Glycaemic variation in Insulin treated Diabetic patients with End stage renal disease on maintenance Haemodialysis and its effect on cardiac electrical activity.

A thesis submitted for the degree of

Doctor of Medicine

By

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Abstract

Diabetic kidney disease remains the single most common cause of renal failure in UK, accounting for 26.9% of patients needing renal replacement therapy (UK renal registry, 2016). Mortality rates on RRT are worse for the diabetes population compared to the non-diabetic population. Diabetic patients on maintenance haemodialysis experience huge variation in their glycaemia, which is not well understood to guide appropriate therapy. ESRD patients are at higher risk of sudden cardiac death and arrhythmia is suspected to be a major cause. However there is no established guideline in detecting at risk patients for preventative therapy.

We aimed to study the glycaemic variation in patients with ESRD on maintenance HD using continuous glucose monitoring for longer periods in order to help understand the variation in relation to dialysis and associated change in cardiac electrical conductivity simultaneously to explore any relation with glycaemia.

In a pilot study we studied glucose variation and cardiac electrical activity using CGM and Holter monitor respectively during 37 weeks in 15 diabetic patients and 5 weeks in 5 non-diabetic subjects.

Diabetic subjects had a significant variation in their glycaemia through the week. There was a significant drop in the interstitial glucose level during HD, followed by a rise in the post-HD period (preHD vs HD vs postHD: 11.4±5.1 vs 8.4±3.6 vs 11.5 ± 4.6mmol/l). There was a significant change in QTc interval from start to end of HD in this population (468 ± 42 vs 481 ± 36 vs 495 ± 49). Short but frequent episodes of arrhythmia were noted throughout the week. All diabetic patients who were prone for arrhythmias had abnormal QTc. Non-diabetic patients also experienced significant variation in IG levels and were noted to have IG in both the hypo and hyperglycaemic range.

CGM helps in understanding the glycaemic variation in this population and real time recording would help in reducing the episodes of hypoglycaemia and hyperglycaemia. There is no relation between glycaemic variation or hypoglycaemia and change in QTc interval or cardiac dysrhythmias, which remain common in this population. Asymptomatic dysrhythmic episodes put these patients at risk of sudden cardiac death. The data suggest that baseline ECG and/or periodic Holter monitoring should be used in clinical care.

Dedicated to my

Family

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Abbreviations

ACR	Albumin Creatinine Ratio
ACEi	Angiotensinogen Converting Enzyme inhibitors
AER	Albumin Excretion Rate
AlbF	Albumin corrected Fructosamine
1,5-AG	1,5-Alpha Glucitol
AGEs	Advanced Glycosylation End products
ARB	Angiotensin Receptor Blockers
AS	Aortic Stenosis
BCT	Broad Complex Tachycardia
Bg	Bigeminy
CABG	Coronary Artery Bypass Graft
CAD	Coronary Artery Disease
CGM	Continuous Glucose Monitoring
CGMS	Continuous Glucose Monitoring System
CKD	Chronic Kidney Disease
CNDP1	Carnosine Dipeptidase 1
CVD	Cardio Vascular Disease
DCCT	The Diabetes Control and Complications Trial
DKD	Diabetic Kidney Disease
DN	Diabetic Nephropathy
ECG	Electrocardiogram
ECM	Extra Cellular Matrix
eGFR	estimated Glomerular Filtration Rate
eGFR-cys	estimated Glomerular Filtrate Rate - cystatin
ESRD	End Stage Renal Disease
FSL	Free Style Libre

FGM	Flash Glucose Monitoring
GA	Glycated Albumin
GDR	Glucose Disposal Rate
GENE1	Genomics England
GFR	Glomerular Filtration Rate
GV	Glycaemic Variability
Hb	Haemoglobin
HD	Haemodialysis
HPLC	High Performance Liquid Chromatography
HRV	Heart Rate Variability
HT	Hypertension
ICD	Implantable Cardiac Defibrillator
IG	Interstitial Glucose
IHD	Ischaemic Heart Disease
IL1	Interleukin 1
IR	Insulin Resistance
ISF	Interstitial Fluid
JR	Junctional Rhythm
KDIGO	Kidney Disease Improving Global Outcomes
LA	Left Atrium
LV	Left Ventricle
LVH	Left Ventricular Hypertrophy
LVSD	Left Ventricular Systolic Dysfunction
MAGE	Mean Amplitude Glycaemic Excursion
MR	Mitral Regurgitation
NCT	Narrow Complex Tachycardia
NF-kB	Nuclear Factor Kappa Beta

NSVT	Non-Sustained Ventricular Tachycardia
PAI-1	Plasminogen Activator Inhibitor-1
PKC	Protein Kinase C
PPM	Permanent Pacemaker
PVD	Peripheral Vascular Disease
QTc	Corrected QT interval
RA	Right Atrium
RRT	Renal Replacement Therapy
RVH	Right Ventricular Hypertrophy
ROS	Reactive Oxidative Species
rt-CGM	real time Continuous Glucose Monitoring
RWMA	Regional Wall Motion Abnormality
SCD	Sudden Cardiac Death
SD	Standard Deviation
Tg	Trigeminy
TGF-β	Transforming Growth Factor- Beta
TNFα	Tumour Necrosis Factor-Alpha
TR	Tricuspid Regurgitation
VPB	Ventricular Premature Beat
UKPDS	United Kingdom Prospective Diabetes Study

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Introduction

Chapter 1: Diabetes and Chronic Kidney Disease

Diabetes is the most common single cause of kidney disease worldwide. The risk of chronic kidney disease (CKD) is increased by about seven fold, in diabetic individuals. It accounts for more than 50% of prevalent cases of end stage kidney disease (ESRD) in the United States of America (USRDS 2015 Annual Data Report).

Diabetic kidney disease (DKD) remains the single most common cause of renal failure in UK, accounting for 26.9% of patients needing renal replacement therapy (RRT) (UK renal registry 18th annual report- Gilg, Caskey and Fogarty, 2016). Mortality rates in patients on RRT are higher for the diabetes population compared to the non-diabetic population. In the age group 18-44 years, 5-year survival was 71% for the diabetic population compared to 89% for the non-diabetic population. Similarly in the age group 45-64 years, 5-year survival was 51% against 68% for the non-diabetic population (UK renal registry 18th annual report- Steenkamp, Rao & Fraser, 2016).

The risk of cardiovascular mortality and morbidity is higher in patients with CKD, with presence of CKD altering the pathology and manifestation of cardiovascular disease and worsening outcomes (Herzog et al., 2011). Cardiovascular disease (CVD) represents the major cause of diabetes-related death. However the presence of CKD (estimated glomerular filtration rate eGFR <60/ml/min/1.73m^2) and albuminuria both independently predict mortality strongly in both type 1 and type 2 diabetes (Russell and Cooper, 2015).

1.1 Development of Diabetic Nephropathy (DN)

1.1.1 Pathophysiology

Nephropathy is a complication of diabetes mellitus. It is well known that long-term poor glycaemic control increases the risk of development of nephropathy leading to CKD and ESRD.

Development of nephropathy is characterised by proteinuria, decline in glomerular filtration and increase in systolic blood pressure (Mogensen, 1989). The structural changes correlate with the functional changes in kidneys in both type 1 and type 2 diabetes (White and Bilous, 2000). Increased glomerular basement membrane width and fractional volume of mesangium and mesangial matrix are the principle abnormalities seen on serial renal biopsies in type 1 diabetes subjects (Drummond & Mauer, 2002).

Multiple pathways are involved in the development of microvascular complications of diabetes including DN. These are shown in figure 1.1 below as reported by Russell and Cooper.

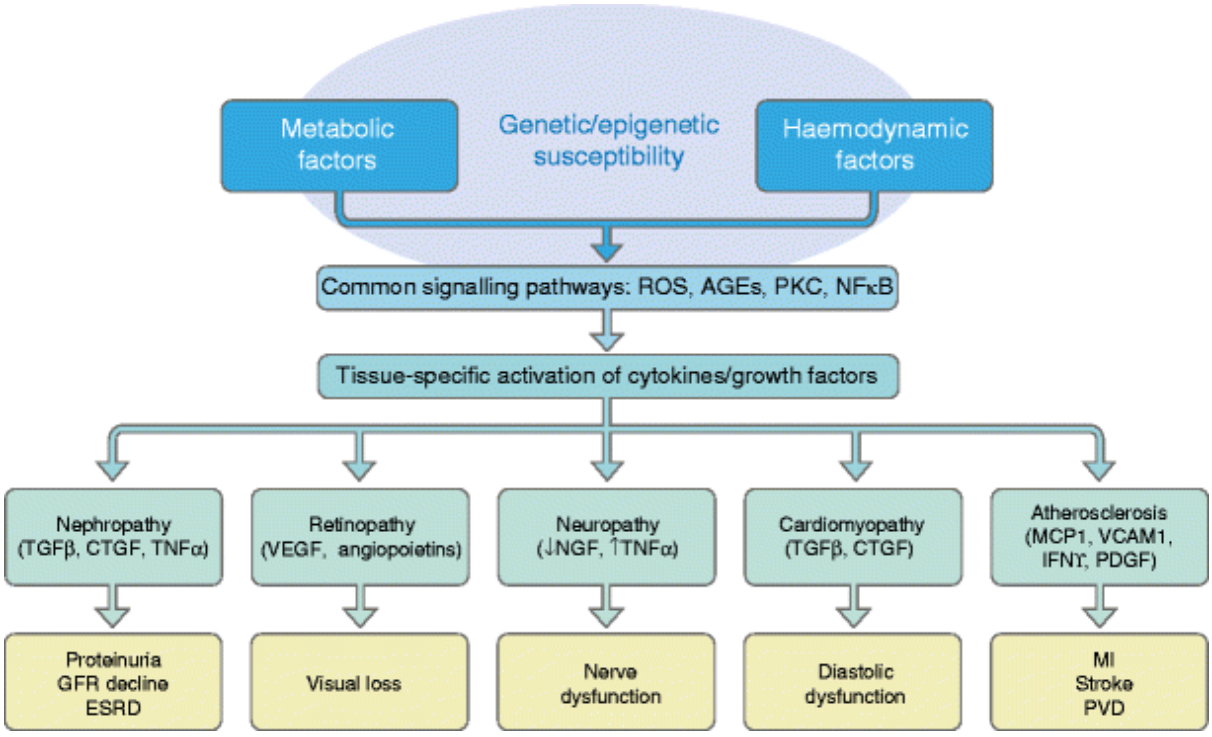


Figure 1.1*: Diagram depicting possible factors in development of diabetic nephropathy. Diagram taken from Russell and Cooper, Diabetologia (2015) 58:1708-1714*

Chronic kidney disease as measured by a fall in glomerular filtration rate (GFR) without proteinuria is not uncommonly seen in the diabetes population (Robles, Villa & Gallego, 2015). The reported prevalence of normoalbuminuric CKD is 36 to 39% in the diabetes population (MacIsaac et al. 2004; Garg et al, 2002). More advanced glomerular lesions are seen in type 1 diabetes patients with normoalbuminuric CKD than in their GFR matched counterparts with albuminuria (Caramori, Fioretto & Mauer, 2003). The Diabetic normoalbuminuric CKD is not closely associated with the presence of retinopathy or hypertension, however it is associated with a significant increase in major CVD events (Bash et al, 2008; Garg et al, 2002).

A progressive decline in renal function is seen in diabetes patients even in the absence of albuminuria but at a slower rate. The decline in eGFR as seen on longitudinal measurement of serum creatinine and cystatin C (eGFR-cys) over 4 – 10 years follow up showed prevalence of decliners, defined as loss of eGFR $\geq$ 3.3%, at 10% in

normoalbuminuric type 1 diabetes patients compared to 32% in those with microalbuminuria (Krolewski, 2015). The observed increase in the prevalence of non-proteinuric diabetic nephropathy in type 2 diabetes could be due to an increase in macroangiopathic rather than microangiopathic lesions, which may reflect changes in treatment improving glycaemic control, reduced lipid levels and blood pressure (Robles, Villa & Gallego, 2015).

Glucose dependent processes are known to be the cause of diabetes complications including nephropathy. Hyperglycaemia leads to increased oxidative stress, which causes DNA damage and contributes to accelerated apoptosis (Giacco & Brownlee, 2010).

Accumulation of advanced glycosylation end-products (AGEs) occurs in the diabetic kidney. This is time dependent (Soulis et al., 1996). Subjects with ESRD secondary to DKD have twice the amount of AGEs in serum compared to diabetic subjects without kidney disease. This is age dependent and strongly correlates with HbA1c and serum triglyceride and cholesterol levels (Galler et al., 2003). AGEs contribute to progressive alteration of renal architecture and loss of renal function. AGE formation on matrix proteins impairs degradation by matrix metalloproteinases, contributing to basement membrane thickening and mesangial expansion (Mott et al., 1997).

Oxidative stress is linked to hyperglycaemia. High glucose induces intracellular reactive oxidative species (ROS) through glucose metabolism and auto-oxidation, and also indirectly through the formation of AGEs (Sano et al., 1998). ROS up regulates transforming growth factor-beta1 (TGF-β1), plasminogen activator inhibitor-1 (PAI-1) and extracellular matrix (ECM) proteins, which can lead to mesangial expansion (Ha and Lee, 2001).

Protein kinase C (PKC) has a central role in hyperglycaemia-induced vascular injury (Wolf, 2004). Several pathways activate PKC, leading to endothelial dysfunction with increased nitric oxide production, increased expression of endothelin-1 and vascular endothelial growth factor (Kanwar et al., 2008). Increased expression of nuclear factor kappa-beta (NF-kB) and PAI-1 induces local tissue inflammatory responses and thrombotic microangiopathy, causing vascular damage (Wolf, 2004). This is further augmented by ROS.

Expression of TGF-beta in the glomerular cells and mesangial matrix is increased by hyperglycaemia, which might contribute to cellular hypertrophy and enhanced collagen synthesis (Lee et al., 2007).

Chronic inflammation has a significant role in the development of diabetes and its complications. Increase in macrophage infiltration and overproduction of leukocyte adhesion molecules occurs in kidneys from diabetic subjects and also in experimental animal models (Galkina, 2006; Nguyen et al., 2006). Pro-inflammatory cytokines such as Interleukin-1 (IL-1) increase vascular permeability and proliferation of mesangial cells and matrix deposition (Rivero et al., 2009). Tumour necrosis factor-α (TNF-α) can impair the balance between vasodilator and vasoconstrictor mediators and up regulate production of ROS, contributing to altered glomerular capillary permeability (McCarthy et al., 1998).

Familial clustering of diabetic nephropathy suggests a genetic influence in the development of nephropathy (Seaquist et al., 1989). This was suspected with the finding of increased risk in developing DN in diabetic siblings of subjects with DN compared to diabetic siblings of diabetic subjects without proteinuria. Varying prevalence of DN in ethnic groups also suggests a genetic influence. Several genetic markers have been reported to predict the development of DN in different ethnic groups. Loci on chromosome 18 have been shown in multiple studies to predict susceptibility to DN. The locus for the 'Carnosine dipeptidase-1' (CNDP1) gene on chromosome 18 has been identified as a marker of susceptibility (Conserva, Gesualdo and Papale, 2016). A recent meta-analysis of 34 studies on the genetic basis of DN involving inflammatory and angiogenesis pathways, has noted significant positive associations of 11 genetic variants in DN (Nazir et al., 2014). Available studies suggest that the genetic influence on the development of DN is polygenic with no one gene having a major influence and with some genetic variations having a protective effect. However, the Genomics England (GENEI) consortium examined the previously reported genetic associations with DN in type 1 diabetes in the largest case-control study yet reported, but was unable to replicate most of the reported genetic variants in DN (Williams et al., 2012).

1.1.2 Markers of diabetic nephropathy

1.1.2.a Biomarkers

A Biomarker is defined as an objective indication of medical state observed from outside the patient that can be measured accurately and reproducibly (Strimbu and Tavel, 2010).

Biomarkers of DN are the metabolites present in urine and/or blood in excess with the onset of DN. These help in early identification of onset of DN and enable early risk stratification. However these should be distinguished from risk markers. A risk marker is an attribute or exposure that is associated with increased probability of disease, but is not necessarily a causal factor (Burt, 2001).

Biomarkers detected in the early stages of DN might help institute preventative and therapeutic measures.

Multiple biomarkers have been evaluated and shown to have clinical importance. They have diverse origin with some being elements of the nephron, some derived from the circulation and some of mixed origin.

One classification groups them under markers of renal dysfunction, inflammatory biomarkers and oxidative stress biomarkers, according to their origin and the pathologic processes (Matheson et al., 2010). They can also be classified as glomerular, tubular and other proteins (Hong and Chia, 1998).

Existing markers

The best known marker used clinically is albuminuria. Presence of albumin in urine beyond normal limits is the basis of a diagnosis of nephropathy. Though the classification based on the level of albuminuria is somewhat arbitrary, it is of practical use and allows staging the progression of DN as normoalbuminuric (A1), microalbuminuric (A2) (moderate) and macroalbuminuric (A3) (severe) (table 1). Albuminuria is thought to be a marker of generalised endothelial dysfunction, which relates the renal complication of diabetes to cardiovascular and cerebrovascular complications. However albuminuria can also occur in other kidney diseases.

Category	**AER (mg/24hrs)**	**ACR (approximate equivalent)**		**Terms**
		(mg/mmol)	(mg/g)	
A1	<30	<3	<30	Normal to mildly increased
A2	30-300	3-30	30-300	Moderately increased
A3	>300	>30	>300	Severely increased

Table 1.1*: Albuminuria categories in CKD (from KDIGO 2012 Clinical Practice guideline for the evaluation and management of chronic kidney disease); AER- albumin excretion rate; ACR- albumin-creatinine ratio*

The presence of microalbuminuria should be confirmed with a repeat test within 3-4 months as per NICE guidelines (Bilous, 2016). This is because transient albuminuria can

occur in the presence of urinary tract infection, vigorous exercise, and contamination by blood in the urine sample and also with concentrated urine.

About half of patients with diabetes have microalbuminuria at some stage (Marshall and Flyvbjerg, 2006). In type 2 diabetes, the prevalence of microalbuminuria varies 26 to 43%, with prevalence being higher in Asian population and also in presence of Hypertension (Newman et al., 2005; Parving et al., 2006; Lu et al., 2007; Ismail et al., 1999; De Cosmo et al., 2016). Microalbuminuria can either progress to severe albuminuria or reverse to normoalbuminuria or remain in microalbuminuric stage (Figure 1.2).

Urinary biomarkers can also be classified as being of glomerular or tubular origin. The various markers studied are shown in the table No.1.2.

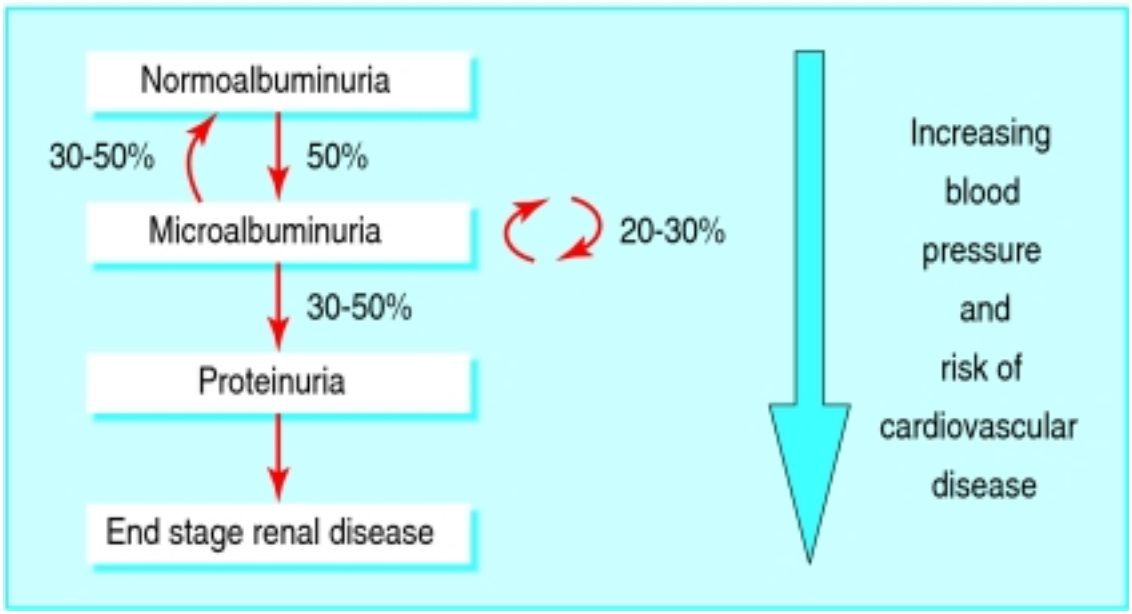


***Figure 1.2**: Progression of microalbuminuria in DN. (Diagram obtained from Marshall and Flyvbjerg BMJ 2006)*

Biomarker	**References**
Glomerular	
Urinary Transferrin	Kanauchi et al. Eur P Int Med.2002;13(3):190-193 Narita et al. Diab Care.2004;27(5):1176-1181 Currie et al. World J Diab.2014;5(6):763-776
Urinary Ceruloplasmin	Yamazaki M et al.Eur J Endocrinol.1995;132(6):681-687 Narita et al. Diab Care. 2004;27(5):1176-1181 Wang et al. Biomarker Res.2013;1:article 9
Immunoglobulin C	Narita et al Diab Care.2004;27(5):1176-1181Cohen-Bucay Int J Nephrol.2012(2012):1-11 web
Type IV collagen	Nelson et al.NEJM.1996;335(22):1636-1642

	Ming et al. Chinese Med J.2002;115(3):389-394 Fiseha T. Biomarker Res.2015;3:article 16
Laminin	Banu et al. Diab Res Clin Pract. 1995;29(1):57-67
Glycosaminoglycans	Torffit O. J Urol Nephrol.1999;33(5):328-332
Fibronectin	Kuboki et al. Diab Res Clin Pract.1993;21(1):61-66
Podocyte markers-Podocalyxin	Shoji et al. Biomarkers. 2015;21(2):164-167 Hara et al. Diabetologia. 2012;55(11):2913-2919 Zheng et al. PLoS ONE.2011;6(5)
Vascular Endothelial growth factor (VEGF)	Kim et al. Diab Med.2004;21(6):545-551 Petrica et al. PLoS ONE.2014;9(11)
Inflammatory biomarkers	
Orosomucoid	Jiang et al. Nephrology.2009;14(3):332-337 El-Beblawy et al. Clin App Thromb/Hemo.2016;22:718-726
Tubular	
Neutrophil gelatinase-associated lipocalin (NGAL)	Bolignano et al. Kidney Blood Pr Res.2009;32(2):91-98 Yildirim et al. J Clin Res Ped Endo.2015;7(4):274-279 Lacquaniti et al. Acta Diab.2013;50(6):935-942
Alpha-1-microglobulin	Weber & Verwiebe. Eur J Clin Chem Clin Bio.1992; 30(10):683-691 Hong et al. Diab Care.2003;26(2):338-342 Wainai et al. J DIab Compl.1991;5(1)160-161 Shore et al. J Ayub Med Coll. 2010;22(4):53-55
Kidney injury molecule-1 (KIM-1)	Petrica et al. Nephr Clin Pract.2011;118(2):c155-164 De Carvalho et al. Clin Biochem.2016;49(3):232-236 Bonventre JV. Trans Am Clin Climatol Asso. 2014; 125:293-299
N-acetyl-β-D glucosaminidase	Bazzi et al. NDT.2002;17(11):1890-1896 Jones et al. Ann Clin Biochem.1995;32(1):58-62 Patel & Kalia. Int J Diab Devel Coun.2015;35(s3):449-457 Ambade et al. Ind J Clin Biochem.2006;21(2):142-148 Assal et al. Clin Med Insigh:Endo Diab.2013;6(7):7 -13
Angiotensinogen	Kamiyama et al. J Pharma Sci.2012;119(4):314-323 Saito et al. Am J Med Sci.2009;338(6):478-480 Zhuang et al. Int J Clin Exp Path.2015;8(9):1464-1469
Cystatin C	Jeon et al. J Kor Med Sci.2011;26(2):258-263 Garg et al. Clin Exp Nephrol.2015;19(5):885-890 Kim et al. Diab Care.2013;;36(3):656-661
Liver-type fatty acid binding protein	Nielsen et al. Diab Care.2010;33(6):1320-1324 Viswanathan et al. Ind J Nephrol.2015;25(5):269-273 Kamijo-Ikemori et al. Diab Care.2011;34(3):691-696
Nephrin	Patari et al. Diabetes. 2003;52(12):2969-2974 Kandasamy et al. Biomark Res.2014;2(1):21
Heart fatty binding protein	Nauta et al. Diab Care.2011;34(4):975-981
Advanced glycation end products	Petrica et al. Int J Clin Exp Med.2015;8(2):2516-2525 Turk et al. Diab Met. 2004:30(2):187-192
Oxidative stress biomarkers	Ha & Lee. Curr Diab Repor.2001;1(3):282-287
8 Oxo 7,8 dihydro-2'-deoxyguanosine	Wu et al. Clin Chem Acta. 2004;339(1-2):1-9 Hinokia et al. Diabetologia.2002;45(6):877-882

	Broadbaek et al. Free Rad Bio Med. 2011;51(8):1473-1479
Other newer markers	
Retinol binding protein 4	Salem et al. Ped Diab.2002;3(1):37-41
Vitamin D binding protein	Shoukry et al. Molecul Cellu Bio.2015;408(1):25-35
Heme Oxygenase-1	Li et al. Nephrology.2016
Periostin	Satiropoj. PLoS ONE.2015;10(4)
Alpha klotho	Lee et al. PLoS ONE.2014;9(8)
Microvesicle-bound dipeptidyl peptidase IV	Sun et al. Diab Vasc Dis Res.2012;9(4):301-308
Micro RNA	Yang et al. Med Hypothe.2013;81(2):274-278 Argyropoulos et al. J Clin Med.2015;4(7):1495-1517
Adipokinesine alpha-2 glycoprotein	Wang et al. J Int Med Res.2016;44(2):278-286 Lim et al. Diab Med.2012;29(7):945-949
Neutrophil to lymphocyte ratio	Huang et al. Clin Endo.2015;82:229-233
Urinary Adiponectin	Panduru et al. Diab Care.2015;38:883-890
sTNFR1 & sTNFR2	Carlsson et al. Cardiovasc Diabetol.2016;15(40):1-8

Table 1.2*: Urinary biomarkers studied as representative of diabetic nephropathy (Gluhovschi et al. 2016)*

Despite the many biomarkers that have been put forward as potential markers for improving the detection of DN at early stages, none to date have shown any better sensitivity than the established marker, albuminuria.

Urinary proteomics is the study of multiple polypeptides excreted in urine. Study of urinary proteomics comparing the polypeptides in nondiabetic and diabetic groups with normo, moderate or severe albuminuria demonstrated that the urinary proteomics were distinct for diabetes, DN and nondiabetic proteinuric renal diseases (Zurbig et al., 2012). Urinary proteomics in patients with moderate albuminuria in this study indicated that proteome analysis could identify the patients at risk of progression to overt nephropathy. A urinary proteomic based classifier, CKD273 shows more promise in predicting the progression of CKD and death. CKD273 of less than 0.55 predicted a better prognosis with patients not needing dialysis or death during follow up, compared to all patients with CKD273 of more than 0.55 needing dialysis or dying (Argilés et al., 2013). This is the first proteomics based classifier tested to predict the progression of CKD.

However, urinary albumin levels remain the best marker for clinical use in detecting the onset and progression of DN.

1.1.3 Prevention of Diabetic nephropathy

Hyperglycaemia and high blood pressure are major contributing factors for the development of DN. Good glycaemic control and blood pressure control has direct effects on the prevention and stabilisation or even reversal of early stages of DN.

The Diabetes Control and Complications Trial (DCCT) in type 1 diabetes showed significant reduction in the risk of moderate albuminuria for both primary (by 34%) and secondary (by 43%) prevention cohorts with intensive glycaemic control for 9 years. The risk of progression to severe albuminuria was reduced by 56% in the secondary prevention cohort (The Diabetes Control and Complications Trials Research group, 1993). Further follow up of subjects from DCCT and its follow up study EDIC, showed continued benefits at 22 years of follow up. The risk reduction of 50% was seen in the intensively treated cohort for the decline in GFR and development of ESRD (The DCCT/EDIC Research Group, 2011)

The UK Prospective Diabetes Study (UKPDS) group in newly diagnosed patients with type 2 diabetes, showed a 25% risk reduction in the microvascular endpoints in the intensively treated group, maintaining an average HbA1c of 7.0% (6.2-8.2) over 10 years. The development of microalbuminuria over the 15 years follow up period was significantly reduced in the intensively treated group (UK Prospective Diabetes Study (UKPDS) Group, 1998).

Hypertension is one of the most common comorbidities in diabetic kidney disease. The UKPDS trial showed a 37% reduction in microvascular events with tight control of blood pressure (BP 144/82 vs 154/87 mmHg). A 13% risk reduction was seen in microvascular events for every 10mmHg reduction in systolic blood pressure (Marshall & Flyvbjerg, 2006). KDIGO guidelines suggest a reduction of blood pressure to <130/80mmHg in diabetic kidney disease. Control of diastolic blood pressure has a stabilizing effect on normo- and microalbuminuria (Estacio et al., 2000).

In type 1 diabetes, loss of nocturnal blood pressure dip in normoalbuminuric patients possibly predicts the onset of microalbuminuria (Lurbe, Redon & Kesani, 2002).

1.1.4 Management

A structured and protocol driven multifactorial approach is important in preventing and managing the complications of type 2 diabetes. A 39% risk reduction in the development

of DN was seen in the Steno-2 trial with an intensive multifactorial approach compared with conventional therapy, including dietary advice to reduce total daily intake of fat including saturated fat, smoking cessation, treatment with angiotensin converting enzyme inhibitors (ACEi) or angiotensin II receptor blockers (ARB's), light to moderate physical activity of 30 minutes 3 to 5 times a week, intensive glucose control and lipid lowering therapy, in specialist secondary care clinics (Gæde et al., 2003). 21 years follow up of this cohort has shown clear benefits in the early intensive therapy group. Progression to severe albuminuria was reduced by 48% in the previous intensive therapy group, despite everyone in the study being offered intensive therapy at the end of initial follow up period of 8 years (Gæde et al., 2016). There was significant cardiovascular benefit in the intensively treated group adding years to life. Continued intensive therapy for the longer term has benefits in reducing the risk of progression of nephropathy.

Good glycaemic control achieved by either oral agents and/or insulin therapy seems to have an equal effect in prevention and stabilization of nephropathy.

ACEi and ARB's have beneficial effect in reducing albuminuria outside their effect on blood pressure. These drugs should be started early and dose titrated as tolerated. These are recommended as first line therapy in patients with hypertension and diabetic nephropathy.

1.2 Other causes of CKD

CKD is defined as abnormalities of kidney structure or function, present for > 3months, with implications for health (KDIGO 2012 Clinical practice guideline). CKD in the diabetic population can occur as a result of other pathological processes similar to the non-diabetic population.

After diabetes, hypertension is the next most common cause for CKD. With the high prevalence of hypertension in the diabetic population, it can be difficult to distinguish CKD due to hypertension and diabetes from CKD due to other causes. Histological changes in the kidneys in the diabetic population with microalbuminuria and coexisting hypertension are variable (Fioretto et al., 1996). This has been classified into three patterns based on light microscopy findings as: C I) suggesting normal or near normal renal structure, C II) with changes typical of DN in type 1 diabetes, and C III) showing atypical patterns of injury sub classified as C III(a) with near normal glomerular structure and tubular basement thickening, tubular atrophy and severe interstitial fibrosis, C III(b) with

mild mesangial thickening and severe arteriolar hyalinosis affecting both afferent and efferent arterioles and C III(c) with some glomeruli showing near normal structure and others severe global sclerosis.

The other causes of CKD can be classified as systemic diseases affecting the kidney and primary kidney diseases (table 1.3). However the occurrence of these pathologies in people with diabetes and CKD is no different from the non-diabetic population.

Classification of CKD is based on presence or absence of systemic disease and location within the kidney of pathologic anatomic findings (KDIGO 2012 Clinical practice guideline).

	Systemic diseases affecting kidneys	**Primary kidney diseases**
Glomerular diseases	Diabetes, autoimmune diseases, systemic infections, neoplasia (including amyloidosis)	Diffuse, focal or crescentic proliferative GN; focal and segmental glomerulosclerosis, membranous nephropathy, minimal change disease
Tubulo interstitial diseases	Systemic infections, autoimmune, sarcoidosis, drugs, urate, environmental toxins (lead, aristolochic acid), neoplasia (myeloma)	Urinary-tract infections, stones, obstruction
Vascular diseases	Atherosclerosis, hypertension, ischemia, cholesterol emboli, systemic vasculitis, thrombotic microangiopathy, systemic sclerosis	ANCA-associated renal limited vasculitis, fibromuscular dysplasia
Cystic and congenital diseases	Polycystic kidney disease, Alport syndrome, Fabry's disease	Renal dysplasia, medullary cystic disease, podocytopathies

Table 1.3: *classification of CKD based on causes and location of pathologic findings*

1.3 Progression of CKD

30-50% of patients with microalbuminuria progress to severe albuminuria and 30-50% can revert to normoalbuminuria (Marshall & Flyvbjerg, 2006). With progression of DN, kidney function declines gradually toward ESRD. CKD is currently classified based upon cause, estimated glomerular filtration rate (eGFR) and albuminuria category.

Reducing eGFR and increasing albuminuria worsens the prognosis of CKD with increasing risk of developing end stage renal disease. The prognosis of CKD progressing to ESRD based upon eGFR and albumin levels is shown in table 1.3.

				Persistent albuminuria categories		
				A1	**A2**	**A3**
				Normal to mildly increased	Moderately increased	Severely increased
				<3 mg/mmol	3-30 mg/mmol	>30 mg/mmol
GFR categories (ml/min/1.73m^2)	**G1**	Normal to high	≥ 90	Low risk	Moderately increased risk	High risk
	G2	Mildly decreased	60-89			
	G3a	Mildly to moderately decreased	45-59	Moderately increased risk	High risk	Very high risk
	G3b	Moderately to severely decreased	30-44	High risk		
	G4	Severely decreased	15-29	Very high risk		
	G5	Kidney failure	<15			

***Table 1.4**: Prognosis of CKD by GFR and Albuminuria categories (KDIGO 2012)*

CKD in diabetes or diabetic kidney disease (DKD) progresses more rapidly with poor or suboptimal glycaemic control, usually assessed by HbA1c measurement. However DKD can also progress due to other modifiable coexisting risk factors such as hypertension, dyslipidaemia, history of cardiovascular disease, smoking, concomitant nephrotoxic therapies, as well as unmodifiable ones such as age, gender, and ethnicity.

KDIGO recommends regular monitoring of kidney function including eGFR and albuminuria in CKD with increasing frequency depending on the risk category, which varies from 1 to 4+ times a year. This helps in instituting or modifying therapy to manage risk factors and stabilize or reduce the speed of progression towards ESRD.

1.4 End stage renal disease

1.4.1 Definition

Progression of CKD to stage 5 when estimated GFR falls below 15ml/min/1.73m^2 is classified as ESRD or kidney failure. This stage of CKD has huge implications for a patient's health and prognosis, and is the time when renal replacement should be planned.

1.4.2 Management

Patients in ESRD need more frequent monitoring of their renal function in order to initiate RRT at the right time. This is based on inability to control volume status or blood pressure, symptoms and signs secondary to kidney failure (including electrolyte & acid-base abnormalities), and deterioration of nutritional status refractory to intervention or cognitive impairment.

Patients with CKD stage 4 & 5 should therefore be referred to specialist renal services

ESRD patients should be monitored for CKD related complications such as anaemia, metabolic bone disease, and acidosis.

Anaemia in patients with CKD is defined as haemoglobin (Hb) concentration <130g/l in men and <120g/l in women. In patients with CKD stage 4 & 5 (ESRD), Hb should be measured at least twice yearly (KDIGO 2012 guidelines). Patients with anaemia may need treatment with Iron replacement and erythropoietin.

In adult patients with CKD stage 3b to 5, serum calcium, phosphate, PTH & alkaline phosphatase levels should be monitored at least once to establish the baseline values and predict progression. Serum phosphate levels should be maintained in the normal reference range. Patients with intact PTH above the upper limit of the reference range should be initially tested for hypocalcaemia, hyperphosphatemia, and vitamin D deficiency. Vitamin D supplementation should be started only when there is documented evidence of deficiency.

In patient with serum bicarbonate levels of <22mmol/L, oral bicarbonate replacement should be started to maintain the level in normal reference range.

1.4.3 Effects of ESRD on glucose metabolism

Uraemia is associated with impaired glucose metabolism. However glucose handling is impaired in CKD/ESRD in several ways.

Insulin resistance (IR) is largely responsible for the abnormal glucose metabolism in patients with ESRD. The mechanisms of IR in CKD are multifactorial, including chronic inflammation, oxidative stress, vitamin D deficiency, adipokine derangement and altered gut microbiome. The other mechanisms that may contribute to IR include increased hepatic gluconeogenesis that does not suppress following insulin administration, reduced

hepatic and/or skeletal muscle glucose uptake and impaired intracellular glucose metabolism due to decreased oxidation or diminished synthesis of glycogen. These changes would lead to significant hyperglycaemia in patients with ESRD and diabetes. IR occurs early in CKD patients, which can be seen even with normal GFR (Kobayashi et al., 2005). It is universally present in all patients with ESRD, which is seen as a complication of ESRD based on data from studies using the hyperinsulinaemic euglycaemic clamp.

IR in turn can accentuate kidney injury by worsening renal haemodynamics by activating the sympathetic nervous system, increasing sodium retention and Na^+-K^+ ATPase activity and increasing GFR (Gluba et al., 2013; Rowe et al., 1981).

IR along with oxidative stress and inflammation is shown to have a role in development of albuminuria and reduced renal function (Gluba et al. 2013).

Glucose disposal rate (GDR) also changes in patients with CKD/ESRD. The GDR is negatively correlated to serum creatinine level and positively with creatinine clearance (Kobayashi et al., 2005). This could lead to worsening hyperglycaemia with progression of CKD and in ESRD patients.

Diabetic subjects with ESRD are also at risk of hypoglycaemia due to the prolonged action of insulin as a result of reduced renal clearance in ESRD (Betônico et al., 2016). As a result of these changes, the risk of both hyperglycaemia and hypoglycaemia is increased in patients with ESRD, leading to a significant variability in blood glucose levels. This makes the management of glycaemia in the diabetic population with ESRD much more complex.

The kidneys play an important role in maintaining plasma glucose levels in the fasting state. Animal studies have repeatedly shown that the kidneys release glucose in the fasting state in order to maintain plasma glucose levels in hepatectomised animals, and simultaneous nephrectomy in these animals leads to increased requirement of glucose infusion to maintain euglycaemia (Bergman & Drury, 1938; Reinecke R, 1943; Drury, Wick & Mackay, 1950). The kidneys can compensate between 50 to 100% for loss of hepatic glucose release (Joseph et al, 2000). Release of glucose from the kidneys is through gluconeogenesis in the renal cortex (Gerich et al, 2001). Increased glucose release from the kidneys occurs during hypoglycaemia (Meyer, Dosto & Gerich, 1999; Cersosimo, Garlick, Ferretti, 1999). Loss of this gluconeogenesis in CKD/ESRD patients makes them more prone to hypoglycaemia (Arem R., 1989).

