucaps	1.05	0.51	2.17	0.90
CCPB	0.81	0.49	1.35	0.42
Lanthanum	0.46	0.21	1.02	0.06
Sevelamer	0.95	0.57	1.59	0.85
RRT Centre				0.38
A		**Reference**		
B	2.95	0.62	14.15	0.18
C	1.36	0.45	4.09	0.58
D	0.84	0.29	2.49	0.76

CCPB, calcium containing phosphate binders; Co., corrected; CI, confidence interval; HD, haemodialysis; HR, Hazard ratio; PD, peritoneal dialysis; PTH, parathyroid hormone; RRT, renal replacement therapy; RT, renal transplant

There was no meaningful data for the PD population due to small numbers.

2.6 Discussion

2.6.1 Summary of findings

Our study is the first that analyses all radiologically proven fractures whether hospitalised or not in patients on all RRT modalities. We found that incidence of fracture of 62.8 per 1000 patient years is almost double that reported in a self-reported health survey from 2002 to 2004 in a sample representative of the general population in England (104) and within the wide range of 3.3 to 99.6 per 1000 patient years in previous reports in the RT population. (101) We found that relative risk of fractures in RT patients was substantially lower compared to the dialysis population. This association remained even when adjusting for associated risk factors including the younger age of transplant patients. Increasing age was independently associated with increased risk of fracture, while prescription of alfacalcidol or lanthanum at baseline was associated with reduced risk and there were no associations with baseline measures of CKD-MBD. The reduced incidence of fractures seen in the RT group is partly explained by selection bias, as the RT population is a healthier population while the HD population was from an older age group with increased frailty and thus potentially increased risks of fall. However even when attempting to adjust for known risk factors, the incidence in RT was reduced compared with dialysis patients despite the likely osteopenic effects of corticosteroids in this population.

2.6.2 Lanthanum & its protective role

CKD is typically associated with hyperphosphatemia, hypocalcemia, increased PTH, reduced activation of Vitamin D, inactivated Vitamin D deficiency and uremia. These abnormalities result in abnormal micro-architectural bone changes, both at cortical and trabecular level (105,106) leading to renal osteodystrophy. Cortical bone plays a key role in providing mechanical strength, (107,108) thus any changes in structure increases risks of fracture. Lanthanum carbonate, a non-calcium containing phosphate binder has been demonstrated to improve renal osteodystrophy compared to

other phosphate binders. (109) It increases mineralisation at the periosteal surface and improves bone mass in HD patients with adynamic bone disease. (110) This has been proposed to reduce fracture risk and our data provide some support for this with lanthanum use at the time of study inception being independently associated with reduced fracture risk. (109,110) Further exploration of this hypothesis would require detailed analysis of total exposure to phosphate binders, which was beyond the scope of this study. It is unlikely that a randomised controlled study of sufficient power will ever be conducted to test the hypothesis that lanthanum reduces fracture risk compared with other phosphate binders. In our cohort lanthanum exposure was not random but was determined by physician and patient preference. The effect of lanthanum was not seen in the RT group but that is likely explained by the fact that few patients in the RT group were on phosphate binders. It is not possible to draw firm conclusions about this potentially protective role of lanthanum as it may be a mere statistical association.

Incident vertebral fractures were identified in 3.2% of our HD cohort in comparison to a prevalence of 55.3% in a study of 387 patients across 18 dialysis centres in Italy by Fusaro *et al*. The authors in the study determined prevalence of historical vertebral fractures in HD patients, irrespective of symptoms identified radiologically using specialised, quantitative vertebral morphology software (MorphoXPress) whereas we identified mainly the incidence of new symptomatic fractures. (111) The marked difference in incidence and prevalence may be a consequence of many vertebral fractures being asymptomatic. There may be other explanations such as the demographics of the individuals studied or differences in their dialysis and medication prescriptions.

2.6.3 Role of Cinacalcet

A recent secondary analysis of the Evaluation of Cinacalcet to Lower Cardiovascular Events (EVOLVE) trial in HD patients revealed an incidence of fractures of 47 per 1000 patient years in the placebo arm (78) compared

with 99.2 in our HD cohort. This may be explained by lower median age in the placebo arm compared to our HD group (54.4 v 63.3 years), exclusion of patients with unstable medical conditions in the EVOLVE trial and fractures being identified by patient-reported clinical events from study centres. (59,78) Cinacalcet use was associated with a 16-29% reduction in fracture. (78) No significant association between fracture and cinacalcet were identified though only 12.1% of the HD cohort were on cinacalcet at study inception.

2.6.4 Role of Vitamin D

The role of Vitamin D supplementation in patients with CKD and fracture prevention is not yet established. Vitamin D administration is associated with increased bone strength in animal models (112) and a prospective, randomised, placebo-controlled double blind study showed improved bone mineral density (BMD) assessed by dual energy X-ray absorptiometry (DEXA) with lowering of plasma PTH levels. (113) Although BMD assessment by DEXA in patients with ESRD has limitations (69,114) the reduced fracture risk in HD patients receiving vitamin D in this study supports the putative protective role of activated Vitamin D in preventing fractures in the HD population.

2.6.5 Role of PTH

The Dialysis Outcome and Practice Patters Study II suggested high PTH levels might be an independent risk factor for fractures in haemodialysis patients. (115) PTH concentrations have a U-shaped relationship with vertebral and hip fractures with no associations for a pelvic fracture. (116) In contrast our results were more consistent with the USRDS Wave 1 data (117), which did not find any such association of fractures with PTH levels.

2.6.6 Albumin & fractures

Our data are also consistent with previous reports that have shown association between risk of fractures in the HD population with lower serum

albumin (115) and no significant fracture association with serum calcium and phosphate. (116) The albumin level could be a surrogate marker for the level of co-morbidity in the population.

It is interesting that we found no higher fracture incidence in females unlike other reports in the RRT and general population. (66,67,115,117) No clear reason for this can be identified at this stage.

2.7 Limitations:

We did not include factors such as race, body mass index, smoking status and ethnicity, which have previously been associated with fracture risks as these data were not available, and nearly all patients were Caucasian. PRD was available for only 1285 patients, which reduced the power of the analysis of this particular association. Exposure to medications was based on the data collected at the point of entry and did not account for cumulative exposure before and after study inception. We may have failed to detect a genuine association between fracture risk and factors such as haemoglobin and phosphate because adherence to treatment guidelines keep these factors within relatively narrow ranges meaning that the conclusions are limited to the ranges studied. We did not evaluate the role of previous RRT modality (other than total duration on RRT before study), previous fractures, reason for receiving a particular phosphate binder and exposure to other potentially relevant medication such heparin. Incidental or silent fractures may have been missed. These limitations are inherent to observational studies of large prevalent cohorts.

Despite these limitations the large population studied from multiple centres, absence of exclusions, access to all radiological reports and averaging of laboratory variables over 3 consecutive readings make our conclusions relevant to patients and clinicians and the data could be used to inform the power calculations of interventional prevention studies.

2.8 Conclusion

The risk of symptomatic bone fracture is high in RRT patients and is approximately 2.5 times higher in HD than in RT patients with the increased risk being independent of baseline factors. In PD the risk was 1.5 times than in RT but this did not reach statistical significance, possibly due to small patient numbers. Fracture risk increases with age and lower serum albumin and is reduced if the PRD is glomerular disease. The possible protective role of Vitamin D and lanthanum in HD patients deserves further exploration. It is clear that the morbidity associated with fractures is a significant factor in all forms of RRT and preventative measures deserve to be considered when assessing the quality of RRT delivered.

Chapter 3

Thrice weekly nocturnal in - centre HDF

3.1 Introduction:

Patients undergoing conventional 4 hour dialysis sessions three times per week exhibit excess mortality that is four times higher than that of the general population for patients under 30, and six times that of the general population for patients above 65. (118) This increased mortality has been attributed to low dialysis dose, (119) but two large randomised controlled trials have failed to show any survival benefit for patients receiving a larger dialysis dose. (120,121)

In an effort to improve outcomes, multiple treatment schedules have been utilised. Online hemodiafiltration (OL-HDF), which combines diffusion and convection, has grown in popularity over the last 20 years. This provides enhanced clearance of toxins (122,123) increased hemodynamic stability (124), and better quality of life. (125) The use of more frequent dialysis has also been shown to be beneficial (126,127) .The advantages in bone mineral profile (128,129), blood pressure control (130), patient experience and improved mortality(131) in programs with extended hours dialysis has been well demonstrated. This is often not an acceptable alternative for patients, and few of the studies of extended hours dialysis are based in centre, as the majority of patients in these studies undertake nocturnal dialysis at home.

The use of convective therapy in combination with extended hours dialysis has been shown to be beneficial (132), but is not frequently described.

This project was set up as an exercise allowing evaluation of small solute clearance by combining convection with long hours dialysis in a thrice-weekly hospital setting. This seemed an excellent compromise for patients who cannot have home dialysis but wanted to benefit from having extended hours. The frequency was kept at thrice-weekly due to patient preference.

3.2 Methods

A single-centred, prospective analysis of patients' electronic records was performed for a two-year period. Patients were self -selected with no stipulated exclusion criteria apart from patients needing to have their own transport due to

the timing of commencement and cessation of treatment. Only ten patients initially came forward.

The duration of haemodiafiltration was increased from a median of 4.5 hours to 8 hours. Dialysis adequacy, biochemical parameters and medications were reviewed on a monthly basis. A reduction in plasma phosphate was anticipated so all phosphate binders were stopped.

3.2.1 Patient characteristics

During the period fourteen patients, 13 male and 1 female started nocturnal OL-HDF with over 2000 sessions of dialysis. Mean age was 45.2 ± 13.1 years (range 23.8 – 66.2) and renal replacement treatment (RRT) vintage was 42.34 ± 40.62 months (range 6.61 –116.94). All patients were on regular OL-HDF for at least six months; mean 30.93 ± 27.68 months, (range 6.67 – 93.44) prior to conversion to the nocturnal regime.

The cause of end-stage renal disease was pyelonephritis/interstitial nephritis, Alports disease, haemolytic uremic syndrome, reflux nephropathy, membranous nephropathy, renovascular disease (1 patient each); adult polycystic kidney disease, IgA nephropathy, rapid progressive glomerulonephritis and unknown causes (2 patients each). All patients except one had a native arterio-venous fistula. Drop out reasons prior to 12 months were planned change in modality (1 patient), transplantation (3 patients) or lifestyle choice (1 patient).

3.2.2 Nocturnal treatment regime

Patients on standard 4- 5 hours thrice weekly OL-HDF were switched to 7-8 hours thrice weekly nocturnal OL-HDF. Patients were dialysed using a Fresenius 5008 machine and a Gambro Polyflux dialyser, without reuse. The blood flow (Qb) was reduced to 200ml/min and dialysate flow (Qd) was reduced to 300ml/min. All patients continued on post-dilution OL-HDF with a dialysate calcium concentration of 1.75mmol/l and potassium adjusted according to pre-dialysis levels. Dialysate bicarbonate concentration was reduced to prevent the development of alkalosis and anticoagulation was increased by 50%.

3.2.3 Biochemical parameters

Monthly review of dialysis adequacy, electrolytes and haemoglobin (Hb) measurements was carried out. The baseline biochemical parameters were calculated from the averages of the last 3 months during standard thrice weekly 4- 5hours OL-HDF. Routine samples (pre-and post-dialysis) were collected on the first weekday of the short intradialytic gap of the month. Small solute clearance was assessed using a standard Kt/V equation (Std Kt/V) (133) with mineral bone disease monitored by plasma phosphate and calcium levels. A reduction in plasma phosphate was expected so all phosphate binders were stopped. All patients were assessed by a qualified renal dietician and advised to relax their phosphate restriction.

Samples for ferritin and vitamin B12 were analysed every three months and parathyroid hormone (PTH), beta-2 microglobulin every six months. Doses of intravenous iron, erythopoetin (darbepoetin-alpha) and blood pressure medication use were recorded. Changes in medication were at the discretion of the responsible physician.

3.2.4 Clinical parameters

Average readings of clinical parameters i.e. blood pressure, weight and hydration were assessed using multi-channel bioimpedance Fresenius ® body mass composition (BCM) monitor and analysed with Fluid Management Tool software version 3.3.0.1637 on quarterly basis.

3.2.5 Statistical analysis

Data was recorded prospectively for a period of 24 months and included in the analysis where 12 months or more of data was available. This was analysed using SPSS software package version 17. A repeated measure of analysis of variance was performed with respect to baseline. Each patient served as his/her own control. As the sample size was small non-parametric statistics were used to compare readings at 24 months with baseline using Wilcoxon signed rank. A value of $p < 0.05$ was considered statistically significant.

3.2.6 Ethical approval

Ethical approval was not sought, as this was a part of quality improvement to services.

3.3 Results

Table 3-1 summarises mean and standard deviation of all parameters measures following change in dialysis regime from thrice – weekly OL-HDF (4-5 hours) to thrice weekly nocturnal HDF (7-8 hours).

Table 3-1: Change from 3/weekly OL-HDF (4 - 5h) to thrice weekly nocturnal HDF (7-8 h) Mean ± SD

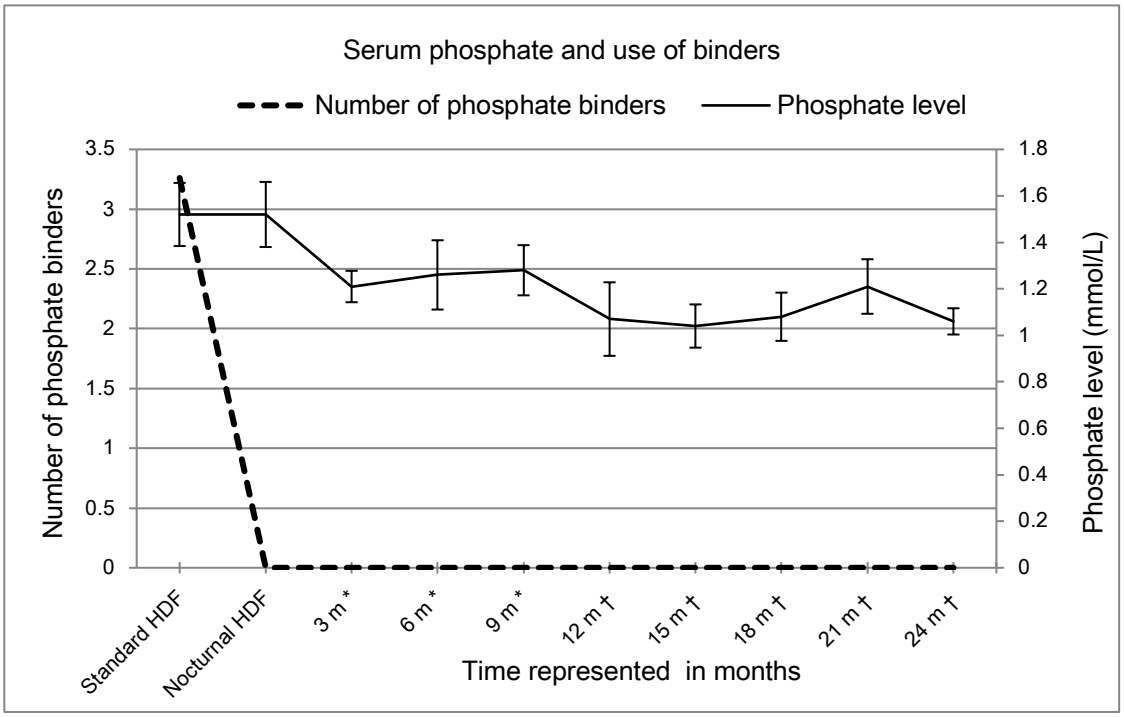
	Baseline	3 months, n=9	6 months, n=9	9 months, n=9	24 months, n=5
Time (min)	277.22±25.80	474.26±9.58 [a]	472.52±7.92 [a]	476.85±5.99 [a]	466.60±13.68 [c]
Volume (litres)	22.54±3.91	27.53±3.19 [b]	27.25±3.34 [b]	28.26±3.79 [a]	28.85±2.22 [c]
URR	0.76±0.08	0.84±0.05 [a]	0.83±0.06 [a]	0.83±0.05 [b]	0.85±0.04 [c]
Std KtV	2.37±0.21	2.68±0.18 [a]	2.62±0.25 [b]	2.61±0.19 [b]	2.68±0.16 [c]
Corr.Ca (mmol/L)	2.34±0.22	2.37±0.17	2.41±0.15	2.39±0.14	2.48±0.19
PO_4 (mmol/L)	1.52±0.41	1.21±0.20	1.26±0.45	1.28±0.32	1.06±0.13[c]
PO_4 Binders	3.26±2.63	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [c]
Hb (g/dL)	11.58±1.35	11.55±0.97	11.19±1.58	12.10±0.72	11.96±0.47
Ferritin (µg/L)	492.85±295.04	463.17±305.99	423.00±247.00	342.33±152.44	321.40±117.27
EPO(weekly) dose)	21.85±21.61	12.96±16.11	18.52±16.92	15.74±13.67	15.00±8.66
Iron dose(mg/week)	50.93±28.40	29.63±32.30 [b]	26.85±23.12 [b]	22.22±23.20 [b]	45.00±32.60
Vitamin B12 (ng/L)	440.07±108.51	480.56±138.48	520±141.55	429.67±128.71	331.80±37.35
Overhydration (litre)	1.53±2.96	1.31±1.48	1.48±1.21	1.08±1.21	1.81±2.44
Systolic (mmHg)	146.63±21.77	139.52±26.68	137.26±25.18	140.30±30.22	137.47±17.30
Diastolic (mmHg)	75.04±12.84	70.20±15.03	67.07±20.09	70.94±17.16	69.40±13.84
BP Medications	1.31±1.04	0.70±1.31 [b]	0.56±1.33 [b]	0.67±1.32 [b]	0.60±0.89 [c]

[a] $p<0.01$ - With respect to baseline, repeated measures analysis of variance (ANOVA) ; b $p<0.05$ - With respect to baseline, ANOVA; c $p<0.05$ - Wilcoxon signed rank test; BP, blood pressure; Corr. Ca, corrected calcium; SD, Standard deviation; EPO, erythropoietin; Hb, haemoglobin; HDF, haemodiafiltration; OL-HDF, online haemodiafiltration; n, number of patients; PO4, phosphate; URR, Urea reduction ratio

3.3.1 Bone profile

Pre-dialysis phosphate level fell from a mean of 1.52±0.41 mmol/L to 1.21±0.2 mmol/l, 1.26± 0.45 mmol/l, 1.28± 0.32 mmol/l and 1.06±0.13 mmol/L at 3, 6, 9 and 24 months respectively, $p<0.05$ at 24 months (Table 1-1 & Figure 1-1). The use of binders dropped from an average of 3.26±2.63 tablets to zero ($p<0.05$) (Table 3-1 & Figure 3-1). None of the patients have required recommencement of a phosphate binder or supplementation during dialysis due to low phosphate levels.

Figure 3-1 Reduction in PO_4 binders & PO_4 levels on conversion from standard HDF to nocturnal HDF



HDF, haemodiafiltration; *n = 9, † n = 5, where n is number of patients
Reduction significant in binders, $p<0.05$ at 3,6,9,24 months & PO_4 levels, $p<0.05$ at 24 months. Data represented as mean ± standard deviation

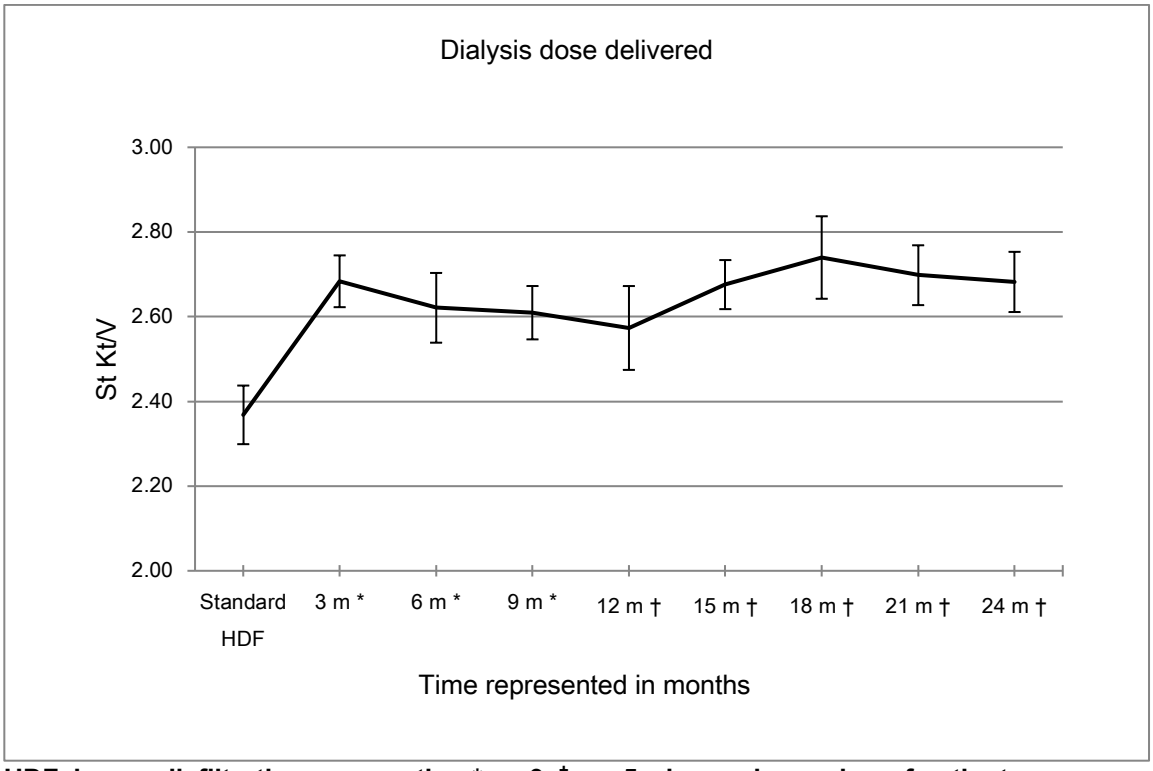
Calcium levels were non-significantly raised at 24 months, which theoretically could lead to a positive calcium balance. PTH however fell from (39.24±31.4) pmol/L at baseline to (23.97±27.1) pmol/L and (26.13±12.6) pmol/L respectively at 12 and 24 months respectively ($p<0.05$, not shown). This failed to reach significance when one patient on cinacalcet

was excluded, p=0.08 at 6 months and p=0.07 at 12 & 24 months. (Table 3-3) None of the patients has required a parathyroidectomy.

3.3.2 Small Solute clearance

Std Kt/V increased from 2.37 ± 0.21 (range 1.93 – 2.65) to 2.68 ± 0.16 (range 2.52-2.92) at 24 months, with p<0.05 at 3, 6, 9 and 24 months compared to baseline. (Table 3-2 & Figure 3-2)

Figure 3-2 Dialysis dose (St Kt/V) delivered on conversion from standard HDF to nocturnal HDF



HDF, haemodiafiltration; m, months; *n = 9, † n = 5 where n is number of patients. Significant improvement in St Kt/V at 3, 6, 9 12 and 24 months, mean ± SD

3.3.3 Middle Molecule clearance

Beta-2 microglobulin levels showed no significant change. Baseline levels were 23.34±9.19 mg/L and levels at 6, 12 and 24 months were 20.88±5.99mg/L, 20.99±5.21mg/L and 24.09±3.13mg/L respectively. (Table 3-2)

Table 3-2 Changes in beta2m and PTH levels on changing from 3/weekly OL-HDF (4 - 5hours) to 3/weekly nocturnal OL-HDF (7-8 hours)

	Baseline	6 months n=9	12 months n=9	24 months n=5
Beta2m (mg/dl)	23.34±9.19	20.88±5.99	20.99±5.21	24.09±3.13
	Baseline	**n=7**	**n=7**	**n=4**
PTH (pmol/l)	30.52±14.55	21.93±22.53	15.45±11.84	20.84±4.82

Beta2m, beta-2 microglobulin; n, number of patients; OL-HDF, online haemodiafiltration; PTH, parathyroid hormone. Data represented as mean ± SD. PTH data with patient on Cinacalcet omitted. No significant reduction over time although changes are almost significant (p = 0.08 at 6 month, p = 0.07 at 12 months & 24 month). Beta2m – no significant change

3.3.4 Filtration volume

OL-HDF substitution volume increased from 22.53±3.91litres (L) per session to 27.53±3.19 L at 3 months and 27.25±3.34, 28.26±3.79, 28.85±2.22 at 6, 9 and 24 months respectively. ($p<0.05$ at 3,6,9 and 24 months)

3.3.5 BP, fluid status and anti-hypertensive medications

BP reduction and changes in over-hydration status did not reach statistical significance, but the use of antihypertensive medications decreased from 1.31 ± 1.04 tablets to 0.6±0.89 tablets at 24 months ($p<0.05$).

3.3.6 Haematological parameters and Erythropoietin dose

Intravenous iron use showed a significant decrease from baseline 50.93±28.4 mg/week to 29.63±32.30, 26.85 ±23.12, and 22.22 ±23.20 mg/week at 3, 6 and 9 months respectively but not at 24 months (45±32.6 mg/week).

Erythropoietin (EPO) use decreased but did not reach statistical significance. Hb levels did not change significantly.

3.4 Discussion

3.4.1 Summary of findings

Thrice-weekly extended-hours nocturnal OL-HDF seems to be an excellent alternative to current modalities of dialysis. It achieves targets for small solute clearance and mineral bone disease with elimination of phosphate binders and no dietary phosphate restrictions. It has been accepted and well tolerated by patients.

The unique finding seen is the complete elimination of phosphate binders without the requirement for intradialytic phosphate supplementation.

3.4.2 Solute & middle molecule clearance

Phosphate control with dialysis alone was first described in 1998 where patients were converted from conventional thrice-weekly dialysis to six nights weekly. Normal phosphate levels were achieved at five months despite a 50% increase in phosphate intake in diet. Blood flow rate was 300 - 350 mls/min and dialysate flow rate of 500 mls/min. (128) A recent paper by Maduell *et al.* to evaluate the beneficial effects of alternate day nocturnal OL-HDF reported a reduction of serum phosphorus from 4.93 ± 1.5mg/dL (1.59 ± 0.48 mmol/L) to 3.74 ± 1mg/dL (1.20 ± 0.32 mmol/L) at 12 months. Phosphate binders were reduced, but not eliminated and intradialytic supplementation was needed in 55% patients. Blood flows were 400mls/min and dialysate flows were 500mls/min (132) The Australian experience of converting patients from conventional regime to home nocturnal 6-9 h sessions 3.5 – 4 x weekly resulted in significant reduction of phosphate levels again with a reduction in phosphate binder consumption, but with 22.7% patients needing supplementation. Blood flow was 200-250mls/min with dialysate flows of 500mls/min. (134) A recent study by a Dutch group comparing conventional (4 hours HD and HDF) and extended (8

hours HD and HDF) dialysis reported 'treatment time' as a major determinant of small molecule clearance and haemodynamic stability. The total solute removal (urea, uric acid, creatinine and phosphate) was higher in the longer duration treatments with no difference noted in the treatment modalities (HD vs HDF). Only acute affects were studied in this randomised cross over trial. Blood flow was 300mls/min and dialysate flows 600mls/min in all study sessions. (135) The improvement in small solute clearance is consistent with other published reports. (132,136)

The unique finding in this study of normal serum phosphate without the need for supplementation, previously not reported, could either be due to the lower dialysate and blood flows employed, or the reduced frequency of 3 times week haemodiafiltration.

We could not however demonstrate a reduction in middle molecule levels (beta-2 microglobulin). If B2M clearance is determined by the convective component of the therapy then this may be due to the already high substitution volumes utilised at baseline in these patients. It does suggest dialysis duration per se is a less important factor in achieving higher beta-2 microglobulin clearance.

3.4.3 Haematological parameters

Other biochemical parameters such as Hb did not change though there was a trend towards a reduction in iron and erythropoietin doses. A more significant lowering in erythropoetin doses may have been achieved if iron doses had not been decreased.

3.4.4 BP & volume control

Charra *et al*. have reported one of the lowest mortality and morbidity statistics in dialysis patients. Absence of cardiovascular complications and good blood pressure was achieved with maintenance of dry weight, low salt diet and no antihypertensive drugs. (130) Despite following a similar regime our BP lowering did not reach statistical significance. There was a reduction

in antihypertensive medications during the study without change in blood pressure which may suggest overall better BP control. Over-hydration (Table 3-1) which theoretically could be from high salt intake, contributing to extracellular volume expansion, may explain why a significant reduction in BP was not demonstrated.

We noted a significantly higher substitution volume, following transfer to the nocturnal program. Theoretically this may confer a survival advantage in the longer term, as implied by post hoc, sub analysis of data from two recent RCT which showed a lower mortality with higher substitution volumes of >17.4 liters and >21.95 litres per session. (119-121)The substitution volumes in this group of patients were already in excess of these numbers at baseline so further study of the effect of increasing beyond this is needed.

3.5 Limitations

Our study has limitations due to the small patient numbers and being single centre and observational in nature. All patients were self-selected thus undoubtedly more motivated and may have less co-morbidity than conventional dialysis patients. Post dialysis phosphate monitoring was not conducted. No food charts or 24-hour urine collections were formally recorded to assess increased dietary intake of phosphate, but it is difficult to imagine that patients advised to follow a relaxed diet would continue to adhere to phosphate restriction. Residual renal function was not measured but as each patient acted as their own control it is unlikely that this would contribute to the benefits seen. Finally, we do not have a parallel comparator group comparing HD and HDF.

3.6 Conclusion

Nocturnal 3 times weekly in centre on-line haemodiafiltration is a practical alternative and is superior to conventional OL-HDF in terms of small solute clearance, control of phosphate and allows a reduction in medication. It has the potential to improve outcomes but this as yet remains unproven. It has

been well tolerated by this patient group and should be considered as a treatment alternative for patients receiving hospital-based haemodialysis therapy. Phosphate control can be achieved without the need for binders or supplementation.

Chapter 4

Phosphate and FGF-23 in peritoneal dialysis

4.1 Introduction

Abnormal bone metabolism, common in CKD and the dialysis population, is measured with surrogate markers such as phosphate, parathyroid hormone (PTH), alkaline phosphatase and calcium. Chronic Kidney Disease - Mineral Bone Disease (CKD – MBD) assessment using bone biochemistry is suboptimal. (17) Phosphate levels vary with circadian rhythm in health and similar diurnal fluctuations are seen in dialysis; PTH responds to acute changes in serum calcium (152)and FGF-23 is unstable (153)and still being used as a research tool. Despite its limitations, FGF-23 measurement over time is believed to be a more accurate representation of renal bone disease though this remains to be proven.

Peritoneal dialysis (PD) is a continuous form of renal replacement therapy in patients with end stage renal disease. Chronic PD is believed to provide a more stable blood chemistry in comparison to conventional thrice weekly haemodialysis. Most patients on PD have a degree of residual renal function that is formally assessed on a quarterly basis as part of standard care. PD utilises the principles of diffusion and convection of solutes across a concentration gradient driven by osmotic and hydrostatic pressure gradients across the peritoneum. It thus forms an ideal model to study the relationship of established biochemical markers to novel laboratory parameters such as FGF-23.

4.2 Aims of the study

1. To study the correlation of phosphate and FGF-23 in a group of PD patients

2. To examine the variability of FGF-23 and phosphate over a period of 9 months

3. To examine the clearances of FGF-23 in PD

4.3 Methods

4.3.1 Study population

All adult patients with end stage renal disease, established on PD and attending the renal services at University Hospital Crosshouse were eligible.

4.3.2 Inclusion criteria

1. Aged > 18 years

2. Able to provide valid consent

3. Established on peritoneal dialysis for at least 3 months

4. No planned change in RRT modality over the next 9 months

4.3.3 Exclusion criteria

Serious co-morbid condition likely to reduce life expectancy to <9 months

4.3.4 End of study

If patient withdraws consent, dies or switches RRT modality i.e. has a transplant or converts to haemodialysis.

4.4 Ethical approvals

Ethical permission was granted by NRES Committee Yorkshire & The Humber - South Yorkshire, REC reference: 14/YH/0095, IRAS project ID: 148296. All patients provided written consent. The study was conducted as per Good Clinical Practice Guidelines, Research Ethics Committee regulations and NHS Board R&D office policies and procedures.

4.5 Baseline characteristics

Data was collected on age, sex, years on renal replacement therapy (RRT vintage), PD vintage and primary renal diagnosis at baseline.

4.6 Laboratory assays

Blood, urine and dialysate samples (from 24-hour collection) were collected during routine care and coincided with clinic visits at time 0 (baseline), 3, 6 and 9 months.

All samples were centrifuged and frozen at -80 C within 4 hours of collection. Samples were analysed after a single thaw at a central processing laboratory in single batch.

1. Urea and phosphate levels (in plasma and urine) were analysed using standard techniques at University Hospital Crosshouse.

2. FGF-23 concentrations in blood (EDTA plasma samples), urine and dialysate were measured in duplicate using Immutopics 2nd generation C-terminal assays (Immutopics Inc., San Clemente, CA, USA) at University of Glasgow. Serial dilution of FGF-23 was performed for levels >1400 RU/ml.

4.7 Calculation of phosphate & FGF-23 clearance

Plasma and 24-hour collections were used to measure FGF-23 (RU/ml), phosphate (mmol/l), urinary and dialysate volumes (ml). Values of creatinine (mmol/l) were obtained from laboratory computer systems as this formed a part of routine investigations. Patients with no urine output were defined as having no residual renal function.

RenalSoft PD Rx management tool (computer software) was used to calculate weekly urea clearance (L/week), creatinine clearance (L/week/1.73m2) and Kt/V.

Clearances were calculated as described below. (Figure 4-1)

Figure 4-1: Formulae to calculate urine and dialysate clearances of phosphate and FGF-23

Dialysate clearance of phosphate (L/day/1.73 m
= [24 hour DV (ml/day)x 0.001
x DP (mmol/L))/ P(mmol/L)]
x1.73 m2

Urine clearance of phosphate (L/day)/1.73 m2)
= [24 hour UV (ml/day)x 0.001
x UP (mmol/L))/P(mmol/L)]
x1.73m 2

Total phosphate clearance (L/day/1.73 m2 BSA)
= Dialysate phosphate clearance + Urinary phosphate clearance

Dialysate clearance of FGF − 23 (L/day/1.73m2/day)
= [24 hour DV (L/day)x
x DFGF − 23 (RU/ml)/FGF − 23RU/ml)]
x1.73 m2

Urine clearance of FGF − 23 (L/day/1.73m2/day)
= [24 hour UV (L/day)x
x UFGF − 23 (RU/ml)/FGF − 23RU/ml)]
x1.73 m2

Total FGF23 clearance (L/day/1.73 m2 BSA)
= Dialysate FGF − 23 clearance + Urinary FGF − 23 clearance

DV, Dialysate volume; DP, dialysate phosphate; P, plasma phosphate; UV, urine volume; UP, urine phosphate; DFGF-23, dialysate FGF-23; UFGF-23, urine FGF-23, FGF-23, plasma FGF-23.

4.8 Statistical methods

Data was analysed using SPSS version 21 for Mac (IBM Corporation, Chicago, IL, USA). Mean ± standard deviation was used to report normally distributed data and median (interquartile range) for non-normally distributed data. Nominal data was represented as total number (n) and percentage (%). Where data was positively skewed further analyses were performed using log-transformed values. P value of <0.05 was considered statistically significant.

Change in levels of FGF-23 over time were analysed using repeated measures ANOVA model with a single 4 level within group repeated factor (time: 0,3,6 & 9

months). Analysis was performed to assess if there was a significant change over time. For patients with missing data or those that decided to withdraw only the relevant available data were analysed. However, for data over time, imputation was used if there was an occasional missing data point in order to maximise the data use.

4.9 Results

4.9.1 Patient characteristics

A total of 19 patients were identified at the start of the study (11 females and 8 males). The average age was 59.6 years at inception (range 26.7 to 84.3 years). Primary renal diagnosis (PRD) was obtained from the ERA-EDTA Coding system using the related web-based PRD search. (103) They were grouped as diabetes, familial/hereditary nephropathies, glomerular disease, systemic disease, tubulointerstitial disease and miscellaneous. Average RRT vintage was over 4 years. Baseline patient characteristics are represented in Table 4-1.

Table 4-1: Patient characteristics at recruitment

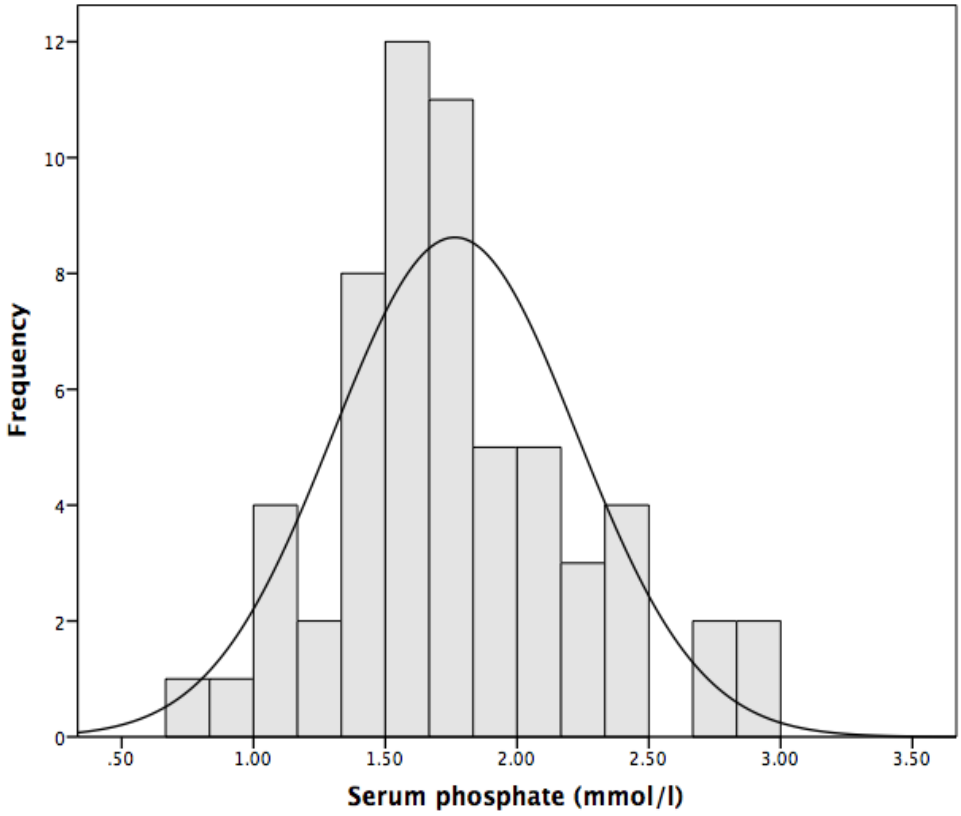
Females, n (%)	11(57.9)
Age in years, Average (range)	59.63(26.7-84.3)
Primary Renal diagnosis, n (%)	
Diabetes	3 (15.8)
Familial/hereditary	2 (10.5)
Glomerular	6 (31.6)
Miscellaneous	1 (5.3)
Systemic	4 (21.1)
Tubulointerstitial	3 (15.8)
PD Vintage, mean (range)*	34.1 (3.0 - 137.8)
RRT vintage, mean (range)*	47.7 (3.0 - 195.3)

PD, peritoneal dialysis; RRT, renal replacement therapy; *data in months

4.9.2 Phosphate in sample population

Phosphate levels over 9 months were normally distributed and ranged from 0.76 to 2.91 mmol/l (mean ± SD, 1.76 ± 0.46, n = 60) (Figure 4-2).

Figure 4-2: Overall serum phosphate levels, normally distributed



4.9.3 FGF-23 in sample population

FGF-23 distribution was positively skewed (plasma, dialysate and urine) and further analyses were performed using log-transformed values. (Figure 4-3, 4-4 & 4-5) FGF-23 levels was not detected in 2 urine samples.

Figure 4-3: Plasma FGF-23

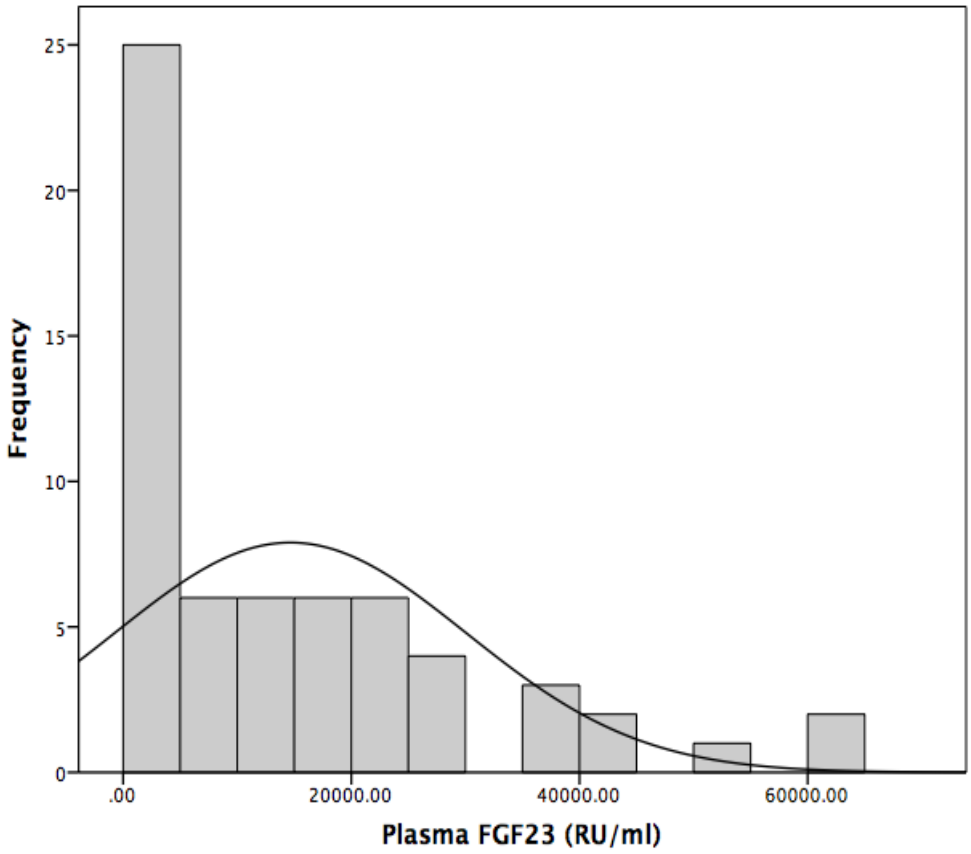


Figure 4-4: Urinary FGF-23, positively skewed

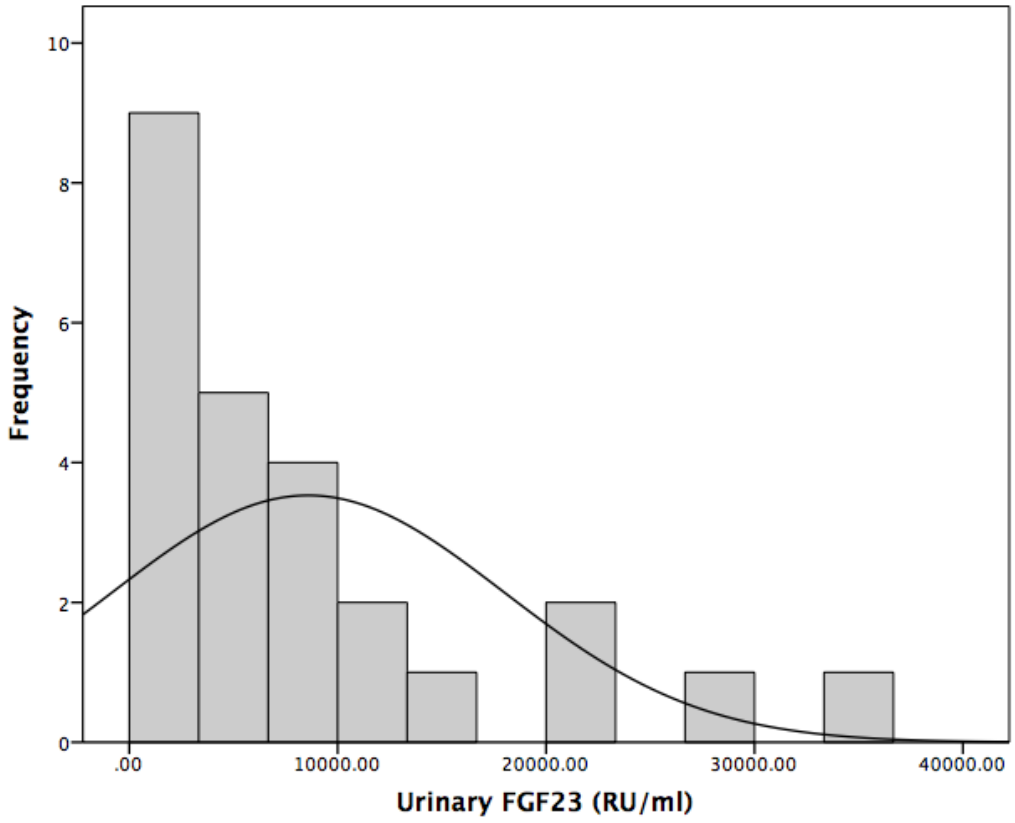
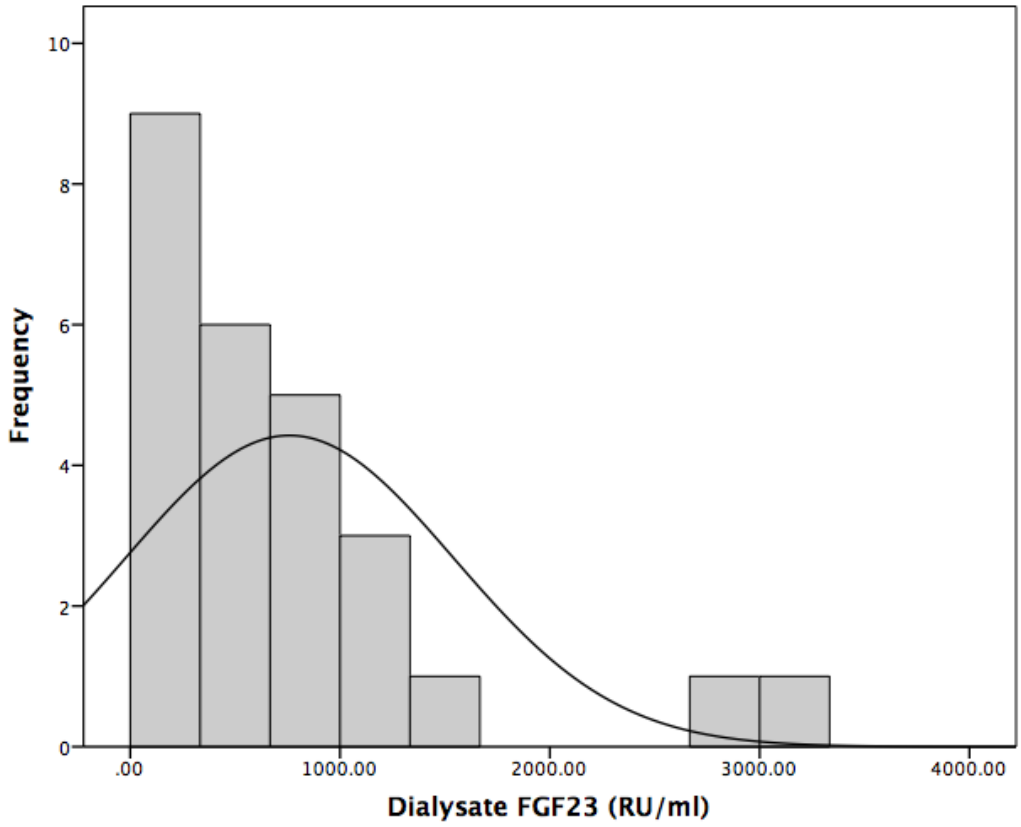


Figure 4-5: Dialysate FGF-23, positively skewed



Median plasma, urinary and dialysate FGF-23 were 8219.2 RU/ml (IQR 2749.2 - 21390.0), n = 61; 5738.0 RU/ml, (IQR 1498.4 – 12019.6), n = 25 and 522.8 RU/ml, (IQR 252-4 – 995.1), n = 26 respectively.