1.4.4 Effects of ESRD on insulin action and its levels.

The kidneys play a central role in Insulin metabolism, although a much smaller role compared to the liver for endogenously secreted insulin. In non-diabetic subjects, 6 to 8 units of insulin equivalent is degraded by the kidneys each day, which amounts to around 25% of endogenously secreted insulin. Metabolism of exogenously administered insulin is mainly by the kidneys, as injected insulin enters the systemic circulation directly, bypassing first pass metabolism in the liver (Duckworth and Kitabchi, 1981; Duckworth, 1988). Insulin action can thus be prolonged due to reduced renal metabolism, thus increasing the risk of hypoglycaemia in insulin treated diabetic patients. Insulin requirement is reduced in diabetic subjects with CKD/ESRD (Biesenbach et al., 2003; Kulozik and Hasslacher, 2013).

Apart from the prolonged action, tissue resistance to insulin is seen in ESRD patients who have diabetes as mentioned above (DeFronzo et al., 1981).

IR is also seen in non-diabetic patients with traits of the metabolic syndrome (Kurella, Lo & Chertow, 2005). IR is suspected to be one of the causative factors of CKD in this cohort.

Due to reduced metabolism, circulating insulin levels can be elevated in patients with ESRD and renal failure. Reduced GFR will decrease the renal insulin clearance rate significantly, which is normally at 190ml/min, higher than a normal GFR of 120ml/min (Chamberlain and Stimmler, 1967). As a result, serum insulin levels can be much higher for both endogenous and exogenous insulin. This necessitates closer monitoring of insulin therapy and blood glucose levels to prevent hypoglycaemia in people with ESRD. There is no established method to assess the variable requirements of insulin in subjects with insulin treated diabetes with ESRD.

1.4.5 Reliability of C-peptide levels in CKD/ESRD

Measuring serum Insulin and C-peptide in the fasting state, along with plasma glucose, is normally a validated indicator of endogenous insulin secretion. However due to the possibility of raised serum insulin and C peptide levels in subjects with ESRD due to reduced renal clearance, the measured concentrations may not truly reflect endogenous secretion. There are limited data to understand the effect of ESRD on C-peptide levels in insulin deficient subjects.

The kidneys play an important role in regulation of circulating plasma C-peptide and also its metabolic clearance. A study in non-diabetic, non-obese individuals showed that renal uptake of C-peptide increased 7 fold more than urinary excretion following a rise in C-peptide secretion, and more than 85% of extracted C-peptide was metabolised in the kidneys (Zavaroni et al., 1987). Renal C-peptide clearance is very high, whereas the urinary excretion is only 14%, suggesting a significant role of the kidneys in the metabolism of C-peptide.

C-peptide levels must therefore be interpreted with caution in renal failure. ESRD with reduced metabolic activity of the kidneys could lead to falsely elevated serum C-peptide levels. This might pose a challenge in assessing endogenous insulin secretion in type 1 and 2 diabetes if there is established CKD/ESRD. One study in ESRD subjects with and without diabetes, including both type 1 & type 2 classified on clinical criteria, found only 70% concordance between clinical categorization into type 1 & 2, and that based upon C-peptide levels (Covic et al., 2000). The mean C-peptide levels were not different between diabetic and non-diabetic populations with ESRD. C-peptide levels were 2.5 fold higher in diabetic subjects with ESRD compared to diabetic subjects without ESRD. These data suggest that C-peptide remains elevated even in the type 1 diabetic ESRD population for prolonged periods making it unreliable to differentiate between the types of diabetes.

1.4.6 Effects of Dialysis on glycaemia

Glucose is an essential part of dialysate fluids for its osmotic effect. However dialysate glucose can diffuse into the circulation and cause hyperglycaemia especially in subjects with diabetes. Dialysate fluids available in the UK may contain 0, 5 or 10mmol/l glucose. The concentration of glucose in the dialysate has varied effect on a subject's blood glucose level.

1.4.6.a Effect in subjects with diabetes

With glucose free dialysates, hypoglycaemia was seen commonly in diabetic subjects. The hypoglycaemic episodes can occur recurrently and are largely asymptomatic. Hypoglycaemia defined as plasma glucose <4mmol/l occurs in approximately 40% of subjects on HD with or without diabetes (Jackson et al., 2000). The increased risk of hypoglycaemia in subjects with diabetes could be due to decreased gluconeogenesis in the remnant kidneys, deranged metabolic pathways, inadequate nutrition due to uraemia, decreased insulin clearance, glucose loss to the dialysate and diffusion of glucose into

erythrocytes during haemodialysis (Abe & Kalantar-Zadeh, 2015). Hypoglycaemia is also seen with dialysates containing 5.5mmol/l glucose compared to dialysate with 11mmol/l (Simic-Ogrizovic et al, 2001).

Frequent hypoglycaemia in diabetic patients on HD could lead to reduction or even cessation of glucose lowering medication either transiently or permanently. Sometimes these patients have an HbA1c lower than 48mmol/mol. This has led to the erroneous concept of 'burnt out diabetes' in the dialysis population (Park et al, 2012).

HD can also cause hyperglycaemia. Dialysate containing a higher glucose concentration (11mmol/l) causes hyperglycaemia due to diffusion of glucose from the dialysate. Higher mean plasma glucose levels are seen with dialysate containing 5.5mmol/l compared to glucose free dialysate in the diabetic patients (Burmeister, Campos and Miltersteiner, 2012). However other factors such as lack of ability to excrete excess glucose by kidneys, increased insulin clearance by HD and secretion of counter-regulatory hormones may also contribute to hyperglycaemia (Abe & Kalantar-Zadeh, 2015).

Paradoxical hyperglycaemia may occur after the completion of HD due to a mechanism similar to the Somogyi effect, along with insulin resistance and insulin removal by dialyzer. HD extracts both plasma glucose and insulin significantly throughout the dialysis period (Abe, Kaizu and Matsumoto, 2007). Insulin extraction by HD could lead to hyperglycaemia depending on the extent of removal, which could vary depending on the dialyzer. For their proven benefits on lipid profile high flux dialyzers are preferred in maintenance HD (Wanner et al., 2004). The dialyzer membrane could vary depending on the dialyzer used. In a well-designed randomized cross over trial using 5 different high flux dialyzers with different membranes, a significant difference was seen in the clearance of immunoreactive insulin between the membranes, except for two which had similar clearance (Abe, Okada and Matsumoto. 2008). This in turn could cause a variation in the plasma glucose levels in patients on HD on the dialysis days compared to non-dialysis days.

Increased glucose variability in diabetic subjects undergoing HD, is associated with excess morbidity and mortality. HbA1c levels of ≥69mmol/mol (8.5%) and ≤36mmol/mol (5.4%) in haemodialysed patients are associated with increased mortality in diabetic patients on HD in a meta-analysis (Hill et al., 2014). An HbA1c target of <69mmol/mol was recommended for this population in this study. However HbA1c as a measure of

glycaemia does not represent blood glucose variation or average blood glucose due to several reasons associated with ESRD and HD. Sudden cardiac deaths (SCD) account for majority of the cardiac deaths in diabetic HD patients. Poor glycaemic control defined as HbA1c >8.0%, which often represents significant glycaemic variation, has a strong association with increased risk of SCD (Dreschler et al., 2009). In this study, every 1.0% increase in HbA1c was shown to increase the risk of SCD by 18%, along with an increase in cardiovascular events and overall mortality. Acute hypoglycaemia with its associated physiological changes (Wright and Frier. 2008) could increase the risk of cardiovascular events and SCD. In the absence of data to understand the effect of glycaemic variation on the mortality in this particular cohort, it can be hypothesised that reduction in the fluctuation in plasma glucose levels would be beneficial in reducing the mortality and morbidity in the dialysis population.

1.4.6.b Effect in subjects without diabetes

Glucose free dialysates cause hypoglycaemia in nondiabetic subjects undergoing HD to the same extent as in diabetic subjects (Burmeister et al., 2007). Dialysates with 5.5mmol/l does not cause hypoglycaemia in this cohort compared to the diabetic group. The mean plasma glucose level in the nondiabetic group was not significantly different between HD with a glucose free dialysate and HD with a 5.5mmol/l glucose dialysate (Burmeister et al., 2007). However the plasma glucose levels can fall in this group using a dialysate with 5.5mmol/l by an average of 1.1mmol/l (Takahashi et al., 2004). Diffusion of glucose from plasma into erythrocytes due to glucose consumption from accelerated anaerobic metabolism could be one of the reasons.

High glucose dialysate (11.1 mmol/l) causes significantly higher intradialytic plasma glucose levels in the nondiabetic group, compared to dialysate with 5.5mmol/l (Raimann et al., 2012). This crossover trial also showed a significant increase in intradialytic serum insulin levels in the nondiabetic group during dialysis with 11.1mmol/l glucose dialysate compared to 5.5mmol/l.

The above literature suggests that there are several reasons for variation in glycaemic control in subjects on HD and maintaining optimum glucose level can be challenging. To understand this glycaemic variation better we need studies that observe the glycaemic pattern in this cohort for longer duration. This would be essential before we can make possible suggestions to insulin regimens in these subjects to reduce the variability.

Currently available studies of continuous glucose monitoring during HD, discussed in further chapters are all of shorted duration and inconclusive.

Chapter 2: Monitoring glycaemic control in CKD and ESRD

Glycaemia is generally measured by capillary blood glucose testing using the ISO certified glucometers available on the market. Depending on the need and a subject's willingness to test, one to 4 tests a day are recommended, going up to 7 tests in certain circumstances. However these readings may not accurately depict the variation in glucose levels occurring throughout the day. Hence continuous glucose monitoring (CGM) would be beneficial in determining the glycaemic variation throughout the day more accurately.

2.1 Role of continuous glucose monitoring

2.1.1 How does CGM work?

CGMS measures glucose in the interstitial fluid (ISF) by glucose oxidase coated sensors or "wired enzyme" sensors, depending on the device. Capillary wall permeability allows glucose to diffuse from the blood into the interstitial space. The glucose level in ISF correlates with blood glucose levels.

Continuous glucose monitoring systems (CGMS) have been in clinical use since late 1999. The first retrospective CGM from Minimed Technologies, 'CGMS Gold' (Medtronic Diabetes, Northridge, CA), was used to measure ISF glucose levels for three days (Hirsch, 2009). This system did not display the glucose level immediately to the patient; results were analysed retrospectively (Liebl et al., 2013). A few years later real-time continuous glucose monitor (rt-CGM) was developed. The Food and Drug Administration have approved these systems for clinical use since 2005. In the last decade, there has been significant improvement both in the technology and in clinicians' understanding of appropriateness of using the rt-CGM for improving glycaemic control.

Currently only a few approved glucose monitoring devices are available in the UK (Table 2.1).

The enzyme embedded in the sensor converts glucose and water in the interstitial fluid to gluconic acid and hydrogen peroxide. The hydrogen peroxide produces a modified charge, which is directly proportional to the concentration of the glucose (Hirsch, 2009).

$C_6H_{12}O_6 + H_2O + O_2 \longrightarrow C_6H_{12}O_7 + H_2O_2$ (Tao et al., 2009)

Manufacturer	Device	System	Mechanism	Sensor life	Sensor site
Medtronic Diabetes	Guardian Real Time	Stand alone	Glucose oxidase embedded sensors	3 to 6 days	Over abdomen
	Paradigm veo	In Minimed 530G and 640G Insulin pumps			
	iPro2	Blinded CGM			
Dexcom	Dexcom G4 Platinum	On their own or in Animas Vibe insulin pump	Glucose oxidase embedded sensors	Up to 7 days	Over abdomen
	Dexcom G5 Mobile				
Abbott Diabetes Care	Navigator II		Glucose Oxidase coupled with Osmium-based mediator molecule	Up to 5 days	Over abdomen
	Freestyle Libre			Up to 14 days	Over upper arm

***Table 2.1**: List of currently available continuous glucose monitor, mechanism and their sensor life*

The devices available from Abbott, Freestyle Navigator and recently Freestyle Libre (FSL), use 'wired enzyme' technology. The sensors in these CGM systems have glucose oxidase coupled with osmium-based mediator molecules anchored on a polymeric backbone film (Feldman et al., 2003). These are 'Flash Glucose Monitoring' (FGM) systems, providing actual ISF glucose concentration on patients scanning the sensor with the handset. The FSL displays the glucose profile over the last 8h.

To measure glucose levels in the interstitial fluid (ISF), a sensor is inserted in the subcutaneous space either on the abdomen or upper arm, depending on the device used. A transmitter attached to the sensor transmits the data to the recorder, which stores 7 to 14 days of readings. Each CGM sensor is devised to last 7 days and that of FGM up to 14 days.

rt-CGM usage allows glucose monitoring and simultaneous insulin dose adjustment by the patient or only glucose monitoring in an individual for later analysis. The CGM readings can be blinded from the patient so they do not act on the glucose readings by modifying their insulin therapy. In this study, the purpose of using CGMS was to understand the

glycaemic variation in individuals on maintenance HD. Hence a CGM with blinding facility was required. To understand the difference in glycaemic variation on dialysis days compared to non-dialysis days, it was essential to study the glycaemic pattern for a whole week. Hence a CGM with sensor lasting up to minimum of 7 days was required.

The Dexcom G4 Platinum CGMS used in my study is a real time monitor with a blinding feature, reading ISF glucose levels every 5 minutes up to maximum of 7 days with each sensor, fulfilling the study requirements.

2.1.2 Use of CGM in Chronic Kidney disease and dialysed population

The availability of rt-CGMS has paved the way to understanding glycaemic variation in individuals with diabetes through any 24 hour period.

Glucose variability (GV) occurs in individuals without diabetes, and is much greater in those with type 1 or type 2. Longer-term measures of glycaemic control such as HbA1c do not provide any information about GV, whilst self-monitored blood glucose levels give limited data as there are only a limited number of estimates. CGMS allows much more precise measurement of GV. A number of parameters are used to describe glucose variability, including ‘mean blood glucose’, ‘standard deviation’ (SD), and ‘mean amplitude of glycaemic excursions’ (MAGE).

Available data and clinical experience suggests extreme variations in blood glucose levels in individuals with end stage renal failure. There are limited data on the use of continuous glucose monitoring in the dialysed population.

It is possible that the glycaemic variation in patients on haemodialysis is significantly different compared to patients not on HD. Also, the inter-day variation between dialysis days and non-dialysis days is not well understood. There are several factors that could increase GV in these subjects, such as variable food intake, higher risk of gastroparesis, and reduced gluconeogenesis from the kidney. Also the glucose concentration of the dialysate can influence GV. Haemodialysate fluids are available at glucose concentration of 0, 5 or 10 mmol/l. Blood glucose levels rise with dialysate containing glucose, being higher with high glucose (HG) dialysate (10mM) compared to low glucose (LG) (0 or 5 mM) dialysate fluid. The risk of hypoglycaemia is higher with dialysate with 0 and 5 mM glucose concentration (Burmeister, Campos and Miltersteiner, 2012.). Asymptomatic hypoglycaemia occurs in people with diabetes on haemodialysis, which may be due to

blunted hormonal responses (Akmal, 2001). The awareness to hypoglycaemia is often reduced with duration of diabetes. People with ESRD secondary to diabetes would generally have had diabetes for long duration which could increase the risk of developing impaired hypoglycaemia awareness.

Significant variation in blood glucose level has been demonstrated in patients with insulin treated type 2 diabetes on haemodialysis. There are data available from few studies on glycaemic variation in haemodialysed population using CGMS, to understand the effect of the HD on blood glucose level. The studies and their key findings are summarised in the table 2.2.

CGMS has been validated to be reliable in patients with ESRD and also accurate and precise in patients on peritoneal dialysis. A significant correlation has been demonstrated between ISF glucose and venous glucose in CAPD patients (Marshall et al., 2003) and also in HD patients (Riveline et al., 2009). Glycaemia can be influenced by the type of glucose concentration in PD dialysate used as well as by peritoneal transport status (Skubala et al., 2010).

In one of the earliest reports of CGMS in diabetic patients on maintenance HD, the large excursions in day to day glucose levels observed were difficult to be managed by adjusting insulin doses (Pitkänen & Koivula, 1979). Blood glucose variation during haemodialysis is not solely due to the pre-existing diabetic state as it occurs even in non-diabetic subjects (Sobngwi et al., 2010).

All of the studies undertaken until now using CGMS on HD patients are of short duration. Though they have shown some significant changes in glucose levels between dialysis days and dialysis free days, they have failed to demonstrate a predictable pattern that could guide an appropriate insulin regimen or dose titration.

Study group & year (Ref)	Cohort & No. of subjects	Duration of CGMS & design	Key findings
Mirani et al. 2010	Diabetes patients on HD N= 12	48 hr HD vs Interdialytic day	Mean 24hr glycaemic value, MAGE, & SD of mean glucose- all significantly higher on HD day
Pitkänen & Koivula. 2010	Diabetes patients on HD N= 4		BG level accurately determined during whole dialysis period. Large day to day variation in pre-HD BG. Frequent hypoglycaemia.
Sobngwi et al. 2010	Non diabetes patients on HD N= 14		Lower BG during HD (5.8± 0.9 mmol/L to a 3-h nadir 4.6± 0.8 mmol/L) ISF glucose mirrored cBG. Mean cBG; Day before HD- 7.1 ± 1.1, during HD 5.2 ± 0.4, post HD 5.8 ± 0.7
Jung et al. 2010	Diabetes patients on HD N= 9	144hr HD vs interdialytic day	MAGE- no significant difference Hypoglycaemia predominantly on HD day Significant drop in glucose on initiation of HD in subjects on treatment for DM despite glucose containing dialysate
Riveline et al. 2009	Diabetes patients on HD vs not on HD N= 19 vs 39	4 days	cBG and ISF glucose- correlated in both HD and non HD groups (p <0.0001) HbA1c & mean ISF glucose- correlated in nonHD (p<0.0001) and HD group (p=0.042)
Kazempour-Ardebili et al. 2009	Diabetes patients N=17	48hr	24h AUC & 24h mean glucose- higher on nonHD day (p=0.02 & p=0.013 respectively) Hypoglycaemia within 24h post-HD Nadir glucose level within 24hr post-HD

Table 2.2*: Summary of available studies on glycaemic variation in HD population. (cBG- capillary blood glucose, ISF- Interstitial glucose, HD- Haemodialysis, AUC- Area Under Curve, MAGE- Mean Amplitude of Glycaemic Excursion)*

With significant change in insulin levels and action in ESRD and haemodialysis, it is difficult to formulate an optimum insulin regimen. In patients with residual endogenous insulin secretion, who are on insulin therapy, it is highly likely that mismatch happens between glucose requirements and insulin doses. There are no data specifically in C-peptide negative or minimally positive patients, who are entirely dependent on exogenous insulin therapy. Hypoglycaemia remains a major risk for this population after HD and the glucose concentration in dialysate is not standardised, however FDA approves the use of dialysate with 100mg/dl (5.5mmol/l) (Locatelli et al., 2015).

Use of CGM can lead potentially to more adaptations to treatment and improved glycaemic control in patients on HD. However given the limitations of the available studies, it is not possible to set standard guidance for insulin dose adjustment for people on HD, in order to reduce glucose variability.

2.1.3 Limitations of CGM

2.1.3.a Technical limitations

Real time-CGM needs calibration usually at 12h intervals to determine the in vivo sensitivity of each sensor and to adjust to the changes in sensor sensitivity over the time it is worn. Calibration is by measuring capillary blood glucose using commercially available blood glucose meters, and the result must be entered onto the CGM receiver within 5 minutes of the test. The analytical measuring accuracy of reliable blood glucose meters is around 3-5%, which can rise to 20% in everyday use. Therefore the calibration of CGM systems can in itself be a significant source of error. Individuals using CGM need to be trained carefully in calibration (Liebl et al., 2013).

Insertion of and changing sensors are complex tasks for which users require detailed training. The Dexcom CGM sensor has a 2h start up period before any ISF glucose levels are recorded. At the end of this period, 2 capillary blood glucose level checks must be performed and entered on to the receiver within 5 minutes before CGM commences. This could lead to a lack of data for the first two hours of dialysis, when CGM is initiated at the start of HD. This can be overcome by inserting the sensor 2h prior to start of HD, but would not be feasible in all patients, especially those having their HD session in the mornings.

The distance between the receiver and the transmitter is an important factor in obtaining continuous recordings. The recommended distance varies between 3 to 6m, depending on the CGM used. Leaving the receiver behind while patients are mobile causes loss of data.

The transmitter battery generally has a one year lifespan, with replacement adding additional costs for long term use.

2.1.4.b Range of measurable glucose levels

There is a restriction in the range of glucose level each CGMS can measure (Table 2.3). Thus patients must monitor their capillary blood glucose if the CGMS reading is out of range.

Device	Lower limit of ISF glucose	Upper limit of ISF glucose
Guardian rt	2.2mmol/l	22.2mmol/l
Dexcom G4 Platinum	2.2 mmol/l	22.2 mmol/l
Navigator II	1.1 mmol/l	27.8 mmol/l

***Table 2.3**: shows the currently available CGMS and their measurable limits*

At blood glucose levels in the hypoglycaemia range, the sensitivity of the CGM is reduced. Hence it becomes less reliable when blood glucose levels are low.

The change in interstitial glucose levels lags behind changes in blood glucose levels. This time lag can vary from 7 to 15 minutes. This renders CGM readings inaccurate when there is a rapid change in blood glucose (Hirsch, 2009).

2.2 Relationship between interstitial glucose level and blood glucose level

The glucose level in the ISF and the blood or plasma is bound to be different due to physiological reasons. Even the plasma glucose is significantly higher than the whole blood glucose level (Holtkamp, Verhoef and Leijnse, 1975). Holtkamp has previously shown the relation between plasma and whole blood glucose as ‘Glu(P) = 1.07 Glu(WB) + 0.11’. It is important to understand this relation while interpreting the glucose variation data recorded on CGM.

The ISF glucose level is based on different fluxes (Aussedat et al., 2000). There is a significant delay in the change in glucose level in subcutaneous tissue and muscle compared to plasma (Moberg et al., 1997). There are physiological variations in the glucose uptake, utilization and elimination in the blood and ISF (Kulcu et al., 2003), which

could lead to difference in the glucose level in these compartments, which are correspondent in time.

Different groups using various ISF glucose monitoring systems have examined ISF glucose levels in relation to blood glucose. ISF levels measured using subcutaneous sensor in non-diabetic rats was at 70% of plasma glucose (Aussedat et al., 2000). The difference in level and the time lag could vary depending on the method or system used.

2.3 HbA1c as a marker of glycaemic control

Glycated haemoglobin A1c is produced by non-enzymatic glycation, on exposure of haemoglobin to plasma glucose, through the life span of red cells. Hence measurement of glycated haemoglobin A1c gives an average level of plasma glucose concentration over a period of 3 to 4 months (the average life span of RBCs). HbA1c measurement is a well-established marker for long-term glycaemic control in diabetic patients and it has been shown to have predictive value for microvascular complications (UKPDS 33, 1998).

2.3.1 Role of HbA1c in managing glycaemic control

HbA1c monitored every 3 to 6 months is clinically used as a guide to modify diabetes therapy. NICE recommends a target level for HbA1c for people with diabetes based on the treatment they require. The cut off values to define good glycaemic control are based on the findings from the hallmark studies like DCCT and UKPDS.

The DCCT study compared intensive blood glucose control to achieve near normal blood glucose levels in patients with type 1 diabetes with conventional control. The target HbA1c of 6.05% (42mmol/mol) or less was set for the intensive therapy group. Although this level was achieved by only 44% of the cohort at least once during the 6.5yr follow up and <5% maintained the target value long term, the benefits gained in terms of significant reduction in diabetes related complications, like retinopathy, nephropathy and onset of neuropathy made clear the value of using HbA1c in managing glycaemic control (The Diabetes Control and Complications Trials Research group, 1993).

The UK Prospective Diabetes Study in subjects with newly diagnosed type 2 diabetes, comparing the effect of intensive therapy achieving an average HbA1c of 7.0% (53mmol/mol) over 10yr compared to 7.9% (63mmol/mol) in the conventionally managed group, showed a significant reduction in the combined diabetes related endpoint. This

study also showed a reduction in diabetes related death and all-cause mortality (UK Prospective Diabetes Study (UKPDS) group, 1998).

The significant reduction in microvascular events in intensively controlled type 2 diabetes has been reproduced in recent trials (The ADVANCE collaborative group, 2008; ACCORD trial group, 2008)

NICE guidelines have set a target HbA1c of 48mmol/mol for good control for subjects on lifestyle and diet only therapy or needing only one oral agent that does not cause hypoglycaemia in type 2 diabetes (NICE guideline NG28, Dec 2015). A level of 53mmol/mol is set as target for individuals on hypoglycaemia causing agents. HbA1c rising to 58mmol/mol is set as a cut off to intensify therapy.

NICE recommends targeting HbA1c of 48mmol/mol or lower for subjects with type 1 diabetes (NICE guideline NG57, Aug 2015).

Tight glycaemic control is associated with an increased risk of hypoglycaemia. Targeting a mean HbA1c of 48mmol/mol or lower caused an increased number of severe hypoglycaemic episodes compared to a mean HbA1c of 56mmol/mol (The ADVANCE collaborative group, 2008). Another trial studying the effect of intensive glycaemic control similarly showed increased risk of severe hypoglycaemia with an average HbA1c of 55mmol/mol or lower (The Action to Control Cardiovascular Risk in Diabetes (ACCORD) study group, 2008)). This study also showed an increased number of cardiovascular deaths, as well as death due to any cause in the intensively treated group.

It is important to set a target HbA1c level to reduce the risk of long term diabetes complications for an individual patient considering all related factors such as, general health, comorbidities, age, amount of diabetes therapy required, risk of hypoglycaemia and also the existence of impaired hypoglycaemia awareness.

2.3.2 Effect of CKD/ESRD on HbA1c

HbA1c levels are affected by various factors that alter red cell survival, e.g. increased turnover or destruction of RBCs, thus making them unreliable as a marker of glycaemic control in such conditions. The landmark trials like DCCT and UKPDS excluded patients with CKD/ESRD. Hence HbA1c is not fully validated in patients with CKD.

Anaemia associated with CKD/ ESRD could potentially cause falsely low HbA1c levels in this population. Haemolysis secondary to HD could lower the HbA1c by reducing the red cell life span (Sam et al., 2015).

Treatment of anaemia either with Iron infusion or erythropoietin lowered the HbA1c values significantly despite no change in mean blood glucose levels measured by 7-point capillary BG levels and CGM (Ng et al., 2010). Despite the improvement in haemoglobin and haematocrit levels with these treatments, the HbA1c level does not change in a linear fashion.

Extremely low or high HbA1c levels should be avoided in patients with CKD/ESRD (Williams et al., 2006; Nakao et al., 1998). A meta-analysis of 10 studies including over 83,000 patients with DKD on HD, showed increased mortality in patients with HbA1c of ≥69mmol/mol (≥8.5%) compared to a group of patients with HbA1c levels of 48 – 57mmol/mol (6.5%- 7.4%) (Hill et al., 2014). There was also an increase in mortality, though not significant, in the group of patients with HbA1c of ≤38mmol/mol (≤5.4%).

The linkage of HbA1c to glycaemic control and diabetic complications in ESRD has been challenged because of the analytical and clinical variability associated with laboratory testing (Ansari, Thomas and Goldsmith, 2003; Holt and Galen, 2004). The immunoturbidimetric assay is more reliable than High performance liquid chromatography (HPLC) in measuring HbA1c by reducing the influence from urea. It is likely that the HbA1c assay is less precise in the HD population because of the reduced red cell life span observed in this population (Holt and Galen, 2004). However HbA1c levels measured by immunoturbidimetric assay in a large cohort of HD patients are unexpectedly similar to those reported for general diabetic population (Williams et al, 2006).

Kidney Disease Outcome Quality Initiative (K/DOQI) guidelines for diabetes and CKD suggests HbA1c targets of around 53mmol/mol to prevent or delay progression of microvascular complications and not to treat to a target of <53mmol/mol in patients at risk of hypoglycaemia (KDIGO 2012 Clinical Practice guidelines, 2013).

HbA1c appears to lack consistent correlation to varying glucose levels unlike in the non-CKD population, with correlation at higher mean glucose levels (>10 mmol/L) but not with glucose variability (Jung et al., 2010; Mirani et al., 2010).

Higher HbA1c in non-CKD population is a good predictor of adverse health outcomes secondary to diabetes related complications including cardiovascular morbidity and mortality. However available data suggests variable results in relation to higher HbA1c and all-cause mortality, as seen with follow up of large cohort of patients (Kalantar_Zadeh et al., 2007; Williams et al., 2010).

2.3.3 Effect of dialysis on HbA1c

The red cell survival is reduced significantly in dialysed patients leading to anaemia despite treatment with erythropoietin and Iron replacement (Vos et al., 2011). Red cell survival in HD is altered partly due to eryptosis or stimulation of suicidal erythrocyte death (Abed et al., 2014; Bissinger et al., 2016). A number of factors like dialyzable plasma components, dialysis procedure, oxidative stress, increased cytosolic Ca^{2+} concentration and ceramide formation are probably responsible.

Hence the reduced red cell survival in haemodialyzed patients could lower the HbA1c level thereby falsely suggesting better glycaemic control.

2.3.4 Reliability of HbA1c as a glycaemic control marker in HD patients

There are variable results in the studies correlating HbA1c to average glucose levels in ESRD/HD patients (Williams et al., 2006; Jung et al., 2010; Mirani et al., 2010). Similarly the results are variable in studies involving large cohort of patients in the relation of HbA1c to all-cause mortality (Kalanter-Zadeh et al., 2007; Williams et al., 2010). Thus HbA1c is less reliable as a measure of glycaemic control and as a predictor of complications in this group of patients.

Glucose variability is an adjunctive risk factor for cardiovascular complications. However there is no proven association between glucose variability and HbA1c in ESRD (Mirani et al., 2010). Significant glucose variability is seen in patients on HD in both diabetic and non-diabetic patients with trough levels in glycaemia during HD (Mirani et al., 2010; Sobngwi et al., 2010).

Chapter 3: Alternative measures of glycaemic control

Fructosamine, glycated albumin (GA) and 1,5-anhydroglucitol (1,5-AG) have been studied as alternative measures of glycaemic control in diabetes patients.

3.1Fructosamine

Fructosamine is formed by the binding of fructose to total serum protein, but mostly albumin and immunoglobulins, unaffected by labile fractions and its concentration correlates with glycation of serum proteins (Mosca et al., 1987). It reflects glycaemia over 2 to 3 weeks preceding the test (Mosca et al., 1987).

3.1.1 In comparison to HbA1c

Glycaemia measured by fructosamine and HbA1c correlates significantly in both diabetic and non-diabetic populations (Pandya et al., 1987). It is possible to estimate HbA1c from the measured fructosamine level in diabetic patients with fair correlation between the two in diabetic population but not in non-diabetic population (r=0.88 for diabetes, r=0.01 for nondiabetics) (Narbonne et al., 2001).

There are limitations to using fructosamine measurements in clinical care in comparison to HbA1c. Due to its higher within subject variability, fructosamine has to be measured frequently. In view of the effect of serum albumin levels, fructosamine values have to be adjusted if serum albumin level is abnormal (Lee, 2015). This could be overcome by using an albumin corrected fructosamine level.

3.1.2 In ESRD and dialysis population

Both HbA1c and albumin corrected fructosamine (AlbF) are well correlated and are significantly associated with glycaemia in patients on haemodialysis (Mittman et al., 2010). AlbF however, was highly correlated with mean glucose values when less than 8.3mmol/l and a more useful predictor of hospitalization and morbidity. AlbF is thus as reliable a measure of glycaemia as HbA1c in diabetic patients on HD at lower glucose concentrations.

Fructosamine may be less reliable in renal failure for similar reasons as HbA1c. AlbF is raised in non-diabetic HD patients undergoing HD and CAPD (Lamb et al., 1993). This could underestimate the glycaemic control in diabetic patients on HD. Presence of other comorbidities causing protein loss could affect the AlbF level making it less reliable.

There are no set reference levels for this population; moreover it is not routinely available in clinical laboratories (Rhee et al., 2014). There are limited data on the association of AlbF with long-term outcomes. Doubling of fructosamine was shown to have a twofold higher risk of all-cause mortality and was also associated with a higher risk of hospitalization with sepsis in one study (Shafi et al., 2013).

3.2 Glycated Albumin

Glycated albumin is formed by the non-enzymatic glycation of albumin (Kovesdy et al., 2010). Like fructosamine, GA estimates glycaemia over the previous 1 to 2 week period (Koga and Kasayama, 2010). It is not affected by anaemia or reduced red cell survival (Rhee et al., 2014). It is expressed, as the serum albumin level, hence does not influence the proportion of serum GA to total albumin as GA%.

3.2.1 In Comparison to HbA1c

GA has a strong correlation with plasma glucose level and is a reliable indicator of glycaemic control over a short period (Tahara and Shima, 1995). Higher GA levels are linked to the presence and severity of cardiovascular disease and impaired renal function (Takahashi et al., 2007). GA has been thought to be a more reliable measure of glycaemic control and predictor of vascular complications in people with diabetes and nephropathy (Vos, Schollum and Walker, 2011).

It has similar limitations like fructosamine with comorbidities acting as confounding factors and with regards to availability and reference values (Rhee et al., 2014).

3.2.2 In ESRD and dialysis population

Plasma glucose and GA are shown to be much higher than HbA1c in patients with diabetes on HD in comparison to patients with diabetes without renal dysfunction (Inaba et al., 2007) suggesting lower HbA1c for the similar plasma glucose levels. GA% is shown to be higher in dialyzed patients in comparison to the non-nephropathy population, whereas HbA1c was paradoxically lower in dialysis patients (Freedman et al., 2010), suggesting underestimation of glucose levels in ESRD and dialysis patients with diabetes using HbA1c.

However, a more recent study using CGM showed that average glucose levels correlated better with HbA1c than GA, though underestimating average glucose, in both HD and non-

nephropathy groups (Hayashi et al., 2016). In this study GA value correlated better than HbA1c with glycaemic variability in the HD group.

Further evaluation is required with larger studies to confirm the benefits of AlbF and GA over HbA1c for monitoring glycaemic control in dialysed patients and also with long term studies for the benefits on reducing complication rates and mortality in this group.

3.3 1,5-anhydroglucitol (1,5-AG)

1,5-AG is a naturally occurring dietary polyol (Dungan et al., 2006) that has been used to monitor short-term glycaemic control for well over two decades in Japan (Fukumura et al., 1994). Blood glucose competitively inhibits renal reabsorption of 1,5-AG and hence even a transient rise in blood glucose level results in an immediate urinary loss of 1,5-AG. Hence it can be used to monitor the glycaemic control over a 24hr period (Buse et al., 2003). Frequent monitoring either daily or weekly can be a substitute for capillary blood glucose monitoring.

1,5-AG is a better reflector of postprandial glucose excursions compared to HbA1c and FA (Dungan et al., 2006), in moderately controlled diabetic patients.

The plasma level of 1,5-AG is decreased in patients with ESRD without diabetes. Plasma levels are reduced in both diabetic and non-diabetic patients on HD with no correlation to plasma glucose, HbA1c or fructosamine levels (Emoto et al., 1992). Hence renal function might be a confounding factor in using 1,5-AG for monitoring glycaemic control. However 1,5-AG levels are not influenced by renal function in mild to moderate impairment, making it a reliable marker in CKD stages 1-3 (Kim et al., 2012).

It appears that 1,5-AG would not a suitable alternative for HbA1c to measure glycaemic control in patients with ESRD and on HD.

Due to limitations with fructosamine, glycated albumin and 1,5-AG in terms of non-availability, need for frequent measurements and inconsistent evidence about their benefit over HbA1c, HbA1c has been continued to be used as the standard marker for glycaemic control in CKD/ESRD patients.

Chapter 4: Cardiovascular complications in CKD/DKD

Diabetic patients are at increased risk of cardiovascular disease by two to four fold. Diabetes is an independent risk factor for CVD, in men and women (Grundy et al., 1999). Development of diabetic nephropathy and CKD increases the risk of CVD further.

4.1 Cardiovascular morbidity and mortality

Cardiovascular morbidity and mortality are significantly increased in the diabetic population with CKD as seen on NHANES III survey (Afkarian et al., 2013).

Patients with diabetes have a higher comorbidity and poorer outcome compared to non-diabetic patients on dialysis. European data shows a 5 year survival probability of only 40.2% unadjusted rate for ESRD patients with diabetes as the cause compared to 48.3% for all patients on dialysis in the 2004-8 period. The 2 year survival probability available for the 2007-11 period cohort shows improvement in the survival probability for diabetes patients on dialysis at 71.1% in comparison to 68.8% for the previous cohort. However this is still lower than survival probability of 73.1% for all patients for the 2007-11 period (ERA-EDTA Registry 2013 annual report- Krammer et al., 2016).

Higher difference is seen in the mortality rates among UK population for patients with diabetes and without diabetes on renal replacement therapy. In the age group 18-44 years, 5-year survival was 71% for the diabetic population compared to 89% for the non-diabetic population. Similarly in the age group 45-64 years, 5-year survival was 51% against 68% for the non-diabetic population (UK renal registry 18th annual report- Steenkamp, Rao & Fraser, 2016).

Similarly, increased mortality and morbidity in diabetes patients with ESRD is seen in US as well. The all-cause hospitalization rate for patients aged 66 or older with CKD stage 4/5 is higher at 1156 per 1000 patient years in the presence of diabetes and CVD, compared to 398 per 1000 patient years for the same group without diabetes and CVD (USRDS annual data report 2016). CV deaths accounts for the majority of mortality in the ESRD diabetes population. Mortality rate for CKD patients with diabetes and CVD is 156 deaths per 1000 patient years compared to around 53 for patients without diabetes and CVD (USRDS annual data report 2016). The death rates increased with progression of CKD stages, with bigger differences between CKD patients with diabetes and CVD and those without diabetes and CVD.

Cardiac deaths including sudden death, myocardial infarction, cardiac arrest, and malignant arrhythmias, are the major causes of death accounting for 43% of all-cause mortality among all HD patients (Kanbay et al., 2010).

4.1.1 Risk factors for CVD in ESRD

Diabetes is a major independent risk factor for CVD in both men and women, including both type 1 and type 2. All major cardiovascular risk factors like hypertension, smoking, and high plasma cholesterol continue to be independent contributors to CVD in people with diabetes (Grundy et al., 1999). Prevalence of hypertension in people with diabetes is higher compared to the nondiabetic population. 50 to 80% of people with type 2 diabetes and 30% of people with type 1 diabetes are reported to have high blood pressure (Landsberg and Molitch, 2004). Other predisposing risk factors like obesity, smoking, reduced physical activity, heredity, advancing age and sex also exacerbate the risks.