Figure 4-6 Distribution of FGF-23 in PD (Plasma)

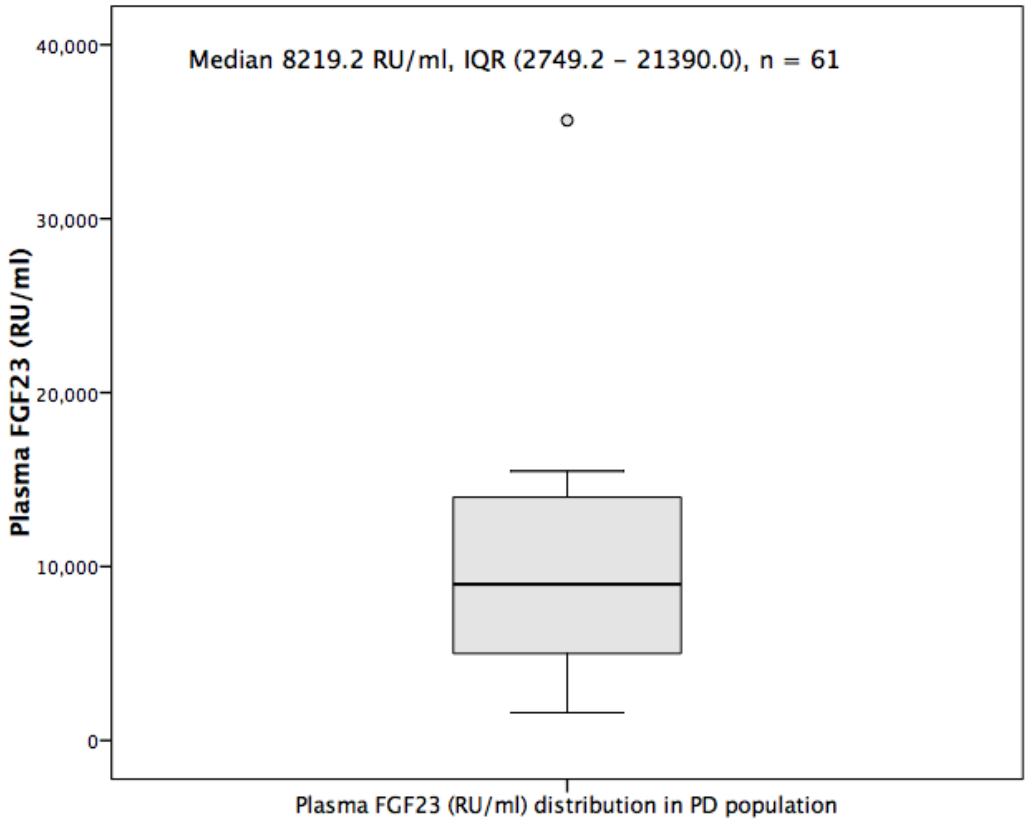
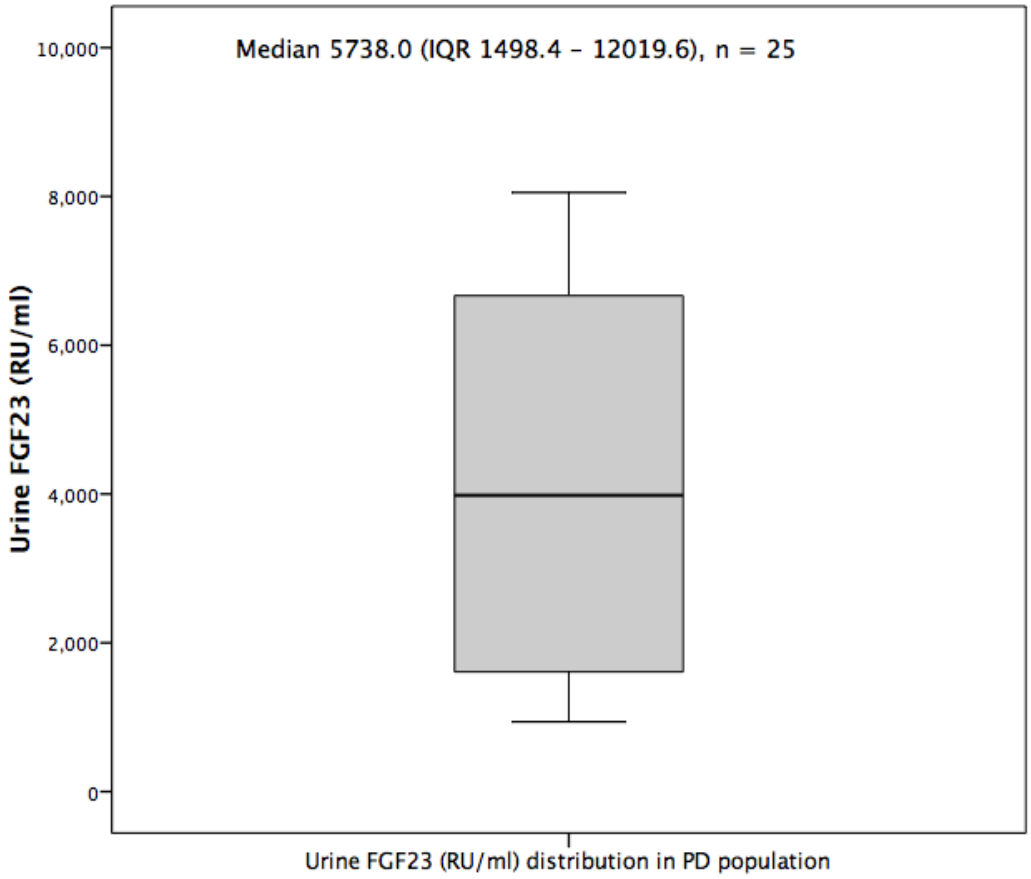
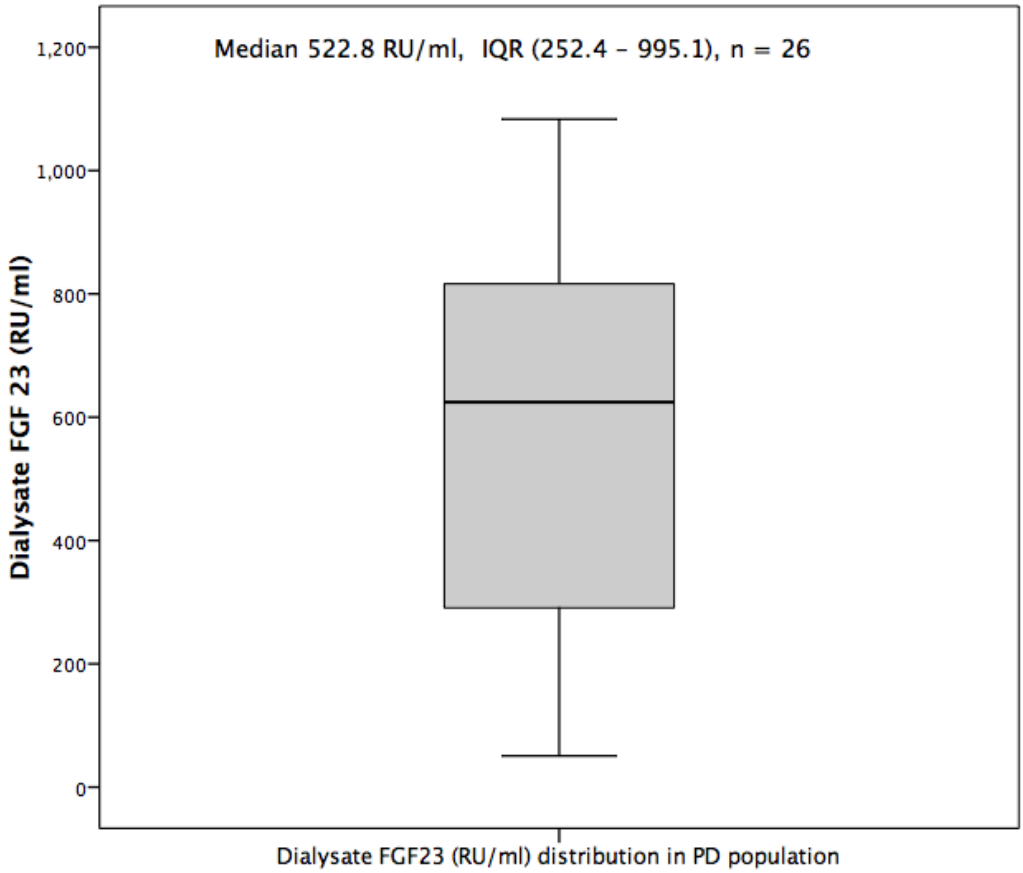


Figure 4-7 Distribution of FGF-23 in Urine & Dialysate



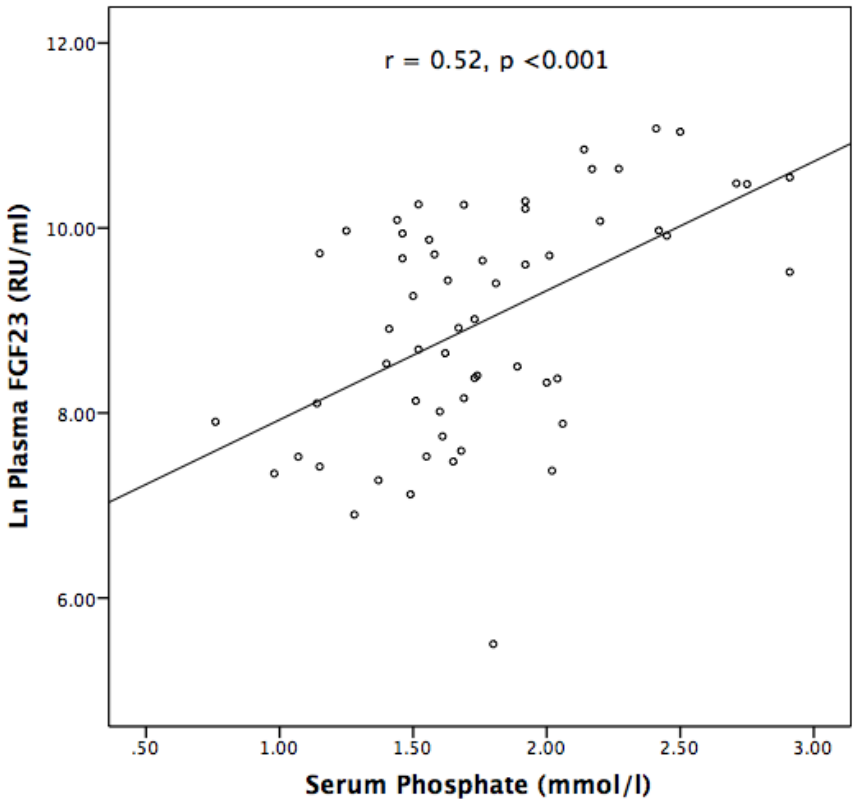


4.9.4 Correlation of plasma FGF-23 and serum phosphate

Across all time periods there were 58 measurements of both FGF-23 and phosphate taken at the same time.

Figure 4-8 is a scatter plot showing the correlation of phosphate to plasma FGF-23 using linear regression and Pearson correlation coefficients. There was a significantly strong association of log plasma FGF-23 with phosphate levels (r= 0.52 (p< 0.001).

Figure 4-8: Scatter plot to show the linear association of FGF-23 and serum phosphate



4.9.5 Correlation of plasma FGF-23 to urinary & dialysate FGF-23

There are significant positive correlations between plasma log FGF-23 with log FGF-23 levels in urine (r= 0.68, p <0.001, figure 4-9) and plasma log FGF-23 with log FGF-23 in dialysate (r=0.89, p <0.001, figure 4-10).

Figure 4-9: Scatter plot to show linear relationship between lnFGF-23 in plasma and urine

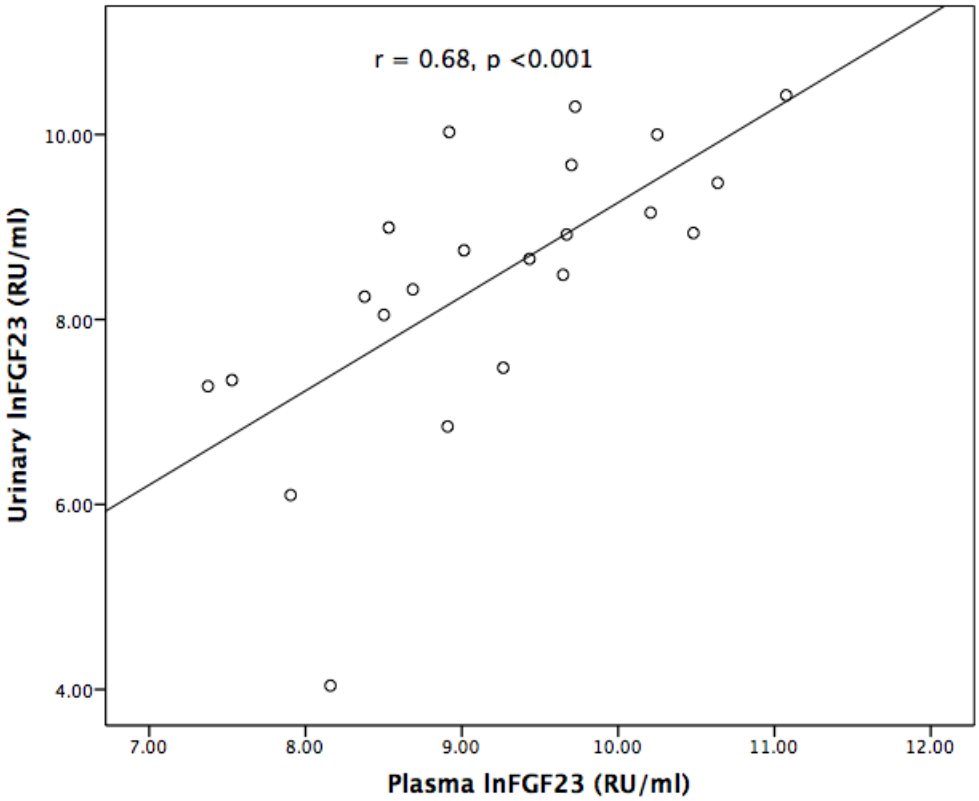
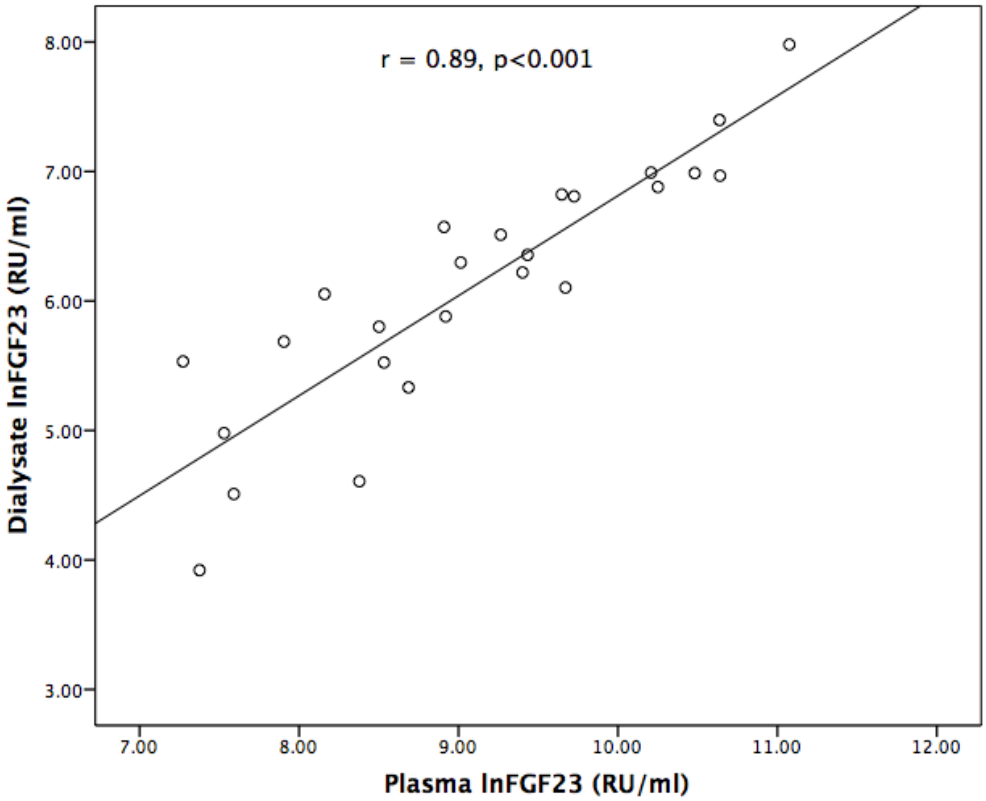


Figure 4-10: Scatter plot to show linear relationship between lnFGF-23 in plasma and dialysate



4.9.6 Changes in FGF-23 and phosphate over 9 months

Changes in FGF-23 and phosphate levels were analysed using paired t test in 14 patients. Mean log FGF23 and phosphate levels at 9 months were 9.09±1.11 RU/ml and 1.68±0.39 mmol/l compared to 8.64± 1.40 RU/ml and 1.75±0.36 mmol/l at baseline (p=0.42) but these changes were not significant.

Table 4-2 : Mean log FGF-23 and average phosphate values at baseline, 3, 6 and 9 months

Plasma samples	n	Mean	SD	Minimum	Maximum
Log FGF-23 (RU/ml)					
Time 0 (baseline)	19	8.64	1.40	5.50	10.64
3 months	14	9.05	1.32	7.35	11.04
6 months	14	9.18	1.12	7.53	10.85
9 months	14	9.09	1.11	7.59	11.08
Phosphate (mmol/l)					
Time 0 (baseline)	19	1.75	0.36	1.28	2.71
3 months	14	1.77	0.58	0.98	2.91
6 months	13	1.88	0.55	1.07	2.91
9 months	14	1.68	0.39	0.76	2.41

n, number of patients; SD, Standard deviation

4.9.7 Repeated measure at 0,3,6 and 9 months (ANOVA)

We further analysed changes in FGF-23 and phosphate over a period of time. There were 9 patients with readings available at 0,3,6 & 9 months. Repeated measures using ANOVA showed no significant change in either markers (FGF-23, F(1,8) = 1.63, P= 0.24; phosphate F(1,8)= 0.09, p = 0.78) over time. In particular there was no linear change (overall increase or decrease). In both cases the mean reading at 3 months were the lowest and the mean reading at 6 months the highest but these differences were not significant (Table 4-3)

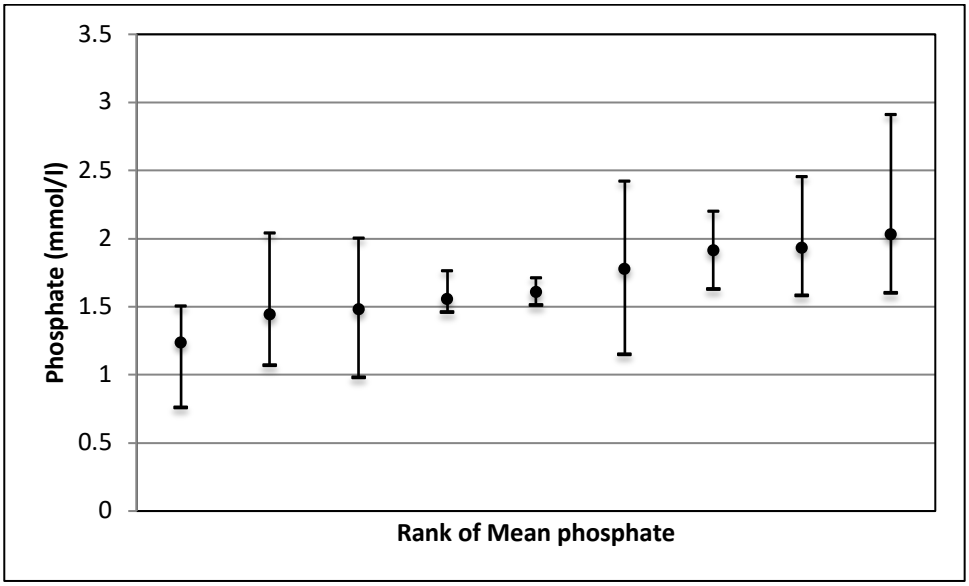
Table 4-3: Repeated measures ANOVA

Log FGF-23 (RU/ml) n = 9	Mean	SD	
Baseline (0 months)	9.13	1.04	
3 months	8.86	1.07	Linear contrast F (1,8)=1.63
6 months	9.57	1.02	P = 0.24
9 months	9.23	1.05	
Phosphate (mmol/l)			
Baseline (0 months)	1.67	0.22	
3 months	1.49	0.31	Linear contrast F (1,8)=0.09
6 months	1.95	0.60	P = 0.78
9 months	1.56	0.39	

4.9.8 Variability of FGF-23 & phosphate

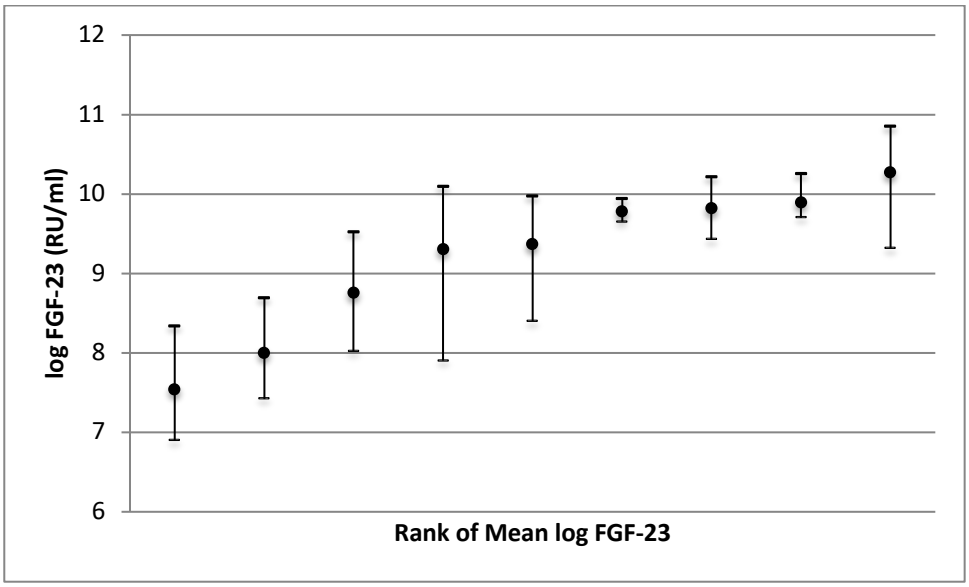
The preceding analysis found that readings over time for both FGF-23 and phosphate did not change. We rank ordered individual participants for each of the 4 measurements to examine within subject means and range of variation. The variations in phosphate and FGF-23 in each individual is represented in Figure 4-11 & 4-12.

Figure 4-11 Variation in phosphate of each individual



Arranged as rank of individual means. Means are represented as black dots and range for upper and lower values as black whiskers.

Figure 4-12 Variation in FGF-23 levels of each individual



Arranged as rank of individual means. Means are represented as black dots and range for upper and lower values as black whiskers.

Assuming that the readings are stable over time it is possible to compare the within to between subject variation using the Intraclass correlation coefficient (ICC). ICC is generally used in reliability analysis when a number of raters measure the same subjects. It is assumed that the underlying subject score remains the same and each rater is estimating the same thing. Thus, in this

analysis the assumption is that the patient's FGF-23 or phosphate level remains constant and the measures at different times are estimates of the same thing. A high ICC indicates that most of the variability is due to between subject differences, hence there is greater stability over time in the repeated measures. This analysis would not be the valid if there were any underlying change over time, for patients. ICC for FGF-23 was 0.90 compared to 0.65 for phosphate. (Table 4-4)

Table 4-4: ICC for FGF-23 and phosphate

	Log FGF-23	Phosphate
Mean	9.2	1.7
Variation		
Between subjects [a]	3.39	0.28
Within subjects [b]	0.37	0.15
Total variance [c]	3.76	0.43
ICC	0.90	0.65

ICC – Interclass correlation coefficient calculated using formulae a/c; Total variance c=a+b

4.9.9 FGF-23 Clearance

Clearances of phosphate and FGF-23 were calculated using formulae as described in section 4-7. The total FGF-23 clearances were the sum of the urine clearance (residual function) and the dialysate clearance. (Table 4-5) Levels of FGF-23 in plasma, dialysate and urine were correlated.

Table 4-5 : FGF-23 clearances in urine and dialysate

Clearance	N	Mean	Median	SD
FGF-23 (ml/min/1.73 m^2)				
Urine	22	0.67	0.5	0.65
Dialysate	25	0.61	0.59	0.37
Total	21	1.34	1.28	0.63
Phosphate (ml/min/1.73 m2)				
Urine	22	4.06	3.5	3.77
Dialysate	21	4.46	3.09	7.14
Total	22	8.73	6.04	7.13

N, Number of samples; SD, Standard deviation

To determine if phosphate clearance was related to the plasma FGF-23 levels we examined the correlation between plasma FGF-23 levels and urinary and dialysate phosphate clearance. Plasma FGF-23 showed a negative non-significant relation to urinary phosphate clearance ($r= -0.192$, $p= 0.39$, $n = 22$). There was no relationship of dialysate phosphate clearance to plasma FGF-23 levels ($r= 0.08$, $p = 0.76$, $n =21$).

Plasma FGF-23 levels exhibited significant positive correlations with FGF-23 in urine ($r = 0.682$, $p < 0.001$, $n =22$) and FGF-23 in dialysate ($r = 0.889$, $p <0.001$, $n = 25$) but a negative non-significant correlation to FGF-23 clearance in urine ($r=-0.334$, $p = 0.12$, $n = 22$) and FGF-23 clearance in dialysate ($r=-0.137$, $p= 0.51$, $n=25$). There was a strongly significant positive correlation between FGF-23 clearance in urine and total FGF-23 clearance ($r = 0.841$, $p <0.001$, $n = 21$). Dialysate FGF-23 clearance showed a moderate, not significant correlation to total FGF-23 clearance ($r= 0.217$, $p = 0.345$).

4.10 Discussion

Our study confirms that FGF-23 is elevated in ESRD(91) and levels correspond to the degree of hyperphosphatemia (154) in a group of PD patients. Previous reports have shown that lower residual renal function is associated with higher FGF-23 levels. (155) We found a negative correlation between plasma FGF-23 levels and urinary clearance despite noting a strong association of plasma levels to urine and dialysate concentrations. Renal FGF-23 clearances correlated to total FGF-23 clearance. These findings suggest that residual renal function has an important role in FGF-23 clearance.

ICC for FGF-23 was 0.90 compared to 0.65 for phosphate over a period of 9 months. A low ICC suggests that the within-subject variation is high while a high ICC indicates variability is explained by between-subject variation. Thus, repeated phosphate measurements over time is likely to yield more heterogenous results in the same individual, while FGF-23 measurements are more likely to be uniform within an individual over time. Our findings, over a longer observation period of 9 months are consistent with reports where FGF-23 demonstrated less within subject variability over a 3-month period compared to PTH and phosphate. (155)

The negative relationship between plasma FGF-23 levels and phosphate clearance is unexplained. It is possible that with loss of residual renal function, FGF-23 levels continue to rise and a threshold is reached beyond which the tubules are unable to excrete phosphate any further.

4.11 Conclusions

The strengths of our study include data collection over a 9-month period and quantification of clearances from both urine and dialysate. Food diaries were maintained and all patients received dietary education. We however did not perform phosphate balance studies or take into account the role of Vitamin D and phosphate binders. Peritoneal membrane characteristics play an important role in clearance of solutes, and this was not formally assessed. Patient numbers were small and like all observational studies this makes the results susceptible to bias. Larger studies in PD are partly limited by the decreasing number of patients choosing this modality of RRT in the developed world.

FGF-23 is a potential biomarker with greater stability over time. Its role in the assessment of CKD-MBD alongside conventional markers deserves to be assessed further. Due to the consistency of plasma levels over time, PD may be a more suited RRT modality to undertake further studies on the characteristics and mechanism of action of FGF-23.

Chapter 5

Effect of single session of dialysis on FGF-23

5.1 Introduction

Increased phosphate levels and deficiency of 1, 25 (OH) Vitamin D has been associated with higher mortality and morbidity in patients with CKD. (91) Fibroblast growth factor (FGF-23), a relatively new biomarker, maintains phosphate haemostasis by increasing urinary excretion of phosphate, and lowering levels of vitamin D. Low FGF-23 levels are associated with hyperphosphatemia and elevated levels of 1,25 (OH) vitamin D. (96) Recent studies have found FGF-23 to be an independent marker of mortality in patients starting haemodialysis. (156) Levels of FGF-23 are 1000 - fold higher in patients with end stage renal disease (ESRD) on dialysis. (96) Any treatment modality which alters FGF-23 is of great interest.

5.2 Aims of the study

To study the characteristics of FGF-23, its association with phosphate and clearances of FGF-23 during a single session of haemodialysis. We also wished to determine if it was possible to study kinetics of FGF-23 and to compare this to existing models for urea and phosphate.

5.3 Fibroblast growth factors - FGFs

FGFs are a group of polypeptides widely present in various adult tissues. They were initially isolated from brain and pituitary in the early 1970s (156,157) as acidic FGF and basic FGF, now known as FGF-1 and FGF-2 (158). FGFs are involved in embryogenesis (159) and play an important role in cell proliferation, angiogenesis, cellular differentiation and repair of cellular injury. (92,160)

The family consist of 22 members and are classified into seven subfamilies (FGF-1, FGF-2, FGF-4, FGF-7, FGF-9, FGF-10, FGF-19) based on phylogenetic analysis. FGF-19 subfamily includes FGF-23, one of the most extensively studied FGF. (161)

5.4 FGF-23

FGF-23 was first isolated from mouse cDNA in the ventrolateral thalamic nucleus of the brain. It was termed as FGF-23 as the 23rd documented FGF. (92) It is now

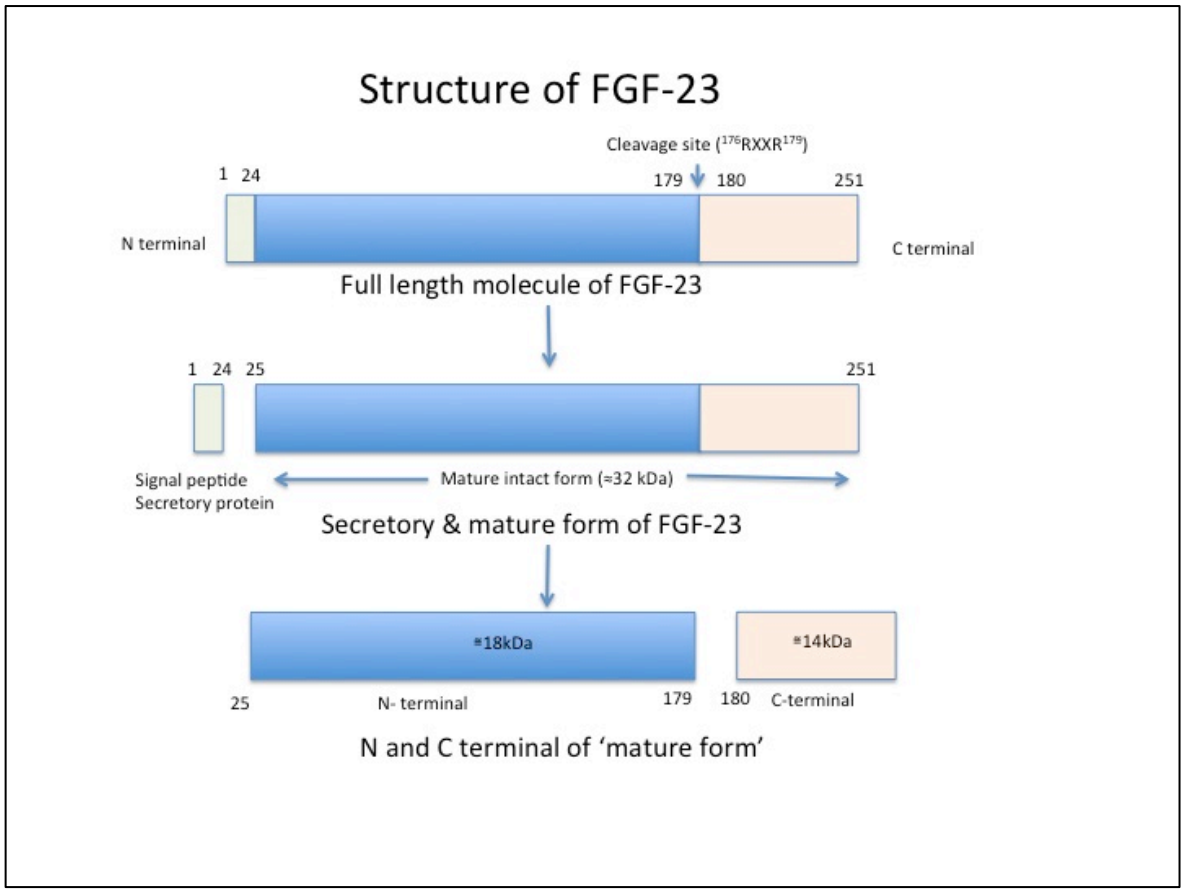
known that FGF-23 is primarily secreted from bone (162,163) and plays a key role in phosphate haemostasis.

5.4.1 Structure of FGF-23

FGF-23 and other members of the FGF-19 subfamily have a similar structure and share a common core region of ß-trefoil. They are distinguished from other subfamilies by the presence of a disulphide covalent bond that is believed to stabilise the core region and result in the important distinctive biological activity affecting phosphate metabolism (164,165)

Human FGF-23 consists of 251 amino acids and is encoded by three exons on the *FGF-23 gene,* located on chromosome 12p13. The first 24 amino acid residues are a signal peptide and appear to be a secreted protein. The following 227 residues (25-251 amino acids) secreted as a 'mature intact form' in the extracellular contains an extended sequence the C-terminal (180-251 amino acids) and a N-terminus. The C-terminus is unique compared to other FGFs and believed to be important in its specific function for phosphate and Vitamin D metabolism. (Figure 5-1) (165)

Figure 5-1 Structure of Fibroblast Growth factor 23



The mature intact form of FGF-23 gains weight following post-translational modification to form a 32kDa molecule. It undergoes cleavage at the consensus site (^{176}RXXR^{179}) by protein convertases and generates an N (≅18kDa) and C-terminal (≅14kDa) fragments. Mutations that impair cleavage increase levels of FGF-23 causing hypophosphatemic rickets (autosomal dominant/X-linked) and tumour-induced osteomalacia. Mature FGF-23 is the biologically active form. FGF-23 without the unique C-terminal fragment domain or the C-terminal fragment on its own is inactive. (161,166,167)

5.4.2 Biological activity of FGF-23

FGF-23 is primarily secreted from osteocytes (162,163) and osteoblasts. It acts via the FGF receptor (FGFR) in the kidneys along with co-factor Klotho. (162,168)

It main functions are:

1. Inducing phosphaturia by down regulation of sodium dependent phosphate co-transporter in the proximal convoluted tubule (PCT) (169) causing decreased phosphate reabsorption.

2. Lowering 1,25 $(OH)_2$ Vitamin D levels by inhibiting 1-α- hydroxylase that converts 25-(OH) Vitamin D to 1,25 $(OH)_2$ Vitamin D (169) and stimulating 24-hydroxylase (170) that catalyses Vitamin D degradation.

5.4.3 FGF-23 assays

In this study Immunotropics 2nd generation assay was utilised to measure FGF-23. This was chosen after reviewing the options commercially available.

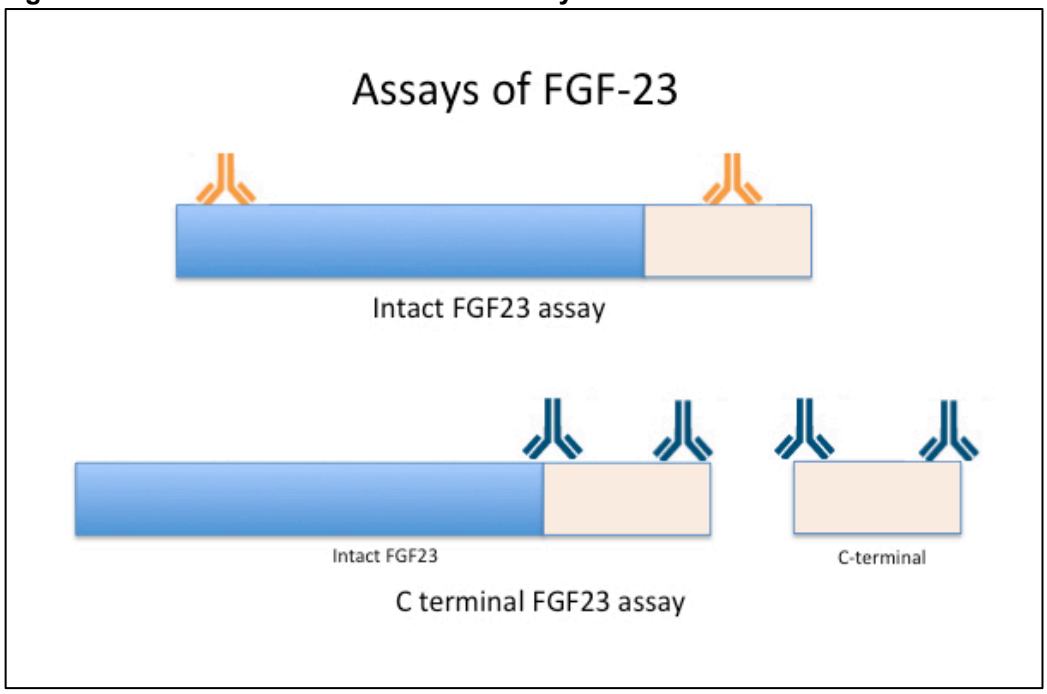
FGF-23 levels have been measured using four different assays.

1. iFGF-23 assay - This measures intact FGF-23 alone and available from Kainos laboratories Inc. (Tokyo, Japan), Merck Millipore (Billerica, MA, USA) and Immutopics Inc. (San Clemente, CA, USA).

2. 2nd generation C-terminal FGF-23 ELISA assay that measures both C-terminal fragments and intact FGF-23 and available from Immutopics Inc. only (Figure 5-2)

Levels are measured using serum (Kainos and Millipore) or EDTA plasma samples (Immutopics Inc.).

Figure 5-2 Fibroblast Growth Factor 23 assays



5.4.3.1 Kainos assay

This uses two specific monoclonal murine anti-FGF-23 antibodies; one used for capture (N-terminal) and the other HRP-conjugated antibodies for detection (C-terminal). The sandwich ELISA complex is then measured with a spectrophotometric reader.

5.4.3.2 Millipore assay

This is a similar sandwich ELISA based assay that captures human FGF-23 molecules utilising polyclonal goat anti-FGF-23 antibody and biotinylated polyclonal goat anti-FGF-23. Streptavidin-horse peroxidase conjugate is added and the enzyme activity measured spectrophotometrically.

5.4.3.3 Immutopics Inc. iFGF-23 assay

This uses a goat polyclonal antibody to capture and recognise epitopes within C-terminal (51-69), and a horseradish peroxidase conjugated goat polyclonal antibody to detect the N terminal (186-206) fragment of the FGF-23 molecule. This recognises the intact FGF-23 fragment alone. (167,171)

5.4.3.4 Immutopics Inc. 2nd generation C-terminal FGF-23 assay

This recognises epitopes within the carboxyl-terminal (E186-206 and G225-244) using two-affinity purified goat polyclonal antibodies, one biotinylated for capture and the other HRP-conjugated to detect epitopes fragment of FGF-23. These bind to both intact and C-terminal fragments of FGF-23. (167,171) The binding of FGF-23 to the capture and detection antibody results in a sandwich complex. Following a period of incubation with a substrate solution the unbound antibodies are washed and analysed spectrophotometrically at 450 nm (primary procedure) and 595 nm - 650 nm (secondary procedure). Values >445 RU/ml are analysed using secondary assays and levels >1400 RU/ml (the value of the highest standard) further diluted to obtain values within the highest standard as per manufacturer instructions.

5.4.4 Intact or C-terminal FGF-23 assays

2nd generation Human FGF-23 (C-terminal) ELISA kits were used to measure plasma samples frozen at -80°C as per manufactures instructions. The choice of FGF-23 assay was determined by the immunoassay stability, repeatability and reproducibility, knowledge about reference range and nature of study.

5.4.4.1 Immunostability

iFGF-23 is an unstable molecule and degrades within 2 hours of sample collection in healthy volunteers and pre-dialysis CKD groups. Conversely cFGF-23 levels increase over the same period of time. The addition of a broad-spectrum protease inhibitor cocktail stabilises the molecule for 4 hours with no significant changes in concentration observed (iFGF-23 and cFGF-23). (153)

The stability in HD patients however varies and levels are based on the analytical methods used. Smith *et.al* measured FGF-23 concentrations in 10 HD patients using whole blood and blood collected using plasma protein preservation systems. Baseline samples were centrifuged and stored at -80C within 10 minutes of collection and remaining samples were allowed to stand at room temperature for 8 hours before centrifugation and storage. Samples analysed using 2nd generation plasma cFGF-23 concentrations remained

unchanged in comparison to baseline (p=0.760). Mean iFGF-23 levels decreased by 5% (Kainos assay), 7% (Millipore) and 23% (Immutopics). (167)

5.4.4.2 Repeatability and reproducibility

The variability of cFGF-23, Immutopics is 2.2–4.4 % for intra-assays and 9-16 % for inter-assays. Comparatively, variability coefficients with other iFGF-23 inter-assays are Immutopics, 22%-61% and manual Kainos 5.7-14%. Intra-assays of automatic washer Kainos are 11-43% and manual Kainos, 5.3-9.7%. (172)

5.4.4.3 Suitability in HD patients

In healthy individuals the assays of iFGF-23 and cFGF-23 show distinct patterns, and don't correlate, a reflection of diurnal, intra-individual variations and presence of both iFGF-23 and cFGF-23 in health. (167,173) iFGF-23 has a higher intra-individual variation compared to cFGF-23 (18.3% versus 8.3%). (173)

In chronic HD patients however, the assays of Immutopics iFGF-23 and cFGF-23 correlate strongly. This possibly is due to the increase in iFGF-23 levels with worsening renal impairment. (167) Torres et al. analysed samples form 14 haemodialysis patients and found an excellent positive correlation has been seen between serum cFGF-23 (Immutopics) and iFGF-23 (Kainos). (174)

5.4.4.4 Reference range

The normal reference range of plasma FGF-23 in 170 healthy individuals using the Immutopics assay was 11.7-48.6 pg/ml for iFGF-23 and 21.6-91.0 RU/ml for 2nd generation cFGF-23. (173) Mayo laboratories immunoassay for cFGF-23 ages 18 years or older is ≤180 RU/mL. (175)

5.4.5 Half life of FGF-23

The half-life (t½) of FGF-23 has been studied in patients with tumour-induced osteomalacia.

Takeuchi *et al.* collected multiple venous blood samples from various sites (femoral veins, common iliac vein, junction of both renal veins, common jugular, inferior and superior vena cava and those from peripheral circulation)

through a catheter inserted in the left femoral vein. T ½ was determined as 21.5 minutes (two sandwich ELISA (176) technique to determine N- terminal and C-terminal) by measuring serum samples before and after removal of the right inguinal sac. (177)

Khosravi *et al*. calculated the t ½ by collecting samples from 3 patients every 30 minutes for up to 2 hours and then less frequently after excision (2 days in 2 patients and 3 days in 1 patient) of tumours in patients with tumour-induced osteomalacia (one phase exponential decay methodology). FGF-23 was measured using 2 assays – cFGF-23 and iFGF-23. Mean T ½ of FGF-23 from the three samples was 46 ± 12 minutes using C-terminal, Immutopics, Inc., assay and 54 ± 34 minutes with iFGF-23 Kainos Laboratories, Inc., assay. (178)

The differing t ½ is possibly due to the methods used - semilog transformation from three time points vs. one phase exponential decay equation. (177,178) Besides FGF-23 undergoes post-translation modification by glycosylation, which may have a role in secretion, metabolism and elimination of FGF-23. These processes are likely to be different in normal physiology and in patients with tumours. (167,178)

5.5 Dialysis modalities and removal of FGF-23

Dialysis is a process of removing toxic solutes and excess fluids from the body across a semipermeable membrane. Conventional haemodialysis or low-flux dialysis (LFHD), is a diffusive process capable of removing small solutes such as urea and creatinine with a molecular weight (MW) of <500 Da. The development of high flux dialysis (HFHD) that combines an element of convection with diffusion arose from the desire to remove larger molecules, such as ß2-microglubin. This modality allows removal of larger molecules up to a MW of 40 kDa.

Haemodiafiltration (HDF) enhances the convective component of the dialysis process and has been defined as - “A blood purification therapy combining diffusive and convective solute transport using a high-flux membrane characterized by an ultrafiltration coefficient greater than 20 mL/h/mm Hg/m2 and a sieving coefficient (S) for ß2-microglobulin of greater than 0.6. Convective

transport is achieved by an effective convection volume of at least 20% of the total blood volume processed. Appropriate fluid balance is maintained by external infusion of a sterile, non-pyrogenic solution into the patient's blood." (179,180) Here we study the effect of different modalities of dialysis on FGF-23 removal.