Apart from the above, factors like left ventricular hypertrophy (LVH), coronary artery disease (CAD), rapid electrolyte shifts, QT dispersion, sympathetic nervous system over activity and CKD related bone mineral disorders are some of the factors associated with the high cardiovascular mortality in this group of patients (Kanbay et al., 2010).

4.1.2 Cardiac arrhythmias

Cardiac arrhythmias are suspected to be very common in people with ESRD (Redaelli et al., 1988). The USRDS database revealed that the single largest cause of death was attributed to arrhythmias. 61% of all cardiac deaths and 27% of all-cause mortality among HD patients were reported to be due to cardiac arrest/ unknown cause or arrhythmia. Cardiac arrhythmias may often be undiagnosed as they may be paroxysmal and asymptomatic.

There is a higher incidence of cardiac arrhythmias in HD population. Sudden shifts in serum electrolytes, especially potassium, have been thought to be one of the reasons (Kanbay et al., 2010). Dialysing with low potassium dialysate of 0 or 1 mmol/l is associated with higher rates of cardiac arrest (Karnik et al., 2001). However there is no consistent association of sudden death rates with dialysates of different potassium concentration. Low potassium dialysate is found to be protective from SCD in patients with pre-HD hyperkalaemia

(Huang et al., 2015). No difference is found in the incidence of death and arrhythmia in all HD between patients dialysed with dialysate of 3 or 2 mmol/l, despite varying pre-HD serum potassium levels (Karaboyas et al., 2016)

4.1.2.a In nonESRD/ nonDialysis population

The presence of hypertension, LVH and myocardial dysfunction are known risk factors for cardiac dysrhythmias. Asymptomatic complex and frequent cardiac arrhythmias increase the risk of all-cause mortality in people without ESRD in the presence of LVH (Bikkina, Larsen and Levy, 1993).

4.1.2.b In the dialysis population

A recent study reported the incidence of arrhythmia events including death to be 7% in all HD patients (Karaboyas et al., 2016). The incidence of asymptomatic supraventricular tachycardia which was self-limiting during HD was found to be 49.3% in one study of patients on HD with or without diabetes (Verde et al., 2016). This study revealed a higher risk of all-cause death, non-fatal CV events and symptomatic atrial fibrillation in patients who had asymptomatic SVT.

4.1.3 Burden of Ventricular premature beats (VPBs)

VPB's detected on routine ECG in people without cardiovascular disease do not predict adverse cardiovascular events or deaths as seen in the NHANES III study (Qureshi et al. 2014). However a more recent study suggested frequent ventricular premature beats (VPB) can be a predictor of the risk for ventricular tachycardia and mortality (Lin et al., 2017), especially when they come in pairs (couplets) or threes (triplets).

The frequency of VPBs is high in the haemodialysed population. A multicentre study reported the frequency of 2 or more VPBs at 29%, with 6% of patients having ventricular triplets on 48hr Holter monitoring (Redaelli et al., 1988). The frequency of arrhythmia was higher in the latter half of HD and for few hours after HD had completed. However there are no consistent data about the relation between HD and frequency of VPBs in patients without coronary artery disease (Quereda et al., 1986; Wizemann et al., 1985). It is likely that patients with DN and ESRD have a higher prevalence of CAD. Together the HD and pre-existing CAD might increase the risk of developing frequent VPBs including couplets and triplets.

4.2 Sudden cardiac death

Sudden cardiac death (SCD) accounts for the majority of cardiac deaths in dialysis patients, particularly those with diabetes. The relative risk of SCD is increased for all patients with chronic kidney disease, but the risk is increased 20- to 30-fold in haemodialysis patients compared to other populations without significant kidney disease. The USRDS report suggests that SCD accounts for about 22-27% of all deaths in haemodialysis patients. Genovesi and his team reported a cumulative incidence of 6.9% for sudden death, representing 19.2% of all deaths in patients on maintenance haemodialysis. The presence of atrial fibrillation, diabetes mellitus, pre-dialytic hyperkalemia, haemodialysis mode and high C-reactive protein are potential risk factors (Genovesi et al., 2009). Sudden death occurs more frequently during the first 24 h of the first short interdialytic interval and during the last 24 h of the long interval, i.e. immediately before and immediately after the first weekly haemodialysis session, suggesting a possible causative role of HD in SCD.

Glycaemic control in diabetic patients on chronic haemodialysis also plays a significant role in the mortality rate. An HbA1c >64mmol/mol (>8%) is said to increase the risk by >2 fold compared with HbA1c ≤43mmol/mol (6%) (Drechsler et al.,2009). Increasing HbA1c is associated with an incremental rise in the risk of sudden death, the SCD risk increases 18% for every 11 mmol/mol rise in HbA1c, suggesting a strong association between poor glycaemic control and sudden cardiac death (Dreschler et al., 2009).

Acute hypoglycaemia causes pronounced physiological responses as a consequence of autonomic nervous system activation, principally of the sympatho-adrenal system, with release of epinephrine. The haemodynamic changes associated with hypoglycaemia include an increase in heart rate and peripheral systolic blood pressure, a fall in central blood pressure, reduced peripheral arterial resistance and increased myocardial contractility, stroke volume and cardiac output (Wright and Frier, 2008). The workload of the heart is therefore temporarily but markedly increased, which may have serious consequences in patients, especially the elderly and those with diabetes. (Frier, Schenthaner and Heller, 2011). Hypoglycaemia is known to cause ECG changes e.g., ST segment changes with prolongation of the QT interval and cardiac repolarization (Judson and Hollander, 1956; Robinson et al., 2003, Koivikko et al., 2008). Hypoglycaemia leads to a reduction in the amplitude of T waves with flattening and lengthening of T waves, which can be quantified by measuring the QT interval (Graveling and Frier, 2010).

Patients on HD are prone for extreme glycaemic variation. Recurrent hypoglycaemia may increase the risk of arrhythmia and in turn to SCD.

There are other potential factors that could lead to the increased risk of sudden cardiac death in diabetic HD patients. Apart from autonomic neuropathy as a consequence of diabetes and uraemia, left ventricular hypertrophy commonly observed in CKD patients and CKD-BMD are potential causes of arrhythmia in this patient group. Uraemic toxins are thought to cause uraemic cardiomyopathy with typical histologic findings of myocardial fibrosis and this could lead to slowing of conduction and increased dispersion of repolarization, which are in turn pro-arrhythmogenic (Mark et al., 2006; Tun et al., 1999). Sudden electrolyte and fluid shifts during haemodialysis sessions can initiate life-threatening arrhythmias in patients with a susceptible myocardium (Yetkin et al., 2000). Several ECG markers such as QRS duration, corrected QT (QTc) interval and QT dispersion, have been suggested as potential predictors of ventricular arrhythmia in dialysis patients. Increased dispersion of QT intervals is known to predispose to ventricular arrhythmias and sudden cardiac death. QTc and QTc interval dispersion can significantly increase following HD (Malhis et al., 2010). There is some evidence for role of routine 12 lead ECG before HD in obtaining prognostic information to add onto standard cardiovascular risk assessment (Krane et al., 2009)

However there is a lack of systematic evidence to support routine cardiac monitoring during haemodialysis sessions.

Implantable cardioverter-defibrillator (ICD) insertion has been proposed as a preventative therapy for people on HD at risk for SCD. However the survival benefits in this population is much lower (Alpert M, 2011; Green et al., 2011).

Risk for SCD has not been well evaluated in this population. Continuous electrocardiographic monitoring to detect changes in QTc intervals and rhythm and echocardiographic evidence of associated risk factors may help in identifying individuals at high risk. Correlation with blood glucose variation and electrolyte levels can hopefully improve our understanding of the underlying mechanisms for the high cardiac mortality in this population.

4.3 Changes in serum electrolyte levels and their effect on cardiac function

During HD electrolytes including calcium, potassium and magnesium are extracted along with urea apart from maintaining acid-base balance. The Change in the levels of electrolytes could depend on their concentration in the dialysate used and the dialyser membrane.

Serum calcium and potassium play an important role in cardiac repolarization. Variation in the electrolyte levels in dialysate used can impact on their serum concentration which can potentially influence the cardiac electrical activity.

4.3.1 Effect on QTc interval

QTc interval is prolonged in patients undergoing HD compared to healthy volunteers (Suzuki et al., 1998). Though pre-existing cardiac defect is a risk factor, prolongation of QTc is seen in nondiabetic individuals with no cardiac dysfunction (Covic et al., 2002). However the prolongation of QTc post-HD is not consistent with some subjects having reduced QTc.

Dialysates with low potassium (2mmol/l) and calcium (1.25mmol/l) levels can significantly increase the QTc interval post-dialysis (Genovesi et al., 2008). Mean QTc is prolonged in 4hours period post HD compared to HD periods and other times of the day or night.

QTc dispersion, which is the maximum QTc minus minimum QTc, is an approximate measure of the abnormality of repolarization (Malik and Batchvarov, 2000). This has been studied often in the HD patients along with QTc to test the effect of HD on cardiac activity. Studies on patients with diabetes and/or hypertension have shown that mean QTc along with QTc interval dispersion are significantly raised after HD compared to before HD based on 12 lead ECG (Malhis et al., 2010; Niaki et al., 2013). However one study showed no effect on QT dispersion by HD in non-diabetic subjects without any cardiac disease (Covic et al., 2002).

4.3.2 Heart Rate Variability (HRV)

Beat to beat variability in the R-R intervals is physiological and is indicative of autonomic nervous system control over heart rate. This has been used as a tool to detect autonomic neuropathy in patients with diabetes in the past (Ewing et al., 1985). Reduced HRV is

associated with increased mortality after myocardial infarction (Wolf et al., 1978) and it is considered as a strong and independent predictor of death following acute myocardial infarction (Kleiger et al., 1987; Bigger et al., 1992). HRV is measured with different variables classified under 'Time domain' measures and 'Frequency domain' measures. Simplest variable is the standard deviation of normal to normal intervals (SDNN).

HRV is shown to be reduced in ESRD patients (Rubinger et al., 2004; Ranpuria et al., 2008). Frequency domain variables can be significantly suppressed in nondiabetic patients on HD (Yang et al., 2010). Higher frequency of HD through the week could increase the HRV suggesting effect of HD on cardiac structure and autonomic function (Chan et al., 2014).

Patients with ESRD are prone for autonomic neuropathy due to uraemia. Diabetes alone is a risk factor for autonomic neuropathy. Diabetic patients with ESRD on maintenance HD could be at higher risk for reduction in HRV, increasing their risk for SCD further. Fasting plasma glucose level appears to impact the HRV significantly in patients on HD compared to other variables in patients with metabolic syndrome, suggesting the role of diabetes (Chang et al., 2016). Measuring the HRV using continuous holter monitoring could help identify patients at higher risk which could be reduced by modulation with available drugs (Nolan et al., 2008).

4.4 Co-existing autonomic neuropathy

Autonomic dysfunction is a common complication of CKD (Vita et al., 1999). This increases the risk of arrhythmia and sudden cardiac death in patients with ESRD on HD (Jassal et al., 1997). Reduced HRV is a consequence of autonomic dysfunction which increases the risk in this population. Autonomic neuropathy in ESRD is largely due to effect of uraemia on parasympathetic system. HD and transplantation are shown to reverse this significantly (Heidbreder, Schafferhans and Heidland, 1985). Poor glycaemic control in diabetes is a known risk factor for autonomic neuropathy. Hence patients with diabetes and CKD have higher risk of autonomic neuropathy, which increases the risk of SCD.

4.5 Blood pressure variability (BPV)

BPV is a risk factor for progression of CKD, stroke and death (Manios et al., 2009; Pringle et al., 2003). It continues to be an existing risk factor for patients on HD due to fluid overload and change in osmolality due to intermittent HD in presence of vascular disease

(Flythe and Brunelli, 2014). Intradialytic BPV is associated with increased risk of all cause and cardiovascular mortality in patients on maintenance HD (Flythe et al., 2013). Older age, shorter dialysis vintage and greater ultrafiltration could cause greater intradialytic BPV.

Chapter 5: Haemodialysis

5.1 Dialysates- difference in electrolyte concentrations

Dialysate fluids are available in various concentrations of potassium, calcium, magnesium, bicarbonate and also glucose. Sodium concentration in the dialysate influences the blood pressure control. Potassium concentration is important to ensure that hyperkalaemia is reduced due to its arrhythmogenic potential. Calcium levels could influence myocardial contractility and repolarization. An adequate bicarbonate level is important to reverse acidosis (Locatelli et al., 2004).

5.1.1 Potassium

Dialysis is considered adequate if predialytic hyperkalaemia is avoided (Locatelli et al., 2004). Due to its arrhythmogenic potential, both hypokalaemia and hyperkalaemia (>6mmol/L) should be avoided. Potassium removal during dialysis occurs by diffusion according to the concentration gradient between plasma and the dialysate (Radaelli, 2001). Post-dialysis serum potassium level depends not only on the pre-dialysis serum potassium and dialysate potassium concentration but also on plasma tonicity and its change following HD (Locatelli et al., 2004).

Low pre-HD serum potassium could be associated with increased QTc dispersion (Covic et al., 2002). QTc prolongation during HD can occur when using dialysate with a K^+ of 2mmol/l, especially in combination with a Ca^{2+} of 1.25mmol/l. This prolongation is seen within the first hour of HD, with continued prolongation throughout and in the post-HD period (Genovesi et al., 2008). In this study by Genovesi et al, reduction in pre-HD QTc was seen when using dialysate with a K^+ of 3mmol/l in combination with a Ca^{2+} of 1.75mmol/l.

Dialysate baths could have 2mmol/l or 3mmol/l of potassium. Current HD practice does not take pre-dialysis serum potassium level into consideration. In the UK, dialysate with 2mmol/l of potassium is used as standard.

5.1.2 Calcium

Calcium is important for myocardial contractility. Patients with CKD are often treated with oral calcium salts as phosphate binders. Hence it is advised to use low calcium dialysate for maintenance HD (Sherman, 1988; Argiles et al., 1993). Dialysate fluids are available

with either 1.25mmol/l or 1.75mmol/l of calcium. It is advised to use dialysate with 1.25mmol/l of calcium (Locatelli et al., 2004).

Changes in serum ionized calcium levels during HD have a significant effect on the blood pressure, which could be due to effect on myocardial contractility (Maynard J, 1986; Fellner et al., 1989; Henrich, Hunt & Nixon, 1984). Low calcium dialysate causes larger reduction in BP compared to high calcium dialysate in patients with no cardiac abnormality (Van Kuijk et al., 1997). Significant reduction in systolic BP is also seen in ESRD patients with cardiac abnormality with low calcium dialysate while high calcium dialysate maintained the mean arterial BP (Van der Sande et al., 1998).

5.2 Glucose concentration of dialysate

Dialysate with 0, 5 and 10mmol/l of glucose have been used for HD. HD affects glycaemic control by altering glucose and insulin levels and increasing insulin sensitivity. Glucose homeostasis in patients on haemodialysis will vary depending on the concentration of glucose in the dialysate.

5.2.1 Effect on blood glucose levels

5.2.1.a Risk of hypoglycaemia and hyperglycaemia

HD with dialysate containing 0mmol/l glucose is associated with significant reduction of plasma glucose levels in diabetic as well as in some non-diabetic patients (Jackson et al., 2000; Akmal, 2001; Jasmin, Mueen and Aljubawii, 2015). Patients on HD may be asymptomatic to hypoglycaemia occurring during HD. Hence glucose free dialysates are no longer used routinely. Dialysate with 3mmol/l of glucose also causes hypoglycaemia compared to dialysate with 5mmol/l (Burmeister, Campos and Miltersteiner, 2012).

Haemodialysis increases the clearance of immunoreactive insulin levels which in turn contributes to hyperglycaemic episodes observed in the post-dialysis period in some poorly controlled diabetic patients (Abe, Kaizu and Matsumoto, 2007). A paradoxical rebound hyperglycaemia may be seen several hours post haemodialysis due to the combined effect of counter regulatory hormones (Somogyi effect) and insulin resistance (Abe and Kalanter-Zadeh, 2015).

Chapter 6: Methodology

6.1 Ethics Approval

This study was approved by 'Newcastle and North Tyneside NRES Committee 2' and South Tees Hospitals Research and Development department.

6.2 Patient Selection

This was a single site study based at James Cook University Hospital, Middlesbrough, where the Department of Nephrology treats patients with ESRD at four haemodialysis centres across the region. These centres include the central dialysis unit at James Cook University Hospital and peripheral dialysis units at North Ormesby health village, Middlesbrough, North Tees University Hospital, Stockton on Tees and Darlington Memorial Hospital, Darlington.

A list of all the patients with diabetes undergoing maintenance haemodialysis was obtained from the Department of Renal Medicine's patient database 'Proton' system. Individual patient's medication list was accessed from the clinic letters stored on the proton system. Patients with diet controlled or managed with oral glucose lowering agents or on combination of oral agents and insulin were excluded.

Eligible patients on insulin therapy only were listed according to the unit for dialysis. Dialysis unit staff were met and appraised about the study design and purpose. Patients were then approached at their respective dialysis units when they arrived for their dialysis. The study was described to individual patients and a copy of the 'Participant information sheet V3.1' (appendix 1) was given. They were encouraged to speak to their family members, their family doctors, their Nephrologist and staff at dialysis units regarding the study. They were re-approached in one to two weeks' time to answer any questions or clear doubts. Patients who agreed to participate were consented using an approved 'Informed consent form V2' (appendix 3). A date was fixed at the same time for a screening sample for C-peptide and plasma glucose levels. Samples were taken either after overnight fasting along with fasting plasma glucose levels or a random pre-dialysis sample for some of the subjects dialysing in the afternoon or twilight session. Samples for C-peptide were collected in a plain vacutainer. Samples were transported to the laboratory

immediately or within 30 minutes for separation of serum and freezing at -20^{0}c. Blood sample for glucose levels were collected in fluoride-oxalate tubes.

A list of non-diabetic patients on maintenance HD was obtained from the departmental data base. Their past medical history, medication and any previous plasma glucose levels and HbA1c available on the region-wide results system 'Webice' were reviewed. 15 patients were randomly selected from among those who did not have any evidence of diabetes or impaired glucose regulation. These patients were approached in a similar way. Informed consent was obtained from 5 patients who agreed to take part after providing 'Participant information sheet- controls V3.2' (appendix 2) and allowing one to two weeks' consideration time. Fasting blood samples were obtained for plasma glucose and HbA1c levels.

6.3 Patient Characteristics

34 patients with diabetes were consented. All patients had fasting or random glucose level checked along with C-peptide after consenting. 5 patients withdrew their consent before their study week. One patient underwent bariatric surgery and became insulin independent before the start of the study. One patient received simultaneous pancreas-kidney transplant and became insulin independent. One patient died before the study was started following voluntary withdrawal from dialysis therapy.

In view of the possibility of C-peptide being falsely positive in this group of patients, C-peptide to glucose ratio was calculated based on the formula shown below in section 6.4.1.c. With the absence of any existing data on the reliability of measured C-peptide levels in defining an absence of endogenous insulin secretion in diabetes patients with ESRD, we selected an arbitrary level of 5ng/mg for C-peptide/glucose ratio as the cut off value. A level of 5ng/mg or over was considered as C-peptide positive for exclusion. Patients with C-peptide/glucose ratio level less than 2ng/mg were grouped as c-peptide negative and those with level between 2 and <5ng/mg were grouped as 'minimally positive. Patients without diabetes were screened following consent with fasting plasma glucose and HbA1c. All five patients consented met the criteria with fasting glucose and HbA1c in the normal range.

Characteristics of patients included in the study are shown in the tables 6.1 and 6.2.

Sub. study No.	Gender	Age (yrs)	Type of Diabetes	Duration of diabetes (years)	Screening glucose level mmol/l (nonfasting-PreHD)	C-pep level nmol/l (non-fasting-PreHD)	C-pep/ glucose	No. of weeks Participated
1	F	45	MODY 3	34	18.8	1.84	1.63	2
3	F	62	2	10	8.8	1.39	2.63	3
4	M	50	1	48	2.4	<0.0	0.0	3
9	M	66	2	19	8.5	1.78	3.5	3
10	F	71	2	13	7.7	0.41	0.89	3
11	M	40	1	18	12.8	0.01	0.01	3
15	F	47	2	25	26.5	1.88	1.84	2
19	F	57	1	35	6.3	0.0	0.0	3
21	M	64	2	25	7.2	0.71	1.64	3
22	M	43	1	29	5.5	0.0	0.0	3
24	F	49	1	36	14.4	0.01	0.01	1
25	F	43	1	23	8.6	<0.03	0.0	3
31	M	58	2	27	13.8	0.57	0.69	2
38	M	51	2	30	5.7	1.28	3.75	1
41	M	72	2	10	7.7	2.3	4.98	2

***Table 6.1**: Characteristics of subjects studied with diabetes.*

Sub. study No.	Gender	Age (years)	Screening fasting Plasma Glucose (mmol/l)	Screening HbA1c (mmol/mol)	No. of weeks participated
32	F	75	4.3	31	1
35	F	74	4.0	30	1
36	F	46	4.4	23	1
37	M	46	5.2	32	1
39	F	57	4.1	31	1

***Table 6.2**: Characteristics of the subjects studied without diabetes*

6.4 Laboratory methods

6.4.1 C-peptide

C-peptide was measured using Roche E411 analyser in South Tees NHS Hospitals laboratory.

6.4.1.a Assay

The C-peptide assay on the Roche E411 analyser employs a Sandwich principle immunometric assay. Procedure involves:

• 1st incubation: 20 µL of sample, a biotinylated monoclonal C-peptide-specific antibody, and a monoclonal C-peptide-specific antibody labelled with a ruthenium complex react to form a sandwich complex.

• 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

• The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

• Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and master curve.

C-peptide levels were interpreted in relation to plasma glucose levels when detectable.

6.4.1.b Laboratory reference range:

0.11 to 0.61 nmol/L, for normal population.

There was no separate reference range for patients with end stage renal disease.

6.4.1.c C-peptide to glucose ratio

$$\text{C-pep/Gluc} = \frac{\text{C-peptide(ng/ml)} \times 100}{\text{Glucose(mg/dl)}}$$ (Fardaji et al., 2007)

Levels from SI units to metric units (Glucose: 1mmol/l =18.016mg/dl and C-peptide: 1nmol/l = 0.333ng/ml)

6.4.1.d Establishing cut off values for screening

In the absence of any available criteria to suggest normal or insufficient C-peptide levels in this population, an arbitrary cut off level was selected. Patients with C-peptide/glucose level of 5.0ng/mg or over were excluded from the study, as at this level we felt endogenous insulin production was ongoing at a reasonable level.

6.4.2 HbA1c

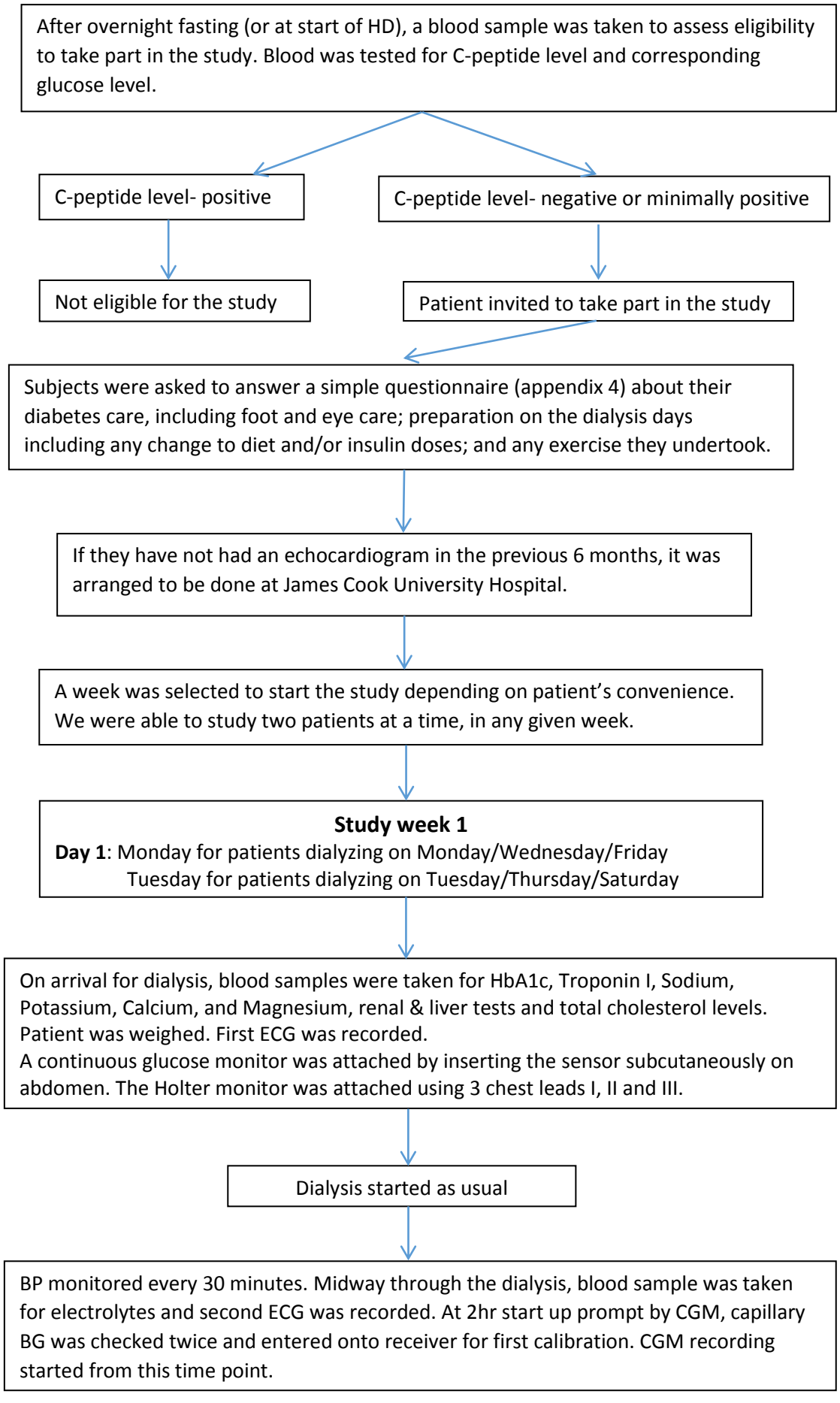
HbA1c was measured using High Performance Liquid Chromatography.

A combination of reverse-phased partition and ion-exchange chromatography was used to elute different haemoglobin fractions. Specimen is first haemolysed and haemolysate is passed through an analytical column. Reversed-phased chromatography first eluted foetal Hb, labile HbA1c and stable HbA1c. HPLC method then switched to ion-exchange which eluted HbA0, HbA2 and any variant of Hb present. The Hb fractions were detected by measuring the absorbance at both 415 and 500nm.

6.5 Study Design

Subjects with diabetes were studied for one to three weeks and subjects without diabetes were studied for one week. Study pathway for diabetes group is shown in the flowcharts 1 & 2.

Flow chart 1: Study pathway for the diabetes group.



At the end of dialysis, a further blood sample was taken for electrolytes and a third ECG was recorded. Patient weighed again.

Patient allowed home, with the CGM and the Holter monitor. These stayed with patients for 7 days, Patients were advised to call the emergency mobile number provided at the end of the leaflet if they had any problem with the monitors. Similar blood samples and ECG recordings were taken at their next two dialysis sessions.

Receiver was set to blind mode and patients were not able to see the IG readings in realtime. Patients advised to check capillary blood glucose levels as usual do and record them and also enter on to the receiver every 12hours for calibration. They were advised to maintain a dairy of your insulin doses, any symptoms of low or high glucose and diet intake.

Study week 2

On arrival for the first dialysis of the week, patients had blood sample taken for glucose control HbA1c, Fructosamine & Glycated albumin, & Troponin I. The CGM & Holter monitors were withdrawn. Food dairy and Holter dairy were collected. Next study week was scheduled to start 3 weeks later.

Study week 5

If patients were happy to proceed, on arrival for the first HD of the week, weight was checked, the CGM and the Holter monitor were attached as before. Dialysis started as usual. CGM and Holter monitors were left in place for 7 days. Patients were advised to continue to monitor capillary blood glucose levels and maintain food and insulin doses dairy. Monitors were withdrawn on arrival for 1st HD the following week and 3rd study week scheduled for 3 weeks

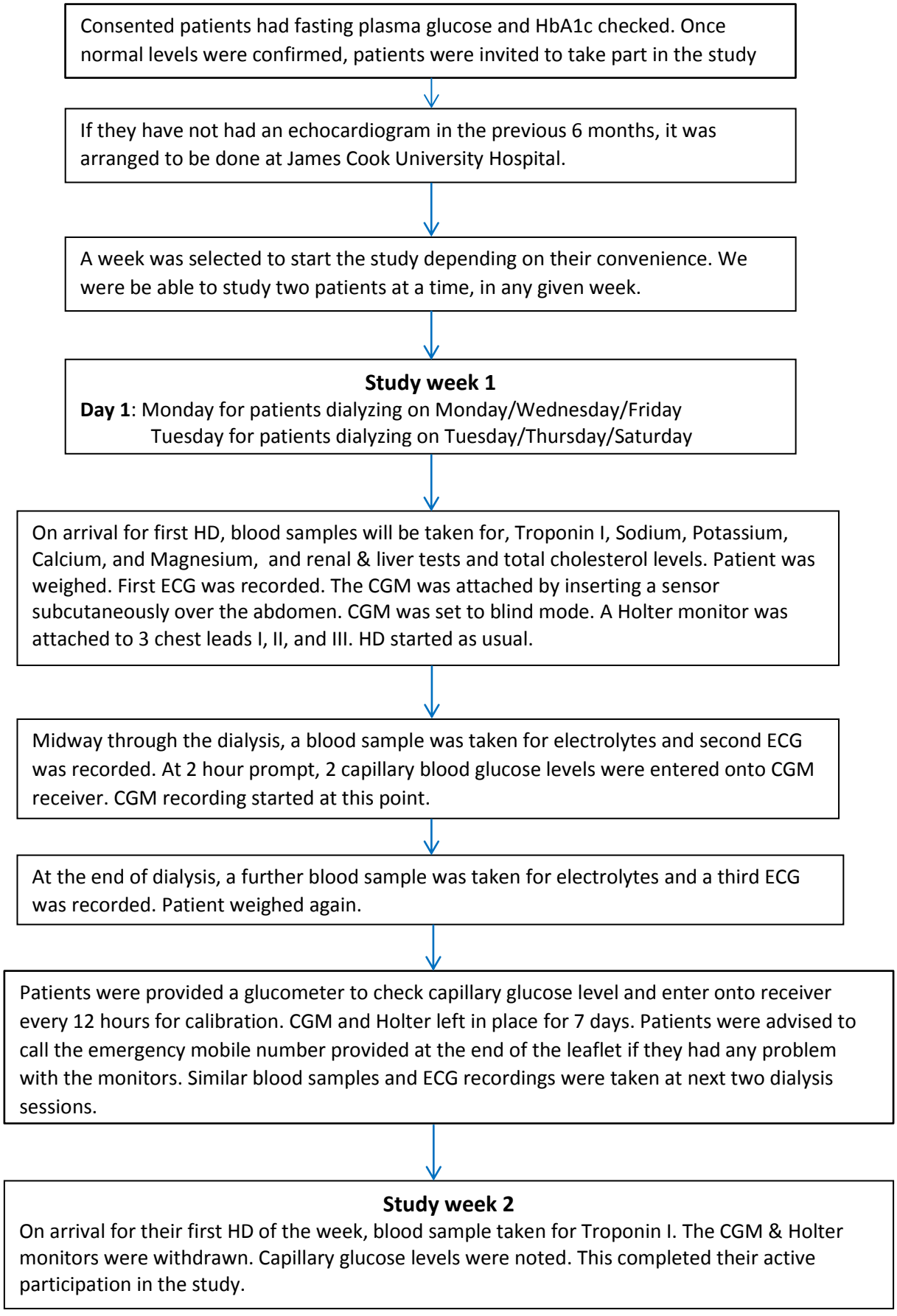
Study week 9

If Patients were happy to proceed, on arrival for first HD of the week, Weigh was checked, the CGM and Holter monitor were attached as above. Dialysis started as usual. CGM and Holter monitors were left in place for 7 days. Patients were advised to continue to monitor capillary blood glucose levels and maintain food and insulin doses dairy. Monitors were withdrawn on arrival for 1st HD the following week.

Study week 10

On arrival for first the HD of the week, blood sample was taken for glucose control measures and Troponin I. Both monitors were withdrawn. This completed the active participation of diabetic patient in the study.

Flow chart 2: Study pathway for the control group.



6.6 CGM

Continuous glucose monitoring system was used to record the glucose levels for one week at a time. Diabetes patients underwent up to three weeks of study with minimum three weeks gap in between.

6.6.1 Device

Dexcom G4 CGMS was used for the study. This device was selected as it was the only available CGM with a blinding option. Patients were blinded from checking the ISF glucose levels in real time, to prevent any alterations to insulin doses as training them in insulin titration was not incorporated in to the study. Dexcom G4 CGMS was designed to record and store data for up to one week.

6.6.2 Calibration

Dexcom CGM required calibration at 12 hourly intervals. At the start of the study, this would need a two hour start up period once the sensor was inserted and the transmitter was linked to the recorder. After the start up period, CGM prompted entry of two capillary blood glucose levels obtained using a commercially available glucometer. These were entered into the CGM receiver immediately and within five minutes gap. CGMS would start ISF glucose monitoring from this time point and recorded ISF glucose levels every 5 minutes. Patients were trained in entering capillary glucose levels every 12 hours for the whole study period and were given a written information guide to follow.

6.7 Assessing measures of glycaemic variation

CGM data were downloaded at the end of the study week and all results from individual patients were imported on to an excel spreadsheet. CGM was then formatted to be ready for the next study.

6.7.1 Glycaemic variation

Variation in glucose levels in relation to dialysis was studied. Average glucose levels for 4 hours before dialysis, during dialysis and 4 hours after dialysis were calculated and compared. Data are shown as mean ± 1SD in mmol/l.

Variation in glucose levels on dialysis days was compared to non-dialysis days. Average glucose levels and amplitude of excursion were calculated for dialysis days and non-

dialysis days. A 24 hour period beginning at the start of HD was taken as the dialysis day and the ensuing 24 hour period ending with start of next HD session was taken as the non-dialysis day. Data were shown as mean ± 1SD in mmol/l.

Amplitude of glycaemic excursion was examined in relation to insulin regimen in patients with diabetes and shown as difference in the mean excursion between different insulin regimens.

6.7.2 Hypoglycaemia

Hypoglycaemia was defined as ISF glucose level ≤3.5mmol/l. The complete glucose data were screened and all episodes with ISF glucose level of ≤3.5mmol/l lasting 20mins or more were selected and recorded separately. The duration of these episodes, their occurrence on dialysis days and non-dialysis days and occurrence during day and night were recorded. The data were examined for frequency of these episodes on dialysis and non-dialysis days and during day time and night time. Day time was defined as 7AM until 11PM and night time was defined as 11PM until 7AM.

6.7.3 Hyperglycaemia

Hyperglycaemia was defined as ISF glucose level ≥13.0mmol/l. The complete glucose data were screened and all episodes with ISF glucose level of ≥13.0mmol/l lasting 20mins or more were selected and recorded separately. The duration of these episodes, their occurrence on dialysis days and non-dialysis days and occurrence during day and night were recorded. The data were examined for frequency of these episodes on dialysis and non-dialysis days and during day time and night time. Day time was defined as 7AM until 11PM and night time was defined as 11PM until 7AM.

6.8 Cardiac Monitoring

Cardiac monitoring was undertaken by both 12 lead ECG and 7 days of Holter monitoring in all study patients.

6.8.1 ECG

6.8.1.a Device

12 lead ECGs were recorded using 1200 CE GM Medical systems.

6.8.1.b Recording

12 lead ECGs were recorded three times during each HD session in the first study week for patients with diabetes and in their one study week for patients without diabetes. They were recorded at the beginning, midway and at the end of the HD session.

6.8.1.c QTc measurement

QTc intervals were calculated manually from each ECG by using the tangent method. Three consecutive normal QRS complexes on lead II were selected. R-R interval for each beat was measured manually and averaged. QT interval was measured using Tangent method (Postema and Wilde, 2014), by drawing a horizontal line along the baseline, vertical line at the upstroke of Q/q/R wave and a tangential line along the down slope of the T wave. Measurement was taken as the distance between the junction of the vertical line with the horizontal line and junction of the tangential line and the horizontal line (diagram 1). Average of three measures was calculated. QTc interval was then calculated using Bezett's formula (QTc = QT/√R-R).

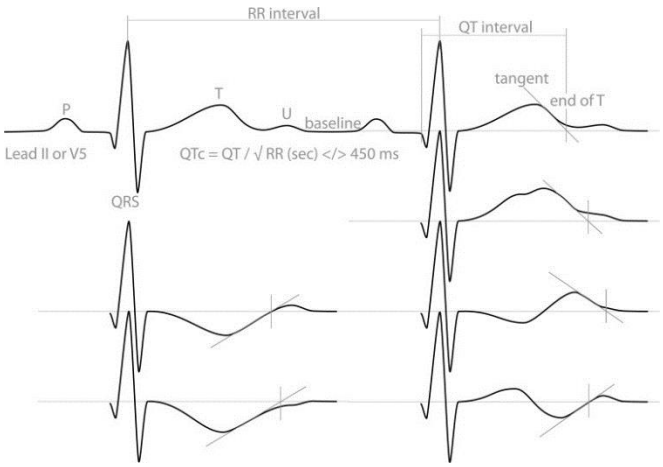


***Figure 6.1**: Tangent method for measuring QT interval (obtained from 'The Measurement of the QT inteval'. Curr Cardiol Rev. 2014 Aug;10(3):287-294)*

6.8.1.d Intra and Inter individual variation

Intra-individual variation in QT measurement was reduced by taking QT measurements from three consecutive QRS complexes and obtaining an average.

Inter-individual variation was examined by having a second observer unrelated to the study to measure QTc using similar methods on 10 randomly selected ECGs from the

study. The second observer was blinded from the initial measurement results to reduce the bias.

6.8.2 Holter monitor

Cardiac rhythm was monitored during the study week in all patients using a Lifecard CS recorder. Patients with diabetes wore the holter monitor for one to three weeks along with CGM and patients without diabetes wore it for one week along with CGM.
Holter monitoring was initiated at the start of the first HD of the week along with CGM and left in place for a full week, until the arrival of the patient for their first HD session in the following week.
Patients were given spare electrodes to change during the week along with an information leaflet to guide them in correct lead placement.
Patients were provided with an event dairy to record any symptoms during the week such as palpitations, near syncope or syncope, black outs, chest pain and shortness of breath, along with date and time. Any recorded symptoms were later correlated with the holter recording.
Holter data were downloaded on to 'Spacelab healthcare' software and was examined by two electrophysiologists, who were blinded to the patient's diabetes status.
Data captured were examined for any arrhythmic episodes and premature complexes and were reported in the standard way. Data were then examined for QTc interval and HRV for the set times, to match with average glucose level before, during and after dialysis sessions.
Arrhythmic episodes are shown as the frequency of different arrhythmia occurring on dialysis and non-dialysis days.
QTc measures are shown as the difference in the mean QTc in relation to dialysis, mean QTc in relation to hypoglycaemia and hyperglycaemia.