5.5.1 FGF-23 removal during dialysis

FGF-23 in acute dialysis has seldom been studied. In a group of 23 patients, Torres *et al*. reported a significant increase in serum FGF-23 levels after dialysis (16241± 14432 RU/ml, pre-HD Vs. 20791 ±19366 RU/ml, post HD, $p<0.0001$). All patients were dialysed for 4-5 hours, three times a week using LFHD with conventional hollow fibre dialysers. Samples were measured using c-terminal FGF-23 ELISA Kit (Immutopics) and collected after a 12 hour fast before dialysis. To ensure that increased levels were not due to by-products, iGFG-23 (Kainos) was compared in a subgroup of 14 patients, and this showed an excellent correlation. Correcting for haemoconcentration did not alter levels. (174)

A randomised cross over trial in 13 patients comparing conventional 4 hours high-flux HD with 4 hours HDF and extended 8 hours high-flux HD with 8 hours HDF reported a higher reduction ratio in HDF compared to HD in both conventional (32.3 HDF vs. 12.1 HD) and extended (48.6 HDF vs. 26.3HD) groups, ($p <0.05$). (181) A similar cross-sectional study measuring pre and post concentrations of FGF-23, in high-flux HD and HDF, found a higher reduction ratio in the HDF group (55.7± 22.2%) compared to the HD group (36.2± 28.6%), ($p=0.0001$). (182) Lower FGF-23 levels were reported in short daily HD, 5-6/week in comparison to conventional HD, 3/week (823RU/mL vs. 2521 RU/mL). Samples were collected immediately before dialysis i.e. 24 hours after previous treatment in the short daily group and 48 hours after the previous treatment in the conventional group. (183)

5.6 Methods

5.6.1 Study population

25 stable patients with end-stage renal disease on haemodialysis were selected from two different centres, University Hospital Crosshouse (UHC) and Glasgow

Royal Infirmary (GRI). UHC dialyses patients using HDF while GRI utilises high-flux HD.

5.6.1.1 Inclusion criteria

1. Patients with end-stage renal disease established on haemodialysis for at least 3 months.

2. Age ≥18years and ≤100 years.

3. Using AV fistula as dialysis access.

5.6.1.2 Exclusion criteria

1. Patients unable to consent.

2. Patients hospitalised with infection or heart failure.

3. Severely malnourished with normalized protein catabolic rate (NPCR) <0.7 and albumin <35 g/L.

4. Evidence of recirculation of > 5% in AV fistula.

Residual renal function of <100mls/ 24 hours was disregarded for purposes of this project.

5.6.2 Laboratory assays

Blood samples were collected during routine dialysis sessions at equally timed intervals with dialysate fluid collected as described below. All samples were centrifuged and frozen at -80 C within 4 hours of collection. Samples were analysed after a single thaw at a central processing laboratory in single batch.

1. Urea and phosphate levels (in plasma and dialysate) were analysed using standard techniques at University Hospital Crosshouse.

2. FGF-23 concentrations (EDTA plasma samples & dialysate) were measured in duplicate using Immutopics 2nd generation C-terminal assays (Immutopics

Inc., San Clemente, CA, USA) at University of Glasgow. Serial dilution of plasma FGF-23 was performed for levels >1400 RU/ml. FGF-23 dialysate samples needed concentrated.

5.7 Ethical permission

Ethical permission was approved by West of Scotland Research Ethics service, REC reference 15/WS/0039. All patients provided written permission for recruitment in the project.

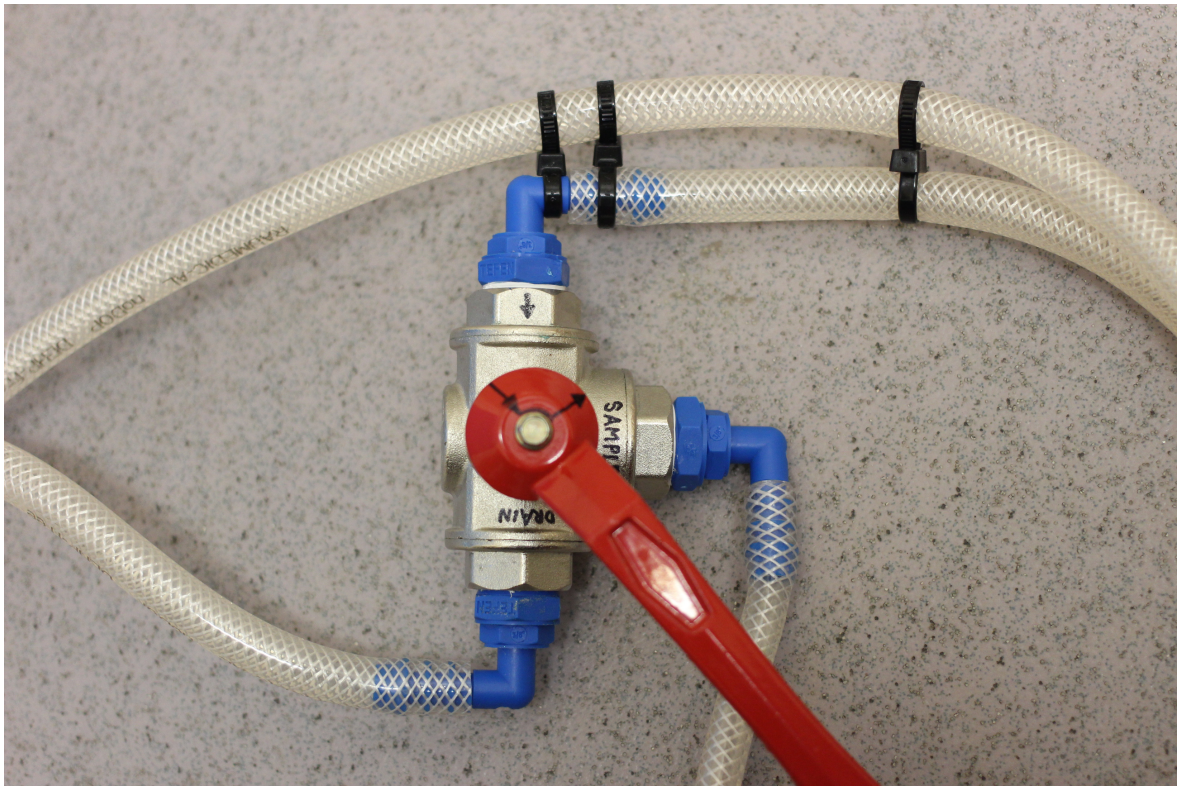
5.8 Sample collection

Plasma samples were collected at previously described. (184) In brief this was at the start (tia), equally timed intervals during dialysis (ti2, ti3, ti4, ti5, ti6) and at the end of treatment (tid). Dialyser clearance was derived from samples collected from dialyser inlet and outlet ports at 20 minutes from start of dialysis. Patients were given a choice to participate until the completion of treatment or stay for an extra hour for further sample collections at 15, 30 and 60 minutes. (184)

All patients had a working fistula with no evidence of recirculation (<5%). Patients were not severely malnourished or hospitalised.

Dialysate samples were collected at 5 different intervals using specially adapted three-way system. (Figure 5-3) Serial concentrations were measured to calculate the total amount of phosphate and FGF-23 removed during dialysis.

Figure 5-3 : Three-way adapted system to collect dialysate effluent samples during HDF at 5 different time intervals



5.9 Baseline characteristics

Data was collected on gender, age, RRT vintage, dialysis vintage, primary renal diagnosis and total body water using Fresenius Body Composition Monitor (BCM) ® (described in section 6.11). Treatment characteristics included dialysis efficiency (URR & St Kt/V), duration of dialysis session (Td), blood flow (Qb) and dialysate flow (Qd) and intradialytic weight changes. Equipment and dialysers used were recorded.

5.10 Statistical methods

Data was analysed using SPSS version 21 for Mac (IBM Corporation, Chicago, IL, USA). Mean ± standard deviation was used to report normally distributed data and median (interquartile range) for non-normally distributed data. Nominal data was represented as total number (n) and percentage (%). Where data was positively skewed further analyses were performed using log-transformed values. A p value of <0.05 was considered statistically significant.

Reduction ratios (RR) of FGF-23 and Urea were calculated from pre (C_{pre}) and post dialysis (C_{post}) concentrations

$$RR = \left(1 - \frac{C_{post}}{C_{pre}}\right) X\ 100$$

Correlation of phosphate and FGF-23 at different time points during dialysis was tested using Pearson's test.

Changes in pre and post dialysis solute levels were analysed using non-parametric Wilcoxon tests. Mass removal of solutes during dialysis (HDF versus HD) was compared using the non-parametric Mann-Whitney U test. P values of < 0.05 were considered statistically significant.

Changes in circulating levels of Urea, phosphate and FGF-23 at different treatment times of dialysis were performed using non-parametric Wilcoxon paired rank test.

5.11 Urea distribution and calculation of Total Body Water (TBW)

The volume of urea distribution (V) was derived from total body water using the Fresenius Body Composition Monitor (BCM) ® before dialysis. BCM uses a physiological three-compartment model of lean tissue mass, adipose tissue mass and over hydration to calculate TBW. The intracellular and extracellular water components are derived using bio impedance spectroscopy. This method of TBW calculation has been validated using gold standard deuterium dilution methods.

5.12 Results

5.12.1 Baseline characteristics

A total of 25 patients (11 HD, 14 HDF) participated; 7 patients agreed to stay on for an extra hour (3 HD, 4 HDF). 1 person could not complete the study.

Baseline and RRT characteristics are depicted in Table 5-1.

Table 5-1:Baseline characteristics of population

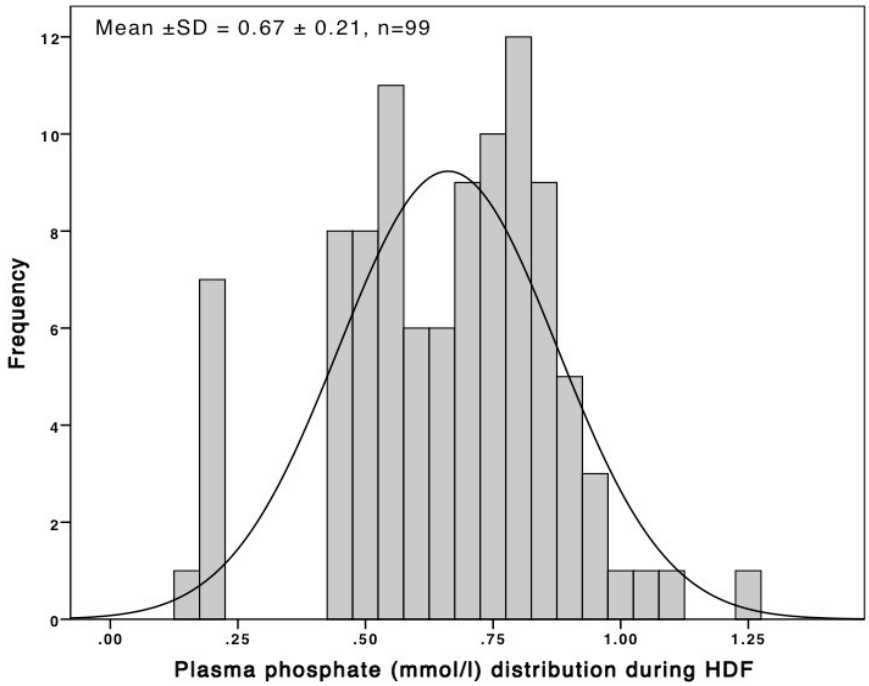
	Haemodiafiltration	Haemodialysis
Number (%)	14	11
Age, years (range)	64.6 (34.2 - 82.7)	66.6 (54.7 - 87.2)
Males	9	8
RRT vintage, months	105	139
HD vintage, months	102	120
Primary Renal Diagnosis, n (%)		
Systemic	4 (28.6)	2 (18.2)
Diabetes	5 (35.7)	1 (9.1)
Familial/Hereditary	2 (14.3)	1 (9.1)
Glomerular	0	3 (27.3)
Tubulointerstitial	2 (14.3)	0
Miscellaneous	1 (7.1)	4 (36.4)
BCM (Vurea)	39.3 (24.6 - 55.3)	36.6(19.8-45.4)
Urea reduction ratio	0.79± 0.07	0.75±0.06
St Kt/V	1.6 (1.1 - 2.5)	1.4(19.8-45.4)
RRT Characteristics		
RRT duration (minutes)	272 (240 - 300)	248(240-270)
Blood flow (millilitres/min)	283 (222-299)	316(281-350)
Dialysate flow (ml/min)	700	500
Weight change (kilograms)	1.4 (0-3.2)	1.5(0.3-2)
Machine & Dialyser characteristics		
Fresenius 5008	14	9
Fresenius 4008	0	2
FX 60	2	0
FX 80	9	5
FX 100	3	6

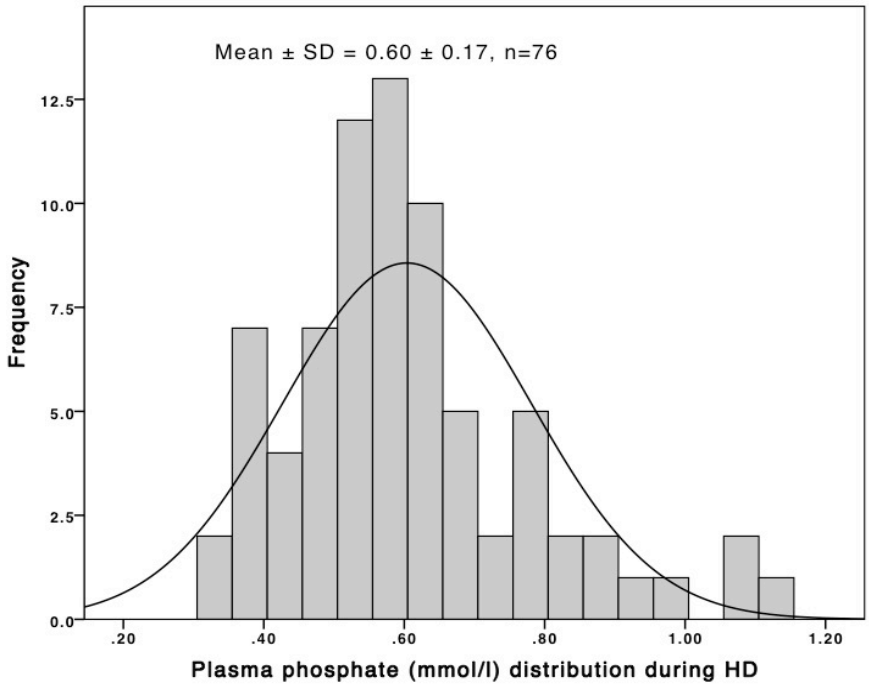
BCM, Body composition monitor; RRT, renal replacement therapy; St Kt/V – standard Kt/V

5.12.2 Phosphate distribution during dialysis

Plasma phosphate measured at all time points during dialysis was normally distributed and ranged from 0.15 to 1.27 mmol/l (mean ± SD, 0.67 ± 0.21) in HDF, n = 99; 0.33 to 1.15 mmol/l (mean ± SD, 0.60 ± 0.17) in HD, n=76. (Figure 5-4)

Figure 5-4: Phosphate distribution during HD & HDF

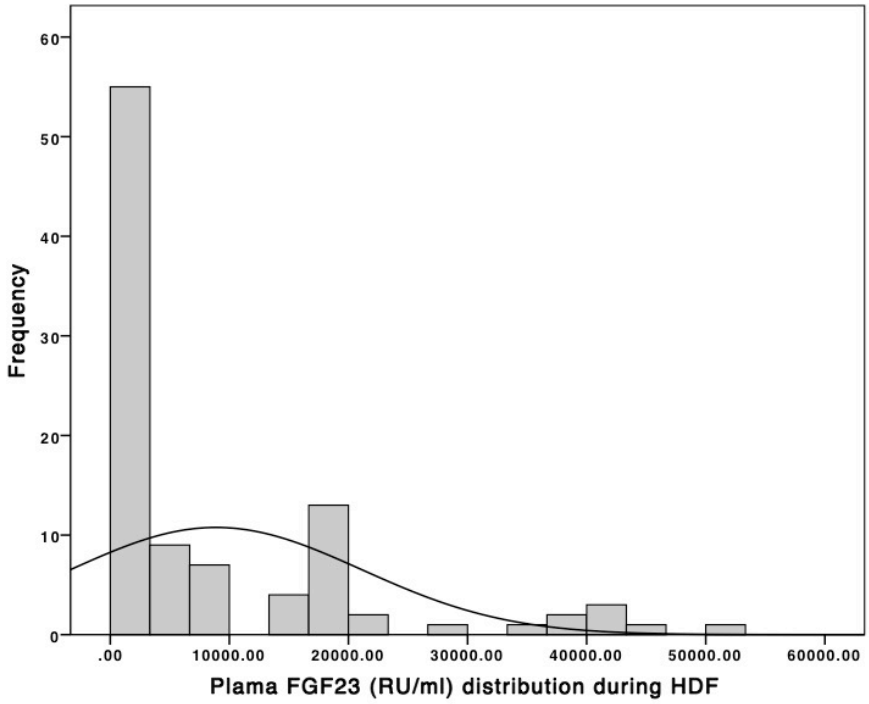


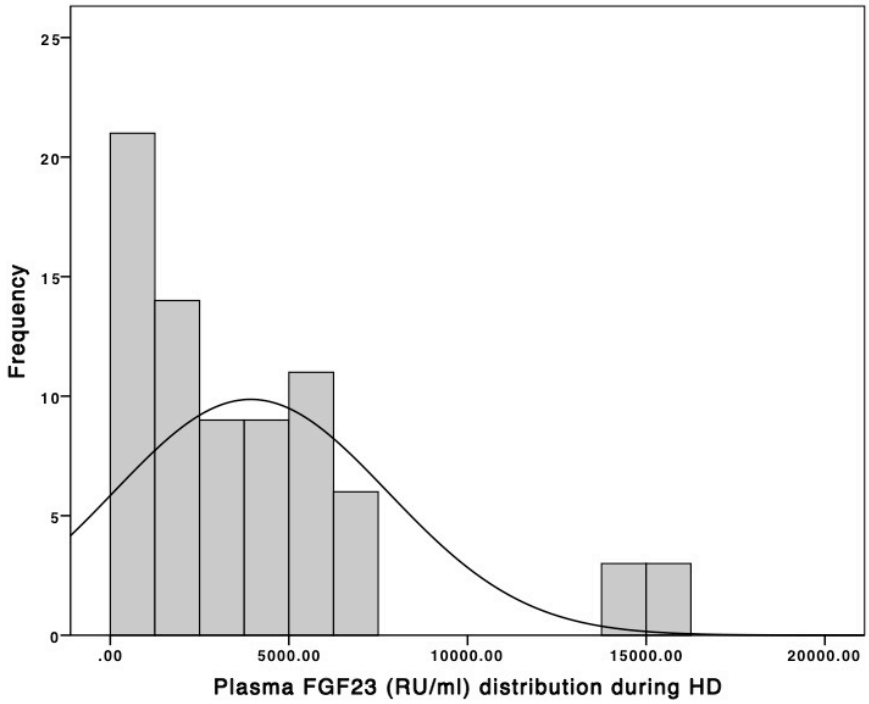


5.12.3 FGF-23 during dialysis

FGF-23 distribution was positively skewed and further analyses were performed using log-transformed values. (Figure 5-5)

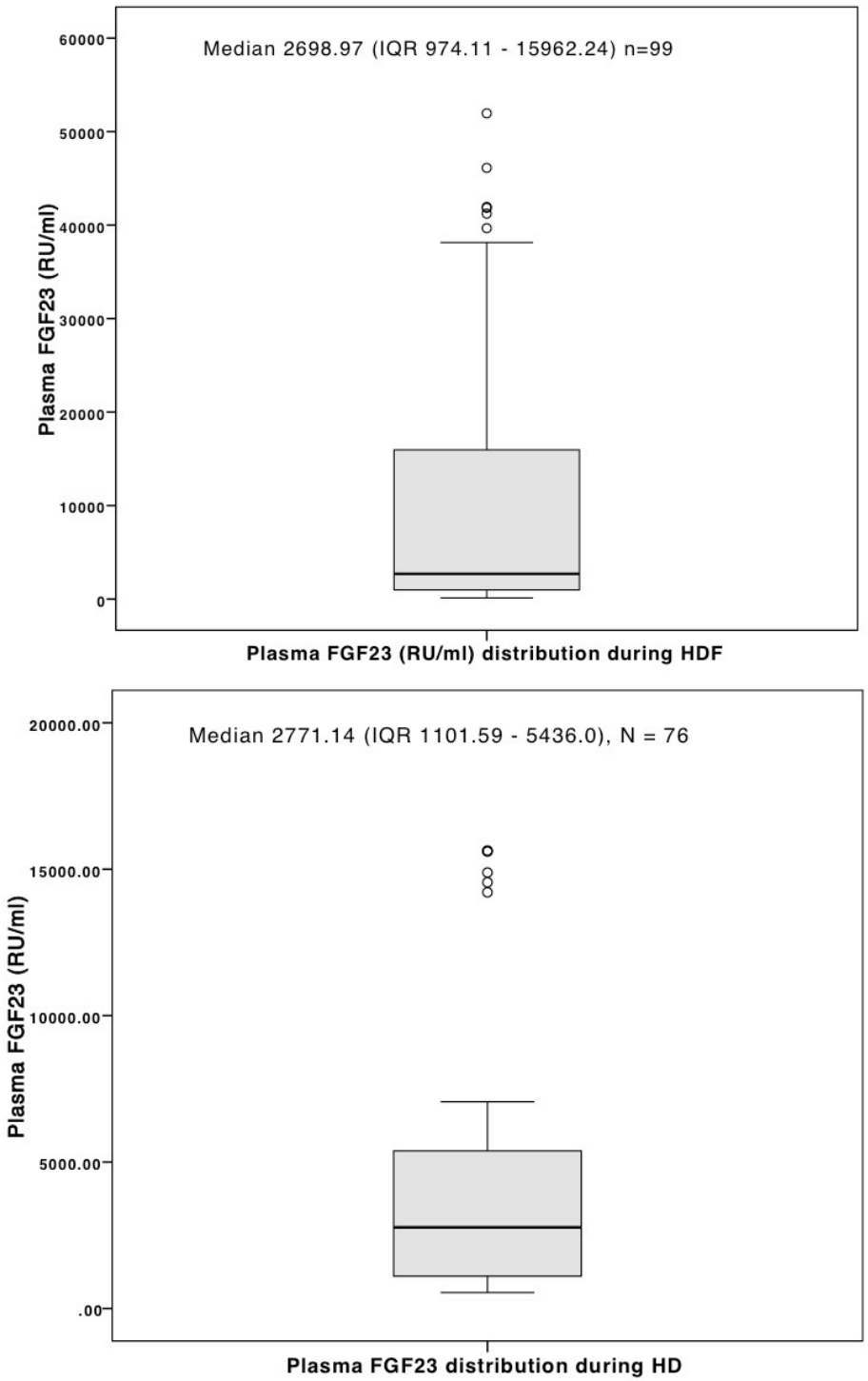
Figure 5-5:FGF 23 distribution during HD & HDF





Median values were 2698.97 RU/ml (IQR 974.11 – 15962.24, n = 99 HDF); 2771.14 (IQR 1101.59 – 5436.0, n = 76 HD). (Figure 5-6)