6.9 Blood pressure

Blood pressure was recorded in all patients at the beginning, midway and at the end of each HD session. The change in BP from start to end of HD was calculated.

6.10 Electrolytes

Serum electrolytes were measured at set points in all the patients.

Blood samples were obtained at the beginning, midway and at the end of the HD session during all HD sessions in one to three weeks of participation of patients with diabetes. Similar samples were obtained for all three HD sessions in one week of study from patients without diabetes.

Samples were analysed for potassium, corrected calcium, and magnesium levels in the central laboratory at James Cook University Hospital.

The average level for each time point was calculated and shown as a difference in mean ± 1SD. The difference in electrolyte levels between each time point in a HD session was calculated and shown as average drop in the first and second halves of the HD session. Change in electrolyte levels during 1^{st}, 2^{nd} and 3^{rd} HD sessions were examined.

6.11 Data analysis

Data were analysed using SPSS 21 software. The tests used are specified in the results section.

Glycaemic variation indices were calculated using EasyGV software V8.8.2.R2 obtained from University of Oxford.

Aims and Objectives

Aims of the study

1. To study the glycaemic variation during haemodialysis in subjects with insulin treated diabetes with nil or minimal endogenous insulin secretion using continuous glucose monitoring and in control subjects without diabetes.

2. To study the variation in cardiac electrical activity in relation to dialysis using Holter monitoring.

3. To explore the relation between glycaemia and cardiac electrical activity**.**

Objectives of the study

1. To understand the variation in glucose levels in diabetic patients in relation to haemodialysis with levels before, during and after dialysis.

2. To understand the variation in glucose levels on dialysis days in comparison to non-dialysis days.

3. To examine the frequency, duration and symptoms of hypoglycaemia.

4. To examine the frequency and duration of hyperglycaemia.

5. To examine the relationship between average glucose level and HbA1c in haemodialysis patients.

6. To explore changes in cardiac rhythm in relation to dialysis.

7. To explore changes in cardiac rhythm in relation to hypoglycaemia.

8. To explore changes in QTc interval in relation to dialysis.

9. To explore changes in QTc interval in relation to hypoglycaemia.

10. To explore changes in QTc interval in relation to hyperglycaemia.

Results

Chapter 7: Glycaemic variation

7.1 Interstitial glucose level recordings in subjects with and without diabetes.

7.1.1 Subjects with Diabetes

Glucose levels in relation to haemodialysis were studied to understand the variation during and after dialysis, in relation to before dialysis. The periods for pre-dialysis and post-dialysis were fixed at 4 hours in view of most dialysis sessions lasting 4 hours. However the dialysis sessions varied in their duration both within individuals and between individuals. The majority (57.3%) of sessions were of 4 hours. All patients had a minimum 3 HD sessions in the week; with only subject 'number 11' having 4 cycles a week regularly. Subjects 21 and 25 had 5 and 4 HD sessions in their 3rd week respectively. Table below shows the duration of all HD sessions for every subject in the diabetes group in each study week (table 7.1).

Subject Number (cycles/week)	**Study Week 1**	**Study week 2**	**Study week 3**
01 (3, 3)	4hr, 4hr, 4hr	4hr, 4hr, 4 hr	Not applicable
03 (3, 3, 3)	4hr, 4hr, 4 hr	4hr, 4hr, 4 hr	4hr, 3hr 30min, 3 hr
04 (3, 3, 3)	3hr, 3hr, 3 hr	4hr, 4hr, 4 hr	3hr 40min, 4hr, 4 hr
09 (3, 3, 3)	4hr, 3hr 50, 3hr 35min	4hr, 4hr, 4 hr	4hr, 4hr, 4 hr
10 (3, 3, 3)	3hr, 3hr, 3 hr	3hr, 3hr, 3 hr	3hr, 3hr, 3 hr
11 (4, 4, 4)	3 hr 10, 3hr 10, 3hr 10, 3hr 30 min	3hr, 3hr 10, 3hr 10, 3hr	3hr, 3hr 5, 3hr 20, 3hr 10min
15 (3, 3)	4hr, 4hr, 4 hr	3hr 30min, 4hr, 4 hr	Not applicable
19 (3, 3, 3)	4hr, 4hr, 4hr	4hr, 4hr, 4hr	4hr, 4hr, 4hr
21 (3, 3, 5)	3hr 45, 4hr, 3hr	3hr 30, 4hr, 4hr	2hr 45, 2hr, 2hr 35, 3hr 45min
22 (3, 3, 3)	4hr, 4hr, 4hr	4hr, 4hr, 4hr	4hr, 4hr, 3hr 15
24 (3)	3hr, 3hr, 3hr	Not applicable	Not applicable
25 (3, 3, 4)	4hr, 4hr, 4hr	4hr, 4hr, 3hr 30min	2hr 30, 2hr, 2hr 30, 3hr, 2hr 30
31 (3, 3)	4hr, 4hr, 4hr	7hr, 7hr, 7hr	Not applicable
38 (3)	4hr, 4hr 10, 4hr	Not applicable	Not applicable
41 (3, 3)	4hr, 4hr, 4hr	4hr, 4hr, 4hr	Not applicable

Table 7.1*: HD duration for individual subjects in each study week*

The CGM was initiated only on arrival of the patients for their first HD session of the week. The start-up capillary glucose levels were checked at 2 hours from the time the transmitter and the recorder had established a link and when prompted by the recorder.

Standard glucose meters available on the ward were used for all patients. Two glucose levels were obtained within 5 minutes and entered onto the CGM receiver. The recording of interstitial glucose (IG) levels started from this point. The recording was ended on the arrival of the subjects to the 1st HD session in the following week. The total duration of recording for individual subjects in all the study weeks in which they participated is shown in table 7.2. The complete recording of IG every 5 minutes should provide 288 readings a day and 1992 readings for the week, taking into account the lack of recording for the first 2 hours of the start-up period.

The glycaemic pattern throughout the recording period of the study week showed a lot of variation with multiple peaks and troughs in all patients with diabetes. The recording of IG levels for one of the study patients with diabetes in all three weeks is shown in the figure below to illustrate this fluctuation.

Subject Number		Study Week 1	Study week 2	Study week 3
01	Days; hours	5 d;	6 d;	DNP[#]
	No. of readings	948	1505	
03	Days; hours	8 d;	8 d;	8 d
	No. of readings	1528	1546	1681
04	Days; hours	6 d;	6 d;	6 d;
	No. of readings	1069	1224	1086
09	Days; hours	8 d;	7 d;	8 d;
	No. of readings	1555	1584	1943
10	Days; hours	8 d;	8 d;	6 d;
	No. of readings	1974	1980	1216
11	Days; hours	8 d;	8 d;	8 d;
	No. of readings	1933	1988	1983
15	Days; hours	8 d;	8 d;	DNP
	No. of readings	1791	1551	
19	Days; hours	8 d;	8 d;	7 d;
	No. of readings	1822	1971	1656
21	Days; hours	8 d;	8 d;	8 d;
	No. of readings	1674	1839	1940
22	Days; hours	8 d;	8 d;	8 d;
	No. of readings	1691	1981	1829
24	Days; hours	8 d;	DNP	DNP
	No. of readings	1983		
25	Days; hours	8 d;	8 d:	7 d;
	No. of readings	1167	1683	1248
31	Days; hours	6 d;	7 d;	DNP
	No. of readings	1374	1984	
38	Days; hours	8 d;	DNP	DNP
	No. of readings	1926		
41	Days; hours	8 d;	8 d;	DNP
	No. of readings	1414	1715	

Table 7.2*: shows the duration of CGM recording in individual study subjects with diabetes in all the study weeks they participated. Days represent calendar days with a maximum of 8 days including starting and ending days. #DNP- Did Not Participate.*

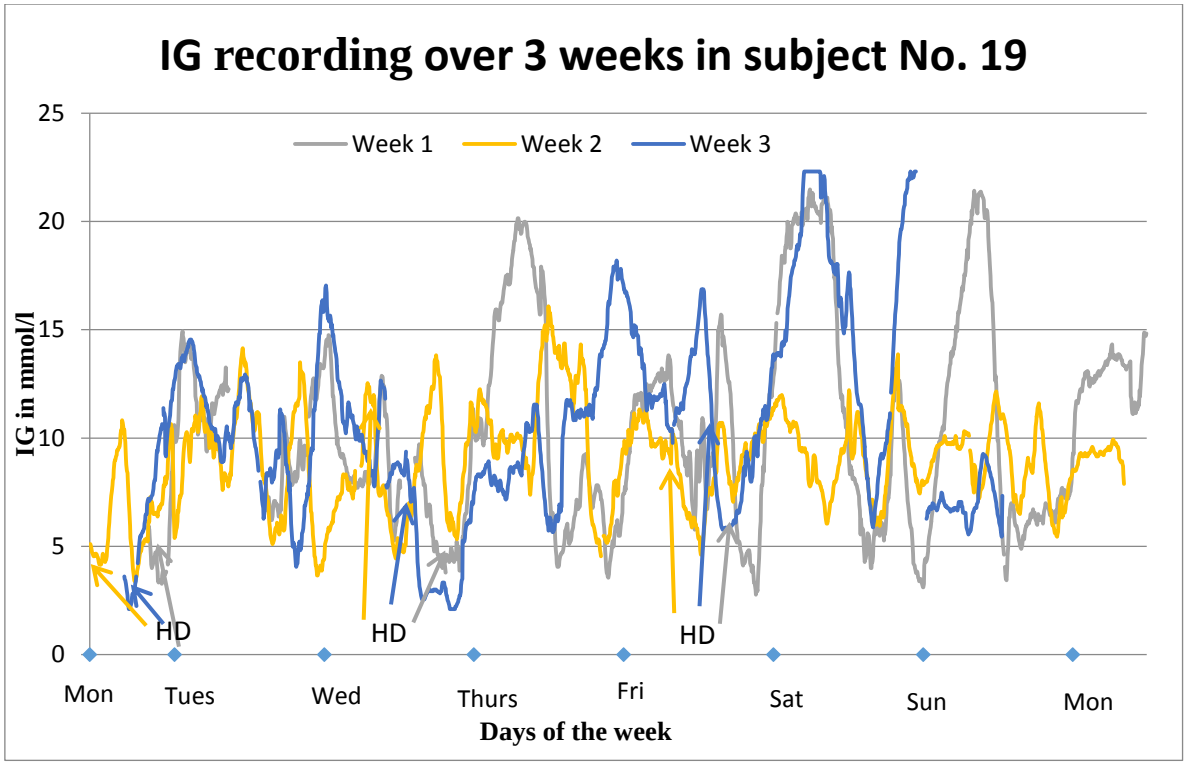


***Figure 7.1**: Shows the recording of IG levels in subject number 19 during 3 study weeks. This is a representative graph to depict the variation in glucose levels through the week. The start time for the HD sessions in each study week varied, as shown with an arrow matching the colour of the graph in the respective week.*

7.1.2 Control group

Each control subject without diabetes on maintenance HD underwent 1 week of study as per the protocol. Four subjects underwent 3 HD sessions a week and one subject underwent 4 HD sessions a week. They were all on standard 4 hourly HD sessions, with one of the dialysis units adding an extra 10 to 15 minutes to ensure a full 4 hours of dialysis. Subject number 39 had the 4th HD session duration shortened due to problems with venous access. The number of HD sessions per week and their duration is shown in table 7.3 and the CGM recording for these weeks is shown in table 7.4.

Subject Number (cycles/week)	**Study Week 1**
32 (3)	4hr 10min, 4hr 10min, 4hr 10min
35 (3)	4hr 10min, 4hr 10min, 4hr 10min
36 (3)	4hr 15min, 4hr 10min, 4hr 10min
37 (3)	4hr, 4hr, 4hr
39 (4)	4hr, 4hr, 4hr, 2hr 15min

***Table 7.3**: number and duration of HD sessions in individual control group subjects*

Subject No		CGM duration
32	Days; hours	8 d;
	No. of recordings	1684
35	Days & hours	8 d;
	No. of recordings	1969
36	Days; hours	8 d;
	No. of recordings	1915
37	Days; hours	8 d;
	No. of recordings	1834
39	Days; hours	8 d;
	No. of recordings	1688

***Table 7.4**: shows the duration of CGM recording available for subjects in control group. Days represent calendar days which could be up to 8 days including starting and end days.*

Fluctuations in IG levels were seen in the non-diabetic group during the study period. The recording of IG levels during the whole of the study week in subject numbered 35 without diabetes is shown in figure 7.2. Subject 35 is chosen as the maximum number of IG levels were recorded (98.8% of expected).

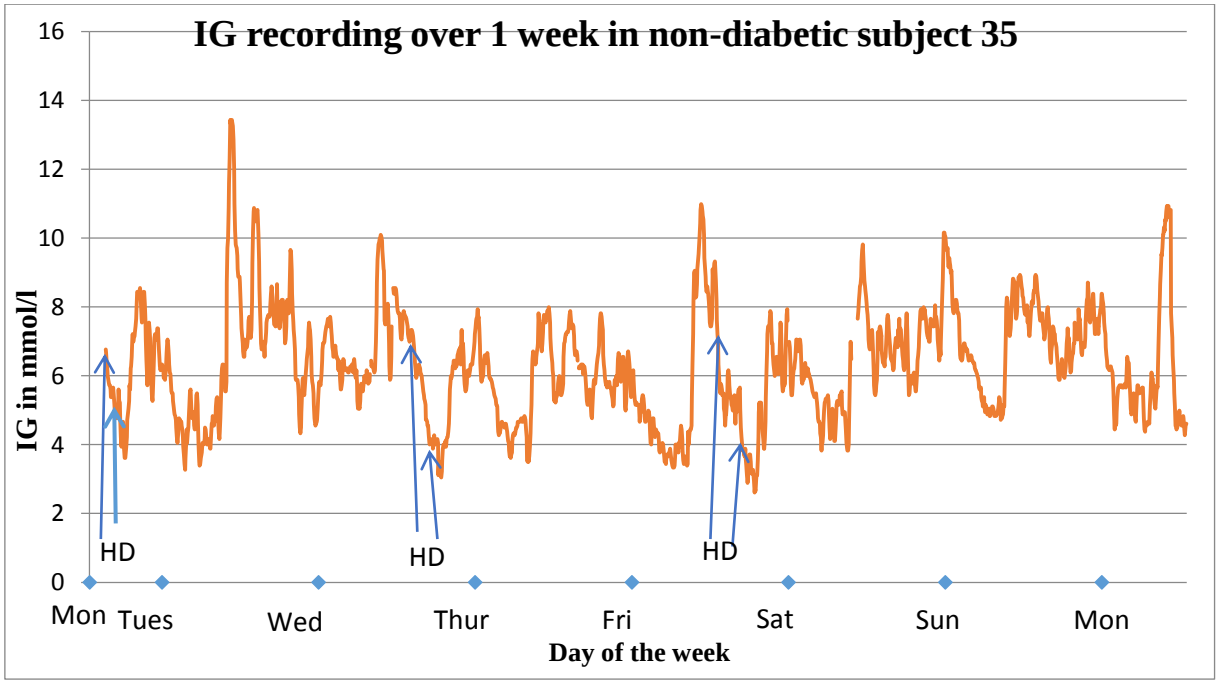


***Figure 7.2**: Shows the recording of IG levels in subject number 35 during the study week as a representative graph of variation in IG levels in subjects without diabetes. HD periods are shown with arrows pointing to start and end of session. Only 1hr 45min of 1st HD is captured.*

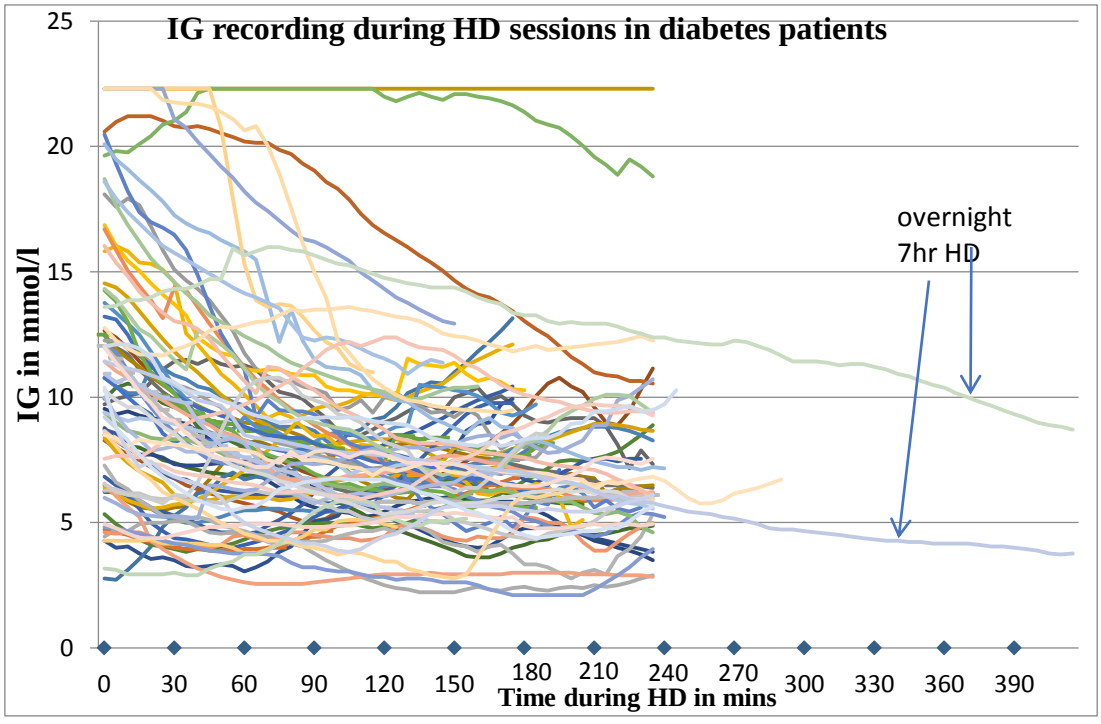
7.2 Glycaemic variation in relation to haemodialysis

The variation of IG levels during pre-dialysis, dialysis and post-dialysis periods in individual subjects with and without diabetes were analysed individually and for the

groups as a whole. The IG levels were erratic without any obvious consistent pattern in individual subjects with diabetes or in the group as a whole.

The pattern of IG levels during the pre-dialysis period of 4 hours, dialysis period with varying duration and post-dialysis period of 4 hours were analysed separately for the diabetes group. There was a trend towards a reduction in IG level as haemodialysis progressed.

The variation in IG levels in subjects with and without diabetes during HD for individual sessions in all study weeks where IG levels were available are shown in figures 3a and 3b. In the diabetes group data were available for 105 HD sessions. In 75 sessions the data were available for more than 75% of HD time. Only these data were selected to represent the changes during HD in the below graph. One subject with diabetes had persistent 'high' recordings throughout the HD session, appearing as a flat line in figure 7.3a. One subject was switched onto overnight HD in the second study week.



***Figures 7.3a**: Shows the IG levels during HD sessions of all subjects with diabetes (n=75)*

There is no consistent pattern, though there is trend towards a drop in IG level in the initial part of the HD session.

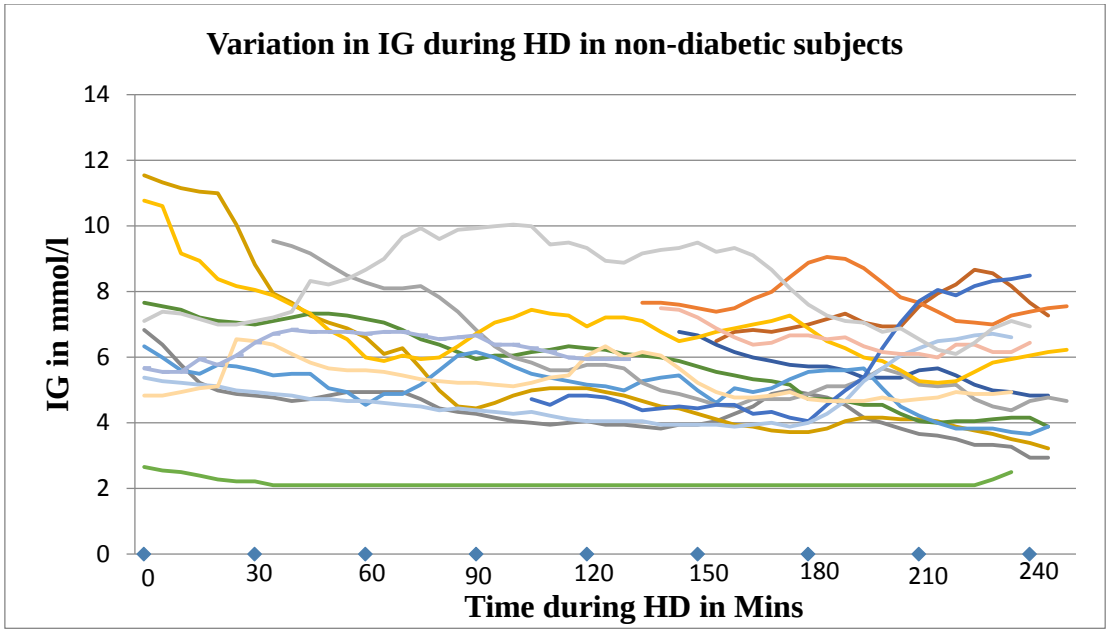


***Figure 7.3b**: Shows the variation in IG levels during HD sessions in all subjects in control group (n=15)*

There was a trend of gradual reduction in IG levels during the first half of the HD in subjects without diabetes, with variable trends in the second half. One subject had persistently low IG during one of the HD sessions.

Data were analysed to examine the variation in average IG levels from the pre-HD to post-HD periods. Paired samples where average IG level was available for all three periods were selected. In the diabetes group paired data were available for 70 out of 117 HD cycles.

There was a trend to drop in average IG from pre-HD period to HD period followed by rise in the average IG level in the post-HD period. The results are shown as change in average IG level expressed as percentage of change from pre-HD to HD period and HD to post-HD period (figure 7.4a). In large majority of HD cycles there is negative change from pre-HD to HD period, whereas a positive change was seen from HD to post-HD period.

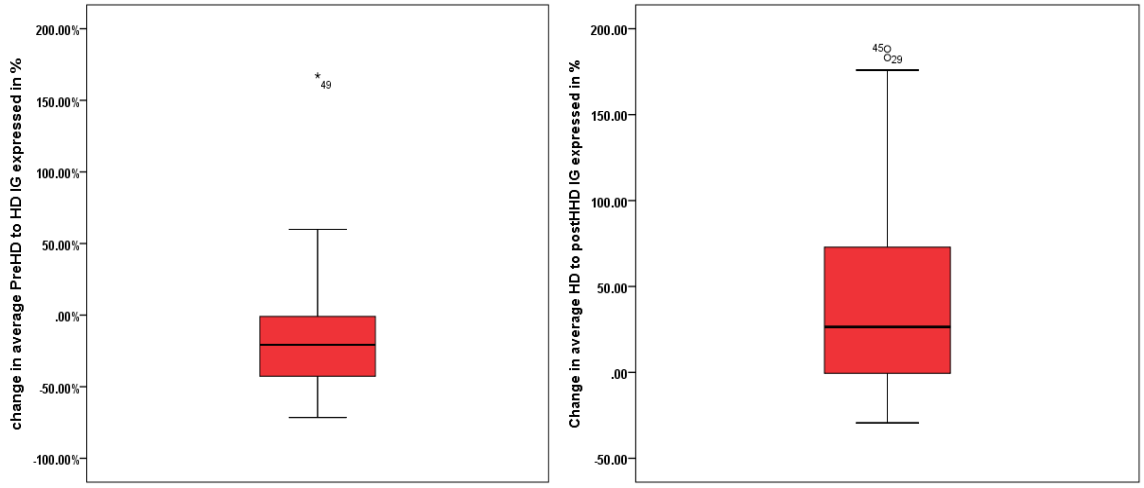


***Figure 7.4a**: shows the change in average IG levels between pre-HD to HD period and HD to post-HD period, as percentage of change in diabetes subjects (n=70)*

Type 1 DM vs Type 2 DM

Data was examined for any difference in the average IG levels around HD between type 1 and type 2 diabetes patients, using the paired samples, where the levels were available for all 3 periods. Mean, SD and 2 tailed significance were obtained using 'Independent-Samples T test'.

There was no difference in mean IG levels between type 1 and type 2 diabetes groups during pre-HD, HD or post-HD periods (Table 7.5).

	Type 1 (n=30)	**Type 2 (n=37)**	**p=**
PreHD IG (mmol/l)	11.5±4.6	11.6±5.2	0.943
HD IG (mmol/l)	8.8±4.0	8.7±3.8	0.931
PostHD IG (mmol/l)	11.9±4.7	10.7±3.7	0.244

***Table 7.5:** shows the comparison of mean IG levels (mean ± SD) during pre-HD, HD and post-HD periods in type 1 and type 2 diabetes*

Paired data were available for 10 HD cycles in the control group. There was variation in the pattern of change in average IG from pre-HD to HD and to post-HD periods. Some cycles showed a similar trend to the diabetes group, whereas other cycles showed a continued reduction in IG levels in the post HD period. Few cycles showed an elevation in IG level through the HD and into the post-HD period (figure 7.4b).

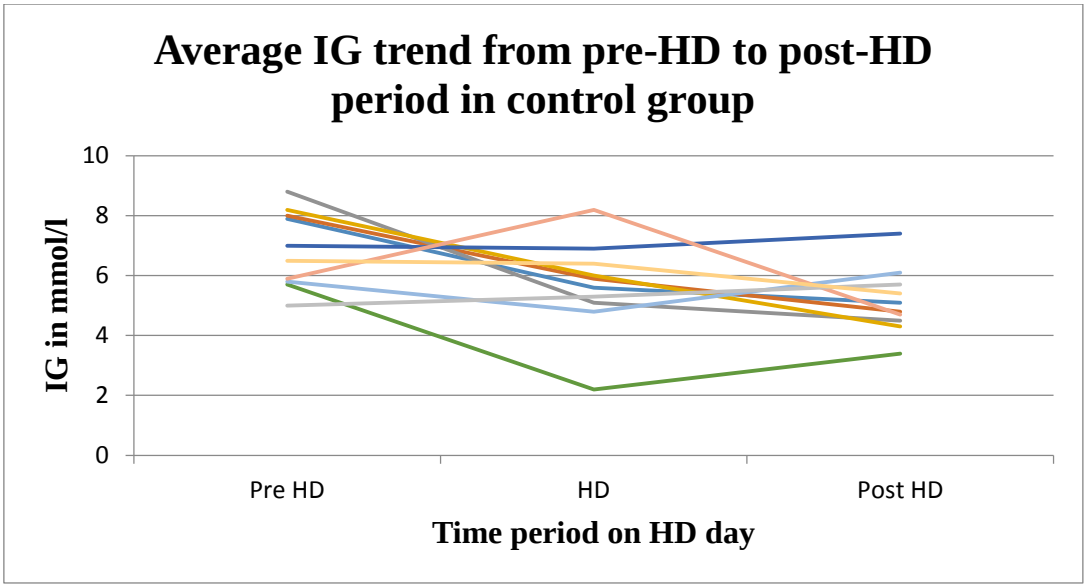


***Figure 7.4b**: shows the trend in the average IG level from pre-HD period to HD period and post-HD period in non-diabetes subjects (n=10)*

7.3 Variation in glycaemic indices on dialysis days vs non-dialysis days

Data were analysed to examine differences in the indices of glycaemic variation on dialysis days in comparison to non-dialysis days. Dialysis days were defined as 24 hours from the start of the haemodialysis session. Non-dialysis days were defined as 24 hours before the start of haemodialysis and with a gap of 24 hours from the start of the previous dialysis session.

Interstitial Glucose (IG) readings for each day were extracted after identifying the start time and 24 hour period from that point for the dialysis days. For the non-dialysis days, they were identified from the end point of the dialysis day for a 24 hour period from that point, or to the start of the next dialysis session, whichever was earlier.

All glycaemic variation indices were obtained using 'EasyGV excel spreadsheet version 8.8.2.R2' obtained with permission from Nathan R Hill, Oxford University.

The following table shows the number of days when the data were available for both subjects with diabetes and control subjects.

Group	Dialysis day	Non-dialysis day	Total
Diabetes	117	126	243
Control	16	19	35
Total	133	145	278

***Table 7.6**: shows the number of dialysis and non-dialysis days where data were available*

The IG data for each day were examined in detail to look for any missing data. IG levels were recorded every 5 minutes as programmed on CGM. Missing values for any expected time or length of time were identified and space was added in the data column equivalent to the length of missing data i.e. 1 row for one missing value. This was to enable interpolation of the data where possible using the software program, in order to calculate the indices of glycaemic variation.

288 readings of IG were expected for each day of complete recording. On the first day of the study week for each subject, a maximum of 264 readings were expected due to the 2 hour start up time from the initiation of CGM.

The table below shows the number of dialysis days and non-dialysis days that subjects experienced in each group during the whole of study period.

Group	**Dialysis days**	**Non-dialysis days**	**Total**
Diabetes	118	141	259
Control	16	19	35
Total	134	160	294

Table 7.7*: shows the number of dialysis and non-dialysis days in each group during study*

There were no available recordings or very minimal data for one dialysis day and 15 non-dialysis days in the diabetes group.

The IG recordings available in terms of number of readings and their percentage of expected numbers are shown below.

Group	**Expected number/day**	**Minimum recordings/day**	**Maximum recordings/day**	**Mean (Range) percentage of expected readings**
Diabetes	288	24	289	88.0% (8.3 to 100)
Control	288	116	292	92.3% (40.0 to 100)

Table 7.8*: shows the expected IG recordings and the available recordings*

In the combined cohort, 261 days of recordings out of 278 days in total had 50% or more readings available. This was higher in the control group with 34 out of 35 days having 50% or more compared to 227 out of 243 days in the diabetes group.

Data were modified where the recording read ‘Low’ or ‘High’ for analysis purpose. ‘Low’ readings were replaced by 2.1 and ‘High’ readings were replaced by 22.3 mmol/l. This

would have affected the estimation of average glucose levels by reducing the average in the episodes where ISF glucose was above the measurable limit and increasing the average in the episodes where ISF glucose was below the limit.

7.3.1 Variation in subjects with diabetes

7.3.1.a Mean glucose levels

The mean glucose levels for an individual day of recording was obtained using 'EasyGV' excel spreadsheet. The mean of these levels on dialysis days was compared to mean of mean glucose levels on non-dialysis days.

The mean glucose levels varied from 3.6 to 22.3 mmol/l for all days (n=243). The overall mean was 11.6±3.7 with no difference between dialysis days (11.5±3.8, n=117) and non-dialysis (11.7±3.6, n=126) days.

7.3.1.b Standard deviation

The SD of glucose levels were obtained similarly using 'EasyGV' spreadsheet. The SDs on the dialysis days were compared to non-dialysis days.

The SD of glucose levels for the dialysis days was significantly higher (3.9±1.6) compared to the non-dialysis days (3.5±1.5) ($p<0.05$), suggesting greater IG variation on dialysis days (figure 7.5).

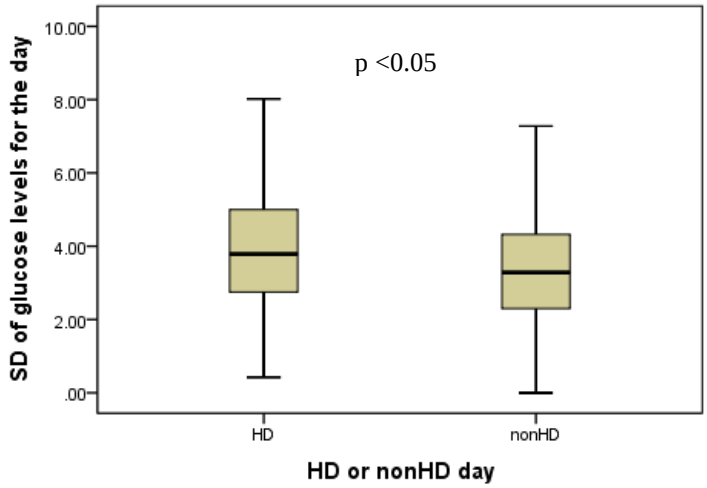


Figure 7.5*: shows the range and median of SD of glucose levels on dialysis and non-dialysis days in diabetes subjects calculated using 'EasyGV' excel spreadsheet.*

7.3.1.c Mean amplitude of glycaemic excursion (MAGE)

MAGE was calculated using 'EasyGV' excel spreadsheet and variation on dialysis days were compared to non-dialysis days.

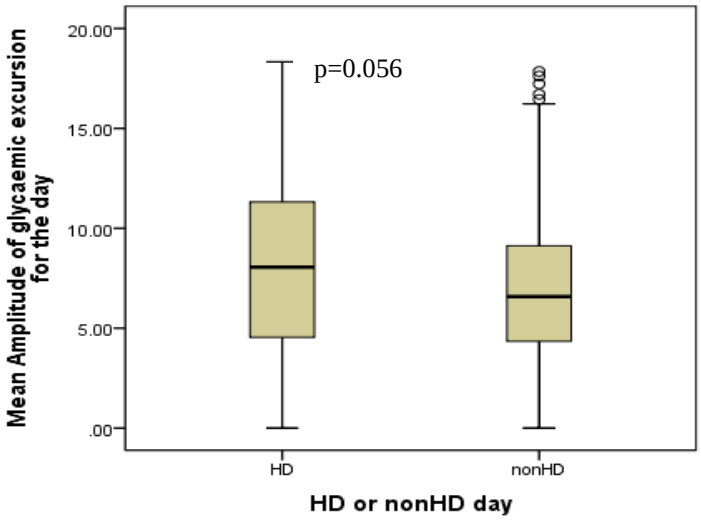


***Figure 7.6**: shows the median and range of MAGE for dialysis and non-dialysis days in diabetes subjects using EasyGV excel spreadsheet*

The mean amplitude of glycaemic excursion was higher on dialysis days (8.1±4.6, n=105) compared to that on non-dialysis days but was not statistically significant (7.0±3.9, n=116) (p=0.056).

Type 1 DM vs Type 2 DM

Data were examined for any differences in mean IG, SD and MAGE on dialysis and non-dialysis days between type 1 and type 2 diabetes groups. Glycaemic excursion (MAGE) is significantly higher in type 1 diabetes group compared to type 2 group on dialysis days but not on non-dialysis days (Table 7.9). However the mean IG level and SD of mean IG levels are significantly higher in type 1 diabetes group, on both dialysis and non-dialysis day.

	Glycaemic variation indices	Type 1	Type 2	p=
HD days	**Mean**	12.3±3.7(n=52)	10.7±3.7 (n=60)	0.028
	SD	4.3±1.7 (n=52)	3.5±1.3 (n=60)	0.010
	MAGE	9.2±5.1 (n=44)	7.3±4.1 (n=56)	0.044
Non-HD days	**Mean**	13.0±3.9(n=53)	10.8±3.2 (n=67)	0.001
	SD	3.8±1.5 (n=53)	3.2±1.4 (n=67)	0.024
	MAGE	7.5±4.4 (n=47)	6.6±3.6 (n=64)	0.216

Table 7.9: *shows the difference in Mean IG, SD and MAGE on HD and nonHD days between type 1 and type 2 diabetes groups. Statistical significance was tested for using 'Summary Independent-Samples T test'.*

C-Peptide negative vs minimally positive

Data were examined to for any difference in the indices of glycaemic variation between C-peptide negative and minimally positive groups as defined for study purpose. Mean and SD levels were significantly higher in C-Peptide negative groups on HD as well as nonHD days. However the glycaemic excursion (MAGE) was not significantly higher in C-Peptide negative group on both HD and nonHD days compared to C-Peptide minimally positive group (Table 7.10).

	Glycaemic variation indices	C-peptide negative	C-peptide Minimally positive	p=
HD days	Mean	12.2±3.8 (90)	9.0±2.2(27)	0.000
	SD	4.1±1.6 (90)	3.1±1.3 (27)	0.003
	MAGE	8.5±4.6 (80)	6.7±4.1 (25)	0.076
Non-HD days	Mean	12.5±3.7(91)	9.5±2.3 (35)	0.000
	SD	3.7±1.5 (91)	2.8±1.3 (35)	0.003
	MAGE	7.3±4.1 (81)	6.2±3.2 (35)	0.142

Table 7.10: *shows the difference in the Mean IG, SD and MAGE, on HD and nonHD days between C-Peptide negative and C-Peptide minimally positive diabetes groups. P values were derived using 'Summary Independent-Samples T test'.*

7.3.1.d Hypoglycaemia and Hyperglycaemia

Data were examined for the duration of time spent in hypoglycaemia and hyperglycaemia on dialysis days compared to non-dialysis days.

The following table shows the percentage of time spent in hypoglycaemia, euglycaemia and hyperglycaemia on dialysis days and non-dialysis days.

Glycaemic state	Dialysis (n=92)	Non-Dialysis (n=114)	P=
Hypoglycaemia	5.9±15.4 (0.0 – 96.9)	3.3±12.4 (0.0 – 98.9)	0.187
Euglycaemia	2.7±2.8 (0.0 – 15.3)	3.8±4.1 (0.0 – 19.4)	<0.05
Hyperglycaemia	91.4±15.9 (0.0 – 100)	92.9±12.8 (0.0 – 100)	0.464

Table 7.11*: Shows the difference in time spent in hypoglycaemia, euglycaemia and hyperglycaemia on dialysis and non-dialysis days, as percentage of time recorded in the 24hr period. Results expressed as mean±SD (range)*

The durations of hypoglycaemia, euglycaemia and hyperglycaemia in minutes were converted to the percentage of the time duration for which IG levels were available. The time duration in minutes of hypoglycaemia and hyperglycaemia varied on individual days depending on the duration of recording available.

There was no significant difference in the mean duration spent in hypoglycaemia or hyperglycaemia on dialysis days compared to non-dialysis days. However more episodes of hypoglycaemia were noted on dialysis days (35.9% vs 25.4%, $p<0.001$) and more episodes of hyperglycaemia were noted on non-dialysis days. Time spent in euglycaemia, however, was significantly less on non-dialysis days compared to dialysis days, although the overall duration was very small.

Mean duration was not available for the three variables on 25 dialysis days and 12 non-dialysis days.

Out of 92 dialysis days where mean duration was calculated, 59 (64.1%) days had no hypoglycaemic episodes. Out of 114 non-dialysis days where mean duration was available, 85 (74.6%) had no hypoglycaemic episodes. Prolonged episodes of hypoglycaemia lasting more than 50% of recorded duration occurred on 3 of 92 dialysis days compared to 2 out of 114 non-dialysis days.

13 out of 92 (14.1%) dialysis days showed persistent hyperglycaemia, compared to 16 (14.0%) out of 114 non-dialysis days.

7.3.2 Variation in subjects without diabetes

7.3.2.a Mean IG levels

Mean IG levels were obtained for dialysis and non-dialysis days using the same software in control group subjects.