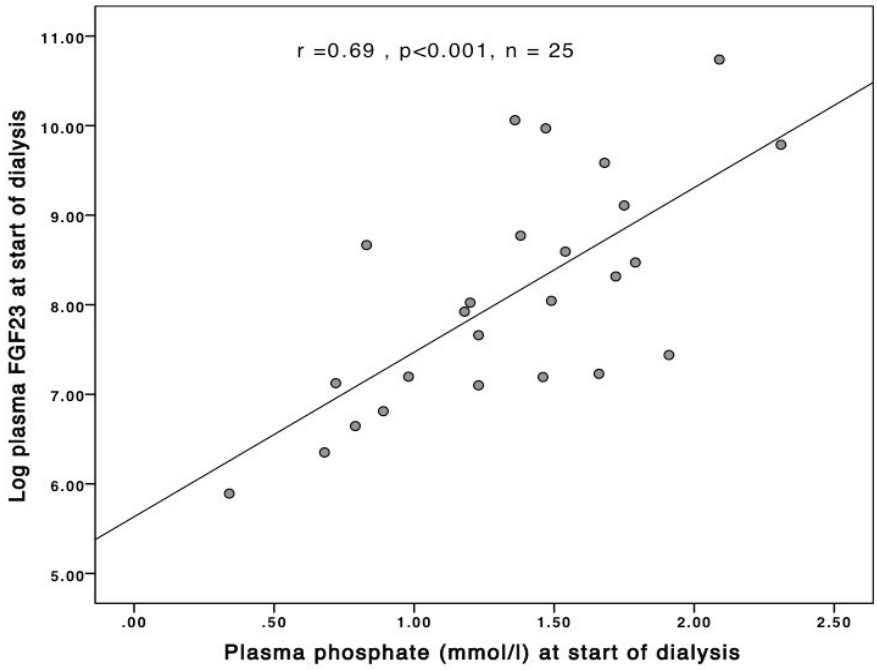
Figure 5-6: Distribution of FGF 23 during HDF & HD



5.12.4 Correlation of FGF-23 and plasma phosphate

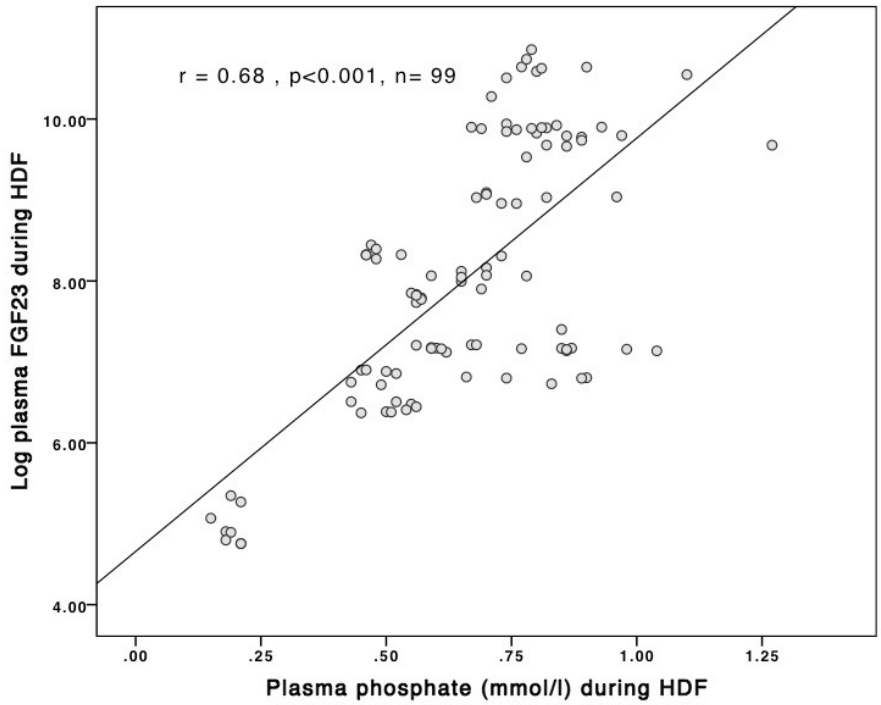
Figure 5-7 is a scatter plot showing the association of phosphate with FGF-23 using linear regression and Pearson correlation coefficients. There was a significantly positive correlation of plasma FGF-23 with serum phosphate at start of dialysis (r= 0.69, p< 0.001, n=25).

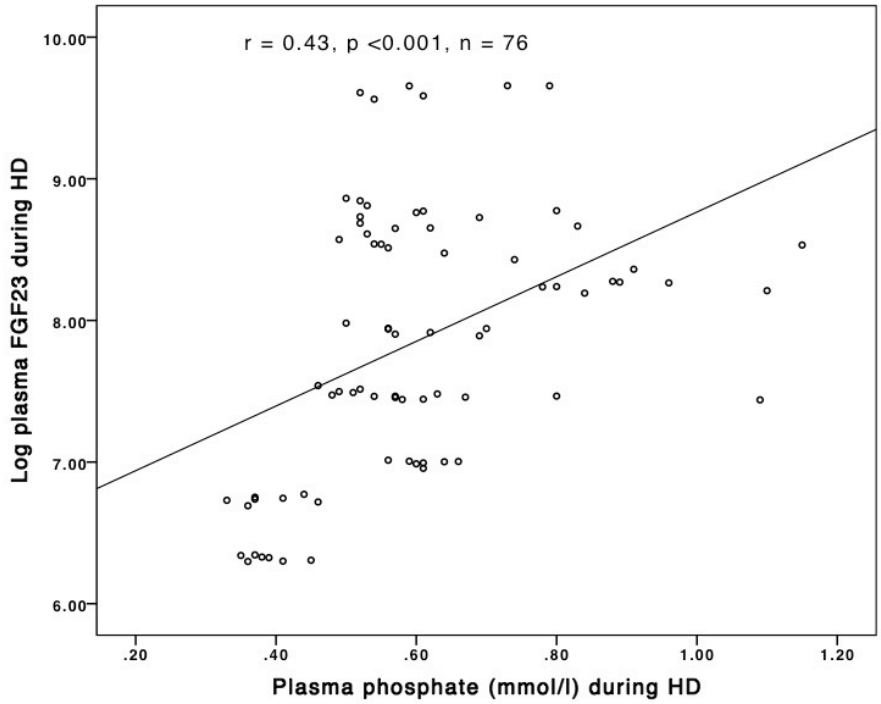
Figure 5-7: Association of phosphate with FGF-23 at start of dialysis



When we extrapolated the data using each modality of dialysis i.e. HDF and HD the results were similar. Serum phosphate strongly correlated to plasma log FGF-23 during both dialysis modalities; r= 0.68, p<0.001, n=99 HDF; r=0.43, p<0.001, n=76 HD. (Figure 5-8)

Figure 5-8: Pearson's correlation of FGF-23 and PO4 during HD & HDF





5.13 Modelling of solute clearance

Kinetic modelling and its application have been extensively studied to measure the 'effective dialysis' dose. Urea a commonly used surrogate marker is almost exclusively excreted by the kidneys and is abundant in renal impairment with good dialysis clearance. It's small molecular properties, distribution and easy measurability, makes it an ideal candidate for clearance measurement. For ease, further references to solute in this chapter imply urea, until specified otherwise.

To understand mathematical modelling, we start with the simple single pool urea kinetic model. In this, the human body is considered a single chamber of fluid with uniform concentration of solutes. There is no generation of urea, with neither removal nor addition of water during dialysis and solute is removed at a constant rate.

In reality, because urea is a water-soluble molecule it is distributed within the total body water distribution in two compartments - intracellular and extracellular. Initial studies on a two-pool mathematical model mostly assumed equal distribution of solute in these two compartments with free flow between the two.

5.13.1 Dialysis adequacy

Dialysis adequacy or the effective dialysis dose is the ability to remove solutes effectively during a session of dialysis. It is an important parameter used by clinicians regularly and often linked to mortality and morbidity. To calculate and understand the 'optimal dialysis dose' dialysis adequacy tools were introduced. Urea reduction ratio and Kt/V (discussed further in the chapter) are two of the most widely accepted dialysis adequacy tools in routine clinical use.

5.13.1.1 Urea reduction ratio (URR)

URR is a simple measure of dialysis adequacy that is mathematically expressed as

$$URR = (U_0 - U)/ U_0$$

or $URR = 1 - U / U_0$ *Equation 1*

where U_0 is the urea concentration pre-dialysis and U the urea concentration post dialysis

5.13.1.2 Kt/V

Kt/V is a dimensionless ratio of the volume of plasma cleared to the urea clearance volume where K is the dialyser clearance (ml/min), t the time on dialysis (ml/min) and V the volume of distribution (ml or litres) of solute. The total volume of distribution of solute is calculated as the total body water (60% in males and 45-55% in females, not relevant when measured using BCM). Thus a Kt/V of 1 is a volume that has been completely cleared of its solute.

We know from first order differential equations, that describes exponential clearance or decay of solutes

$$V \frac{du}{dt} = -KU$$

where $\frac{du}{dt}$ is the first derivative of concentration of solute with time, V the volume of distribution of solute, K the dialyser clearance and U the urea concentration at the end of dialysis. The dialyser clearance (K) or the ability of the dialyser to clear a solute is based on the size, membrane permeability, blood and dialysate flow rate.

Thus

$$\frac{1}{U}\frac{du}{dt} = -\frac{K}{V}$$

Integrating both sides

$$\int \frac{1}{U}\frac{du}{dt} = \int -\frac{K}{V}$$

$$\int \frac{du}{U} = \int -\frac{K}{V}\, dt$$

$$\log U = -K\frac{t}{v} + constant$$

Thus $$U = e^{-\frac{Kt}{v}+constant}$$

Where *e* is the natural logarithm

$$U = U_o\, e^{-Kt/V}$$

Where U_o is the concentration at start of dialysis

$$\frac{Kt}{V} = log\frac{U_o}{U}$$ Equation 2

We know from Equation 1 $URR = 1 - U / U_0$

Thus $1 - URR = U/U_0$ Equation 3

5.13.1.3 URR in relation to Kt/V

The mathematically relationship of Kt/V to URR can be derived by substituting the values from equation 3 in equation 2

Thus because $$\log\frac{U}{U_0} = -\log\frac{U_0}{U}$$

The equations can be rewritten as $$\frac{Kt}{V} = -\log(1 - URR)$$

The single pool model however does not account for solute generation and ultrafiltration. It assumes a linear decline in solute concentration during dialysis. A simplified second-generation equation was subsequently developed that could be applied to thrice weekly 2.5-4 hours dialysis that corrected for ultrafiltration and fluid removal as depicted below. (133)

$$SpKt/V = -\log(R - 0.008\,Xt) + (4 - 3.5\,XR)\,X\,UF/W \quad (133)$$

R, post dialysis/predialysis ratio; t, time on dialysis (hours); UF, ultrafiltration (litres); W, post dialysis weight (kilograms)

Solute rebound occurs post dialysis to reach a steady state of equilibration in the various chambers of the body.

To address the urea rebound (difference in solute concentration immediately post dialysis and the time taken to attain full equilibration of urea, usually 30-60 minutes after dialysis) $equilibrated\ Kt/V\ \ (eKt/V)$ has been suggested. This recognises solutes are not confined to a single chamber.

eKt/V is calculated as follows based on the access used and depicted as follows.

Arterial access $eKt/V = spKt/V - \ (0.6\ x\ spK/V) + 0.03$ (185)

Venous access $eKt/V = spKt/V - \ (0.47\ x\ spK/V) + 0.02$(185)

Other commonly used formulae to predict eKt/V includes the Schneditz rate equation(186), Smye method (187)and the Tattersall equation(188).

Since patients now dialyse more frequently and follow different treatment regimes a $weekly\ Std\ Kt/V$ has been developed. The weekly Std Kt/V is not a product of the SpKt/V by the sessions of dialysis, since urea decreases with each session of dialysis.

Thus the weekly urea clearance is derived as follows -

$Weekly\ Std\ Kt/V = 7\ x\ 1440\ [(0.184(PCRn - 0.17)]/$
$predialysis\ urea\ weekly$(189)

$Std\ Kt/V$, standard Kt/V; PCRn, normalised protein catabolic rate, 7 is number of days X 1440 minutes/ day = 10,080 minutes per week.

KDOQI recommends a minimum $SpKt/V$ of 1.2 or a URR of 65% in dialysis patients on 3 times/week with residual renal function of <2ml/min/1.73m^2. To achieve this target, the delivered $SpKt/V$ should be 1.4 or a URR of 70%. (190) UK Renal Association clinical practice guidelines on haemodialysis recommend a $SpKt/V$ of >1.3, a $epKt/V$ of 1.2 or a URR of 65% during a session of dialysis. The minimum targets are an URR of 70% or $epKt/V$ of 1.3 to achieve these goals.

5.14 Phosphate kinetics

Phosphate unlike urea is a complex molecule distributed in various components in the body. It's primarily found in bone as hydroxyapatite as a complex with calcium. (191) Serum phosphate measures the inorganic phosphorus while various other forms as dihydrogen phosphate ($H_2PO_4^-$) and hydrogen phosphate (HPO_4^{-1}) exist. Diurnal variation in phosphate, the amount of phosphate intake, effects of other hormones and residual renal function play an important role in serum phosphate levels. Levels are difficult to control in CKD and ESRD despite removal during dialysis. To better understand the role of phosphate and its distribution various kinetic models with 2 to 4 compartment assumptions have been proposed. These aim to improve our understanding of phosphate balance during dialysis and help assess phosphate status better.

A recent systematic review of phosphate kinetic models in haemodialysis published before August 2016 looked at 1964 studies and included 11 with 9 different models. (192)These were assessed using the modified version of the Newcastle-Ottawa scale that includes 14 quality indicators based on model approach, treatment setup, design, validation and conclusions. 3 of the 11 studied models were considered high quality with scores of 10.5 – 11 (6 medium quality, scores of 6.5 to 9.5 and 2 low quality, scores of 2 to 4 on a scale of 0-14) but none of them have gained clinical acceptance. (192)

Physiologically some of these models are plausible but complex. Spalding *et al.* four pool model is based on a standard 2 pool model that proposes a third compartment which allows an additional flux of phosphate into the extracellular space to maintain targets. A fourth compartment comes into play when intracellular phosphate falls below a certain level. (184)Other simple models are theoretical and some have assumed phosphate as equivalent to the TBW. (193)The role of dietary phosphate, phosphate binders, hormonal influences and dialysate composition adds to the complexity in our ability to build a phosphate kinetic model. Despite these limitations and the absence of a phosphate kinetic model that is widely accepted it is these initial works that prompt further investigation into phosphate behaviour.

The emerging role of FGF-23 in phosphate regulation and clearance in dialysis may help better understand mineral bone metabolism in CKD.

5.15 Mathematical modelling of FGF-23

Sample collection and methods are previously described in section 6.8. Dialytic clearances were calculated from the total solute (FGF-23) removed divided by dialysis duration and the log means of the pre and post dialysis concentration of the FGF-23 calculated. Total solute removed was the sum of the dialysate volume, ultrafiltration volume and substitution volume (only in HDF) multiplied by the solute concentration. Urea, phosphate and FGF-23 concentrations in the dialysate were calculated from the fluid collected at the 5 different time points via the three-way system.

Means of urea and phosphate concentration and medians of FGF-23 were recorded for patients on both HD and HDF. Data for an hour post dialysis were available for only 4 on HD and 5 on HDF. Separate plots were created for this subgroup of patients.

5.15.1 Results

Figures 5-9 and 5-10 shows the fall in urea concentration during dialysis followed by a rebound in the subgroup of patients that agreed to stay for an hour extra. Similar findings are noted in phosphate concentrations in Figure 6.13 and 6.14.

Figure 5-9: Mean urea concentration during dialysis

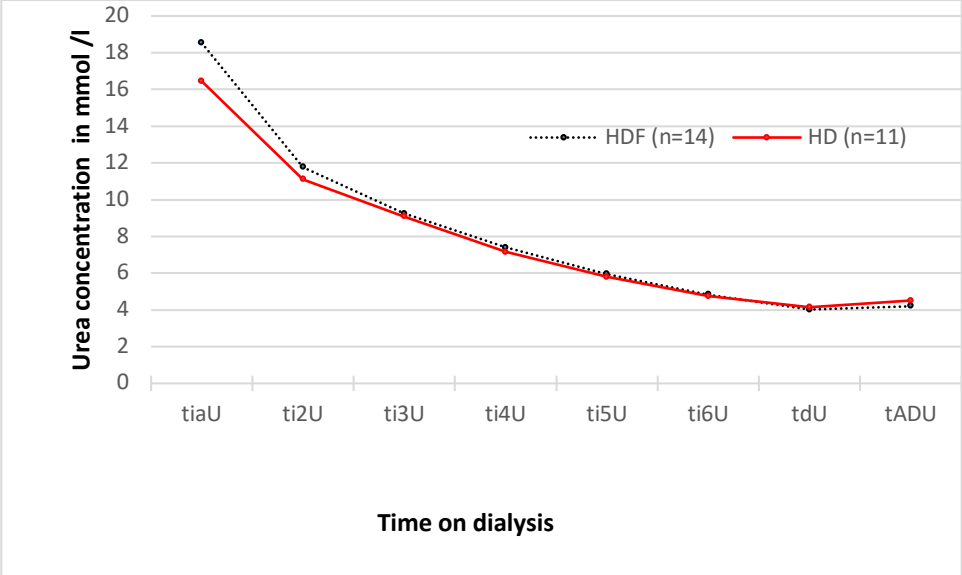
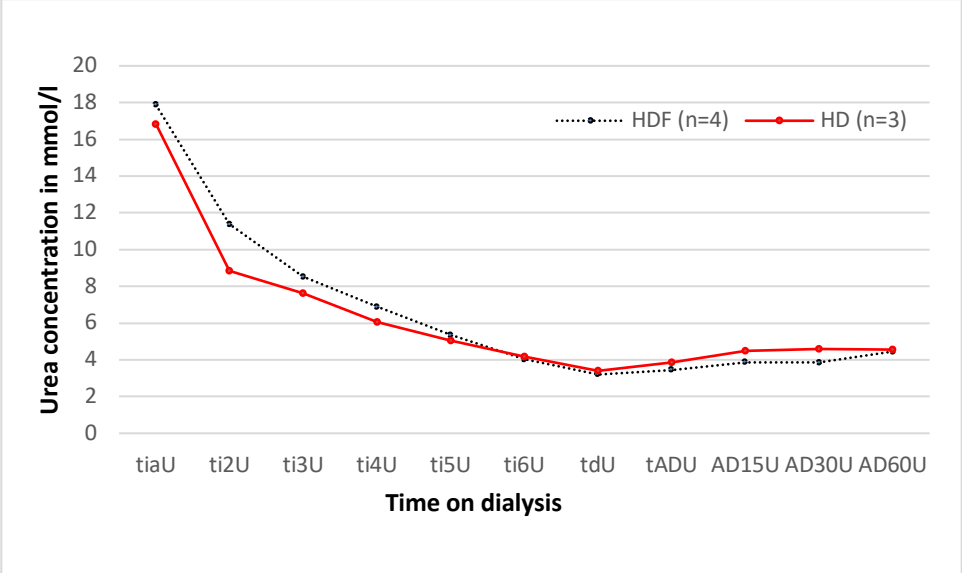


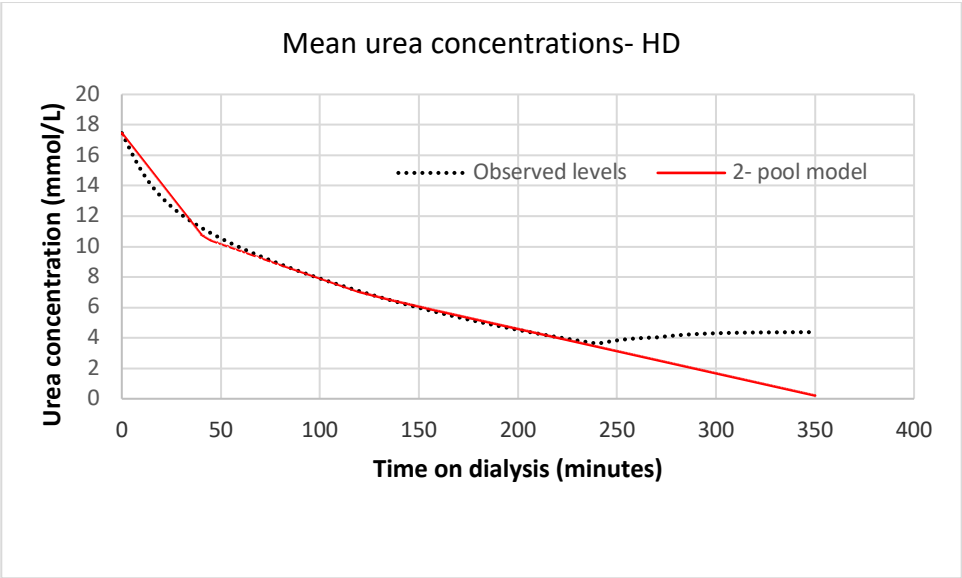
Figure 5-10: Mean urea levels in a subgroup, to include data 1-hour post dialysis



tia, start of dialysis; ti2 – ti6, equally timed intervals between dialysis; Td, end of dialysis; tAD, after dialysis; tAD15 – tAD60 are 15, 30 and 60 minutes post dialysis concentrations; U after each time signifies urea concentration at that time interval

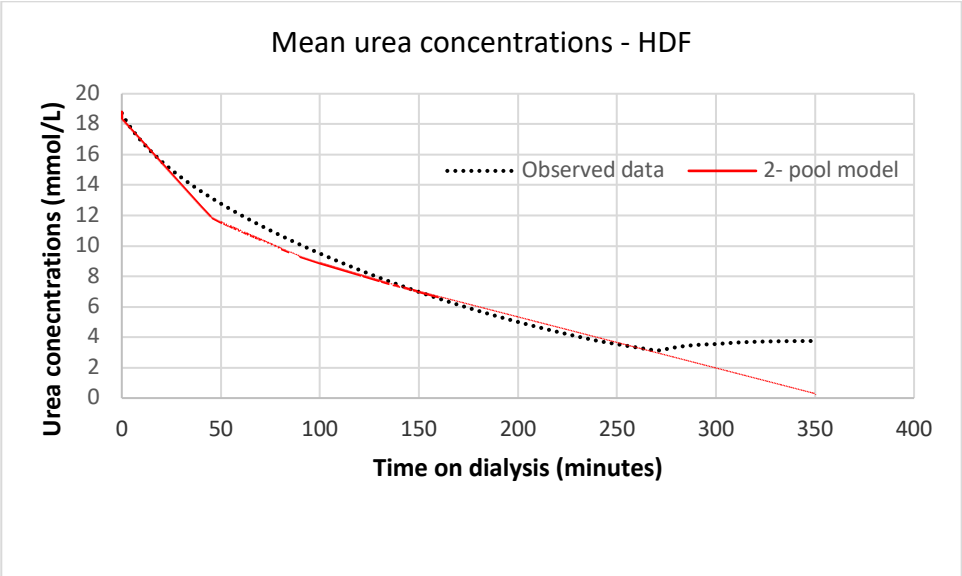
Samples for urea were further analysed using the single pool model to ensure accuracy of data collection. Figure 5-11 & 5-12 shows observed and predicted 2-pool models in both modalities of dialysis.