Data were available for 16 dialysis days and 19 non-dialysis days. The mean of the individual day means on dialysis days was compared to non-dialysis days.

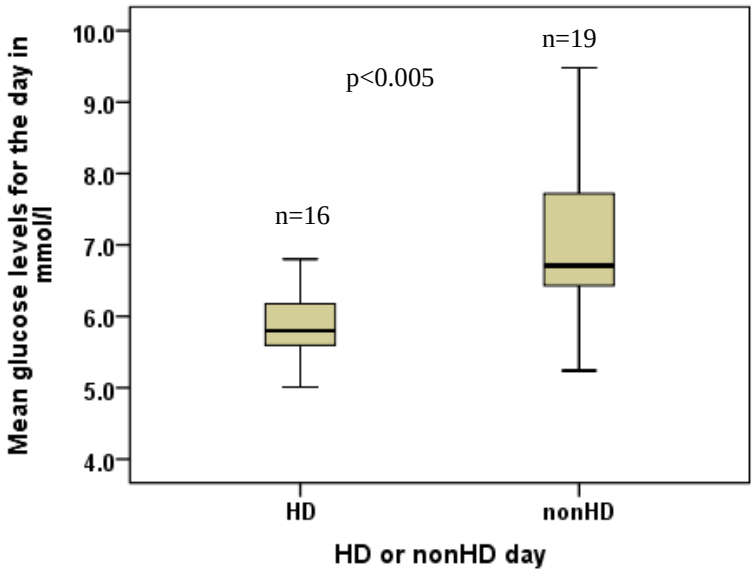


***Figure 7.7**: shows the median and the range of mean IG levels for dialysis and non-dialysis days in control subjects*

The overall mean of mean IG levels on dialysis days (5.9±0.7 mmol/l) was significantly lower than mean of mean IG levels on non-dialysis days (7.1±1.3 mmol/l) ($p<0.005$)

7.3.2.b SD of IG levels

Standard deviation for each day was calculated using the EasyGV software. Mean SD on dialysis days was compared to non-dialysis days.

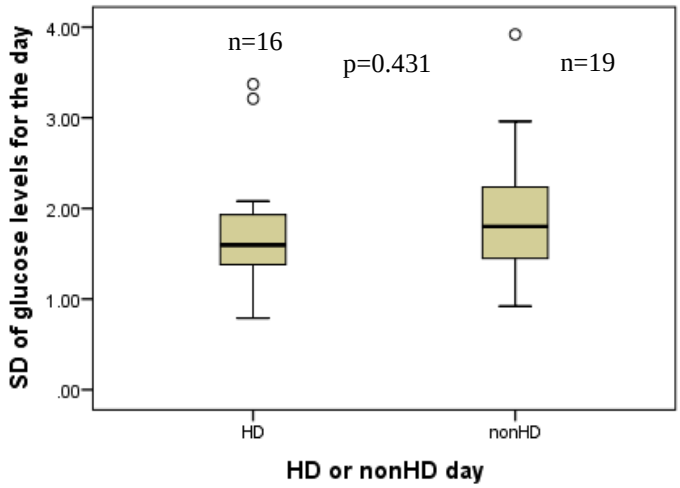


***Figure 7.8**: shows the median and range of SD of IG levels on dialysis and non-dialysis days in control subjects.*

The mean of SD of IG levels on dialysis days (1.76±0.68) was not significantly different from that of non-dialysis days (1.95±0.73) (p=0.431)

7.3.2.c Mean Amplitude of Glycaemic Excursion

MAGE for individual dialysis days (n=15) and non-dialysis days (n=19) was calculated using EasyGV excel spreadsheet.

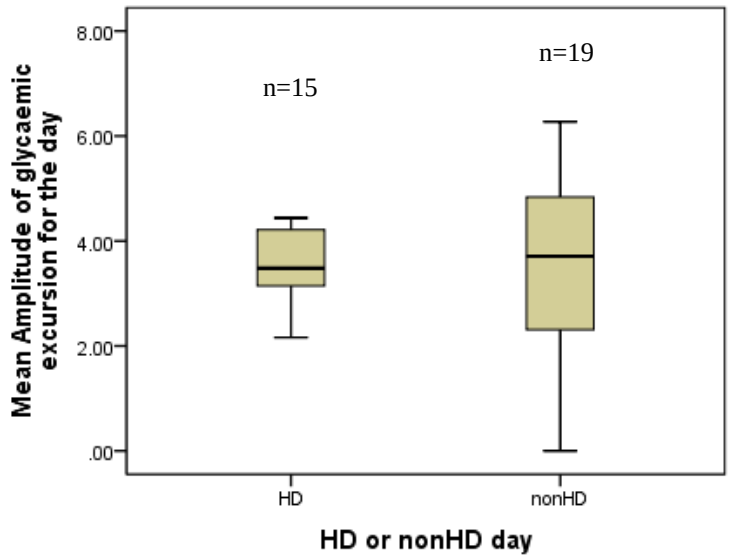


***Figure 7.9**: shows the median and the range of MAGE for dialysis and non-dialysis days in control subjects calculated using EasyGV excel spreadsheet*

The overall mean of MAGE on dialysis days (3.8±1.1) was not significantly different from that on non-dialysis days (3.7±1.7).

7.3.2.d Hypoglycaemia and Hyperglycaemia

Out of 16 dialysis days, both hypoglycaemic and hyperglycaemic episodes were recorded on only one day. This showed 54.1% of time being spent in hypoglycaemia and 38.0% of time spent in hyperglycaemia, with a euglycaemic period of 8.0%.

Out of 19 non-dialysis days, 3 days had documented hyperglycaemia. There was no hypoglycaemia on these days. The time in hyperglycaemia ranged between 89.8% and 97.5%.

Hypoglycaemia and hyperglycaemia are discussed in more detail in the following chapters.

7.3.3 Comparison of glycaemic variation between subjects with diabetes and the control group

The individual glycaemic variation indices were compared between the two groups for dialysis and non-dialysis days separately.

7.3.3.a Difference on dialysis days

As expected, the difference in the glycaemic variation indices including mean IG level, MAGE and SD on both the dialysis days and non-dialysis days were significantly higher in the diabetes group compared to the control group. These are shown in the table below.

		Diabetes group	**Control group**	**P=**
Overall Mean IG (mmol/l)	Dialysis day	11.5±3.8 (n=117)	5.9±0.7 (n=16)	p<0.0001
	Non-dialysis day	11.7±3.6 (n=126)	7.1±1.3 (n=19)	p<0.0001
Mean SD	Dialysis day	3.9±1.5 (n=117)	1.8±0.7 (n=16)	p<0.0001
	Non-dialysis day	3.4±1.4	1.9±0.7	p<0.0001
Mean MAGE	Dialysis day	8.1±4.6 n=105)	3.8±1.1 (n=15)	p<0.0001
	Non-dialysis day	7.0±3.9 (n= 116)	3.7±1.7 (n=19)	p<0.0001

***Table 7.12**: Shows three indices of glycaemic variation in the diabetes and control groups. Data are expressed as the mean ± SD for each value.*

As expected, all three indices of glycaemic variation were significantly higher in the diabetes compared to the control group.

Chapter 8: Hypoglycaemia

Hypoglycaemia was defined as an IG level ≤ 3.5mmol/L for this study. All periods of hypoglycaemia lasting more than 20 minutes occurring during the study periods were noted. Hypoglycaemia occurring in relation to dialysis was defined as episodes occurring during the 4 hour Pre-HD period, during HD and the 4 hour Post-HD period. Persistent hypoglycaemia was defined as IG levels ≤3.5mmol/l without any reading above 3.5mmol/l for the whole length of the pre-HD, HD or post-HD periods.

Hypoglycaemia in relation to dialysis was calculated as percentage of time recorded, i.e. duration of hypoglycaemia / duration of recording for the given period x100. This was in view of the absence of recording for part of the time period analysed on some dialysis days.

8.1 Hypoglycaemia in diabetes subjects

8.1.1 Hypoglycaemia in relation to dialysis

The 15 subjects with diabetes underwent 117 cycles of HD during the study period. The selected pre-HD and post-HD duration was fixed at 4 hours (240 minutes). HD duration was dependent upon the length of dialysis of individual cycles which varied. Over half (55.6%) of HD cycles were of 4 hours duration. However 44 (37.6%) HD sessions were shorter than 240 minutes ranging from 120 to 230 minutes. 5 cycles were slightly longer at 245 to 250 minutes and 3 cycles were of 420 minutes in one subject who was changed from usual daytime dialysis to overnight dialysis by his 2nd study week. Hence data were noted for the given HD periods based upon the length of the dialysis session. The total duration of CGM recording available for every session (PreHD/HD/PostHD) was noted. The percentage of available recording to expected recording was calculated.

Period	Expected number of recordings	Actual Number of recordings	Percentage (Mean (SD)) of expected period recorded	Minimum length of recording (minutes)	Maximum length of recording (minutes)
Pre-HD	83	82	87.1 (26.2)	10	240
HD	117	107	84.0 (26.4)	40	420
Post-HD	117	114	92.2 (17.9)	10	240

Table 8.1*: Shows the number of periods of pre-HD, HD and post-HD expected to be recorded based upon time of initiation of CGM and number of HD cycles in the study period. 1st column shows the different periods on dialysis days, 2nd column shows the number of periods expected to be recorded, column 3 shows the number of periods where any recording was available, column 4 shows the percentage of period recorded in relation to expected length, column 5 shows the minimum length of recording available and column 6 shows the maximum length of recording available.*

There was a wide variation in the length of available recordings of IG on CGM. Hence the periods of hypoglycaemia were analysed for duration in relation to recorded duration rather than expected duration. There were 7 episodes of hypoglycaemia occurring during pre-HD, 11 episodes during HD and 9 episodes during the post-HD period. The length of hypoglycaemia during these episodes is shown in table 2.

Period	**No. of episodes**	**Time length (minutes)**		**Median Time (mins)**	**Median time (Min-Max) Hypo duration in % of time recorded**
		Minimum	Maximum		
Pre-HD	7	5	155	45	19.0 (2.1 – 92.8)
HD	11	10	205	40	37.5 (8.3-72.7)
Post-HD	9	15	210	65	27.1 (6.4 – 87.5)

Table 8.2*: Shows the frequency of hypoglycaemia on dialysis days at pre-HD, HD and post-HD periods.*

The length of hypoglycaemia recorded during the Pre-HD, HD and Post-HD periods varied widely. The mean and median duration was longer during the Post-HD period compared to the HD and Pre-HD periods when the absolute duration in minutes was considered. However when the data were analysed in percentage of the duration recorded, hypoglycaemic episodes appeared to be longer during HD compared to the pre-HD and post-HD periods as seen in table 2. However, given the small number of events statistical tests for significance in the difference in duration of hypoglycaemia in these periods were not undertaken.

8.1.2 Hypoglycaemia on dialysis and non-dialysis days

Occurrence of hypoglycaemic episodes on dialysis days (24hrs from starting of HD), and on non-dialysis days (24hours from start of previous HD or to the start of next HD) were examined for the difference in frequency and patterns of hypoglycaemia.

71 episodes of hypoglycaemia were recorded in 13 of 15 diabetic subjects, with 2 subjects having no episodes during the study period. The frequency between subjects varied from 1 to 12 episodes.

The hypoglycaemic episodes were nearly twice as common on dialysis days compared to non-dialysis days ($p<0.001$) (figure 8.1).

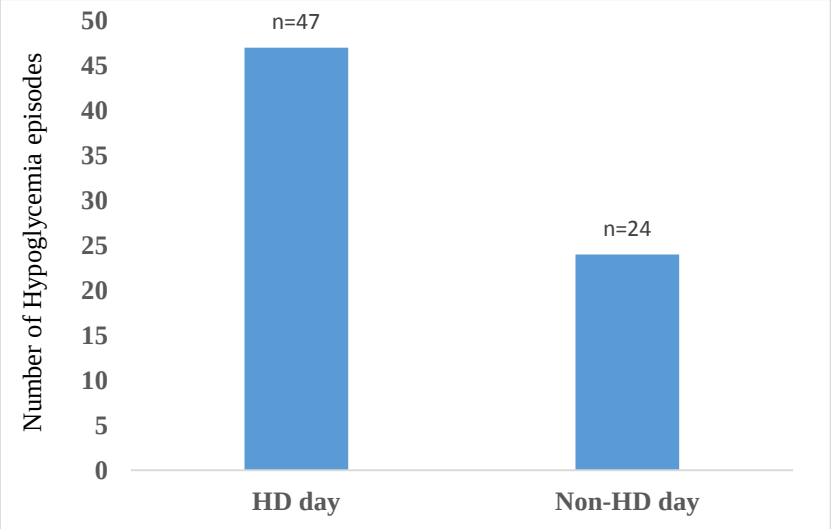


Figure 8.1*: Shows the frequency of occurrence of hypoglycaemia on dialysis vs non-dialysis days*

There was no difference in the mean duration of episodes between dialysis days and non-dialysis days ($p=0.982$) (figure 8.2). Median duration was 75minutes (20 to 420) on HD days compared to 67.5 minutes (20 to 320) minutes on non-HD days.

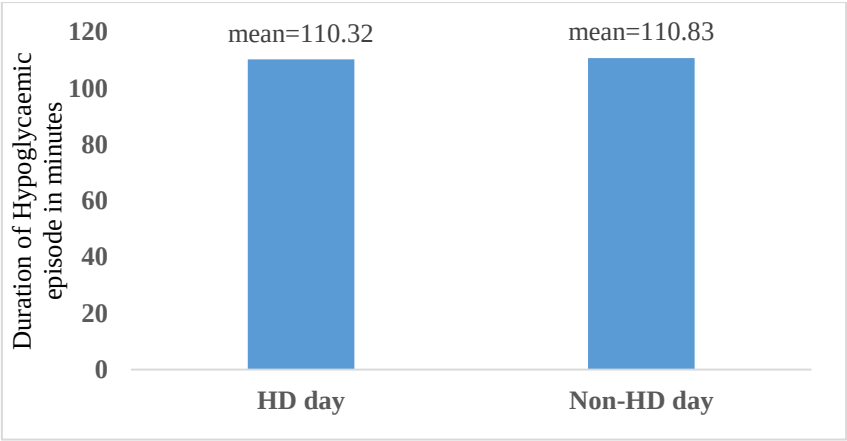


***Figure 8.2**: Mean duration of hypoglycaemic episodes on dialysis and non-dialysis days.*

The longest period of hypoglycaemia was recorded on an HD day lasting 420 minutes compared to 320 minutes on a non-dialysis day. There were 4 episodes of persistent hypoglycaemia lasting from 325 to 420 minutes on dialysis days. However excluding these outliers, the duration of hypoglycaemia appeared longer on non-HD day (figure 8.3).

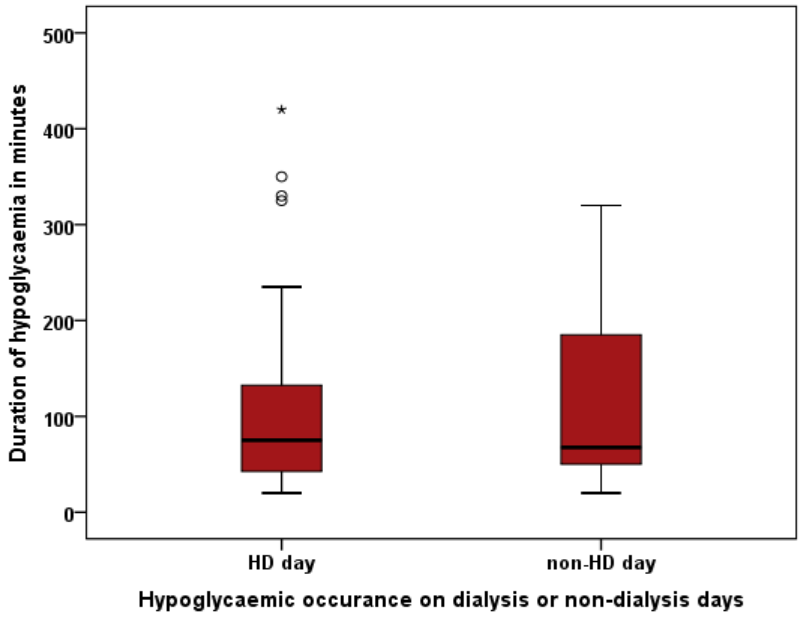


***Figure 8.3**: Shows the range, inter-quartile range and median of hypoglycaemic episodes in minutes occurring on dialysis and non-dialysis days. There are 4 outlier episodes on dialysis days lasting 325, 330, 350 and 420 minutes.*

8.1.3 Diurnal variation in occurrence of hypoglycaemia

The episodes of hypoglycaemia were studied for the time of occurrence for daytime and nocturnal frequency. Day time was defined as 7AM to 11PM and night time as 11PM to 7AM. Episodes continuing from day to night or vice versa were considered as either daytime episodes or nocturnal episodes based upon the maximum duration of that episode occurring in day or night time respectively.

The frequency of hypoglycaemia was higher during daytime than night time ($p<0.001$) (figure 8.4).

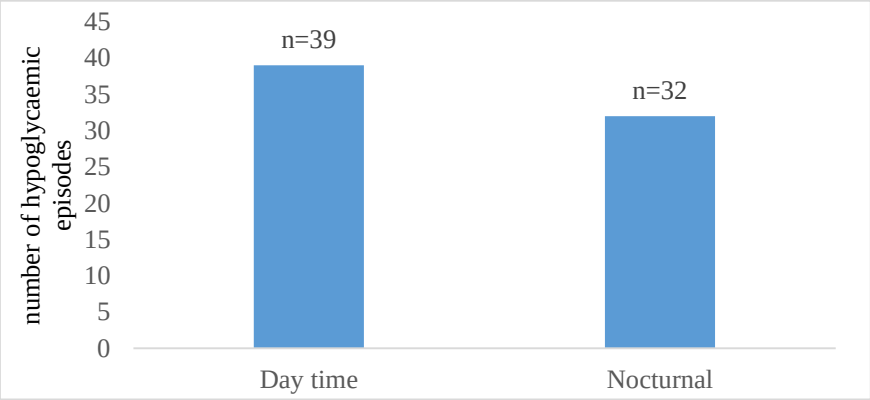


***Figure 8.4**: Frequency of episodes of hypoglycaemia occurring during daytime (7AM till 11PM) and night time (11PM till 7AM).*

The duration of hypoglycaemia was examined for differences between day time and nocturnal episodes. Though the mean duration of hypoglycaemia was longer during night time compared to day time episodes (mean ± SD: 119.84 ± 100.92 vs 102.82 ± 82.25), it was not statistically significant ($p=0.436$). The longest duration recorded during day time was 420 minutes compared to 350 minutes at night (figure 8.5).

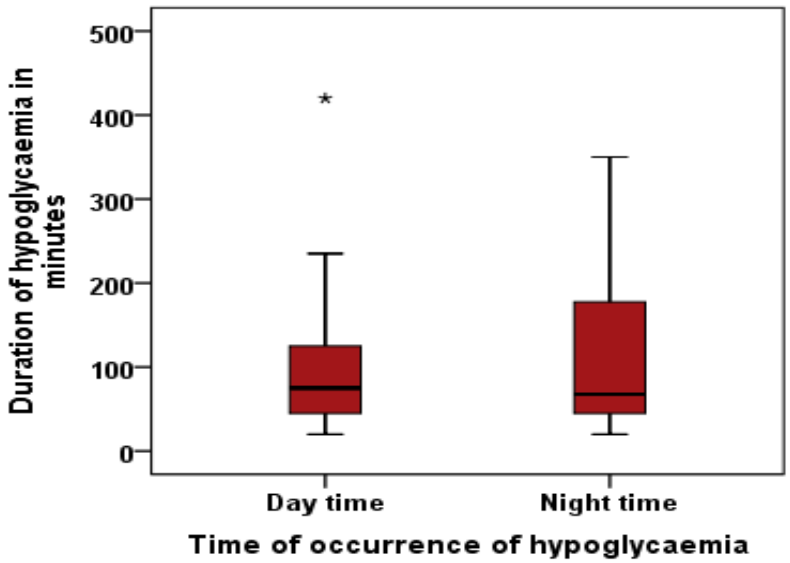


Figure 8.5*: Shows the range, interquartile range and median of hypoglycaemic episodes occurring during day time (7AM to 11PM) and night time (11PM to 7AM).*

The duration of hypoglycaemic episodes was examined for any difference in diurnal occurrence between dialysis and non-dialysis days.

The data were further analysed to look at the difference in frequency and duration of hypoglycaemia during day and night on HD and non-HD days. There were only few more episodes of hypoglycaemia during the day compared to night time on both dialysis and non-dialysis days (Table 8.3)

Dialysis Day Time of the Day	HD day	Non-HD day	Total
Day time	25	14	39
Night	22	10	32
Total	47	24	

Table 8.3*: shows the frequency of occurrence of these episodes in relation to time and dialysis.*

The difference in mean duration of the episodes in relation to time and dialysis was analysed. This was longest during the night time on non-dialysis days, though with a fewer number of episodes (Table 8.4).

	HD day	Non-HD day
Day time	103.0 ± 86.6 (20 - 420)	102.5 ± 76.9 (20 – 230)
Night time	118.6 ± 103.1 (20 – 350)	122.5 ± 101.1 (25 – 320)

Table 8.4*: shows the difference in duration [mean ± SD (range) in minutes] of hypoglycaemia in relation to time and dialysis.*

8.2 Hypoglycaemia in the control group

IG ≤3.5mmol/l was used as a definition of hypoglycaemia in the control group as in the diabetes group. All episodes of hypoglycaemia lasting 15minutes or more were recorded. 18 episodes of hypoglycaemia were noted in total occurring in all 5 participants in the control group. All recordings reading 'low' were converted to 2.1 for analysis purposes. This could have affected the mean IG level for that episode and the overall mean. 'Low' readings were recorded in one subject persistently for 3hrs 20 minutes during one episode and for 2hrs 25 minutes during another episode. In another subject, 'low' readings were recorded only for 10 and 20 minutes separately as part of a prolonged episode.

The duration of these episodes varied between 20 to 390 minutes with a mean duration of 103.6 minutes. Overall mean IG was 3.1±0.3 mmol/l (range: 2.4 to 3.4 mmol/l).

14 of these 18 episodes occurred on HD days, including HD and in the 24 hours from the start of HD. One of these episodes started in the pre-HD period and continued through the HD and in the post-HD periods. Hence this was counted as occurring on the HD day. Only 4 episodes occurred in the non-HD days. One episode on a HD day continued into the non-HD day. Due to a small number of events on overall and specifically on non-HD days, statistical significance was not calculated.

Hypoglycaemic episodes occurring on HD days were prolonged with a mean duration of 126±132 minutes (range 20 to 390). 5 of these episodes lasted 190, 200, 290, 345 and 390 minutes. Hypoglycaemic episodes on non-HD days were of shorter duration with an average duration of 25 mins (range 15 to 35 minutes).

Data were examined to analyse the time of occurrence of these episodes with day and night defined in the same way as for the diabetes group. 12 episodes occurred in the day and 6 episodes occurred in the night. Two of the prolonged episodes of 200 and 390 minutes occurred during the night and the rest occurred during the day. The average duration of these was not different between day and night (day vs night: 114±118 vs 116±151 minutes). However the average length in both periods is skewed due to fewer episodes of very prolonged hypoglycaemia.

Chapter 9: Hyperglycaemia

Hyperglycaemia was defined as an interstitial glucose level ≥ 13.0mmol/l on the CGM readings for the study purpose.

Occurrence of hyperglycaemia in relation to dialysis was looked at by examining the time spent with hyperglycaemia during a pre-HD period of 4 hours, the HD period and a post-HD period of 4 hours.

Duration of recording available for analysis for the above periods varied. Hence the percentage of recording available out of the expected duration was calculated. Hyperglycaemic duration was then calculated as percentage of available duration of recording.

9.1 Hyperglycaemia in diabetes subjects

There were 117 cycles of HD in the 15 diabetic subjects during the study period. Only 83 HD cycles were expected to have any recording for the Pre-HD period given that the initiation of the CGM recording was at the beginning of the first dialysis session. All 117 HD cycles were expected to have some recording for that period. However only 116 cycles were expected to have post-HD periods recorded (Table 1).

The time period examined for pre-HD and post-HD remained constant at 240 minutes (4 hours). However, the duration of HD varied between 120 to 420 minutes with a mean ± SD duration of 222.95 ± 44.93 minutes. One patient had 3 cycles of 420 minutes (7 hours) during his second study week after changing over from 4 hourly daytime cycles to 7 hourly overnight cycles.

Period	Expected number of cycles	Number of cycles with available recording	Ratio of available recording to expected length (mean ± SD)
Pre-HD	83	82	87.02 ± 26.2
HD	117	107	83.97 ± 26.4
Post-HD	116	114	92.15 ± 17.9

Table 9.1: *Shows the number of cycles expected to be recorded, number of cycles where any length of recording of IG is available and percentage of the available recording in relation to expected duration*

306 episodes of hyperglycaemia were noted. The average IG during these episodes was 16.2±2.3 mmol/l (range 13.0 to 22.1).

9.1.1 Hyperglycaemia in relation to dialysis

The frequency of hyperglycaemia was much higher during the post-HD period compared to the pre-HD and HD periods. The median duration of hyperglycaemia was also longer during the post-HD period. However compared to the HD period the median duration was much longer during the pre-HD and post-HD periods (Table 9.2).

Period	Frequency	Median duration (minutes)	Minimum duration (minutes)	Maximum duration(minutes)
Pre-HD	41	125	5	240
HD	29	50	5	240
Post-HD	65	140	10	240

Table 9.2: Shows the frequency, median, minimum and maximum duration of hyperglycaemia in relation to HD

Given the variation in the length of the available recording for these periods, the duration of hyperglycaemia was analysed as a proportion of the available recording. The proportion of the available recording in the hyperglycaemic range was similar during the pre and post-HD periods. This was much longer compared to the proportion of the hyperglycaemic periods during HD (Table 9.3).

Period	Median duration (in %)	Minimum duration (in %)	Maximum duration (in %)
Pre-HD	64.8	2.1	100
HD	29.2	2.8	100
Post-HD	64.6	4.2	100

Table 9.3: Shows the median and the range of proportion of the available recording during pre-HD, HD and post-HD periods spent in hyperglycaemia.

There were periods where IG persisted in the hyperglycaemic range in all three periods. The duration of hyperglycaemia was significantly longer in the pre HD compared to the HD period (p= 0.002). Similarly, the duration of hyperglycaemia was significantly longer in the post-HD compared to HD period (p=<0.001).

9.1.2 Hyperglycaemia in comparison between dialysis and non-dialysis days

306 episodes of hyperglycaemia lasting 20minutes or more were noted in all subjects.

A greater number of episodes of hyperglycaemia occurred on the non-HD day (n=158) compared to the HD day (n=126) (51.6% vs 41.2%). 7.2% (n=22) of these episodes overlapped into both periods.

However the duration of the hyperglycaemic episodes in minutes on non-HD days was not statistically significant compared to the duration of episodes on HD days (257.9±277.3 vs 266.7±263.3, p=0.786).

The mean duration of hyperglycaemia as percentage of recording was longer on HD days compared to non-HD days (21±18.5 vs 19.6±20.6), but this difference was not significant (p=0.560).

9.1.3 Occurrence of hyperglycaemia in relation to time of the day

The majority of these episodes occurred in the day time with 188 recorded in the day compared to 50 episodes at night. 68 episodes were spread through both periods, either starting in the day time and continuing into night, or vice versa.

The percentages of recorded time spent in hyperglycaemia during day, night and combined periods are shown in the table below.

Time of occurrence	N=	Mean duration (in %)	SD (in %)
Day (7AM to 11PM)	187	14.1	14.9
Night (11PM to 7AM)	50	17.3	14.1
Combined	67	43.0	20.2

Table 9.4*: shows the duration of hyperglycaemia as the percentage of time recorded in relation to time of occurrence.*

The duration of hyperglycaemic episodes in minutes were longer during the night than day (mean±SD: 203.9±154.3 vs 169.2±146.0). However this was not significant (p=0.141). The difference in the proportion of recording in hyperglycaemic range during night and day was also not significant (p=0.193).

9.2 Hyperglycaemia in control group

IG ≥13.0mmol/l was set as a definition of hyperglycaemia in the control group in order to match the criteria set for the diabetes group. All episodes with IG ≥13.0mmo/l lasting 20 minutes or more were analysed.

9 episodes of hyperglycaemia were recorded in the control group occurring in 4 of 5 subjects. The frequency of episodes was 1 - 5 episodes/patient in one week.

The average of mean IG during these episodes was 14.8±1.1 mmol/l (range of means: 13.8 to 17.0). The mean duration was 76.6±51.1 minutes (range: 20 to 195). 7 out of 9 episodes

lasted 60 minutes or more, ranging from 60 to 195 minutes. There was no 'High' reading recorded in any control patient.

7 out of 9 episodes were recorded on a non-HD day and 7 out of 9 episodes occurred during daytime. Out of 2 nocturnal episodes, one occurred on an HD day and another one on a non-HD day.

Due to the small number of events, the relation between time of occurrence of hyperglycaemia and dialysis could not be correlated. Statistical significance could not be calculated for the same reason.

Chapter 10: Changes in serum electrolyte levels

Blood samples were obtained at the start, midway and at the end of HD in all study patients during all of the HD sessions in a week. Blood samples were obtained from the diabetes patients in their first study week.

Blood samples were analysed for potassium, calcium and magnesium.

There were 46 HD cycles in the diabetes group in the first week of study and 16 cycles in the control group. Samples were obtained only during the three HD cycles in patients in both groups as planned. The number of available results for pre-HD, HD and post-HD samples in both groups is shown in the table below.

	Diabetes group	Control group
No. of HD cycles Studied	46	16
No. of pre-HD results available	43	15
No. of mid-HD results available	44	15
No. of post-HD results available	44	15

Table 10.1*: shows the number of samples obtained at different time points during HD sessions*

Changes in serum electrolyte levels from the start of HD to end of the HD were analysed. Data were examined for differences in serum levels in the first half and second half of the dialysis period. Paired-sample T test was used to examine the change in first half and second half of HD, in all subjects where paired samples were available for the respective periods.

Data were examined for any difference in the level of electrolyte levels at each time point between the three HD cycles of the week using One-way ANOVA. Data were also examined for any difference between diabetes and control groups at each time point for all HD cycles together using One-way ANOVA.

10.1 Changes in serum potassium

The serum potassium level reduced during all HD sessions in all subjects (Fig 10.1). The majority of the drop in serum level happened in the first half of the dialysis session in all subjects together (table 10.2). A similar pattern of reduction was seen in both the groups (Fig 10.2 & 10.3). One subject in the diabetes group was hypokalaemic at the start of a session and remained so without any change throughout that session.

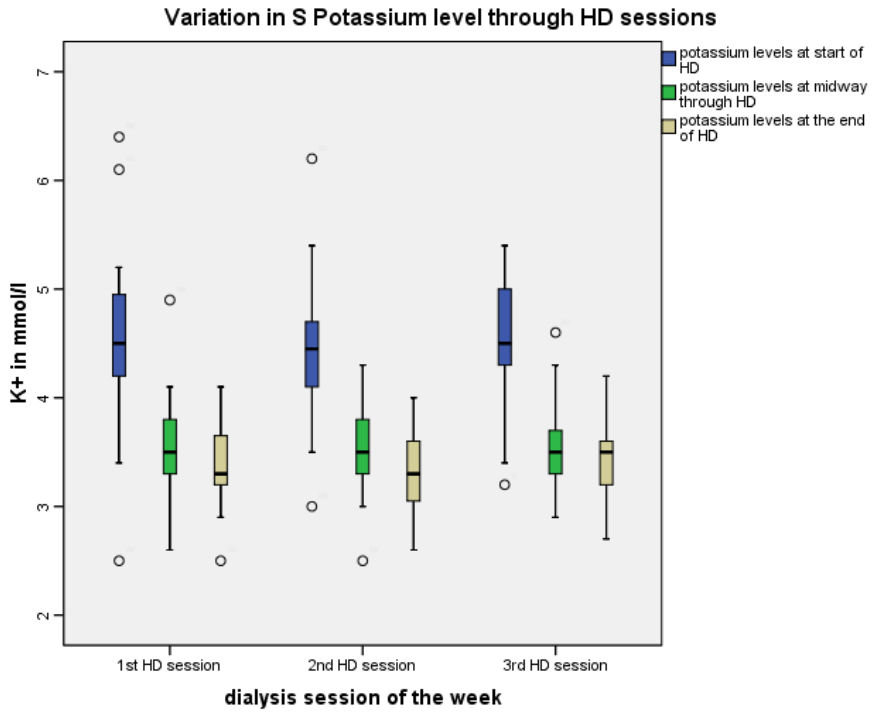


***Figure 10.1**: Box plot shows the median and inter-quartile range in the serum potassium levels in all subjects at three time points in 1st, 2nd and 3rd HD session separately.*

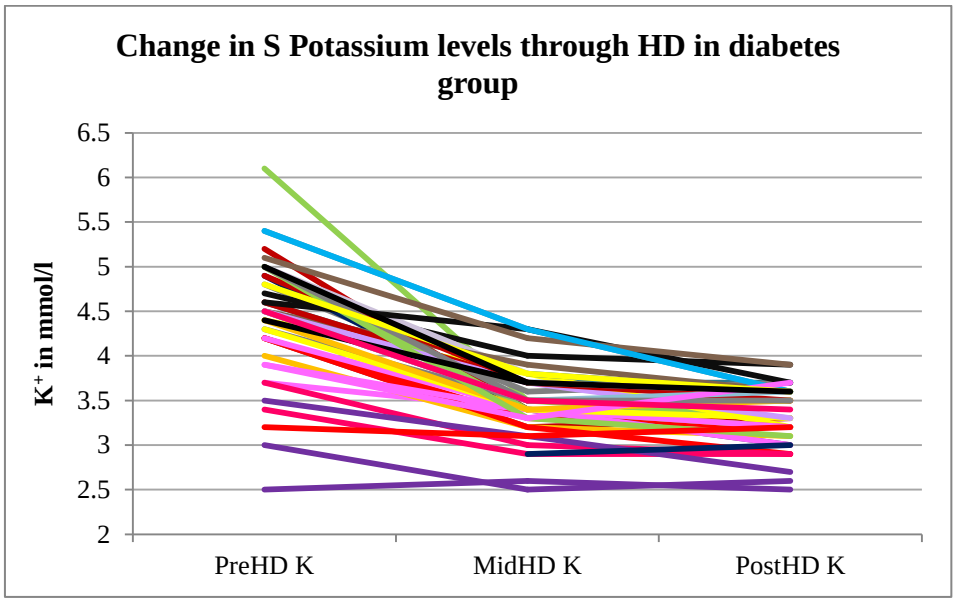


***Figure 10.2**: shows the change in serum potassium levels in individual HD sessions in the diabetes group. Each patient is represented by single colour.*

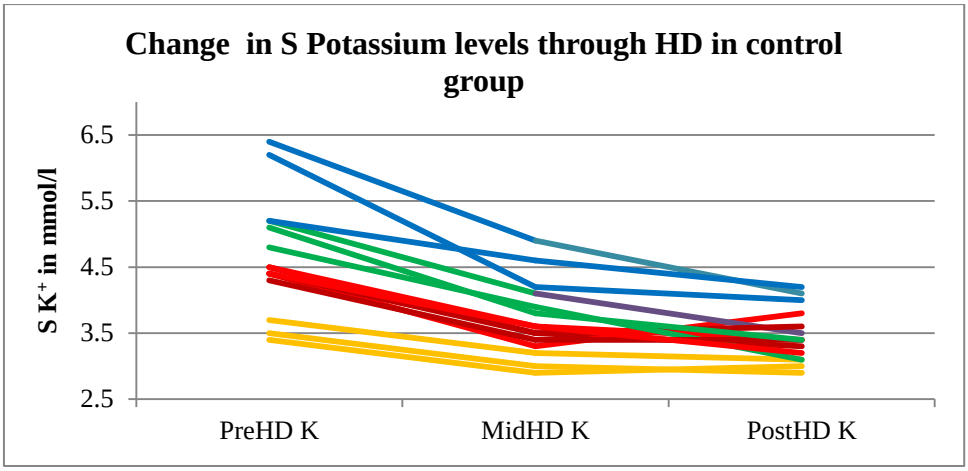


***Figure 10.3**: shows the change in serum potassium levels in individual HD sessions in the control group. Each subject represented by single colour (Sub 32- brown, Sub 35- red, Sub 36- orange, Sub 37- green, Sub 39- blue)*

Paired samples	Mean ± SD (mmol/l)	P=
PreHD vs MidHD (n=57)	4.5 ± 0.7 vs 3.5 ± 0.4	<0.001
MidHD vs PostHD (n=58)	3.5 ± 0.4 vs 3.4 ± 0.3	<0.001
PreHD vs PostHD (n=58)	4.5 ± 0.7 vs 3.4 ± 0.3	<0.001

***Table 10.2**: shows the difference in mean S Potassium levels in each half of HD and in total in all subjects derived using Paired –samples T test.*

The difference in serum levels at these three time points between the 1st, 2nd and 3rd HD sessions were not significant (start of HD p=0.814, midway p=0.941, end of HD p=0.634).

Mean ± SD levels in the two groups are shown in the table 10.3. There was no significant difference in the levels at any time point between the groups.

Time point	Diabetes group (mmol/l)	Control group (mmol/l)	p=
K^+ at start of HD	4.4±0.7 (n=42)	4.7±0.9 (n=15)	0.288
K^+ at middle of HD	3.5±0.4 (n=43)	3.7±0.6 (n=15)	0.123
K^+ at end of HD	3.3±0.3 (n=43)	3.5±0.4 (n=15)	0.204

***Table 10.3**: shows the serum potassium level in diabetes and control groups at three time points during HD derived using One-way ANOVA*

10.2 Changes in serum calcium

The reduction in serum calcium level during HD sessions varied between subjects. The median level reduced from the start to the end of HD (Fig 10.4). However there was a rise in serum calcium levels in some individuals in the second half of HD (fig 10.5 & 10.6). The difference was similar in both groups. The mean level was reduced significantly from

the pre-HD to the post-HD period (table 10.4). The reduction happened in the first half of the dialysis session. Similar pattern of reduction were seen in both the groups (Fig 10.5 & 10.6).