Figure 5-11: Mean urea concentrations during HD



HD- Haemodialysis

Figure 5-12: Mean urea concentrations during HDF



HDF - Haemodiafiltration

Figure 5-13: Mean phosphate level during dialysis

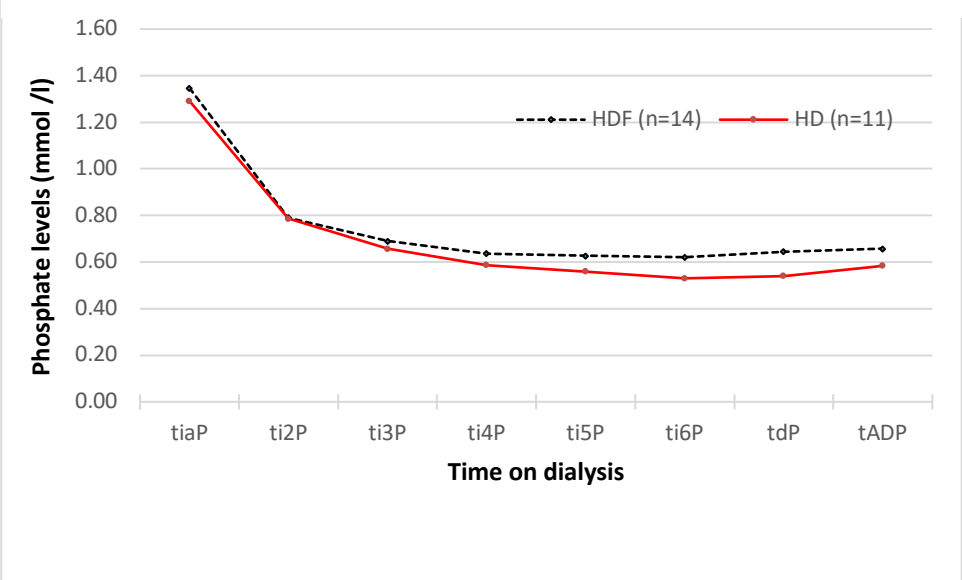
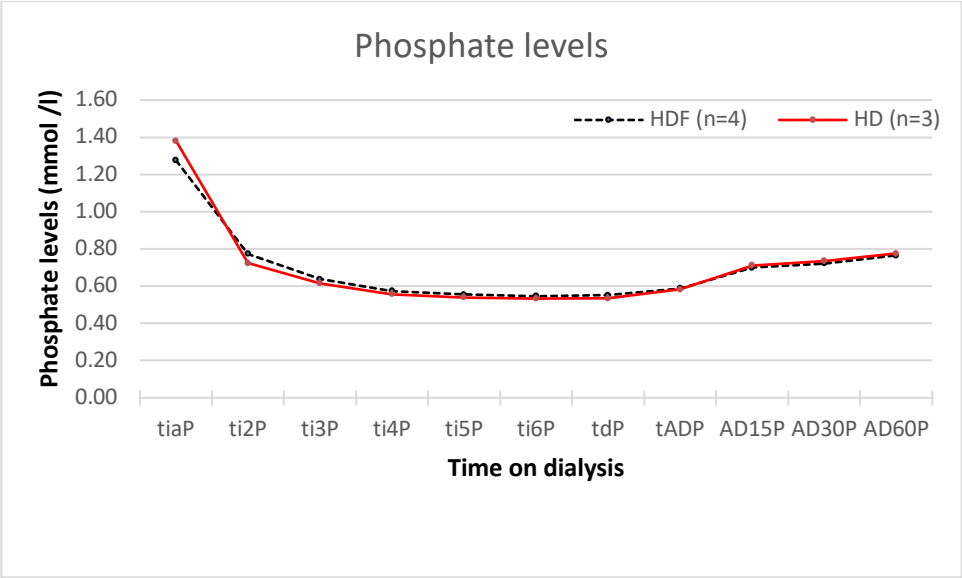


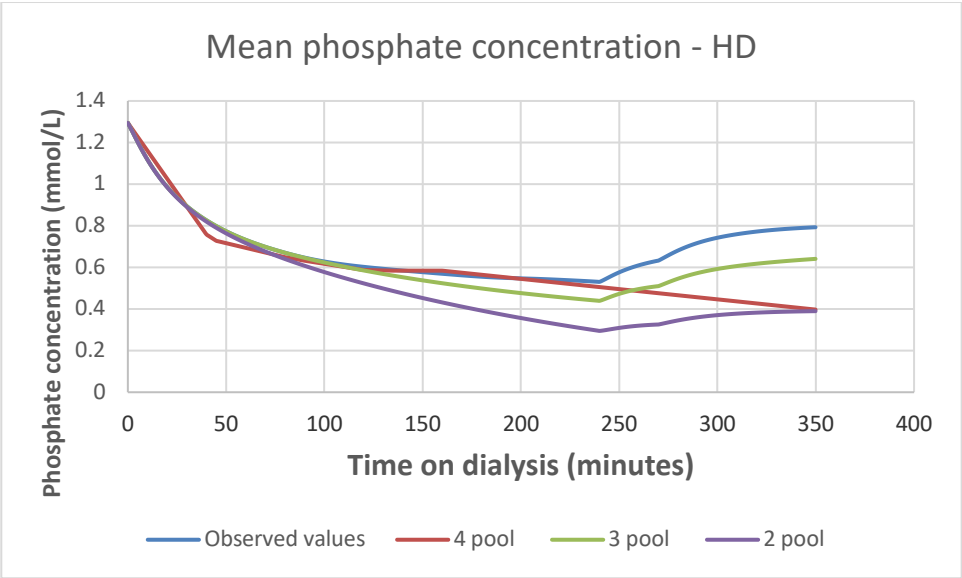
Figure 5-14: Mean phosphate levels in a subgroup, to include data 1-hour post dialysis



tia, start of dialysis; ti2 – ti6, equally timed intervals between dialysis; Td, end of dialysis; tAD, after dialysis; tAD15 – tAD60 are 15, 30 and 60 minutes post dialysis concentrations; P after each time signifies urea concentration at that time interval

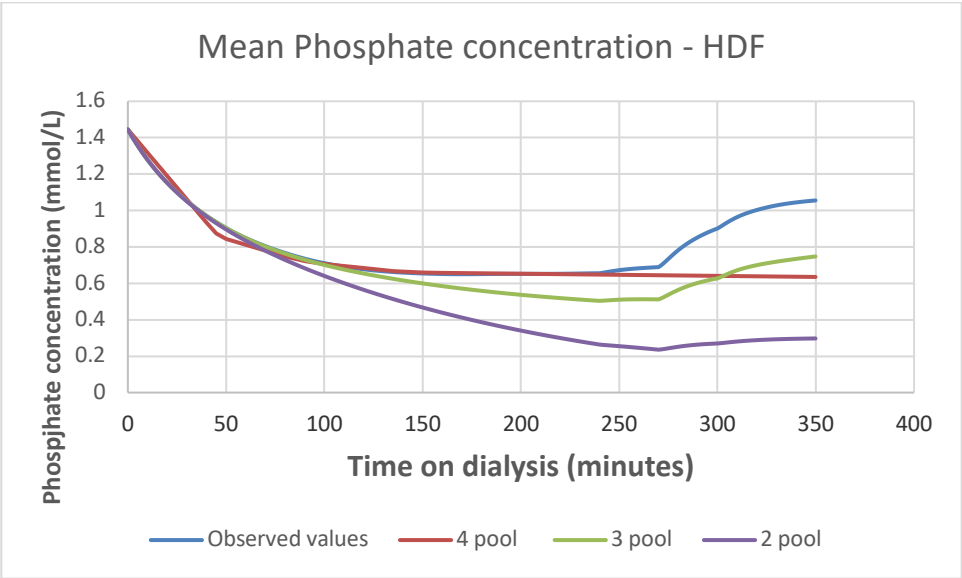
Samples for phosphate were further analysed using the 2 pool, 3 pool and 4-pool models. Figure 5-15 & 5-16 shows observed and predicted models in both modalities of dialysis.

Figure 5-15: Mean phosphate concentrations during HD



HD, Haemodialysis

Figure 5-16: Mean phosphate concentrations during HD



HDF, Haemodiafiltration

Figure 5-17: Median FGF-23 levels during dialysis

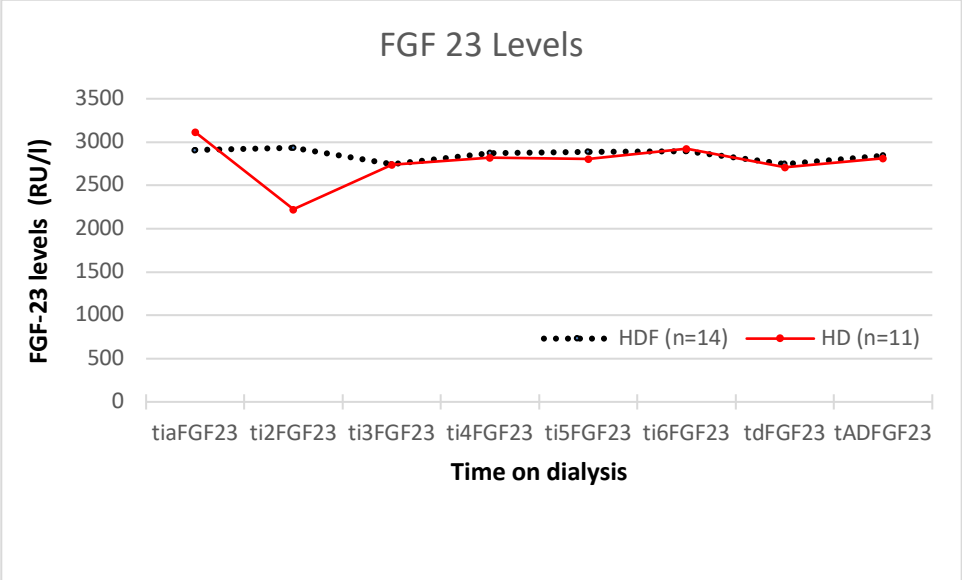
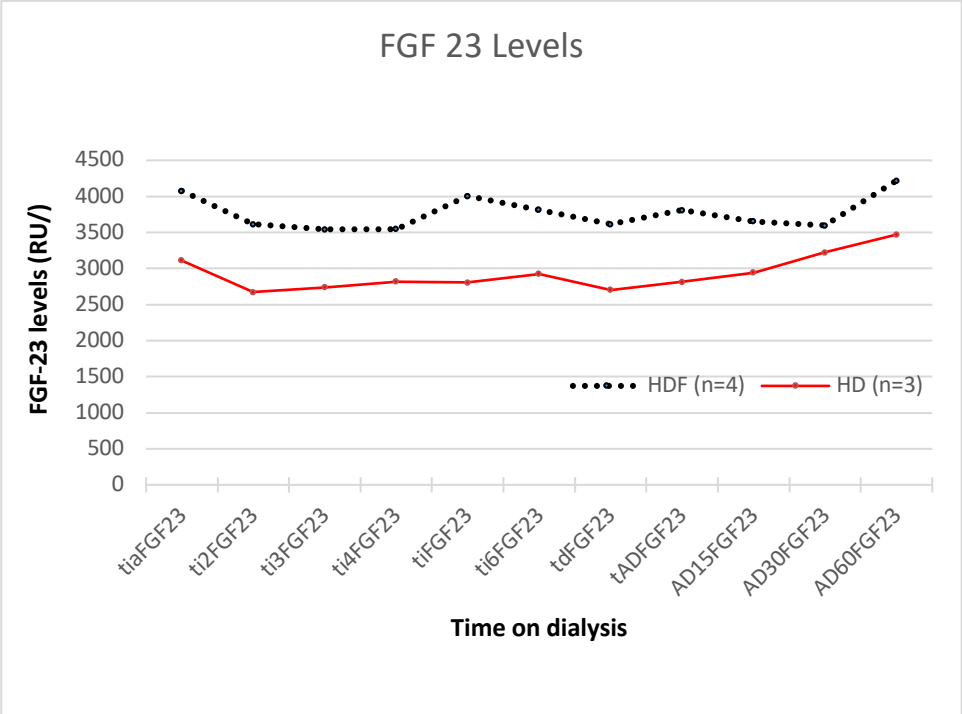


Figure 5-18: Median FGF-23 levels in subgroup, to include data 1-hour post dialysis



tia, start of dialysis; ti2 – ti6, equally timed intervals between dialysis; Td, end of dialysis; tAD, after dialysis; tAD15 – tAD60 are 15, 30 and 60 minutes post dialysis concentrations; FGF after each time signifies FGF-23 concentration at that time interval

5.15.2 Urea Reduction Ratio (URR)

Urea clearance was calculated using formulae described in Section 1.13.1.1, equation 1. URR for HDF was 0.79±0.07 and HD 0.75±0.06. Mean urea reduced

from 18.52 ± 3.41 mmol/L during HDF to 4.02 ± 1.72 mmol/L, n = 13; For HD mean values fell from 16.44 ± 5.67 mmol/L to 4.1 ± 1.09 mmol/L, n = 11. 1 HD patient could not complete the test.

5.15.3 FGF-23

Post dialysis FGF-23 concentrations were corrected for haemoconcentration assuming single pool distribution

$$[FGF\text{-}23corrected = FGF\text{-}23post/[1+\Delta BW/(0.2XBWpost)]$$

Where ΔBW is the difference between pre and post dialysis treatment weight and BW post the post dialysis body weight.

It is clear from the above data that a one or 2- pool model cannot explain FGF23 removal. The maintenance of a steady level throughout dialysis suggests FGF-23 generation, either through acute changes in phosphate concentrations or alterations in PTH in response to ionized calcium.

During HDF median FGF-23 decreased from 2906.1 RU/ml (IQR 1307.4 - 18678.3) to 2608.7 (IQR 1212.0 – 5802.2), p = 0.002, n = 13; For HD median values fell from 3113.54 RU/ml (IQR 1212.0 – 5802.2) to 2461.9 RU/ml (IQR (958.3-5438.9), p= 0.003, n = 11.

Total FGF-23 mass removal during dialysis by HDF was greater compared to HD (HDF, 183987.82 RU (IQR 36603.84 – 969224.16); HD 180464.71 RU (IQR 72321.30 – 323473.56), but this was not significant (HDF, n = 13; HD, n =11; p = 0.8, 2 tailed).

5.15.4 Phosphate

During HDF phosphate decreased from a mean of 1.35 ± 0.53 mmol/L to 0.65 ± 0.19 mmol/L, n = 13; For HD mean values fell from 1.34 ± 0.41 mmol/L to 0.58 ± 0.14 n = 11. 1 HD patient could not complete the test.

Mass phosphate removal by HDF was greater compared to HD (HDF, 28352.70 ± 11661.66 mmol; HD 26229.89 ± 7299.08 mmol), though this was not significant (HDF, n = 13; HD, n =11; p = 0.8, 2 tailed).

5.16 Discussion

This observational study confirms FGF-23 is removed during dialysis and its levels are 1000- fold higher in ESRD. HDF is superior to HD in mass phosphate and FGF-23 removal and there is a strong correlation of phosphate to FGF-23 during dialysis, both in HD and HDF.

A recent study comparing short daily dialysis with conventional dialysis did not find any differences in time averaged phosphate (3 serum samples collected prior to dialysis levels over 3 months) and PTH in the 2 groups. Levels of serum phosphate in the conventional group were 5.0±1.3 mg/dL versus 5.0±1.2 mg/dL in the short group. In comparison median plasma FGF-23 levels were lower (short daily, 823; IQR 263 – 2169 RU/mL versus conventional, 2521; IQR 909 – 5556 RU/mL, p <0.01) in the short daily group despite equivalent levels of phosphate. The correlation of phosphate to FGF-23 concentrations was similar to our findings (r= 0.42, p <0.01; conventional and r= 0.52, p <0.01; short daily). (194)

In contrast, a cross sectional study by Knap *et al.* reported similar levels of FGF-23 on long nocturnal HD with standard dialysis (mean 2677.2 ±4010.1 RU/ml, n=10, Nocturnal; 4134.4 ±6310.7 RU/ml, n=40, standard; P= 0.28). Patients in the nocturnal were younger (48±10 years versus 58.5 ± 15.7 years), dialysed for longer (24hours/week versus15hours/ week), had longer RRT vintage (mean 15.4 years versus 7.6 years) and had lower phosphate levels (1.2± 0.25 mmol versus 1.54±0.55 mmol/L, P= 0.05) (195) The high standard deviations in the FGF-23 levels and small patient numbers may have had an effect on the unexpected results.

Yamamoto *et al.* studied acute changes in circulating FGF-23 levels in 45 clinically stable patients from Brazil. Using, reused cellulose acetate or derivatized cellulose membrane they recorded a -19% reduction of FGF-23 levels during a single session of HD. They speculated the close association of phosphate

to FGF-23 explained the drop. Whether the reuse of dialysers or germicides to contributed to these results are unknown. (196)

Mass FGF-23 removal, a better assessment of the total FGF-23 reduction during dialysis was greater in the HDF group in comparison to HD arm in our study population. The HDF group was younger and had a lower RRT vintage. This may have contributed to the higher median FGF-23 levels in the HD group.

What is not clear is if FGF-23 removal has any positive effect on the overall outcome of patients. It may be, like phosphate and PTH, therapeutic measures to reduce FGF-23 in the future will only advisable in extreme cases. This question still remains unanswered for now.

Our attempts to create a kinetic model for FGF 23 was met with various inherit problems. FGF 23 is a large unstable molecule and concentrations in dialysate fluid were low, with some samples needing concentrated by factors of 10, 100 and 1000. Its secretion primarily from the bone and distribution in the multiple pools of the body is unknown and unlikely to be constant. Levels are affected by phosphate removal. The effect of haematocrit, dialyser membranes, ultrafiltration and duration of dialysis on FGF-23 is unclear.

5.17 Limitations

Our study was limited by small patient number and being observational in nature. Phosphate balance from diet, medications and hormonal influences were not taken into consideration.

5.18 Conclusion

The strengths of our study include robust data collection that was validated by the urea kinetic model. Subjects were from two different centres and used different models of dialysis.

Plasma FGF-23 levels strongly correlate to phosphate before and during a session of dialysis. Haemodialysis (HD or HDF) is an effective treatment in reducing FGF-

23 levels. Further kinetic studies should be undertaken to examine the elimination of FGF-23 during dialysis.

Chapter 6

Conclusion & Discussions

6.1 Introduction

The work in this thesis was designed to narrow the gap and improve our understanding of the management of CKD- MBD. The initial chapter identifies the problem and the next chapter explores possible solutions to improve care in the present health care setting. The use of IT to develop new technology and it's effect on quality of life has been explored and the study of novel biomarkers in relation to PD and HD is covered in the subsequent chapters.

6.2 Symptomatic fractures in RRT population

Fractures are common in the CKD population and remain a significant cause of mortality and morbidity. The incidence of fractures and their association with surrogate bone markers in the RRT population is poorly defined. The principal aim of this chapter was to identify radiological proven bone fractures and study the relationship to risk factors.

Retrospective data collection was carried out over a 3-year period, across 4 different health boards and included all radiology reports from 20 different hospitals. Fractures were classified using the ICD 10 nomenclature defined by the WHO and Primary renal disease using the ERA-EDTA Coding system. Covariates known to affect bone parameters (demographic, biochemical and medications) were identified.

The data was analysed using Cox-proportional hazard models and time to first fracture calculated by Kaplan Meier survival curves.

A total of 340 fractures were identified with an overall incidence of 62.8 per 1000 patient years. This was almost double that of a self-reported health survey from 2002-2004, in England (104)and within the wide range of 3.3 to 99.6 per 1000 patient years of previous reports. (101)

The highest incidence of fractures was recorded in the HD population and the lowest in the RT group. Risk ratio for HD and PD were 2.6 and 1.7 respectively for all fracture types. Further analysis was carried out for major fractures i.e. hip, pelvis and femoral. In the Univariable analysis age, female gender, HD,

diabetes, serum phosphate and exposure to sevelamer were associated with reduced time to first fracture. Age and HD were independent risk factors for fractures in multivariable analysis. These results are perhaps unsurprising, given that the RT patients are a self-selected healthier population compared to the HD group.

Exposure to alfacalcidol and lanthanum and increased serum albumin were associated with decreased risk. Lanthanum has been reported to improve renal osteodystrophy (109) and increase mineralisation at the periosteal surface in HD patients with ABD, (110) while Vitamin D has shown improvement in DEXA BMD in CKD. (113) Our data supports the role of Lanthanum and Vitamin D at reducing fractures. Low albumin is a surrogate marker of poor nutrition and its association with fracture risk (115) is consistent with previous reports.

There was no site effect for each of the RRT modalities, which meant the findings were across the board.

Despite being retrospective in nature our study was robust and the first reported study that looked into radiological proven bone fractures in a large CKD population. Data was collected over 3 years and included average reading of 3 consecutive values. It included relevant parameters that are used in daily clinical practice.

The model of this study could be used to design future prospective trials with inclusion of associated risk factors such as FGF-23. The role of DEXA as recently defined in the KDIGO guidelines and impact of Cinacalcet could be explored further.

6.3 Thrice weekly nocturnal in-centre HDF

This was a prospective study over a 2-year period from electronic records on a self-selected group of 14 patients. They were converted from conventional 4-5 HDF to nocturnal 8 hour HDF in an in-centre setting. Changes in clinical and biochemical parameters were recorded at 3,6, 9 and 24 months. Analysis was carried out only for data that was available for more than 12 months. Each patient served as his or her own control.

The use of phosphate binders was altogether eliminated and small solute clearance improved significantly. Phosphate (128,132) and better small solute control (132,135) with increased dialysis has been reported since 1998. The unique finding in our study was normalisation of phosphate without need for supplementation and complete elimination of all binders. This could perhaps be explained with the lower dialysate and blood flows and the reduced frequency of dialysis.

There was a reduction in blood pressure with lesser use of antihypertensive medications that would suggest better blood pressure. Charra *et al.* have previously reported one of the lowest mortality with excellent blood pressure with extended hours of dialysis. (130)

A trend towards the lesser use of intravenous iron and erythropoietin was recorded though no changes in beta-2 microglobulin was noted.

A major limitation of this study was small patient numbers in a self-selected group. There were no recordings of food charts to assess phosphate balance and we did not have a comparator group.

This study could be the basis for conducting large multicentre trials especially in patients with difficult phosphate and fluid management. It also forms an opportunity to explore the effects of long hours of dialysis on novel markers such as FGF-23.

6.4 Phosphate and FGF-23 in PD

The chapter focussed on the study of novel biomarkers and their correlation to phosphate in a group of PD patients. PD was chosen as the RRT modality of choice due to it being a continuous form of RRT. The formal assessment of residual renal function is a part of routine clinical care for all patients on PD which was an additional advantage.

In this prospective study over 9 months data was collected on phosphate and FGF-23 from plasma and dialysate. Clearances were calculated and scatter plots created using linear regression and Pearson correlation coefficients. Changes in

levels of phosphate and FGF-23 were analysed using ANOVA. Intraclass correlation coefficients were measured to compare the within to between subject variations.

The study confirms FGF-23 is elevated in a group of PD patients and that levels are strongly correlated to phosphate. There was a negative correlation between FGF-23 and urinary clearance suggesting residual renal function had an important role in FGF-23 clearance. FGF-23 measurements are likely to be less variable over a longer period compared to phosphate. These findings are consistent with previous reports (91,155) though the negative relationship between plasma FGF-23 and phosphate clearance needs further exploration.

The strength of the study was the data collection over a 9-month period, maintenance of food dairies and quantification of clearances. This chapter will hopefully help future researchers to undertake studies on FGF-23 and phosphate clearances in PD patients.

6.5 Effect of single session of dialysis on FGF-23

FGF-23 is a novel biomarker that is believed to play a critical role in CKD- MBD. Despite extensive research it remains an elusive molecule that is yet to make its way into routine clinical practice. This chapter aims to demystify FGF-23 and study its function, the role it plays in phosphate, Vitamin D and PTH regulation. The commercially available assays are reviewed and the t ½ examined.

I studied the association of phosphate to FGF-23 during two different modalities of dialysis, HD and HDF. A basic understanding of Kinetic modelling, URR and its association to Kt/V is examined in relation to urea and phosphate clearance. Finally, we explored the possibility of creating a model to study the kinetics of FGF-23.

This prospective study consists of 25 patients from 2 different centres on HD and HDF. Blood and dialysate samples are collected during a session of dialysis at equally timed intervals. In a subgroup, sample collection was extended to 1-hour post dialysis.

The results confirmed a 1000- fold increase in FGF-23 levels in dialysis patients with ESRD. Phosphate had a strongly positive correlation to plasma FGF-23 during dialysis and total FGF-23 (mass removal) by HDF was greater compared to HD.

Though I was able to replicate previous urea and phosphate kinetic models, (184) a tight fit for a FGF-23 model could not be established. While it establishes the strong association of phosphate to FGF-23 (183) in a HD cohort it raises more questions about its clearances. Some authors have reported a reduction of FGF-23 during a session of HD (196) while others have had no changes between standard dialysis and long nocturnal HD. (195)

It is clear that the rate of removal of FGF-23 by the dialysis process is being matched by FGF-23 generation as dialysis continues. The stimulus for this could be related to the demonstrated changes in phosphate concentration or could be due to alterations in PTH in response to changes in ionised calcium. Further studies may be able to elucidate this more clearly particularly if the analysis of FGF-23 concentrations becomes easier through the development of commercially viable FGF-23 assay.

In conclusion, it is clear that while some answers have been provided by this research, there are many unanswered questions and controversies to be resolved in the field of CKD-MBD.

The studies included in this thesis were designed to further define some of the morbidities associated with CKD-MBD. I have sought to understand some potential ways of improving metabolic control and have investigated the potential role and associations of novel biomarkers in relation to conventional markers of CKD-MBD. The results have added to the pool of knowledge in this field but have also opened up a range of new challenges and questions that need to be addressed. Undertaking this research has enabled me to expand my own knowledge, gain experience in clinical research and has given me a secure foundation to further develop my interest in the field of CKD-MBD in my clinical career.

Appendices

Chapter 1

1.1 Summary of 2019 & 2009 KDIGO CKD- MBD recommendations

Summary and comparison of 2017 updated and 2009 KDIGO CKD-MBD recommendations

2017 revised KDIGO CKD-MBD recommendations	2009 KDIGO CKD-MBD recommendations	Brief rationale for updating
3.2.1. In patients with CKD G3a–G5D with evidence of CKD-MBD and/or risk factors for osteoporosis, we suggest BMD testing to assess fracture risk if results will impact treatment decisions (*2B*).	3.2.2. In patients with CKD G3a–G5D with evidence of CKD-MBD, we suggest that BMD testing not be performed routinely, because BMD does not predict fracture risk as it does in the general population, and BMD does not predict the type of renal osteodystrophy (*2B*).	Multiple new prospective studies have documented that lower DXA BMD predicts incident fractures in patients with CKD G3a–G5D. The order of these first 2 recommendations was changed because a DXA BMD result might impact the decision to perform a bone biopsy.
3.2.2. In patients with CKD G3a–G5D, it is reasonable to perform a bone biopsy if knowledge of the type of renal osteodystrophy will impact treatment decisions (*Not Graded*).	3.2.1. In patients with CKD G3a–G5D, it is reasonable to perform a bone biopsy in various settings including, but not limited to: unexplained fractures, persistent bone pain, unexplained hypercalcemia, unexplained hypophosphatemia, possible aluminum toxicity, and prior to therapy with bisphosphonates in patients with CKD-MBD *(Not Graded)*.	The primary motivation for this revision was the growing experience with osteoporosis medications in patients with CKD, low BMD, and a high risk of fracture. The inability to perform a bone biopsy may not justify withholding antiresorptive therapy from patients at high risk of fracture.
4.1.1. In patients with CKD G3a–G5D, treatments of CKD-MBD should be based on serial assessments of phosphate, calcium, and PTH levels, considered together (*Not Graded*).		This new recommendation was provided in order to emphasize the complexity and interaction of CKD-MBD laboratory parameters.
4.1.2. In patients with CKD G3a–G5D, we suggest lowering elevated phosphate levels toward the normal range (*2C*).	4.1.1. In patients with CKD G3a–G5, we suggest maintaining serum phosphate in the normal range (*2C*). In patients with CKD G5D, we suggest lowering elevated phosphate levels toward the normal range (*2C*).	There is an absence of data supporting that efforts to maintain phosphate in the normal range are of benefit to CKD G3a–G4 patients, including some safety concerns. Treatment should aim at overt hyperphosphatemia.
4.1.3. In adult patients with CKD G3a–G5D, we suggest avoiding hypercalcemia (*2C*). In children with CKD G3a–G5D, we suggest maintaining serum calcium in the age-appropriate normal range (*2C*).	4.1.2. In patients with CKD G3a–G5D, we suggest maintaining serum calcium in the normal range (*2D*).	Mild and asymptomatic hypocalcemia (e.g., in the context of calcimimetic treatment) can be tolerated in order to avoid inappropriate calcium loading in adults.
4.1.4. In patients with CKD G5D, we suggest using a dialysate calcium concentration between 1.25 and 1.50 mmol/l (2.5 and 3.0 mEq/l) (*2C*).	4.1.3. In patients with CKD G5D, we suggest using a dialysate calcium concentration between 1.25 and 1.50 mmol/l (2.5 and 3.0 mEq/l) (*2D*).	Additional studies of better quality are available; however, these do not allow for discrimination of benefits and harms between calcium dialysate concentrations of 1.25 and 1.50 mmol/l (2.5 and 3.0 mEq/l). Hence, the wording is unchanged, but the evidence grade is upgraded from 2D to 2C.
4.1.5. In patients with CKD G3a–G5D, decisions about phosphate-lowering treatment should be based on progressively or persistently elevated serum phosphate (*Not Graded*).	4.1.4. In patients with CKD G3a–G5 (*2D*) and G5D (*2B*), we suggest using phosphate-binding agents in the treatment of hyperphosphatemia. It is reasonable that the choice of phosphate binder takes into account CKD stage, presence of other components of CKD-MBD, concomitant therapies, and side effect profile (*Not Graded*).	Emphasizes the perception that early "preventive" phosphate-lowering treatment is currently not supported by data (see Recommendation 4.1.2). The broader term "phosphate-lowering" treatment is used instead of phosphate binding agents since all possible approaches (i.e., binders, diet, dialysis) can be effective.

(*Continued on next page*)

2017 revised KDIGO CKD-MBD recommendations	2009 KDIGO CKD-MBD recommendations	Brief rationale for updating
4.1.6. In adult patients with CKD G3a–G5D receiving phosphate-lowering treatment, we suggest restricting the dose of calcium-based phosphate binder (*2B*). In children with CKD G3a–G5D, it is reasonable to base the choice of phosphate-lowering treatment on serum calcium levels (*Not Graded*).	4.1.5. In patients with CKD G3a–G5D and hyperphosphatemia, we recommend restricting the dose of calcium-based phosphate binders and/or the dose of calcitriol or vitamin D analog in the presence of persistent or recurrent hypercalcemia (*1B*). In patients with CKD G3a–G5D and hyperphosphatemia, we suggest restricting the dose of calcium-based phosphate binders in the presence of arterial calcification (*2C*) and/or adynamic bone disease (*2C*) and/or if serum PTH levels are persistently low (*2C*).	New evidence from 3 RCTs supports a more general recommendation to restrict calcium-based phosphate binders in hyperphosphatemic patients across all severities of CKD.
4.1.8. In patients with CKD G3a–G5D, we suggest limiting dietary phosphate intake in the treatment of hyperphosphatemia alone or in combination with other treatments (*2D*). It is reasonable to consider phosphate source (e.g., animal, vegetable, additives) in making dietary recommendations (*Not Graded*).	4.1.7. In patients with CKD G3a–G5D, we suggest limiting dietary phosphate intake in the treatment of hyperphosphatemia alone or in combination with other treatments (*2D*).	New data on phosphate sources were deemed to be included as an additional qualifier to the previous recommendation.
4.2.1. In patients with CKD G3a–G5 not on dialysis, the optimal PTH level is not known. However, we suggest that patients with levels of intact PTH progressively rising or persistently above the upper normal limit for the assay be evaluated for modifiable factors, including hyperphosphatemia, hypocalcemia, high phosphate intake, and vitamin D deficiency (*2C*).	4.2.1. In patients with CKD G3a–G5 not on dialysis, the optimal PTH level is not known. However, we suggest that patients with levels of intact PTH above the upper normal limit of the assay are first evaluated for hyperphosphatemia, hypocalcemia, and vitamin D deficiency (*2C*). It is reasonable to correct these abnormalities with any or all of the following: reducing dietary phosphate intake and administering phosphate binders, calcium supplements, and/or native vitamin D (*Not Graded*).	The Work Group felt that modest increases in PTH may represent an appropriate adaptive response to declining kidney function and has revised this statement to include "persistently" above the upper normal PTH level as well as "progressively rising" PTH levels, rather than "above the upper normal limit." That is, treatment should not be based on a single elevated value.
4.2.2. In adult patients with CKD G3a–G5 not on dialysis, we suggest that calcitriol and vitamin D analogs not be routinely used. (*2C*) It is reasonable to reserve the use of calcitriol and vitamin D analogs for patients with CKD G4–G5 with severe and progressive hyperparathyroidism (*Not Graded*). In children, calcitriol and vitamin D analogs may be considered to maintain serum calcium levels in the age-appropriate normal range (*Not Graded*).	4.2.2. In patients with CKD G3a–G5 not on dialysis, in whom serum PTH is progressively rising and remains persistently above the upper limit of normal for the assay despite correction of modifiable factors, we suggest treatment with calcitriol or vitamin D analogs (*2C*).	Recent RCTs of vitamin D analogs failed to demonstrate improvements in clinically relevant outcomes but demonstrated increased risk of hypercalcemia.
4.2.4. In patients with CKD G5D requiring PTH-lowering therapy, we suggest calcimimetics, calcitriol, or vitamin D analogs, or a combination of calcimimetics with calcitriol or vitamin D analogs (*2B*).	4.2.4. In patients with CKD G5D and elevated or rising PTH, we suggest calcitriol, or vitamin D analogs, or calcimimetics, or a combination of calcimimetics and calcitriol or vitamin D analogs be used to lower PTH (*2B*). • It is reasonable that the initial drug selection for the treatment of elevated PTH be based on serum calcium and phosphate levels and other aspects of CKD-MBD (*Not Graded*). • It is reasonable that calcium or non-calcium-based phosphate binder dosage be adjusted so that treatments to control PTH do not compromise levels of phosphate and calcium (*Not Graded*). • We recommend that, in patients with hyper-calcemia, calcitriol or another vitamin D sterol be reduced or stopped (*1B*).	This recommendation originally had not been suggested for updating by the KDIGO Controversies Conference in 2013. However, due to a subsequent series of secondary and *post hoc* publications of the EVOLVE trial, the Work Group decided to reevaluate Recommendation 4.2.4 as well. Although EVOLVE did not meet its primary endpoint, the majority of the Work Group members were reluctant to exclude potential benefits of calcimimetics for G5D patients based on subsequent prespecified analyses. The Work Group, however, decided not to prioritize any PTH-lowering treatment at this time because calcimimetics, calcitriol, or vitamin D analogs are all acceptable first-line options in G5D patients.

2017 revised KDIGO CKD-MBD recommendations	2009 KDIGO CKD-MBD recommendations	Brief rationale for updating
	• We suggest that, in patients with hyperphosphatemia, calcitriol or another vitamin D sterol be reduced or stopped (*2D*). • We suggest that, in patients with hypocalcemia, calcimimetics be reduced or stopped depending on severity, concomitant medications, and clinical signs and symptoms (*2D*). • We suggest that, if the intact PTH levels fall below 2 times the upper limit of normal for the assay, calcitriol, vitamin D analogs, and/or calcimimetics be reduced or stopped (*2C*).	
4.3.3. In patients with CKD G3a–G5D with biochemical abnormalities of CKD-MBD and low BMD and/or fragility fractures, we suggest that treatment choices take into account the magnitude and reversibility of the biochemical abnormalities and the progression of CKD, with consideration of a bone biopsy (*2D*).	4.3.3. In patients with CKD G3a–G3b with biochemical abnormalities of CKD-MBD and low BMD and/or fragility fractures, we suggest that treatment choices take into account the magnitude and reversibility of the biochemical abnormalities and the progression of CKD, with consideration of a bone biopsy (*2D*).	Recommendation 3.2.2 now addresses the indications for a bone biopsy prior to antiresorptive and other osteoporosis therapies. Therefore, 2009 Recommendation 4.3.4 has been removed and 2017 Recommendation 4.3.3 is broadened from CKD G3a–G3b to CKD G3a–G5D.
	4.3.4. In patients with CKD G4–G5D having biochemical abnormalities of CKD-MBD, and low BMD and/or fragility fractures, we suggest additional investigation with bone biopsy prior to therapy with antiresorptive agents (*2C*).	
5.5. In patients with G1T–G5T with risk factors for osteoporosis, we suggest that BMD testing be used to assess fracture risk if results will alter therapy (*2C*).	5.5. In patients with an estimated glomerular filtration rate greater than approximately 30 ml/min/1.73 m^2, we suggest measuring BMD in the first 3 months after kidney transplant if they receive corticosteroids, or have risk factors for osteoporosis as in the general population (*2D*).	2009 Recommendations 5.5 and 5.7 were combined to yield 2017 Recommendation 5.5.
	5.7. In patients with CKD G4T–G5T, we suggest that BMD testing not be performed routinely, because BMD does not predict fracture risk as it does in the general population and BMD does not predict the type of kidney transplant bone disease (*2B*).	
5.6. In patients in the first 12 months after kidney transplant with an estimated glomerular filtration rate greater than approximately 30 ml/min/1.73 m^2 and low BMD, we suggest that treatment with vitamin D, calcitriol/alfacalcidol, and/or antiresorptive agents be considered (*2D*). • We suggest that treatment choices be influenced by the presence of CKD-MBD, as indicated by abnormal levels of calcium, phosphate, PTH, alkaline phosphatases, and 25(OH)D (*2C*). • It is reasonable to consider a bone biopsy to guide treatment (*Not Graded*). There are insufficient data to guide treatment after the first 12 months.	5.6. In patients in the first 12 months after kidney transplant with an estimated glomerular filtration rate greater than approximately 30 ml/min/1.73 m^2 and low BMD, we suggest that treatment with vitamin D, calcitriol/alfacalcidol, or bisphosphonates be considered (*2D*). • We suggest that treatment choices be influenced by the presence of CKD-MBD, as indicated by abnormal levels of calcium, phosphate, PTH, alkaline phosphatases, and 25(OH)D (*2C*). • It is reasonable to consider a bone biopsy to guide treatment, specifically before the use of bisphosphonates due to the high incidence of adynamic bone disease (*Not Graded*). There are insufficient data to guide treatment after the first 12 months.	The second bullet is revised, consistent with the new bone biopsy recommendation (i.e., 2017 Recommendation 3.2.2).

25(OH)D, 25-hydroxyvitamin D; BMD, bone mineral density; CKD, chronic kidney disease; CKD-MBD, chronic kidney disease–mineral bone disorder; DXA, dual-energy x-ray absorptiometry; PTH, parathyroid hormone; RCT, randomized controlled trial.
Changes to above summarized recommendations resulted in renumbering of several adjacent guideline statements. Specifically, 2009 Recommendation 4.1.6 now becomes 2017 Recommendation 4.1.7; 2009 Recommendation 4.1.8 now becomes 2017 Recommendation 4.1.9; 2009 Recommendation 4.3.5 now becomes 2017 Recommendation 4.3.4; and 2009 Recommendation 5.8 now becomes 2017 Recommendation 5.7.

Chapter 2

Chapter 3

Chapter 4

4.1 Consent form

Centre Number:

Study Number:

Patient Identification Number for this trial:

CONSENT FORM

Title of Project: To study the effect of a phosphate reduction program on novel biomarkers - (Fibroblast Growth factor - 23, Klotho) and quality of life in a group of peritoneal dialysis patients

Name of Researcher: Dr Vishal Dey

Please initial all boxes

1. I confirm that I have read and understand the information sheet dated …… (Version ….) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from NHS Ayrshire & Arran, from regulatory authorities or from the NHS Board, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I agree to my GP being informed of my participation in the study. ☐
5. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
Name of Person taking consent.	Date	Signature

1 for participant; 1 for researcher; 1 (original) to be kept in medical notes

Chapter 5

5.1 Patient Information sheet

Contact Vishal Dey
Renal Office, Level 2 East
Crosshouse Hospital
Kilmarnock KA2 OBE

Telephone 01563 825177
Email vishal.dey@nhs.net

Participant Information Sheet

Kinetics of FGF-23 during dialysis
(To study the removal of a protein called FGF-23 during dialysis)

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. This should take about 20 minutes. Talk to others about the study if you wish.

Part 1 tells you the purpose of this study and what will happen to you if you take part.

Part 2 gives you more detailed information about the conduct of the study.
Ask us if there is anything that is not clear

What is the purpose of the study?

To study the removal of a protein called FGF-23 (Fibroblast Growth factor -23) during a session of dialysis.

Phosphate is a mineral normally found in your bones. This with calcium and Vitamin D helps you remain healthy and build strong bones. In kidney failure the levels of phosphate tend to go up and can cause damage to your bones and blood vessels. New tests as FGF-23 are believed to play an important role in phosphate regulation.

The kidney teams, at hospitals, across West of Scotland want to study the mechanism of FGF-23 clearance during dialysis to have a better understanding of phosphate regulation

Why have I been invited?

You have been invited to take part because you have kidney disease and are currently on haemodialysis.

Do I have to take part?

No. If you decide to join the study we will describe the study and go through this information sheet. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care you receive.

If you have given informed consent and lose capacity to consent during the study you will be withdrawn from the study. Identifiable data or tissue already collected with consent would be retained and used in the study. No further data or tissue would be collected or any other research procedures carried out on or in relation to you

What will happen to me if I take part?

During the study we will collect 13 extra samples of blood on dialysis. 10 of these samples will be done during dialysis and 3 an hour after completion of dialysis. In case you do not wish to have the 3 tests after completion of dialysis you will have only 10 tests done. The tests will be done at the same time as routine dialysis and will not involve extra hospital visits. We will also collect samples from the fluid that is discarded after dialysis.

Prior to your tests, we will ensure there is no recirculation in your fistulae and measure your total body water (TBW). You may already be having these tests routinely, as part of your treatment.

Measurement of TBW is like having an electrocardiogram (ECG) and takes 2 minutes.

The doctors who are running the project will access your hospital record to review your results, hospital admissions and diagnosis. This will allow us to see what happens to your health, without having to contact you regularly.

You will not receive any specific additional treatment as a part of this study. You will continue to receive your usual treatment as agreed with the dialysis team, and we will keep a record of this.

What will I have to do?

Consent to have your blood and dialysis fluid being tested along with your routine tests

What are the possible benefits of taking part?

The information collected from this study will give us a better understanding of newly developing blood tests and its role in the future in detecting/ treating patients with kidney disease in the future. Otherwise you will receive no direct benefit from taking part in the study.

What happens when the research study stops?

You will continue to receive your regular care.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. The details are included in Part 2.

This completes part 1.

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision

Part 2 of the information sheet

What if relevant new information becomes available?

Sometimes we get new information about the treatment being studied. If this happens, your research doctor will tell you and discuss whether you should continue in the study. If you decide not to carry on, your research doctor will make arrangements for your care to continue. If you decide to continue in the study he may ask you to sign an agreement outlining the discussion.

What will happen if I don't want to carry on with the study?

You can withdraw from having further tests. Information collected may still be used. Any stored blood or tissue samples that can still be identified, as yours will be destroyed if you wish.

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions [Vishal Dey, Phone 01563 825177]. If you remain unhappy and wish to complain formally, you can do this with Patient Relations and Complaints Department, NHS Ayrshire & Arran, Eglinton House, Ailsa Hospital, Dalmellington Road, Ayr KA6 6AB, Phone 01292 513 620

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against NHS Ayrshire & Arran but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Will my taking part in this study be kept confidential?

All information, which is collected, about you during the course of the research will be kept strictly confidential, and any information about you which leaves the hospital/surgery will have your name and address removed so that you cannot be recognised.

Involvement of the General Practitioner/Family doctor (GP)

We will inform your GP about participation in the study

What will happen to any samples I give?

Your blood samples will be taken and stored in a freezer. Your details will be removed from the samples. Some of the tests will be done at Crosshouse hospital, NHS Ayrshire & Arran and others will be performed at Glasgow University. Any extra samples after the necessary tests will be disposed in accordance with the current legislation (Human Tissue Authority Code of Practice).

Will any genetic tests be done?

No

What will happen to the results of the research study?

The results will be presented at conferences and published in medical journals. The first set of results will be published around 12-18 months after your study. You can contact us, if you would like a copy of the published results. The results will be anonymous and no individual person will be identified in the publications.

Who is organising and funding the research?

The research is supported NHS Ayrshire and Arran along with University of Glasgow

Who has reviewed the study?

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by West of Scotland Research Ethics Service (WoSRES).

5.2 Consent Form

Centre Number:

Study Number:

Patient Identification Number for this trial:

CONSENT FORM

Title of Project: **Kinetics of FGF-23 during haemodialysis**
(To study the removal of a protein, FGF-23 during dialysis)

Name of Researcher: Dr Vishal Dey

Please **initial** all boxes

1. I confirm that I have read and understand the information sheet dated …… (Version.....) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that relevant sections of my medical notes, electronic records and data collected during the study may be looked at by individuals from NHS Ayrshire & Arran, from regulatory authorities or from the NHS Board, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I agree to have my blood and dialysis fluid tested along with my routine tests at Crosshouse Hospital, NHS Ayrshire & Arran and Glasgow University. ☐
5. I agree to my GP being informed of my participation in the study. ☐
6. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
Name of Person taking consent.	Date	Signature

1 for participant; 1 for researcher; 1 (original) to be kept in medical notes

Consent form date of issue: **02/03/2015**
Consent form version number: **2.1**

5.3 Dialysis visit record

NHS Ayrshire & Arran

Participant Dialysis Visit Record

Study ID …… Date of Visit ……..

Age ☐

Consent form signed Y/N

No evidence of recirculation Y/N

BCM performed Y/N

Blood collection date: ……

5 ml blood samples at each of the time intervals (Tick box)

5 mls of dialysate fluid in plain sterile vial at each of the time intervals

Stage 1

Time ti2 - ti6 are 5 equally spaced intervals during dialysis	**Time from start of dialysis Record time below**	**Blood Sample √ Box**	**Dialysate effluent Sample √ Box**
tia Before dialysis	e.g. 0 minutes		
ti2			
ti3			
ti4			
ti5			
ti6			
td (End of dialysis)			
After Dialysis	2 minutes **after** dialysis		

Divide blood samples into 2 vials EDTA / Li-Heparin

Stage 2

Patient agreeable to have further samples after completion of dialysis Y/N

Perform wash back after taking 2 minute sample (routine practice)

If **YES leave A needle** in AV fistulae

(Only proceed if patient agrees to wait an hour post dialysis)

5 ml samples at each of the following time intervals (Tick box Yes/No)

Time after stopping dialysis	**Sample Blood √Box**
15 minute	
30 minute	
60 minute	

Completed by (PRINT NAME) …… Date of completion……..

Signature of Researcher

5.4 Letter to GP

RENAL DEPARTMENT ROOM 244 EAST
CROSSHOUSE HOSPITAL CROSSHOUSE KA2 OBE
TELEPHONE 01563 521133

Enquiries to: **Vishal Dey**
☎ *Direct Line: 01563 825177 or Ext 25177*
Fax Number *01563 577987*

Email: vishal.dey@nhs.net

Date……..

Dear Dr

Kinetics of Fibroblast growth factor (FGF-23) during dialysis

Re: Name
Date of Birth
CHI

This patient is participating in the above study. This will involve extra investigations in the form of blood and dialysate samples, but is an observational study with NO specific therapeutic intervention.

A copy of the patient information sheet is enclosed for your information.

Yours sincerely

Dr Vishal Dey
On behalf of the study investigators

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