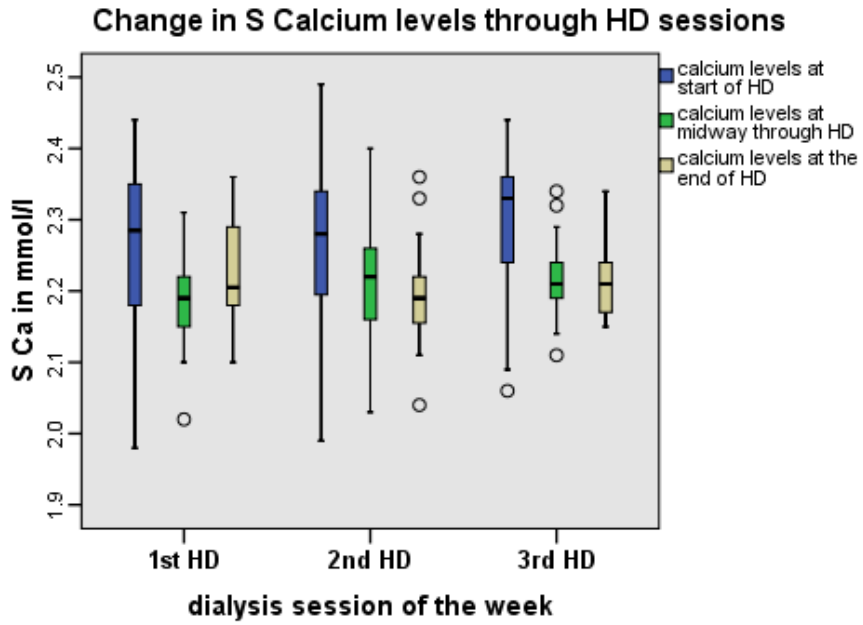


***Figure 10.4**: Box plot shows the median and inter-quartile range in the serum calcium levels in all subjects at three time points in 1st, 2nd and 3rd HD session separately.*

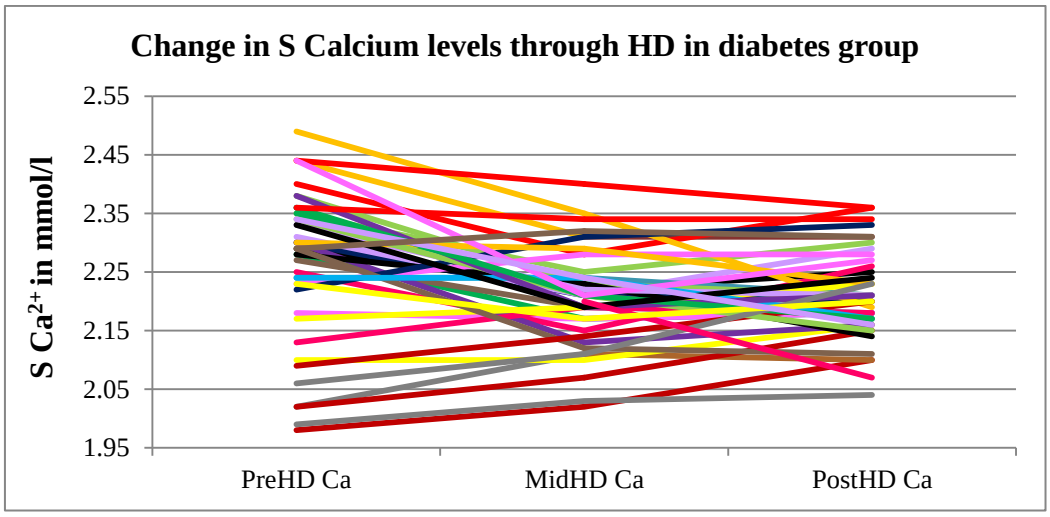


***Figure 10.5**: shows the change in serum calcium levels in individual HD sessions in the diabetes group. Each patient is represented by single colour*

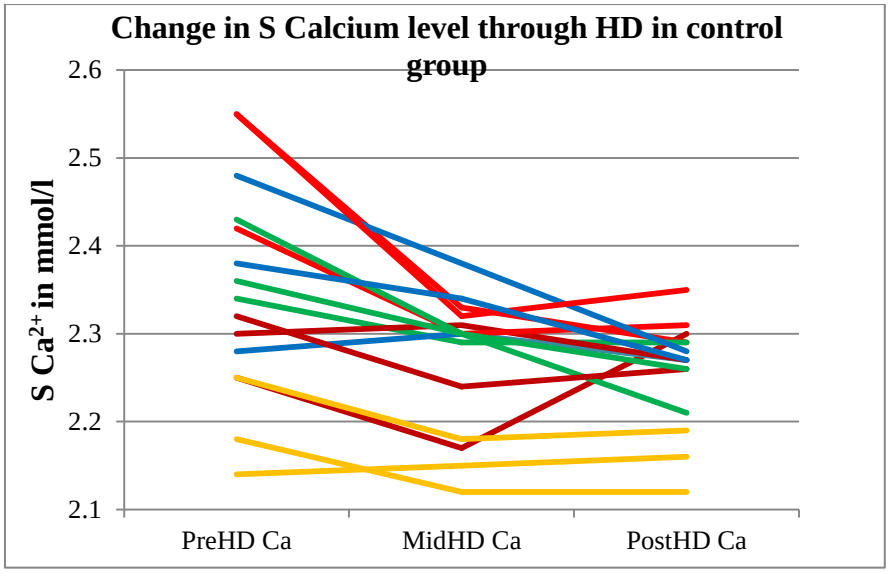


***Figure 10.6**: shows the change in serum calcium levels in individual HD sessions in the control group. Each subject represented by single colour (Sub 32- brown, Sub 35- red, Sub 36- orange, Sub 37- green, Sub 39- blue)*

Paired samples	Mean ± SD	P=
PreHD vs MidHD (n=57)	2.29 ± 0.13 vs 2.22 ± 0.08	<0.001
MidHD vs PostHD (n=58)	2.22 ± 0.08 vs 2.22 ± 0.07	0.929
PreHD vs postHD (n=57)	2.29 ± 0.13 vs 2.22 ± 0.07	<0.001

***Table 10.4**: shows the difference in mean S calcium levels in each half of HD and in total in all subjects*

The difference in serum levels at these three time points between 1st, 2nd and 3rd HD sessions were not significant (start of HD p=0.533, midway p=0.328, end of HD p=0.571).

The calcium levels at each time point were significantly different in the diabetes group compared to the control group (table 10.5)

	Diabetes group (mmol/l)	**Control group (mmol/l)**	**P=**
Ca^{2+} at start of HD	2.26 ± 0.13	2.35 ± 0.12	0.033
Ca^{2+} at middle of HD	2.20 ± 0.08	2.27 ± 0.08	0.011
Ca^{2+} at end of HD	2.21 ±0.07	2.25 ± 0.06	0.037

Table 10.5: shows the serum calcium level in the diabetes and control groups at three time points during HD

10.3 Changes in Magnesium

Serum magnesium levels were reduced during HD significantly in all three HD sessions of the week (fig 10.7). The majority of the fall in the level occurred in the first half of the session (figures 10.8 & 10.9). However the reduction was significant in both halves of the session (table 10.6).

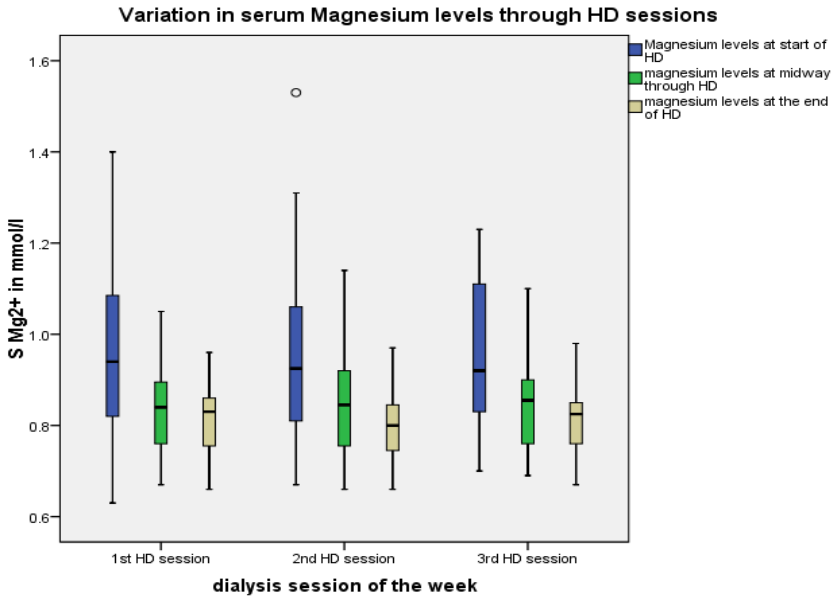


***Figure 10.7**: Box plot shows the median and inter-quartile range in the serum magnesium levels in all subjects at three time points in 1st, 2nd and 3rd HD session separately.*

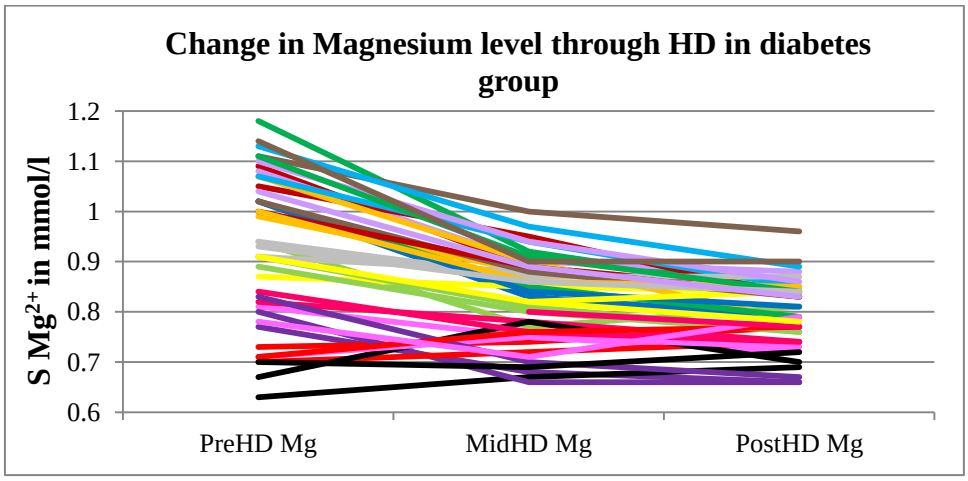


***Figure 10.8**: shows the change in serum magnesium levels in individual HD sessions in the diabetes group. Each subject represented by single colour*

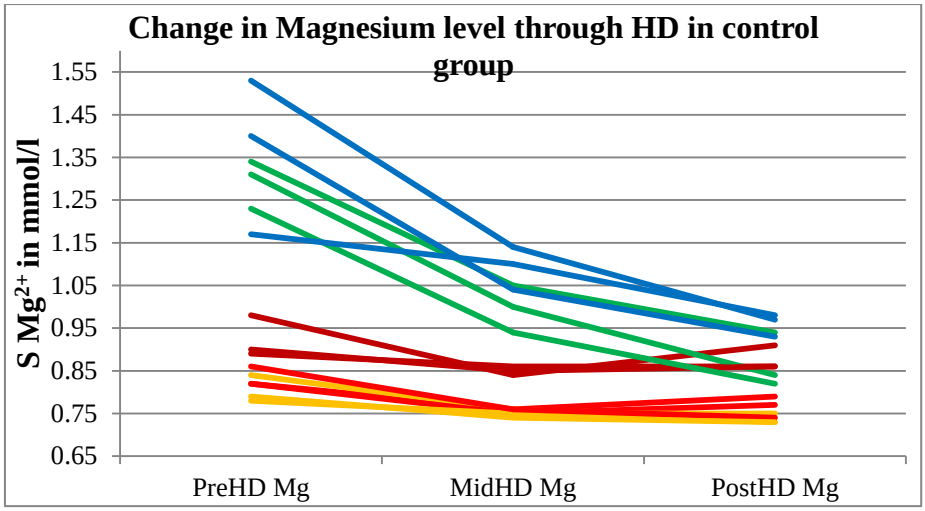


***Figure 10.9**: shows the change in serum Magnesium levels in individual HD sessions in the control group. Each subject represented by single colour (Sub 32- brown, Sub 35- red, Sub 36- orange, Sub 37- green, Sub 39- blue)*

	Mean ± SD (mmol/l)	**P=**
PreHD vs MidHD (n=57)	0.96 ± 0.19 vs 0.84 ± 0.11	<0.001
MidHD vs PostHD (n=58)	0.84 ± 0.11 vs 0.81 ± 0.07	<0.001
PreHD vs PostHD (n=59)	0.96 ± 0.19 vs 0.81 ± 0.07	<0.001

***Table 10.6**: shows the difference in mean S Magnesium levels in each half of HD and in total in all subjects*

The mean serum magnesium levels were not significantly different at any time point between the 1st, 2nd and the 3rd HD sessions (start of HD p=0.973, mid HD p= 0.970, end of HD p= 0.780).

However serum magnesium levels were significantly lower at the start and end of HD in the diabetes group compared to the control group (table 10.7) and non-significantly at middle of HD compared to the control group.

Paired samples	**Diabetes group (mmol/l)**	**Control group (mmol/l)**	**P=**
Mg^{2+} at start of HD	0.93 ± 0.15	1.04 ± 0.26	0.039
Mg^{2+} at middle of HD	0.83 ± 0.09	0.89 ± 0.14	0.051
Mg^{2+} at end of HD	0.79 ± 0.07	0.84 ± 0.09	0.046

***Table 10.7**: shows the serum magnesium level in diabetes and control groups at three time points during HD*

Chapter 11: Cardiac rate, rhythm and conductivity

11.1 Changes in Corrected QT (QTc) Interval.

QTc interval and its change in relation to dialysis and glycaemic variation was studied in 12 subjects with diabetes and all 5 subjects in the control group.

Two subjects in the diabetes group had a permanent pacemaker in situ, and thus could not participate in the study of cardiac rate and rhythm with 12 lead ECG and Holter monitor. One other subject in the diabetes group developed an allergic reaction to 2 different types of Holter monitor leads, hence was studied using only 12 lead ECGs during dialysis.

12 lead ECGs were recorded at the start of HD (1st), midway (2nd) and at the end of HD (3rd). The numbers of ECGs recorded in all subjects and in individual groups at these times are shown in the table below (table 11.1).

HD Session of the week	N=	Diabetes group	Control group
1st	53	38	15
2nd	54	39	15
3rd	54	39	15

***Table 11.1**: shows the numbers of ECGs recorded in the diabetes and control groups at different time points during HD, along with the number of HD sessions*

One ECG in Subject number 31 at the beginning of the 3rd HD session was not recorded due to unplanned and un-informed early start to dialysis.

11.1.1 Change in QTc during HD from 12 lead ECGs

The QT interval was measured on 12 lead ECGs manually and corrected for heart rate using Bezett's formula ($QTc = QT/\sqrt{RR}$). The Tangent method was used to measure QT interval.

The normal QTc interval was considered as per defined standards for each gender. For men it is up to 440 msec and women it is up to 460 msec.

Mean QTc was significantly higher in the diabetes group compared to the control group at the start, midway and at the end of HD (table 11.2). Some individuals with diabetes had very prolonged QTc at all three time points compared to the control group.

Time point during HD	**Diabetes Group**	**Control group**	**Diabetes vs control**	**All Subjects**
	Mean ± SD (Range) in msec	**Mean ± SD (Range) in msec**	**P=**	**Mean ± SD (Range) in msec**
Start of HD	465 ± 39 (391 – 569)	414 ± 30 (367 – 457)	<0.001	451 ± 43 (367 – 569)
Midway	478 ± 42 (415 – 647)	426 ± 37 (384 – 505)	<0.001	464 ± 47 (384 – 647)
End of HD	492 ± 46 (414 – 604)	451 ± 44 (406 – 526)	0.004	481 ± 49 (406 – 604)

Table 11.2*: shows the mean, SD & range of QTc interval in all subjects and in two groups determined from 12 lead ECGs.*

The change in QTc interval from start to midway, midway to end and from start to end of HD was significant in the whole cohort together (table 11.3, figure 11.1). The changes were significant in the diabetes group alone between each time point. There was no significant change in QTc from start to midway of HD in the control group; however there was a greater change in QTc in the second half of the HD compared to the diabetes group (table 11.4).

Paired samples	**All subjects**		
	Mean ± SD	**Mean change**	**p=**
Start to Midway	451 ± 43 vs 463 ± 47	12 ± 35	<0.05
Midway to end	464 ± 46 vs 481 ± 49	17 ± 26	<0.001
Start to end	451 ± 43 vs 480 ± 49	29 ± 36	<0.001

Table 11.3*: shows the change in the QTc (from 12 lead ECGs) between time points in all subjects.*

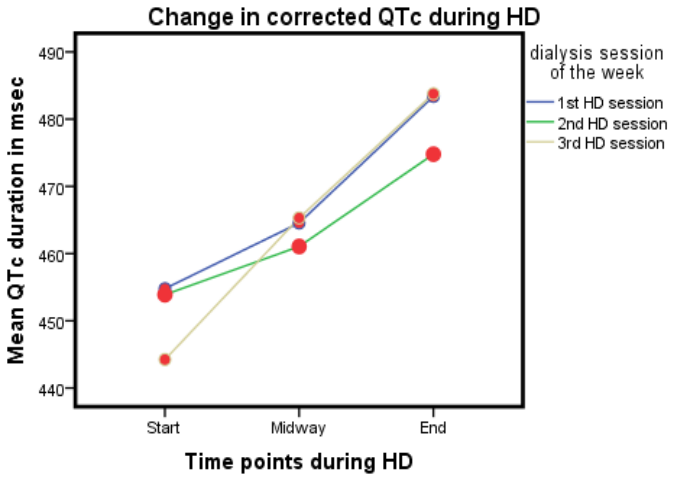


***Figure 11.1**: shows the change in mean QTc duration in **all subjects** (from 12 lead ECGs) during all HD sessions from start to midway & to end of HD*

Paired samples	**Diabetes group**			**Control group**		
	Mean ± SD	**Mean change**	**p=**	**Mean ± SD**	**Mean change**	**p=**
Start to midway	465 ± 39 vs 478 ± 42	-12.9 ± 37.7	<0.05	414 ± 30 vs 426 ± 36	-11.5 ± 29.1	0.147
Midway to End	478 ± 41 vs 492 ± 46	-13.9 ± 28.9	<0.01	426 ± 36 vs 451 ± 43	-24.8 ± 17.7	<0.001
Start to End	465 ± 39 vs 492 ± 47	-26.8 ± 37.2	<0.001	414 ± 30 vs 451 ± 43	-36.3 ± 33.0	<0.005

***Table 11.4**: shows the change in QTc (from 12 lead ECGs) between each time point*

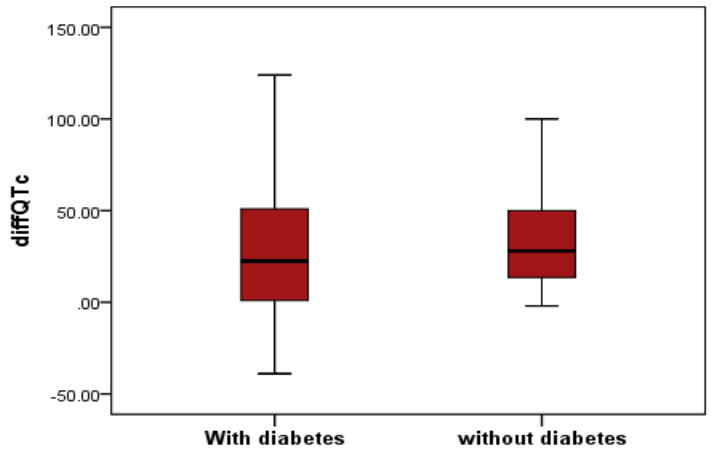


***Figure 11.2**: Shows the median and the range of difference in QTc interval (from 12 lead ECGs) from start to the end of HD in diabetes and control groups.*

The range of difference in QTc at the end of HD from the start was much wider in the diabetes group compared to controls. However in some subjects in the diabetes group, QTc

at the end of HD was shorter. In the control group, QTc at the end of HD was longer in all subjects and sessions (figure 11.2).

11.1.2 Difference in QTc duration between HD sessions

The data were examined for any difference in the QTc intervals at different time points of HD between 1st, 2nd and 3rd HD sessions in each group. The change in QTc in individual HD sessions between time points was analysed to look for any differences in the range of change in the 1st HD compared to subsequent HD sessions.

Diabetes group

The mean QTc was prolonged from start to mid and to the end of the HD in each session. The mean QTc at the three time points in the 1st HD session was not significantly different from the corresponding time points in the subsequent HD sessions (tables 11.15 & 11.6)

	1st HD	**2nd HD**	**3rd HD**
	Mean ± SD (range)	**Mean ± SD (range)**	**Mean ± SD**
Start of HD	468 ± 42 (408 – 569)	470 ± 45 (391 – 552)	458 ± 29 (422 – 518)
Midway	481 ± 36 (436 – 568)	476 ± 31 (424 – 584)	479 ± 56 (415 – 647)
End of HD	495 ± 49 (414 – 591)	484 ± 47 (426 – 584)	498 ± 44 (437 – 604)

Table 11.5: *shows the comparison of Mean QTc (from 12 lead ECGs) at different time points in 3 HD sessions in* ***the diabetes*** *group.*

Time point during HD	**P=**	
	Diff 1st to 2nd HD	**Diff 1st to 3rd HD**
Start of HD	0.926	0.467
Midway	0.723	0.922
End of HD	0.576	0.881

Table 11.6*: shows the level of significance in the difference in mean QTc (on 12 lead ECGs) at different time points of HD between 1st & 2nd and between 1st & 3rd HD in the* ***diabetes*** *group.*

The median and range of QTc duration during individual HD sessions at the three time points is shown in figure 11.3.

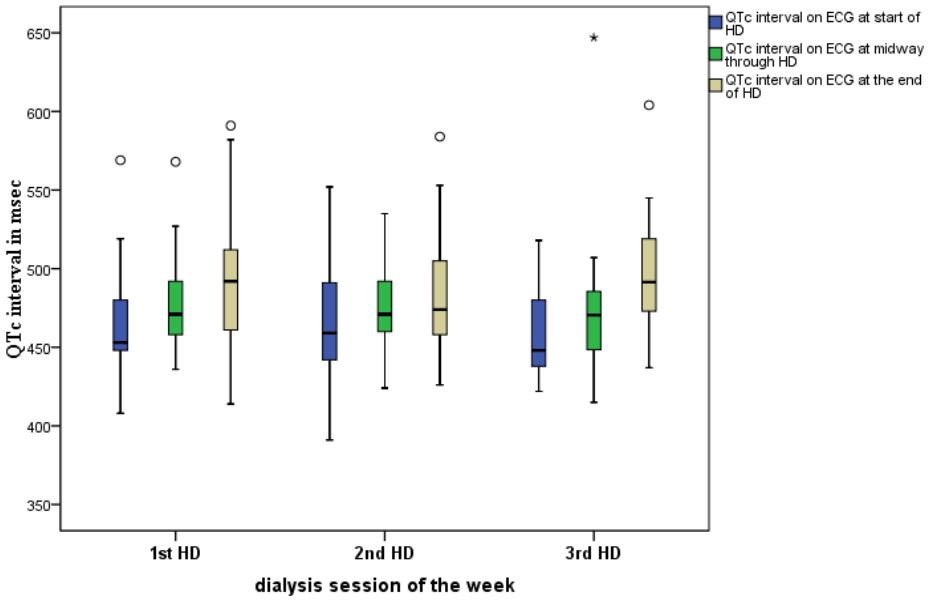


***Figure 11.3**: The median and range of change in QTc from start to midway and to end of HD (on 12 lead ECGs) in the **diabetes** group.*

Control group

There was prolongation of QTc from start to end of HD on 12 lead ECGs during all the three HD sessions in the control group (table 11.7), as in the diabetes group. The mean QTc at the three time points was not significantly different between the 3 HD sessions (table 11.8).

	1st HD	**2nd HD**	**3rd HD**
	Mean ± SD (range)	**Mean ± SD (range)**	**Mean ± SD (range)**
Start of HD	419 ± 30 (378 – 457)	412 ± 33 (369 – 457)	412 ± 33 (367 – 456)
Midway	422 ± 35 (387 – 473)	422 ± 35 (384 – 480)	433 ± 46 (399 – 505)
End of HD	453 ± 46 (406 – 518)	450 ± 48 (411 – 518)	449 ± 46 (408 – 526)

***Table 11.7**: Comparison of Mean QTc (from 12 lead ECGs) at different time points in 3 HD sessions in the **control** group*

Time point during HD	**P=**	
	Diff 1st to 2nd HD	**Diff 1st to 3rd HD**
Start of HD	0.721	0.718
Midway	0.993	0.698
End of HD	0.923	0.890

Table 11.8: shows the level of significance in the difference in mean QTc (on 12 lead ECGs) at different time points of HD between 1st & 2nd and between 1st & 3rd HD in the **control** group.

The median and range of QTc in individual HD sessions at the three time points is shown in figure 4.

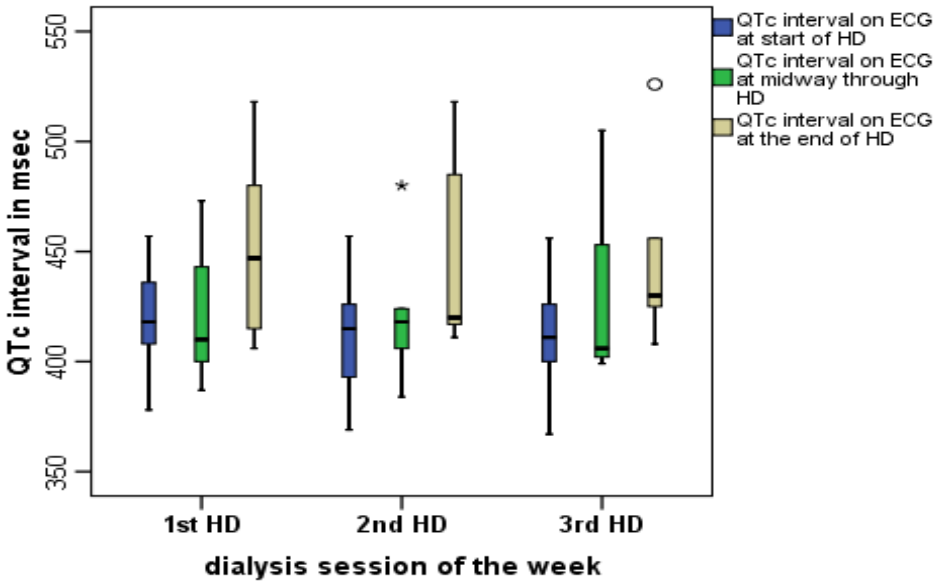


Figure 11.4*: Shows the median and range of change in QTc (on 12 lead ECGs) from start to midway and to end of HD in* ***control*** *group in individual HD sessions.*

11.1.3 Effect of change in electrolytes on QTc interval from 12 lead ECGs

Data were examined for any effect of change in serum electrolyte levels on QTc interval. The change in individual electrolyte levels and change in QTc at the end of HD were correlated, individually and in combination.

Serum K^+, Mg^{2+} and Ca^{2+} dropped significantly in both groups from start to end of HD. Serum K^+ and Mg^{2+} levels fell significantly in both halves of the dialysis, with the majority of the drop occurring in the 1^{st} half. However Ca^{2+} levels dropped only in the first half. In the 2^{nd} half of HD, Ca^{2+} level changed in different ways in different HD sessions. The average levels for each time point were calculated for the whole cohort and the values at each time points at start, midpoint and end of HD were plotted (figures 11.5, 11.6 & 11.7).

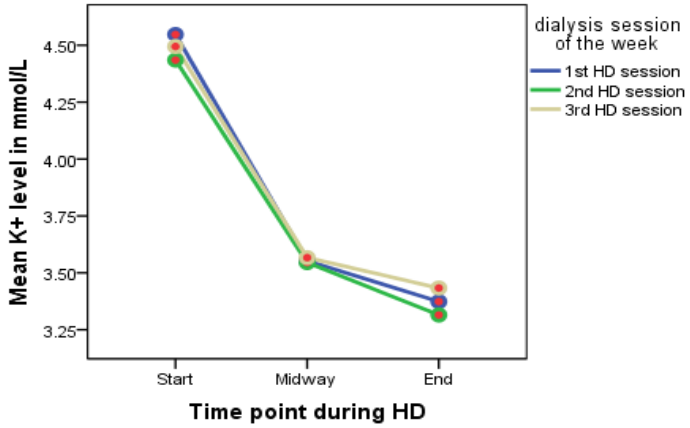


Figure 11.5: *shows the change in serum K^+ levels during HD in all subjects, showing the drop in average level from start to midpoint and to end in 1st, 2nd & 3rd HD cycles*

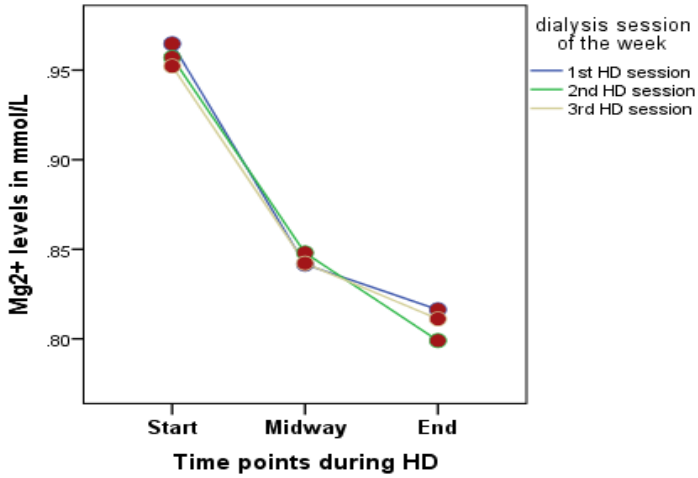


Figure 11.6*: shows the change in serum Mg^{2+} levels during HD in all subjects, showing the drop in average level from start to midpoint and to end in 1st, 2nd & 3rd HD cycles*

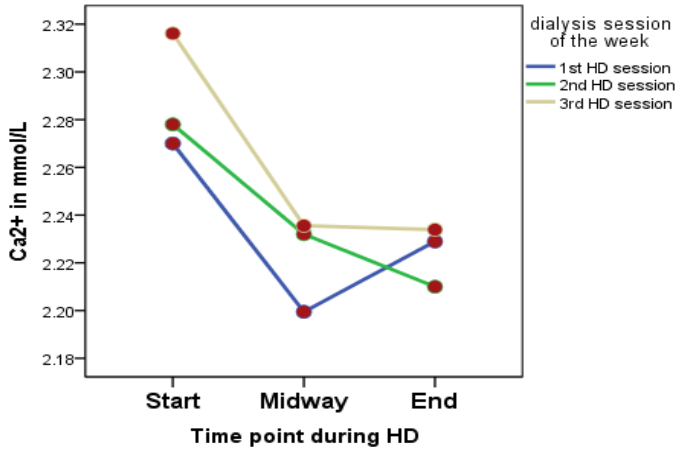


Figure 11.7*: shows the change in serum Ca^{2+} levels during HD in all subjects, showing the drop in average level from start to midpoint and to end in 1st, 2nd & 3rd HD cycles*

Change in serum K^+, Mg^{2+} and Ca^{2+} levels did not correlate with change in QTc interval from 12 lead ECGs when analysed for the whole cohort (table 11.9).

Electrolyte	**Correlation with diffQTc R=**	**p=**
$diffK^+$	0.138	0.331
$diffMg^{2+}$	0.159	0.261
$diffCa^{2+}$	0.004	0.980

Table 11.9*: shows the Pearson correlation value and 2 tailed significance between change in electrolyte levels and change in QTc interval. (diffQTc- difference in QTc from start to end; $diffK^+$ - difference in K^+ from start to end; $diffMg^{2+}$ - difference in Mg^{2+} from start to end; $diffCa^{2+}$ - difference in Ca^{2+} from start to end)*

Linear regression analysis with difference in QTc interval from start to end as a dependent variable did not show any significant correlations between change in electrolyte levels and change in QTc interval when tested for the whole cohort (table 11.10).

	Mean square	df	F	p=
Regression	656.886	3	0.483	0.696

Table 11.10*: Linear regression with difference in QTc (from 12 lead ECGs) as a dependent factor with difference in K^+, Mg^{2+} & Ca^{2+} as covariates.*

Diabetes group

The correlation between difference in QTc from start to end of HD on 12 lead ECGs to difference in serum K^+, Mg^{2+}, Ca^{2+}, change in glucose from start to end and glucose variation during HD i.e. maximum – minimum levels were analysed.

The change in Mg^{2+} level, but not the change in K^+ or $Ca^{2+,}$ correlated significantly to change in QTc interval on univariate ANOVA (table 11.11)

	Mean square	**df**	**F**	**p=**
$diffK^+$	2162.174	1	1.549	0.222
$diffMg^{2+}$	7349.992	1	5.890	**<0.05**
$diffCa^{2+}$	79.201	1	0.054	0.817
Gluc_change	3.518	1	0.002	0.963
Gluc_variation	497.277	1	0.313	0.580

Table 11.11*: shows the results of univariate ANOVA in the **diabetes group** with change in QTc as a dependent factor.*

There was no significant correlation between change in glucose levels from start to midway or to end of HD, or in glucose variation taken as difference between maximum

and minimum glucose levels during HD, and change in the QTc interval on 12 lead ECGs in the diabetes group.

Control group

Change in electrolyte levels and glucose levels did not correlate with change in QTc interval on the 12 lead ECGs in the control group (table 11.12).

	Mean square	**df**	**F**	**p=**
diffK^+	109.014	1	0.094	0.764
diffMg^{2+}	549.707	1	0.488	0.497
diffCa^{2+}	832.289	1	0.753	0.401
Gluc_change	494.739	1	0.437	0.520
Gluc_variation	1697.488	1	1.634	0.223

Table 11.12*: shows the results of univariate ANOVA in* ***control group*** *with change in QTc as a dependent factor*

11.1.4 Holter derived QTc before and after dialysis in relation to glucose levels

Average QTc intervals were obtained for 4 hours before the start of dialysis, for the duration of dialysis and for 4 hours after the end of dialysis from Holter monitoring. The exact time of HD was identified on the Holter recording for each HD session and QTc durations were obtained along with the mean for the pre, during and post dialysis periods in order to explore for continued change in QTc after the end of HD.

Mean glucose levels were obtained for the same period from the CGM. For calculation purposes, 'Low' glucose reading was defined as ≤ 2.1 mmol/L and 'High' glucose reading was defined as ≥ 22.3 mmol/L.

Diabetes group

The average QTc interval was prolonged in the 4hour post-HD phase in the diabetes group compared to the Pre-HD and HD phases. Though the mean of average QTc was significantly prolonged during the post-HD period, the range was not significantly different compared to the pre-HD and HD period (table 11.13, figure 11.8). However the change in the average QTc interval did not follow the pattern as seen with average glucose levels which fell significantly during HD and recovered during the post-HD period (table 11.13, figure11.9). The average QTc intervals obtained from the Holter monitor were notably lower than the QTc intervals obtained from 12 lead ECGs in all patients.

Time period	QTc interval (msec)			Glucose level (mmol/l)		
	N=	Mean ± SD	Range	N=	Mean ± SD	Range
Pre-HD	57	421 ± 18	369 – 465	77	11.4 ± 5.1	2.3 – High
HD	86	424 ± 19	382 – 466	104	8.4 ± 3.6	2.7 – High
Post-HD	86	429 ± 17	384 – 470	112	11.5 ± 4.6	2.9 – High

***Table 11.13**: Mean and range of average QTc (on Holter) and average glucose levels for pre-HD, HD and post-HD periods in the **diabetes** group*

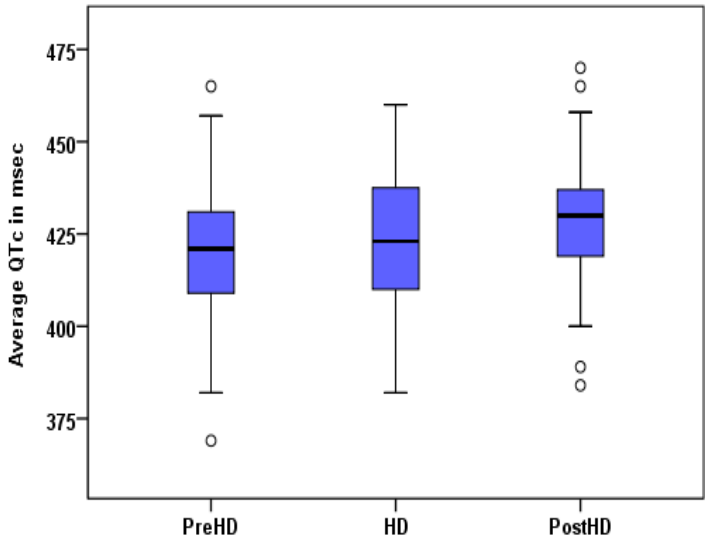


***Figure 11.8**: Median and the range of average QTc interval (from Holter) during the pre-HD, HD and post-HD periods in the **diabetes** group (n=55, for all 3 periods).*

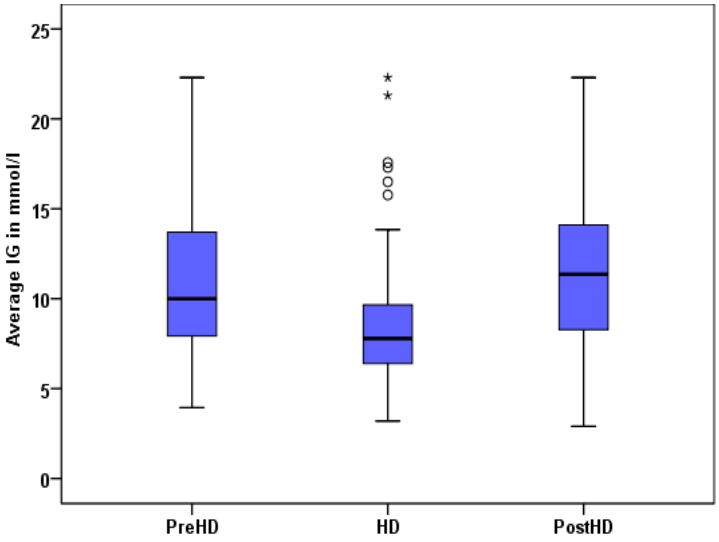


***Figure 11.9**: Median and the range of average glucose levels for pre-HD, HD and post-HD time periods in the **diabetes** group (n=70, for all time periods).*

Paired samples were analysed for the significance of the observed prolongation of average QTc interval on Holter recording. Although this was not significant from the pre-HD to

HD period, it was significant from the HD to the post-HD period and from the pre-HD to the post-HD period (table 11.14).

Paired samples	N=	Mean ± SD	Differences	p=
Pre-HD vs HD	56	420 ± 18 vs 423 ± 19	2.7 ± 16.9	0.234
HD vs Post-HD	84	424 ± 19 vs 429 ± 17	5.0 ± 12.5	<0.001
Pre-HD vs post-HD	56	421 ± 18 vs 428 ± 17	7.6 ± 16.8	<0.005

Table 11.14*: Results of the paired samples test for average QTc interval (from Holter) between 3 time periods in the* ***diabetes*** *group.*

Control group

The average QTc interval (from Holter) was reduced during the HD compared to the pre-HD period in the control group. However it was prolonged in the post-HD period with the mean QTc being longer than during the pre-HD period (table 11.15, figure 11.10). Average glucose levels appeared to follow a different pattern compared to the diabetes group with a significant drop in levels during HD from the pre-HD period and a continued drop in the post-HD period (table 11.15, figure 11.11).

Time period	QTc interval (msec)			Glucose level (mmol/L)		
	N=	Mean ± SD	Range	N=	Mean ± SD	Range
Pre-HD	11	408 ± 13	383 - 426	10	6.9 ± 1.3	5.0 – 8.8
HD	16	404 ± 19	367 - 428	15	5.8 ± 1.5	2.2 - 8.2
Post-HD	16	411 ± 19	372 - 439	16	5.6 ± 1.3	3.4 – 8.3

Table 11.15: Mean and range of average QTc (from Holter) and average glucose levels for pre-HD, HD and post-HD periods in the **control** group

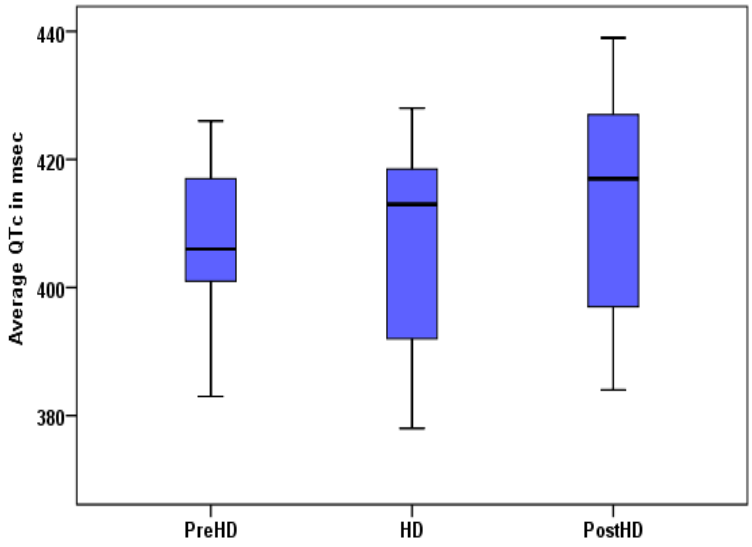


Figure 11.10*: Median and the range of average QTc interval (from Holter) during pre-HD, HD and post-HD periods in the* ***control*** *group (n=11 for all time periods).*

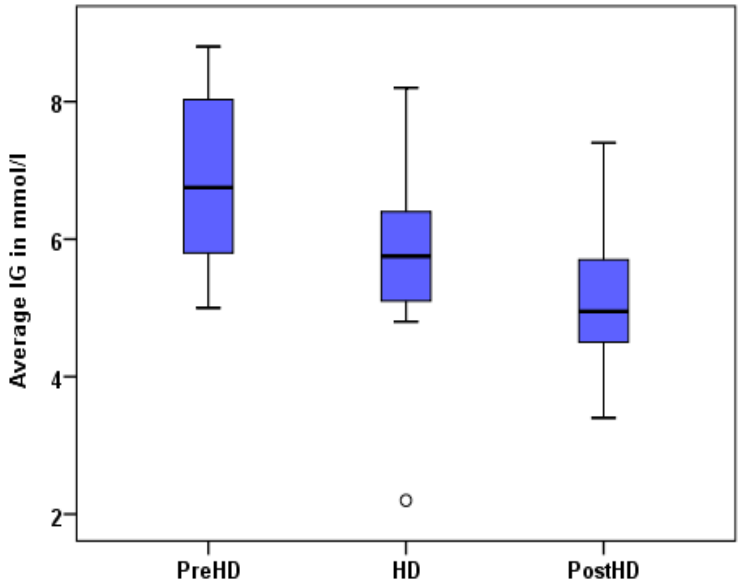


Figure 11.11*: Median and the range of average glucose levels for pre-HD, HD and post-HD time periods in the* ***control*** *group (n=10 for all time periods).*

In the controls, paired sample analysis showed significant prolongation of average QTc (on Holter recording) from HD to post-HD periods, as in the diabetes group. However in contrast to diabetes group, the prolongation from pre-HD period to post-HD period was not significant (table 11.16).

Paired samples	**N=**	**Mean ± SD**	**Differences**	**p=**
Pre-HD vs HD	11	408 ± 13 vs 407 ± 17	1.2 ± 14.9	0.798
HD vs Post-HD	16	404 ± 19 vs 411 ± 19	-7.3 ± 8.2	<0.005
Pre-HD vs post-HD	11	408 ± 13 vs 413 ± 18	-5.0 ± 17.7	0.370

Table 11.16*: Mean and the range of average QTc interval for pre-HD, HD and post-HD time periods in the* ***control*** *group.*

11.1.5 Effect of hypoglycaemia on QTc interval on Holter recording

Data were examined to assess any effect of hypoglycaemia on the QTc interval recorded on Holter. 60 episodes of hypoglycaemia were studied. Time of hypoglycaemia in relation to day or night, duration in minutes and average IG level were recorded. Average QTc ± 1SD matched in time for the duration of hypoglycaemia was obtained from the Holter recording. Periods on CGM with near euglycaemia (IG: 4.0 - 13.0 mmol/l) matching each hypoglycaemic period in time of the day and duration were selected from the same study week. Average QTc was obtained from Holter recordings for these episodes and compared to explore for any effect of hypoglycaemia on QTc interval.

Out of 60 episodes, 33 episodes of hypoglycaemia occurred in the day and 27 were nocturnal. Average duration of hypoglycaemia was 102±85 minutes (range 20 to 420mins). Average duration of hypoglycaemia was not significantly different between day time episodes and nocturnal episodes (101±85 vs 103±86, p=0.928). Mean QTc on Holter was not prolonged during hypoglycaemia. Overall mean of QTc interval during these episodes was 426±19 msec. There was no significant difference in the overall mean duration between the episodes of hypoglycaemia occurring during day and night (427±15 vs 425±22, p=0.694). The length of average QTc from Holter did not correlate to the duration of hypoglycaemia (p=0.781) (figure 11.12). There was no correlation between average IG level during the hypoglycaemic episodes and the length of average QTc on Holter (p=0.152) (figure 11.13). There was no difference in the average QTc on Holter between the hypoglycaemic episodes and the matched euglycaemic periods (427 ± 15 vs 430 ± 13, p= 0.248). The median and range of mean QTc for the hypoglycaemic periods was not different in comparison to their matched euglycaemic period (figure 11.14 & 11.15).

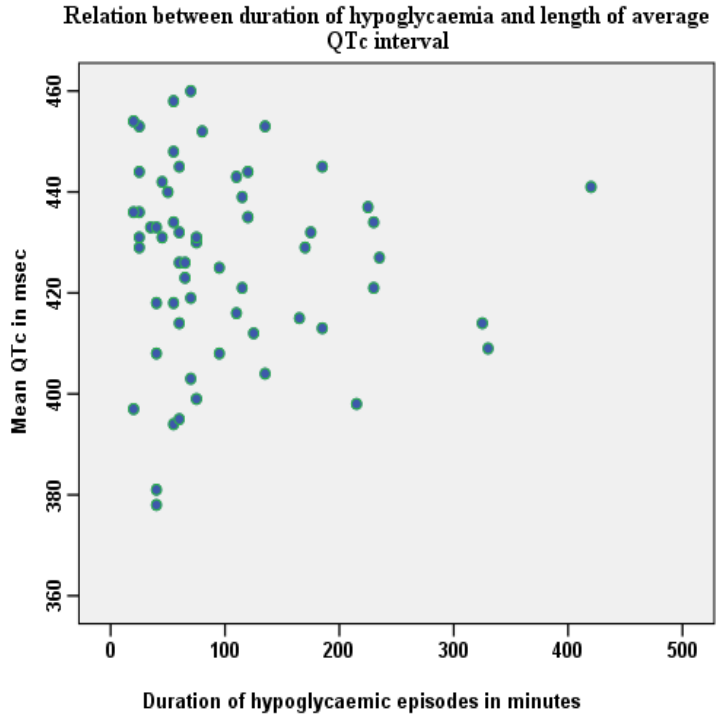


Figure 11.12*: shows lack of correlation between duration of hypoglycaemia and average QTc (from Holter) in diabetes subjects.*

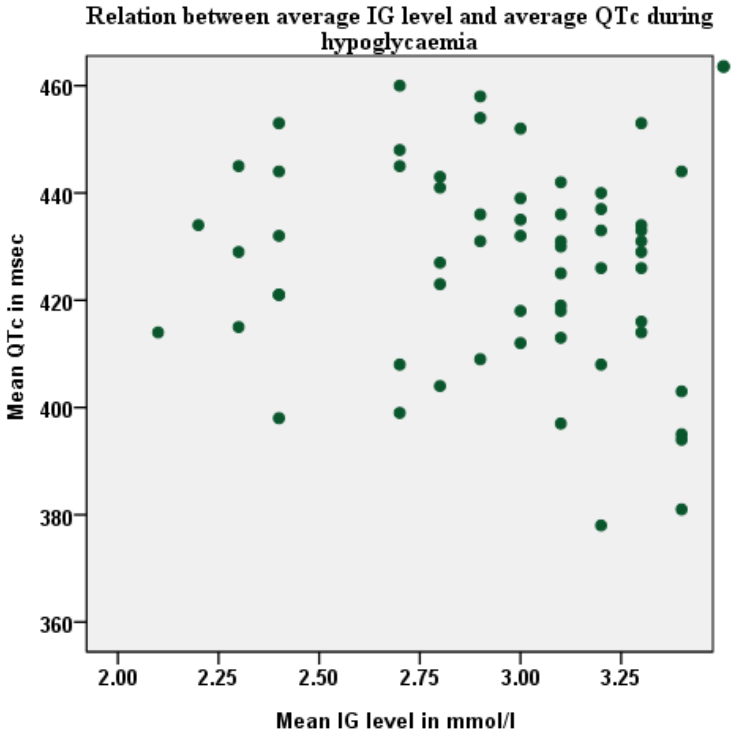


***Figure 11.13**: shows lack of correlation between mean IG level and mean QTc (from Holter) during hypoglycaemia.*

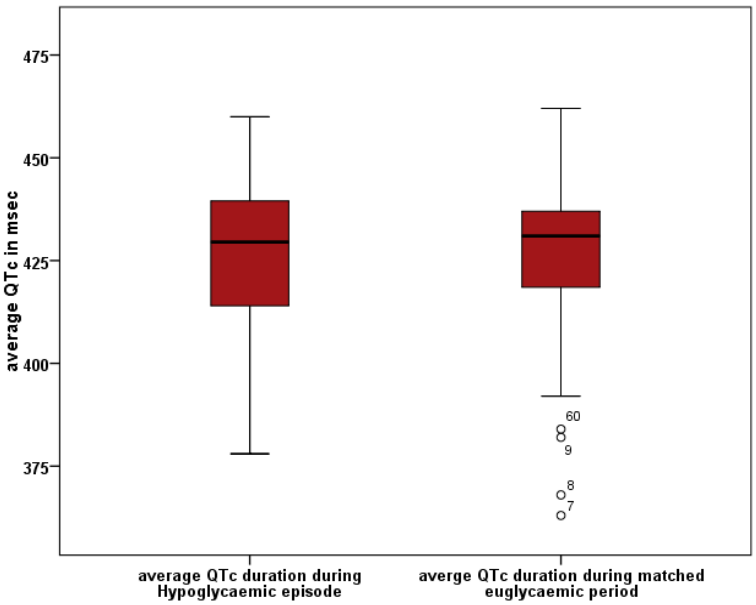


***Figure 11.14**: shows the median and range of average QTc intervals (from Holter) during hypoglycaemia and matched euglycaemic periods.*

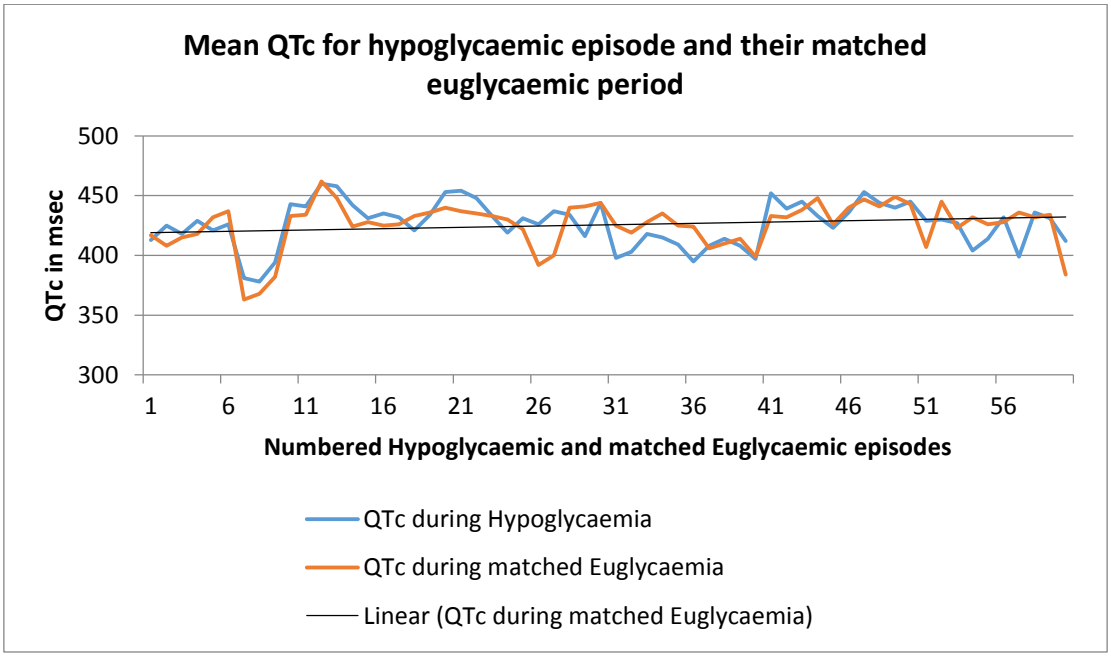


Figure 11.15*: shows the average QTc (from Holter) for each episode of hypoglycaemia with it's time matched euglycaemic period with a linear correlation trend line in black.*

11.2 Heart rate and rhythm on 12 lead ECGs

Changes in heart rate and rhythm during dialysis were examined on serial 12 lead ECGs during HD. Heart rates in individual ECGs were noted and examined for changes at midway and at the end of HD compared to the start of HD.

3 ECGs were available from each HD session of the week for 13 out of 15 subjects in the diabetes group except one subject who did not have an ECG at the start of the 3rd HD session. 3 ECGs were available for all 5 subjects in the control group for all HD sessions.

There was no change in mean heart rate from start to midway and to the end of HD in the whole cohort (77±11 vs 77±11 vs 78±10). There was no change to mean heart rate when examined for diabetes and control groups separately (Diabetes group: 76±10 vs 76±10 vs 77±8; Control: 80±13 vs 80±12 vs 82±13).

Rhythm change during HD was seen only in one patient in the diabetes group and two patients in the control group. Changes observed in these patients are shown in table 11.17 individually.

Sub No.	1st HD			2nd HD			3rd HD		
	Start	**Mid**	**End**	**Start**	**Mid**	**End**	**Start**	**Mid**	**End**
DM 15	SR	Bi/Tri geminy	Couplets/ Triplets	SR	Bi/Tri geminy	Couplets /Triplets	VEs	Couplets/ Triplets	VEs
Non DM 35	SR	SR	Bi/Tri geminy	SR	SR	SR	SR	SR	SR
Non DM 39	VEs	SR	SR	SR	SR	SR	SR	SR	SVEs

Table 11.17*: shows the occurrence of arrhythmias on 12 lead ECGs during HD in individual subjects. VEs- Ventricular ectopics, SVEs- Supraventricular ectopics*

Changes in rhythm observed in one diabetic subject were seen consistently in all 3 HD sessions and occurred after starting HD in the first two sessions. However the changes observed in the non-diabetic subjects were not consistent.

Diabetic subject 15 with changes in rhythm could not participate in Holter monitoring due to allergic reaction to the Holter leads. Hence no further observation could be undertaken.

Changes noted in the non-diabetic subjects on Holter monitor are mentioned below in the relevant sections.

11.3 Arrhythmias on Holter recording

Holter recording was reported by cardiac physiologists, including all episodes of rhythm changes including any occurrence of ventricular premature beats (VPB), complex VPB such as couplets, triplets, bigeminy, trigeminy and ventricular salvos; non-sustained ventricular tachycardias (NSVT) or broad complex tachycardia, sinus bradycardia, and junctional or idioventricular rhythm and any episodes of ST depression.

The time and day of occurrence of these episodes were noted in terms of HD/non-HD to examine the frequency of arrhythmic episodes in relation to hypoglycaemia and HD.

11.3.1 Diabetes group

60 episodes of hypoglycaemia were recorded in 12 out of 15 subjects who underwent Holter monitoring. 27 weeks of (4206 hours) Holter recording were obtained. Occurrence of arrhythmia in this group was examined for any relation with hypoglycaemia by testing the frequency of arrhythmia during hypoglycaemic episodes and time matched near euglycaemic episodes in 10 of 12 subjects who underwent Holter monitoring. Periods of near euglycaemia (IG: 4.0 to 13.0 mmol/l) matched in duration, time of the day and HD or non-HD day were selected from the same study week.

Holter recordings were examined for the occurrence of arrhythmias during these periods. Mean duration (range) of hypoglycaemia was 102 minutes (20 to 420). Mean (range) IG level during hypoglycaemia was 2.9 mmol/l (<2.1 to 3.4) and during matched near euglycaemic period was 8.5 mmol/l (4.2 to 12.5).

Out of 12 subjects who participated in Holter monitoring, 5 had previous history of myocardial infarction, ischaemic heart disease or heart failure and 6 had a history of hypertension and/or peripheral vascular disease. 8 subjects had echocardiographic abnormalities including mild abnormalities (table 11.18).

Sub No.	Echocardiogram findings	Risk factors/ previous intervention	Arrhythmias noted	Complex VPB count
01	Normal	ICD in situ, coronary stenting	1 NCT	None
03	Mild RVH	PVD	19 Bg/Tg, 7 BCT	210 couplets 41 triplets
04	Mod to severe AS, Severe LVSD, widespread RWMA	IHD, CABG & coronary stents, CVA, Severe PVD	52 TG, 10 NCT, 6 BCT	361 couplets 10 triplets
09	Mild LVH, mildly dilated LA		1 BCT	23 couplets
10	Mild LVH	IHD, Coronary stenting, Hypertension, PVD	DNP (PPM in situ)	DNP
11	Mild septal LVH, Severe RV dilation, Bi-atrial dilation	Hypertension	1 NCT	1 couplet
15	Severe LVSD, Mildly impaired RV, Significant Pulmonary HT, Global hypokinesia	Hypertension, Obesity	DNP (allergic to Holter leads)	DNP
19	Moderate apical and septal hypertrophy, Mild LVSD	IHD, Heart failure	2 NCT, 1BCT	3 couplets 1 triplets
21	Moderate global LVH, Mild to moderate dilated LA, Mild to moderate TR	Hypertension, PVD	DNP (PPM in situ)	DNP
22	Moderate LVSD, RWMA (DST) Mild to moderate TR, Pulmonary HT	IHD	3 SB, 9 Bg/Tg, 2 JR, 10 BCT	306 couplets 21 triplets
24	Moderate LVH	None	1 Bg, 1 NCT, 1 JR	1 couplet
25	DNP	Hypertension	1 SB, 4 JR	8 couplets
31	Normal (DST)	Hypertension	20 Tg	None
38	Mild LVSD, Septal hypokinesia (DST)	IHD, CABG, Hypertension	1 Tg	None
41	None significant (mild MR and AR)	Hypertension	None	None

Table 11.18: *shows the echocardiogram abnormalities, cardiovascular risk factors other than diabetes, different arrhythmic episodes and complex ventricular premature beats recorded in individual patients in diabetes group. (LVH- Left ventricular hypertrophy, RVH- Right ventricular hypertrophy, LVSD- Left ventricular systolic dysfunction, RWMA- regional wall motion abnormality, LA- left atrium, RV- right ventricle, AS- aortic stenosis, MR- mitral regurgitation, TR- tricuspid regurgitation, AR- aortic regurgitation, HT- Hypertension, DST- dobutamine stress echocardiogram, IHD- ischaemic heart disease, CABG- coronary artery bypass graft, ICD- implantable cardiac defibrillator, PVD- peripheral vascular disease, NCT- narrow complex tachycardia, Bg- bigeminy, Tg- trigeminy, SB- sinus bradycardia, BCT- broad complex tachycardia, JR- junctional rhythm, DNP- did not participate, PPM- permanent pacemaker, VPB- ventricular premature beat)*

153 short episodes of arrhythmia were noted on Holter in 11 of 12 subjects who took part in Holter monitoring. 3 episodes of nocturnal ST depression were recorded in one subject. 4 subjects had a single episode of arrhythmia and the remaining 149 episodes were noted in 7 subjects. Types of arrhythmia recorded included: narrow complex tachycardia (atrial/ supraventricular tachycardia), junctional rhythm, sinus bradycardia (HR <40bpm),

bigeminy, trigeminy, triplets, and non-sustained or broad complex tachycardia (NSVT/BCT/salvos).

Bigeminy/trigeminy and NSVT/BCT were the commonest rhythm abnormality noted (table 11.19). Corresponding IG level was available for 83 episodes of arrhythmia. The mean IG was 9.8 ± 4.1 mmo/l (2.3 to 20.2). Majority of arrhythmic episodes occurred when IG was in the defined euglycaemic range (table 11.20). Occurrence of arrhythmic episodes was not different between matched periods of hypoglycaemia and near euglycaemia (Mean: 1.97 vs 1.92, p=0.26).

Type of arrhythmia & VPB	**Frequency**
Bigeminy/Trigeminy	102
NSVT/BCT	25
Narrow complex tachycardia	14
Junctional rhythm	8
Sinus bradycardia	4
Triplets	103
Couplets	913

Table 11.19: *shows the frequency of different types of arrhythmias and complex VPBs*

IG range	**Frequency of arrhythmia**
Hypoglycaemia (≤ 3.5mmol/l)	4
Euglycaemia (4.0 to 13.0mmol/l)	61
Hyperglycaemia (≥ 13.5mmol/l)	18
IG not available	70

Table 11.20*: shows the frequency of arrhythmia in relation to IG range*

Time of occurrence of arrhythmia was noted in relation to the HD sessions of the week to examine the frequency on different days. The highest number of episodes were noted to occur before the 1st HD session; the second most frequent period was following the 3rd HD session of the week (figure 11.16). Combining the frequency of arrhythmia in post HD1 (~20hrs) and pre HD2 (4hrs) periods shows an increased propensity for the occurrence of arrhythmia during this period.

VPB and complex VPB counts during hypoglycaemia were examined for change in frequency between hypoglycaemia and matched euglycaemia. No significant difference was found in average VPB count/hr (29 ± 44 vs 25 ± 35, p=0.227) (figure 11.17) or average complex VPB count/hour (0.74 ± 1.42 vs 0.81 ± 2.11, p= 0.832) between the matched periods.

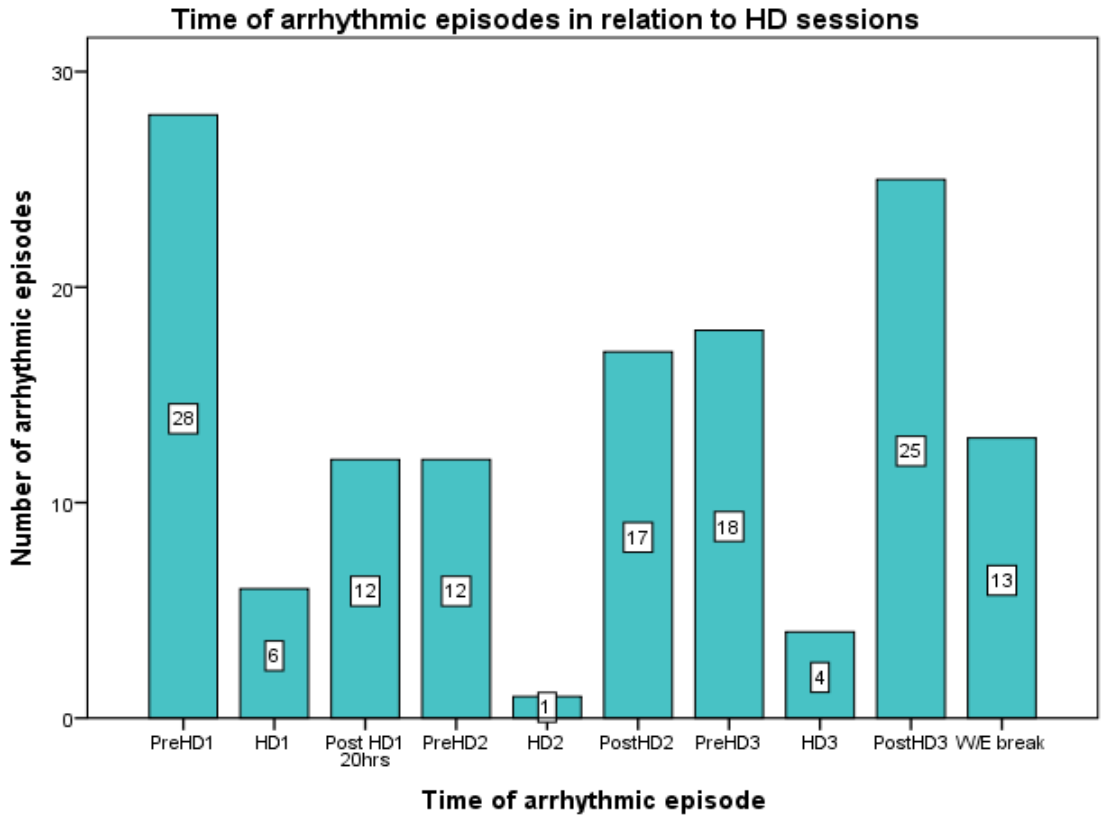


Figure 11.16*: shows the frequency of arrhythmic episodes on Holter on different days of the week in relation to 1st, 2nd and 3rd HD sessions.*

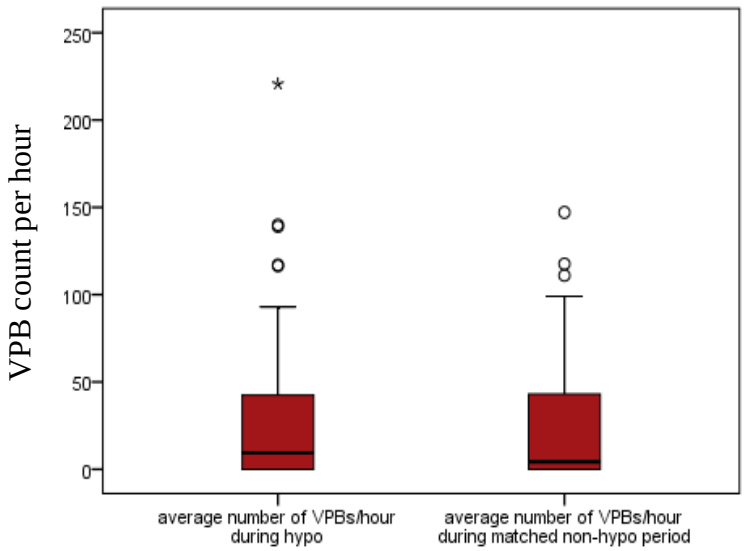


Figure 11.17*: Box plot shows the median and range of mean VPB count/Hr during hypoglycaemic episodes and their time matched euglycaemic episodes.*

11.3.2 Control group

Occurrence of arrhythmias on Holter was examined in the control group separately. 36 episodes of arrhythmia including triplets and 15 episodes of ST depression were recorded in 4 of 5 subjects in this group.

The frequency varied between these subjects with 2 subjects having 2 episodes each and the rest occurred in the other 2 subjects. There was a difference in the types of arrhythmias between subjects. All 15 episodes of ST depression were recorded in one subject and all 11 episodes of NSVT/BCT were recorded in one subject (table 11.20). Hence correlation with HD sessions was not undertaken. Table below shows the echocardiogram findings and existing cardiovascular risk factors in control group subjects.

Sub No.	**Echocardiogram findings**	**Risk factors/ previous interventions**	**Arrhythmias noted**	**Complex VPB & ST depression**
32	None	IHD, Hypertension, Dyslipidaemia	None	None
35	Decreased LV size, Mild LVH, Mild to moderate AR	Hypertension	1 Bg/Tg	1 Triplets
36	Mild to moderate LVH	Hypertension	None	2 Triplets
37	Mild global LVH, moderate LA dilation	Hypertension	None	15 ST depression
39	Low normal LV systolic function	Dyslipidaemia	21 Bg/Tg 11 NSVT	None

Table 11.21*: shows the frequency and types of arrhythmia and ST depression in subjects in the control group. (LV- left ventricle, LVH- left ventricular hypertrophy, AR- aortic regurgitation, LA- left atrium, IHD- ischaemic heart disease, Bg- bigeminy, Tg- trigeminy, NSVT- nonsustained ventricular tachycardia)*

11.4 Heart rate variability

Heart rate variability which is a measure for variation in beat to beat interval was examined for any effect of hypoglycaemia by comparing to time and duration matched near euglycaemic periods.

Time domain measures were obtained with the available software. Frequency domain measures could not be obtained due to lack of software. Time domain measures including SDNN (SD of Normal to Normal), SDANN (SD of average Normal to Normal), Total sNN50 (number of interval differences of successive NN intervals greater than 50msec), RMSSD (square root of the mean squared differences of successive NN intervals) and

SDNNi (the mean of the 5-minute standard deviations of NN intervals) were obtained for the selected time periods.

SDNN and SDANN were significantly higher during hypoglycaemia compared to near euglycaemic periods (table 11.21).

Measures	Hypoglycaemia	Near euglycaemia	P=
SDNN (msec)	40 ± 24	35 ± 20	<0.05
SDANN (msec)	34 ± 22	30 ± 19	<0.05
Total sNN50	454 ± 590	563 ± 1028	0.449
SDNNi (msec)	15.9 ± 9. 8	16.0 ± 9.6	0.993
RMSSD	12.6 ±7.8	15.0 ± 13.5	0.13

Table 11.22*: shows the difference in measured time domain HRV measures during hypoglycaemia and matched near euglycaemia.*

Discussion

12.1 Glycaemic Variation

Diabetic patients on maintenance HD experienced significant variation in their glycaemia throughout the week. The IG levels were in either the hypoglycaemic or hyperglycaemic range equally on both dialysis and non-dialysis days. Time spent with IG in hypoglycaemic, euglycaemic and hyperglycaemic range respectively were 5.9±15.4 vs 2.7±2.8 vs 91.4±15.9 % of recorded times on dialysis days and 3.3±12.4 vs 3.8±4.1 vs 92.9±12.8% of recorded times respectively on non-dialysis days. Significant variation in IG levels was observed on each day through the week, with mean IG levels varying between 3.6 mmol/l and 'High' (≥22.3 mmol/l) in diabetic subjects. The mean IG level was not different between dialysis and non-dialysis days, however the variation as calculated by SD of the mean was significantly higher on dialysis days than non-dialysis days (3.9±1.6 vs 3.5±1.5, $p<0.05$). Similarly the variation as calculated by 'mean amplitude of glycaemic excursion' was higher on dialysis days compared to non-dialysis days (8.1±4.6 vs 7.0±3.9, $p=0.056$) although this was not significant. This suggests that diabetic patients undergoing haemodialysis experience significantly greater glycaemic excursions on dialysis days compared to non-dialysis days.

More episodes of hypoglycaemia were observed on dialysis days compared to non-dialysis days (35.9% vs 25.4%, $p <0.001$) in the diabetic subjects. However, the mean duration of hypoglycaemia was not different between dialysis and non-dialysis days due to episodes with longer duration being frequent on non-dialysis days. Hypoglycaemia occurred through the day and night following HD. Occurrence of hypoglycaemia during daytime (7AM to 11PM) and night time (11PM to 7AM) was not different during HD and non-HD days (64.1% vs 68.7% for day; 35.9% vs 31.3% for night). None of the subjects in our cohort altered their insulin doses before or during dialysis. There was no restriction of food intake during dialysis. All subjects were dialysed with standard dialysate containing 5.0mmol/l of glucose.

A trend of fall in IG level during dialysis was noted in the majority of diabetic subjects with the mean IG of all HD sessions dropping significantly from pre-HD period ($p<0.001$) followed by significant rise in the post-HD period from HD period ($p<0.001$) (mean±SD: 11.4±51 vs 8.4±3.6 vs 11.5±4.6 mmol/l during pre-HD, HD and post-HD period respectively). The post HD IG levels were not different to pre-HD levels. The reduction in

IG levels during dialysis occurred in the initial half of the dialysis in majority of the sessions.

Patients with diabetes experienced frequent and longer duration of hyperglycaemia in the post-HD period comparatively (Pre-HD vs HD vs Post-HD = 41 vs 29 vs 65 episodes; mean duration 125 vs 50 vs 140 minutes). However when IG readings were examined as proportion of available recordings, the proportion of time spent in the hyperglycaemic range was the similar for the pre-HD and post-HD period (median: 64.8 vs 64.6%). Time spent in hyperglycaemia was much less during dialysis (median time of 29.2%) due to the significant drop in IG levels during HD.

Out of 306 episodes of hyperglycaemia recorded in all diabetic subjects, 51.6% (n=158) occurred on the non-HD day compared to 41.2% on HD day. This is not explained by the lower frequency of hyperglycaemia during HD period on dialysis days as the mean IG for dialysis days and non-dialysis days was not different. It is possible that the effect of HD reduced the rise in IG in the ensuing hours. However the mean duration of hyperglycaemia in minutes was not different between dialysis and non-dialysis days (257.9±277.3 vs 266.7±263.3) and the proportion of recorded IG spent in the hyperglycaemic range was higher on dialysis days compared to non-dialysis days (21±18.5 vs 19.6±20.6) suggesting higher fluctuation in IG levels on dialysis days than non-dialysis days. 7.2% of hyperglycaemic episodes were prolonged and overlapped between dialysis and non-dialysis days.

Hyperglycaemia occurred more frequently during the daytime compared to night (187 vs 50 episodes). However the duration in minutes (203.9±154.3 vs 169.2±146.0, p=0.141) and as proportion of recorded time (17.3±14.1 vs 14.1±14.9) was not significantly longer at night.

Glucose and insulin are extracted during dialysis (Abe, Kaizu and Matsumoto, 2007). Jackson et al showed that hypoglycaemia occurred in 40% of patients undergoing HD with or without diabetes (Jackson et al., 2000). Hypoglycaemia occurs more frequently with dialysate concentrations of 5.5mmo/l of glucose compared to 11mmol/l (Simic-Ogrizovic et al., 2001). With dialysate fluid used in our patients containing 5.5mmol/l, it is possible that a significant amount of extraction of glucose occurred during HD leading to a significant drop in IG level. Insulin extraction by dialysis combined with consumption of

food and counter-regulatory hormone response to a fall in glucose levels and blood pressure during HD could be contributing to a rise in IG levels in the post HD period.

The increased frequency of hypoglycaemia seen in our study is similar to that previously reported. Jung et al (2010) found an increased occurrence of hypoglycaemia on HD days, and Kazempor-Ardebili et al (2009) reported a nadir IG level within 24 hours of HD in 14 of their 17 patients. The study by Jung et al used CGM for 6 days in patients on maintenance HD. There was no difference in mean amplitude of glycaemic excursion between HD and non-HD days in their study, whereas in our study MAGE and SD of mean IG were higher on HD days. Kazempour-Ardebili used CGM for only 48 hours in type 2 diabetic patients. Both these studies were therefore of shorter duration compared to our study. The recently reported DIALDIAB study (Joubert et al., 2015) used CGM durations similar to our study i.e. 3 studies at 2 week intervals but with 5 days CGM; the intention was to assess the effectiveness of CGM in improving glycaemia compared with SMBG. This study also noted lower median and interquartile range (IQR) of IG during HD similar to the drop in mean IG during HD in our study. The median and IQR of IG on non-dialysis days was higher than IG at similar times during HD in an example shown in this publication (Joubert et al., 2015). However comparison could not be made in relation to change in glycaemia between HD and non-HD days or between pre-HD, HD and post-HD periods as done in our study, due to a lack of information in the publication. This study was designed to examine the improvement in glycaemic control with iterative CGM in comparison to SMBG with CGM data being reviewed by an expert after every study week and treatment modification being advised, albeit remotely through a nephrologist.

Though other studies have reported on glycaemic variation using CGM in diabetic patients on HD, no other study has specifically included C-peptide negative or minimally positive patients. Variation in IG seen in our study on dialysis and non-dialysis days suggest the need for closer monitoring of individual patients in order to assess glycaemic variation using CGM in this population, and managing insulin therapy accordingly. Though it was not possible to draw an exact pattern of change in glycaemia through the dialysis week even in our fully insulin dependent patients in clinical practice, there was a pattern of high IG level in the pre-HD period followed by significant drop in IG level during HD and a rebound high IG level in the post-HD period. Our study was conducted as a non-interventional observational study where patients were blinded to real-time IG level and

hence no changes were made to their insulin doses. It is a close reflection of glycaemic variation in these patients in clinical practice.

Assessing the variation in IG level in relation to food intake and insulin regimen might help understand the glycaemic variation better in these patients and thereby help formulate more suitable insulin dose titration. A real time CGM study would need to be performed to test this assumption.

There were limitations to the assessment of glycaemia with CGM in our cohort. Dexcom G4 CGM used in our study has limitations to the range of IG level it can record (2.2 to 22.2 mmol/l). Our CGM data revealed a number of episodes with IG level persistently below 2.2mol/l read as 'Low,' or persistently above 22.2mmol/l read as 'High'. These readings could either be a true reflection of patient's glycaemic status or due to lack of calibration using capillary blood glucose level during these episodes. These levels might have had some effect on some of the mean IG levels calculated. Dexcom G4 CGM was chosen for this study, as this was the only CGM available with blinding facility which could also record ISF glucose data for 7 days, at the planning stage of the study. With the limitations of Dexcom G4 CGM, especially the need for patients to enter capillary glucose levels 12 hourly for calibration and paracetamol consumption potentially affecting the ISF glucose reading, Medtronic iPro2 CGM could be a better alternative. iPro2 CGM is designed to record ISF glucose for 7 days and is blinded from patients. Though it requires patients to check their capillary glucose levels at least twice a day for downloading the data at the end of the study week, patients do not need to enter the levels on to CGM unlike Dexcom CGM.

Glycaemic variation in non-diabetic patients showed a variation in the pattern through the post-HD period compared to that in diabetic patients. IG levels dropped significantly during HD even in non-diabetic patients but continued to drop further in the post-HD period (6.9 ± 1.3 vs 5.8±1.5 vs 5.6 ± 1.3) in contrast to diabetic patients where it rose to pre-HD levels. The drop during HD in non-diabetic patients has been reported previously by Jackson et al (2000), but the further trend in post-HD period has not been described. Insulin clearance is increased by dialysis even in non-diabetic patients (Jorgensen et al., 2015). This should in turn cause an elevation in glucose levels post-dialysis. Lack of renal gluconeogenesis along with loss of blood glucose during HD and poor appetite may potentially keep the glucose level low in the post-HD period in non-diabetic subjects.

However closer observation of the trend in IG levels on individual HD days in this group revealed a later rise in IG levels after a 4 hour post-HD period on most HD days. This again was variable between the subjects.

Hypoglycaemia was also noted in non-diabetic subjects, with 18 episodes using the IG cut off ≤3.5mmol/l. The majority of these episodes (14 of 18) occurred on dialysis days as seen in the diabetes group. These episodes were often prolonged (mean 103.6, range 20 to 390 minutes) with episodes on HD days being longer (126±132 minutes).

Surprisingly our non-diabetic cohort experienced hyperglycaemia, despite setting a cut off value as IG of ≥ 13.5mmol/l to match the diabetes group. 9 episodes of hyperglycaemia lasting 20 minutes or more were recorded in this group with an overall mean IG of 14.8±1.1 mmol/l (range of means: 13.8 to 17.0). Interestingly 7 episodes lasted 60 minutes or more (range 60 to 195minutes). The majority of these episodes occurred on the non-dialysis days suggesting possible significant effects of glucose extraction by HD preventing a rise in IG levels in the immediate post-dialysis period. Our control group subjects all had normal fasting plasma glucose and normal HbA1c at recruitment. The rise in IG level could not be explained by post-prandial rise as they have remained elevated for longer periods than physiologically expected in the immediate post-meal period. This again cannot be explained by the glucose containing dialysate as the majority of these episodes occurred on non-dialysis days. This needs further investigation to understand the effect of ESRD and dialysis on glucose metabolism in non-diabetic subjects. This has not been reported in any other studies.

12.2 Change in electrolytes

Serial measurements of serum potassium, calcium and magnesium during HD revealed a significant drop in their levels through the dialysis. However there was a difference in the changes between these electrolytes through the HD.

Serum potassium levels dropped in both halves of the dialysis session significantly (from start to midway; 4.5 ± 0.7 vs 3.5 ± 0.4 and midway to end: 3.5 ± 0.4 vs 3.4 ± 0.3; $p<0.001$ for both halves). The majority of the drop in levels through HD occurred in the first half. Though mean serum potassium level was lower in the diabetes group, the difference was not significant. Closer examination of changes in individual patients in the diabetes group showed that the potassium level dropped in the majority of patients in both halves, whereas

one patient who was hypokalaemic at the start remained stable without a further drop. There was a slight rise by 0.1 to 0.4mmol/l in the second half in 7 out of 43 sessions in the diabetic group. There was no difference in serum levels at the start of the HD between 1st, 2nd and 3rd HD sessions. In the control group, 3 sessions showed some rise in potassium level in the 2nd half by 0.1 to 0.5 mmol/l. However at the end of HD serum potassium was always lower than at the start.

Change in serum calcium levels differed in the pattern in comparison to potassium. There was a significant drop in the serum calcium level in the 1st half (2.29 ± 0.13 vs 2.22 ± 0.08, p <0.001), but there was no change to mean level in the 2nd half (2.22 ± 0.08 vs 2.22 ± 0.07, p=0.929). The drop in the calcium level was not consistent in all patients in the diabetes group. Interestingly in all patients who had serum calcium <2.2mmol/l (ranging 1.98 to 2.18mmol/l) at the start of HD, the serum calcium levels rose or remained stable through the dialysis (ranging from 2.1 to 2.18 mmol/l at the end). The drop in serum calcium levels in the control group was inconsistent and different from the diabetes group. The mean level was significantly lower in the diabetes group compared to the control group at the start (2.26±0.13 vs 2.35±0.12, p=0.033), midway (2.20±0.08 vs 2.27 ±0.08, p=0.011) and at the end of HD (2.21±0.07 vs 2.25±0.06, p=0.037). None of the control group subjects were hypocalcaemic at the start of any HD session (range 2.14 to 2.55mmol/l) and the change in levels in the 1st and 2nd were inconsistent.

The correction of low serum calcium levels could be influenced by the dialysate containing acetate or citrate enriched bicarbonate (Šafránek et al., 2015). In the study by Šafránek et al, use of traditional acetate enriched dialysate resulted in improvement in serum calcium levels in those patients with levels <2.33 mmol/l at the start of HD. KDOQI guidelines suggest using dialysate with a calcium concentration 1.25mmol/l for patients on calcium-based phosphate binders and dialysate containing 1.25 - 3mmol/l of calcium for patients not on any calcium containing phosphate binders based on serum calcium levels. Our patients were dialysed using standard dialysate containing of 1.25mmol/l calcium.

Similar to potassium, there was no significant difference in the serum calcium levels at the start or midway or end of HD between the 3 HD sessions. The difference in change in calcium levels through HD and also levels at set time points during HD between diabetic patients and non-diabetic patients has not been reported previously. However poor glycaemic control has been suspected to play a role in the development of

hypoparathyroidism seen in patients with diabetes and CRF (Martinez et al., 1998). In their study of 326 patients with various stages of CRF without previous parathyroidectomy or hyperparathyroidism, Martinez et al compared 58 patients with diabetes and 268 patients without diabetes. Diabetic patients had significantly lower PTH levels (p=0.0003). However total or ionized calcium levels were not different between the groups. None of their patients were mentioned to be on renal replacement therapy.

Serum magnesium levels dropped significantly through HD similar to potassium. The drop in the serum level was significant in both 1st (0.96 ± 0.19 vs 0.84 ± 0.11, p<0.001) and 2nd (0.84 ± 0.11 vs 0.81 ± 0.07, p<0.001) halves of HD in the whole cohort. The majority of the drop occurred in the 1st half of HD as seen with potassium. The serum magnesium levels were not significantly different at any of corresponding time points between 3 HD points. However the mean calcium levels were lower at all time points in the diabetes group compared to control group (0.93±0.15 vs 1.04±0.26, p=0.039 at start; 0.83±0.09 vs 0.89±0.14, p=0.051 at midway and 0.79±0.07 vs 0.84±0.09, p= 0.046 at the end). Martinez et al reported lower magnesium levels in diabetes patients with CRF (p=0.02) compared to nondiabetic patients with CRF.

Similar to improvement in low calcium levels, serum Magnesium levels improved in diabetic subjects who had low serum calcium at start (ranging 0.67 to 0.73mmol/l at start and 0.69 to 0.77mmol/l at the end). None of the subjects in control group had serum calcium below 0.78mmol/l.

Improvement in low serum magnesium in our study subjects was similar to study reported by Šafránek et al (2015). In their study, patients dialysed with acetate enriched bicarbonate solution showed improvement in serum magnesium levels in all patients with a pre-dialysis magnesium of <0.76mmol/l.

The results of our study show that diabetic patients tend to have lower serum calcium and magnesium levels compared to non-diabetic subjects. Our non-diabetic cohort was smaller compared to our diabetic cohort. The study was not powered to evaluate these differences and hence it is difficult to derive any conclusion with regards to differences in the electrolyte levels between the two groups. Changes in electrolytes were examined to explore any effect on cardiac electrical activity in both groups, which is discussed later.

12.3 Changes in cardiac electrical activity

QTc interval measured by 12 lead ECGs at start, midway and at the end of HD showed significant prolongation from start to end of HD in both the diabetic and non-diabetic groups. The mean QTc for diabetes group at the three time points was 465±39 vs 478±42 vs 492±46 msec. Mean QTc for the control group was 414±30 vs 426±37 vs 451±44msec. The QTc was significantly higher at each time point in the diabetes group compared to the control group. The baseline QTc was prolonged in 30 out of 38 ECGs at the start of HD in the diabetic patients, being above 440msec (range 391 – 569 msec) whereas in the control group they were mostly normal (range 367 – 457msec). EURODIAB IDDM complication study group has shown the higher prevalence of prolonged QTc in insulin treated type 1 diabetic patients (Veglio et al., 1999). Abnormal QTc prolongation was seen in 16% of patients in the study of 3250 patients. They found the QTc being more prolonged in women then in men. In our sample, the baseline QTc in diabetic patients was not different between men and women (466.6±40.1 vs 464.3±39.0, p=0.898).

The prolongation in QTc during HD was significant from the start to end of HD for the whole cohort together. The change was significant in both halves of the dialysis session with the change being more in the second half than in the first (mean change 12±35 vs 17±26 msec). When examined in the individual groups, the change in QTc in the first half was not significant in the control group (p=0.147) compared to diabetes group (p=<0.05). The range of change of QTc from start to end of HD was much wider in the diabetes group, with QTc being shorter at the end of HD compared to the start in some subjects whereas all non-diabetic subjects showed longer QTc at the end of the HD compared to the start. The pattern of change in QTc in the control group does not match the change in electrolytes. A study in 68 non-diabetic HD patients with normal cardiac status ascertained by extensive testing showed a clear pattern between change in QTc and the electrolyte concentration of the dialysate (Covic et al., 2002). QT interval was calculated similarly to our study as the average of 3 consecutive complexes on 12 lead ECGs 10 minutes pre and post-HD and corrected using Bezett's formula. This study also reported a significant increase in QTc between pre-HD to post-HD ECGs (421±26 vs 434±29 msec, p=0.005). Prolonged QTc above 440msec was found in 34% of subjects on pre-HD ECG and in 46% of subjects in the post-HD ECG. In our small cohort of non-diabetic subjects, one patient (20%) had abnormal QTc at baseline before all 3 HD sessions (456-457 msec). Abnormal QTc at the end of HD was seen in 7 out 15 HD sessions in 3 subjects in this cohort of 5

subjects. 2 subjects had consistently abnormal QTc at the end of all HD sessions including one subject with abnormal QTc at the start, and one subject had abnormal QTc only at the end of one HD session. However the subject with abnormal QTc at baseline showed further prolongation in only 2 post-HD ECGs, whereas QTc remained the same as in pre-HD ECG though abnormal in one of the HD session. High pre-HD calcium and larger drop in calcium correlated with a higher increase in QTc interval in the study by Covic et al. However in our study there was no correlation between difference in calcium and difference in QTc from start to end of HD ($r=0.004$, $p=0.980$). Also there was no correlation between serum calcium level at the end of HD and difference in QTc ($r= 0.112$, $p=0.428$). There was no correlation between change in QTc at the end of HD and change in serum potassium level or magnesium level or change in IG level or glycaemic variation (max-min) in our whole cohort. In our diabetes cohort 34 out of 39 (82%) ECGs at the end of HD showed abnormal QTc (>440msec). With 30 out 39 (76.9%) ECGs recorded at the start of HD already having abnormal QTc, further prolongation at the end of HD was seen in 32 out of 34 ECGs which had abnormal QTc at the beginning of HD. Our results suggest that a very high proportion of diabetic patients have abnormal QTc at baseline, and an even higher proportion have further prolongation of QTc with HD. There was no correlation between change in QTc and glucose variation (max- min IG) during HD or with difference in IG level from start to end of HD. Further prolongation of QTc in our cohort is likely due to multifactorial reasons, which might include existing cardiac health status, baseline electrolyte levels and their change through dialysis.

Average QTc obtained from Holter recordings showed a continued prolongation in the 4hr post-HD period compared to 4hr pre-HD period and HD period in the diabetes group. These QTc intervals were notably shorter compared to QTc calculated from the ECGs. It is well recognised that QTc interval from Holter does not correspond qualitatively to QTc obtained from 12 lead ECGs (Goldenberg, Moss and Zareba, 2006). This is due to various factors such as signal filtering, difference in the method of recording and some R-R intervals not taken into consideration for calculation on Holter (Lutfullin et al., 2013; Badilini and Maison-Blanche, n.d)

The abnormal QTc puts these patients at higher risk of arrhythmia and SCD. Routine ECG or periodic Holter monitoring is not undertaken as a part of routine clinical care to detect at risk patients. Undertaking these measures might help in risk stratifying these patients and initiate any preventative therapy.

12.4 Cardiac arrhythmias

Sudden cardiac death and arrhythmias are some of the major causes for cardiac deaths in patients on HD and cardiac arrhythmias are very common in people with ESRD (Kanbay et al., 2010; Redaelli et al., 1988). Sudden deaths were noted to be more frequent on the first day of the week around first HD session (Foley et al., 2011; Karnik et al., 2001). Rapid electrolyte shifts, left ventricular hypertrophy, dialysis with low potassium dialysate and QT dispersion have been reported often as some of the causes predisposing the patients on dialysis to SCD (Kanbay et al., 2010; Pun et al., 2011). Karnik et al showed that presence of diabetes was associated with an increased incidence of cardiac arrest in dialysis patients. However the factors associated with diabetes such as glycaemic variation or hypoglycaemia has not been specifically reported in relation to the incidence of arrhythmias or SCD.

Incidence of arrhythmias including burden of ventricular premature beats has been reported. Kimura et al in their study of 100 patients on maintenance HD using 72hour Holter monitor, reported ventricular arrhythmias including VPBs in 18 patients (Kimura et al., 1989). High burden of VPBs were noted during and for 4 hours after HD in this study. Ramirez reported 40% incidence of cardiac arrhythmias in patients on haemodialysis (Ramirez, Brueggemeyear and Newton, 1984). Shapira and Bar-Khayim reported clinically significant arrhythmia in 12 of their 39 patients studied, which included supraventricular, ventricular and that of combined origin (Shapira and Bar-Khayam, 1992). These were recorded on 12 lead ECG and 24hour Holter recordings. Verde et al, in their study of 77 patients (29.9% with diabetes) reported supraventricular arrhythmias in 49.3%, which were short and asymptomatic (Verde et al., 2016).

In our study, we recorded 153 episodes of short arrhythmias in 11 out of 12 (91.67%) subjects in the diabetes group including junctional or idioventricular rhythms, sinus bradycardia and episodes of ST depression apart from ventricular and supraventricular arrhythmias. The incidence in our study is much higher than that reported in the literature. Kimura et al reported 8 patients with supraventricular, 3 patients with combined supraventricular and ventricular, and 1 patient with ventricular arrhythmias among 18 patients noted to have arrhythmia. In our study 5 patients (33.3%) had recurrent episodes of arrhythmias and ventricular arrhythmias formed the large majority. 127 episodes of recorded arrhythmias were of ventricular origin including ventricular bigeminy/trigeminy,

non-sustained ventricular tachycardia/broad complex tachycardia. Only 14 episodes were of atrial origin. All episodes were short and asymptomatic. In addition 913 episodes of couplets and 103 episodes of triplets were seen. There was no relation between hypoglycaemia and frequency of dysrrhythmic episodes, as large majority occurred with IG level in euglycaemic range. Where corresponding IG level was available matched in time for occurrence of arrhythmic episode, the mean IG was 9.8±4.1 mmol/l. The frequency of arrhythmia during hypoglycaemic episode was not different compared to time matched euglycaemic period. Our results suggest that there is no demonstrable relation between hypoglycaemia and arrhythmia in this dialysis cohort. The highest incidence of arrhythmias was found in the 24 hours prior to 1st HD of the week (20.6%). The literature suggests that there is increased risk of SCD before and after 1st HD of the week. Increased frequency of arrhythmia during these periods might be contributing to this increased risk of SCD. In our study, another 17.6% of episodes occurred in the immediate 20hours post 1st HD period. 18% of episodes were noted after the 3rd HD. Interestingly all diabetic patients in our study with ventricular arrhythmias had QTc prolongation on baseline as well as on post-HD ECG. 14 of 15 diabetes patients who underwent echocardiography, 3 had normal or only mild abnormalities. The findings showed presence of moderate or severe abnormalities in subjects who also had higher number of arrhythmic episodes. This could suggest presence of structural cardiac abnormality could increase the risk of arrhythmias in this population.

Higher frequency of ventricular premature beats (VPB) and complex VPBs are considered to be risk factors for ventricular arrhythmias. VPB burden is noted to be higher in the post-HD period. It has not been reported, if burden of VPB or complex VPB is associated with glycaemic variation or hypoglycaemia in diabetic patients on HD. We did not find any significant difference in the VPB or complex VPB burden during hypoglycaemic episodes in comparison to time matched euglycaemic periods.

Conclusions

1. We observed a significant pattern in the variation in IG levels in relation to haemodialysis in diabetic patients who are insulin dependent. There was a significant drop in mean IG level during HD followed by rebound hyperglycaemia in the post-HD period. The pattern was different in non-diabetic patients. The mean IG level continued to drop in the immediate post-HD period.
2. The glycaemic variation was higher on dialysis days compared to non-dialysis days as measured by the SD and MAGE, though the mean IG level for the two days was not significantly different.
3. Hypoglycaemia was very common in our insulin dependent diabetic population, which occurred more commonly on dialysis days. These episodes were all asymptomatic and often prolonged.
4. Hyperglycaemia was more frequent on non-dialysis days compared to dialysis days. Our patients spent the majority of time either being hypoglycaemic or hyperglycaemic on both dialysis and non-dialysis days.
5. Changes in cardiac rhythm during dialysis were not frequent and occurred consistently only in one diabetic patient.
6. Our study did not demonstrate any relationship between hypoglycaemia and occurrence of dysryhythmias.
7. Our study did not demonstrate any relationship between hypoglycaemia or electrolyte changes on QTc interval during dialysis.
8. There was a high frequency of ventricular dysrhythmias in patients with diabetes and end stage renal disease and all of these occurred in those with a prolonged QTc on their ECG recorded at the start of dialysis. Hence we conclude that routine baseline ECGs before dialysis should be part of regular clinical care.
9. These results suggest that careful monitoring of cardiac rhythm may be justified in those with an abnormal QTc on their resting ECG.

Future Directions:

A real time CGM study would be essential in larger number of patients on different insulin regimen to understand the relation between glycaemic variation and insulin therapy to guide us formulate an appropriate regimen/dose titration for HD.

Appendices

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Participant information sheet for people with diabetes

Study of glucose control during haemodialysis in C-peptide negative diabetic end stage renal disease population in Teesside

Chief Investigator: Professor R Bilous

We would like to invite you to take part in a research study in people with diabetes and end stage renal disease needing maintenance haemodialysis. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish. Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part.

What is the purpose of the research?

It is known that people with diabetes and kidney disease needing haemodialysis have difficulty in controlling their blood glucose level adequately. It is difficult to make very effective changes to Insulin doses during this period to help patients improve glucose control based on finger prick tests alone. We would like to use a 'continuous glucose monitoring system' to assess glucose levels, which records changes in glucose levels in a real time fashion at set intervals and find out if we can make helpful recommendations for Insulin dose change for people on dialysis.

Patients undergoing haemodialysis have a higher risk of severe adverse consequences, more so in patients with diabetes. It is thought that there are possible sudden changes to heart rate and rhythm, which we can detect from small changes on heart tracings. Low blood glucose levels could be associated with these changes. We would like to use a 7 day heart trace monitor in order to examine any changes and link them with blood glucose levels. We will also check various salt levels in the blood to see if these are linked to heart rate and rhythm.

Why have I been invited?

We are inviting all patients in Teesside with diabetes treated with Insulin undergoing haemodialysis for kidney failure, who have evidence of having no insulin production of their own. We are looking to involve 15 to 20 patients with diabetes to give us adequate knowledge. We are inviting some patients in the region undergoing haemodialysis without diabetes to understand the variation in blood glucose levels without the effect of insulin treatment. We are looking to involve 4 to 5 patients in this group.

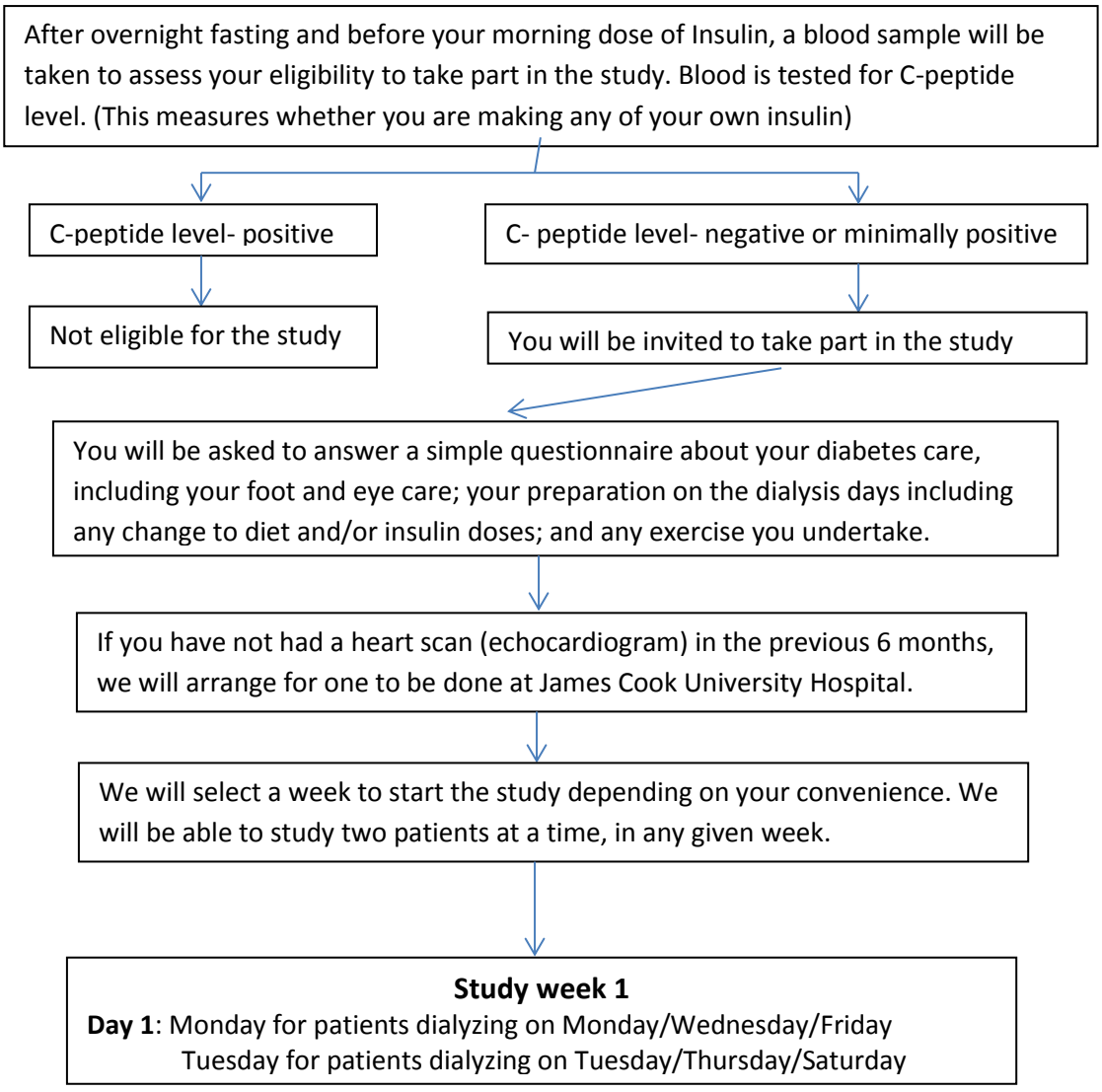
Do I have to take part?

It is up to you to decide. We will describe the study and go through this information sheet, which we will then give to you. If you are happy to proceed, we will then ask you to sign a consent form to show that you agree to take part. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care you receive.

What will happen to me if I take part?

The study is a pilot study to understand the changes in blood glucose levels before, during and after haemodialysis together with any variation in heart rate and rhythm. To improve blood glucose levels, it is important to understand their variation, so effective changes to insulin dose or regimen can be made. Also it is important to understand any changes to heart tracings during haemodialysis, which could potentially cause life threatening rhythm problems.
There will be two groups of patients depending on whether they have diabetes or not.
The study will be carried out over one to two years, but you will be involved up to 10 weeks.

This flow chart below explains what it involves for you as a study subject, ***if you have diabetes.***



On your arrival for dialysis, blood samples will be taken for average blood glucose (HbA1c), heart enzyme (Troponin I), salt levels (Sodium, Potassium, Calcium, and Magnesium), Kidney & liver tests and cholesterol levels. You will be weighed. A heart trace (ECG) will be recorded.
A continuous glucose monitor will be attached by inserting a small needle under the skin on your tummy. There would not be any tubes between the monitor and the needle. A heart monitor (Holter) will be attached via 3 wires attached to 3 sticky pads on your chest.

Dialysis started as usual

Midway through the dialysis, a blood sample will be taken for salt levels and second heart trace will be recorded

At the end of dialysis, a further blood sample will be taken for salt levels and a third heart tracing will be recorded. You will be weighed again.

You will be allowed home or your place from where you arrived, with the glucose monitor and the heart monitor. These will stay with you for 7 days. If you have any problem with these monitors, you should call the emergency mobile number provided at the end of this leaflet.
Similar blood samples and ECG recordings will be made at your next two dialysis sessions.

You will not be able to see the glucose readings on the monitor. You are expected to check your glucose levels by finger prick tests as you do and record them. You are expected to maintain a dairy of your insulin doses, any symptoms of low or high glucose. We will copy these on to our notes when you come for dialysis the week after.

Study week 2

On arrival for your first dialysis of the week, you will have a blood sample taken for glucose control measures (HbA1c, Fructosamine & Glycated albumin) & heart enzyme (Troponin I). The glucose & heart monitors will be taken away. We will see you again after a 3 week gap. Your recordings on your blood glucose & insulin dairy will be noted.

Study week 5

If you are happy to proceed, on your arrival for the first dialysis of the week, the continuous glucose monitor and the heart monitor will be attached as above. You will be weighed. Dialysis started as usual. Glucose and heart monitors will stay with you for 7 days. You are expected to continue to check your blood glucose levels and record your insulin doses as before.

Study week 6

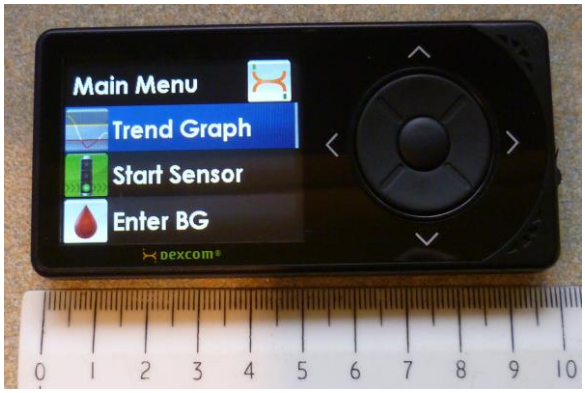
On your arrival for the first dialysis of the week, you will have blood sample taken for glucose control measures and heart enzyme. Both monitors will be taken away. Your entries in your glucose dairy will be noted.

Study week 9

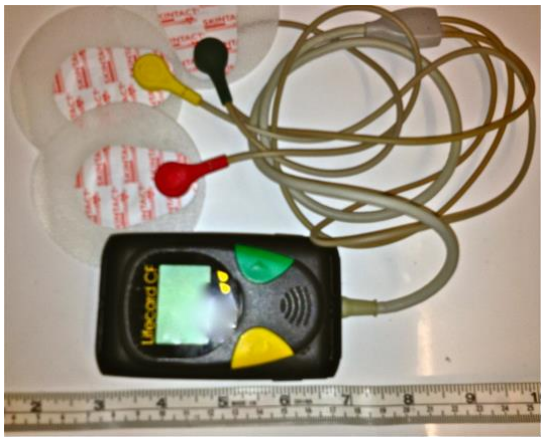
If you are happy to proceed, on your arrival for first dialysis of the week, the continuous glucose monitor and the heart monitor will be attached as above. You will be weighed. Dialysis started as usual. Glucose and heart monitors will stay with you for 7 days. You are expected to continue to check your blood glucose levels and record your insulin doses as before.

Study week 10

On arrival for your first dialysis of the week, you will have a blood sample taken for glucose control measures and heart enzyme. Both monitors will be taken away. This finishes your active participation in the study. We will arrange to see you at a later date at the dialysis unit, to explain the results of the monitors.



This picture shows the glucose monitor (black oval instrument). This links with a small transmitter (small grey instrument below) attached to you on top of the needle inserted under the skin. The monitor needs to stay in your pocket/bag/room.



This picture shows the heart monitor, which can be kept in your pocket or tucked in to your belt. This will be attached via 3 wires to 3 sticky pads on your chest

What will I have to do?

During the study,
1. You should fill in the questionnaire attached and return it to the doctor.
2. You should continue to check your blood glucose levels with finger prick tests and document along with Insulin type and dose you take.
3. You will be expected to keep the glucose monitoring system and heart monitor attached for the full period of study in that week.
4. You should continue to attend your dialysis sessions as usual and any other appointments with your GP or hospital.
5. You should carry on usual activity through this period.

What are the possible disadvantages and risks of taking part?

You will have the continuous glucose monitoring system attached to you with a tiny needle under your skin, on your tummy for full week and a Holter (heart trace) monitor attached via three cables to sticky pads on your chest and tummy for 7 days.
The potential risks anticipated are;
1. Mild pain or discomfort due to the needle (similar in size to your insulin pen needle), inserted under the skin.
2. Minor inconvenience with some of the routine activities you undertake on a day to day basis.
3. We will not be changing your Insulin dose or type during this study, unless done so by your doctor looking after your diabetes. You are free to adjust your dose as usual.
4. This kind of monitoring system has been available for many years and used in different patient groups. The safety of these systems has been proven and there are no side effects other than minor discomfort.
5. You will be provided with a contact telephone number to ring anytime of the day, if you happen to suffer any untoward adverse effect due to the monitoring system.

What are the possible benefits of taking part?

Understanding the changes in your blood glucose levels during and in between haemodialysis could help in suggesting changes to your Insulin type and doses. Your week long test results will be studied later and results discussed with you and passed on to your doctor in charge of your diabetes care. Depending on these results, your doctor may consider changing your Insulin type or dose to improve your control.
Your heart tracings will be studied in detail later for any changes in rate or rhythm, specifically looking for changes during periods of low glucose levels. If any are found, results will be discussed with you and passed on to your doctor and a cardiologist (heart doctor) for <u>further investigation or treatment</u>.
If this monitoring is found helpful, we aim to suggest similar monitoring to be taken up as regular care of similar patients.
This will also help us to understand the possible glucose changes before, during and after haemodialysis in patients. We expect to use this knowledge to improve the care of patients with diabetes undergoing haemodialysis, in terms of reducing time they spend with both high and low blood glucose levels.

What happens when you finish your study period?

When you finish your study period, you will continue with your dialysis and diabetes care as before. Your continuous glucose readings will be studied later and conveyed to you and your doctor in charge of diabetes care. As a result your Insulin type and dose may be changed by your doctor as deemed necessary. Your ECGs and 7 day long heart tracing will be studied especially in relation to your salt and glucose levels. The results will be discussed with you and passed on to your doctor and also a cardiologist if required.

Will my taking part in the study be kept confidential?

Yes. We will follow the ethical and legal practice and all information about you will be handled in confidence.
All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognized from it, unless it is passed on to your family doctor with your consent. All information stored about you will be labelled anonymously, with a study number rather than your name. We will store all information gathered in the study for fifteen years in the archive unit of the hospital. The only people with access to the information will be the researchers and representatives from the Trust in order to make sure that the research in being carried out correctly.

What if relevant new information becomes available?

Sometimes we get new information from published research work carried out elsewhere regarding similar method of glucose monitoring. If this happens we will tell you and discuss any relevant findings. We do not foresee any possible reports about any disadvantages or untoward effects of this method of glucose monitoring.

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

You can withdraw from the study at any time if you wish, without giving any reason. Your care will continue as usual, even if you withdraw from the study.

What if there is 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. If you remain unhappy and wish to complain formally, you can do this through NHS complaints procedure. Details can be obtained from the hospital; Patient Advice and Liaison Service, The James Cook University Hospital, Marton Road, Middlesbrough, TS4 3BW, Email pals@stees.nhs.uk or call 0800 0282451 at The James Cook University Hospital and 0800 0282462 at the Friarage Hospital
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 legal action against South Tees Acute NHS Trust, but you have to pay legal costs. The normal National Health Service complaints mechanisms will still be available to you.
There is no cover for harm (caused by unexpected event associated with taking part in the study), which is no one's fault. If a no fault injury occurs the trial sponsors, South Tees Acute NHS Trust, will not be held responsible. It will not be possible to claim damages against the trust.

Will my GP (family doctor) know I am in the study?

Yes. If you agree to take part in this study, we will write to your GP to let him/her know that you are taking part with your consent.

What will happen to results of the research study?

The results of the study will be published in the medical journals. At the end of the study we will send you information about the final results.

Who is organizing and funding the research?

The research has been organized by South Tees Acute NHS trust. Your doctor or nurse will not be paid other than his / her usual salary.

Who has reviewed this study?

All research in the NHS is looked at by an independent group of people called the, 'Research Ethics Committee', to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given a favourable opinion by 'Newcastle and North Tyneside 2 Research Ethics Committee'.

Further information
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Participant information sheet for non- diabetic control subjects

Study of glucose control during haemodialysis in C-peptide negative diabetic end stage renal disease population in Teesside

Chief Investigator: Professor R Bilous

We would like to invite you to take part in a research study in people without diabetes but with end stage renal disease needing maintenance haemodialysis. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish. Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part.

What is the purpose of the research?

It is known that people with diabetes and kidney disease needing haemodialysis have difficulty in controlling their blood glucose level adequately. It is difficult to make very effective changes to Insulin doses during this period to help patients improve glucose control based on finger prick tests alone. We would like to use a 'continuous glucose monitoring system' to assess glucose levels, which records changes in glucose levels in a real time fashion at set intervals and find out if we can make helpful recommendations for Insulin dose change for people on dialysis. In order to make sense of this information we also need to test a small number of people who are on haemodialysis but who do not have diabetes.

Patients undergoing haemodialysis have a higher risk of severe adverse consequences, more so in patients with diabetes. It is thought that there are possible sudden changes to heart rate and rhythm, which we can detect from minute changes on heart tracings. Low blood glucose level could be associated with these changes. We would like to use a 7 day heart trace monitor in order to examine any changes and link them with blood glucose levels. We will also check various salt levels in the blood to see if these are linked to heart rate and rhythm.

Why have I been invited?

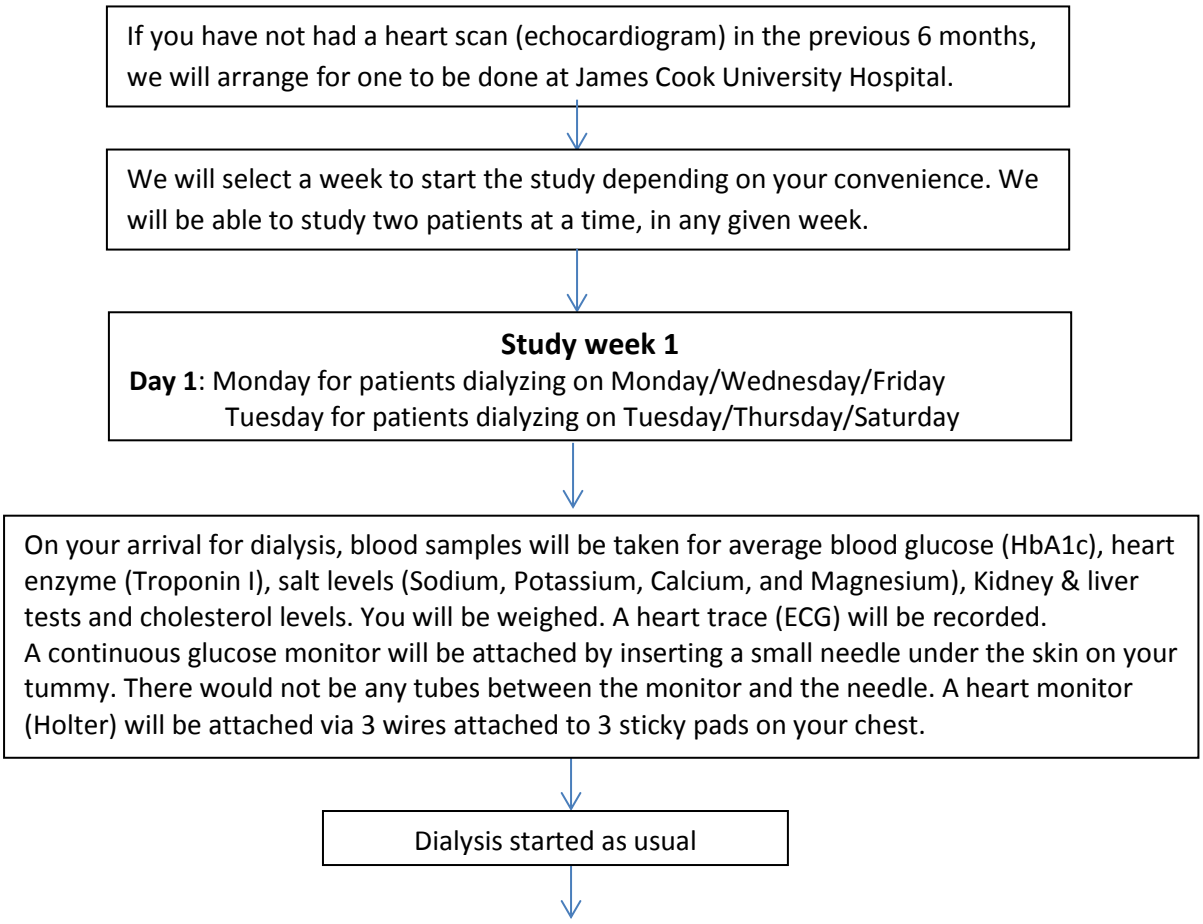
We are inviting patients in the region undergoing haemodialysis without diabetes to understand the changes in blood glucose levels without the effect of insulin treatment. We are looking to involve 4 to 5 patients in this group.

Do I have to take part?

It is up to you to decide. We will describe the study and go through this information sheet, which we will then give to you. If you are happy to proceed, we will then ask you to sign a consent form to show that you agree to take part. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care you receive.

What will happen to me if I take part?

The study is a pilot study to understand changes in blood glucose levels before, during and after haemodialysis together with any variation in heart rate and rhythm. To improve blood glucose levels, it is important to understand their variation, so effective changes to insulin dose or regimen can be made. Also it is important to understand the changes to heart tracings during haemodialysis, which could potentially cause life threatening rhythm problems.
There will be two groups of patients depending on whether they have diabetes or not.
The study will be carried out over one to two years, but you will be involved up to 2 weeks.
This flow diagram below explains what it involves for you as a study subject, ***if you do not have diabetes*.**



Midway through the dialysis, a blood sample will be taken for salt levels and second heart trace will be recorded

At the end of dialysis, a further blood sample will be taken for salt levels and a third heart tracing will be recorded. You will be weighed again.

You will be given a regular glucose meter to do finger prick tests at home for glucose. You will be allowed home or your place from where you arrived, with the glucose monitor and the heart monitor. These will stay with you for 7 days. If you have any problem with these monitors, you should call the emergency mobile number provided at the end of this leaflet. Similar blood samples and ECG recordings will be made at your next two dialysis sessions.

Study week 2

On arrival for your first dialysis of the week, you will have a blood sample taken for heart enzyme (Troponin I). The glucose & heart monitors will be taken away. We will note down your finger prick test results. This finishes your active participation in the study. We will see you at the dialysis centre at a later date to explain the results of the monitors.

You will not be able to see the glucose readings on the monitor.

This picture shows the glucose monitor (black oval instrument). The transmitter (small grey instrument shown below) will be attached to you on top of the needle inserted under the skin. The monitor needs to stay in your pocket/bag/room.

This picture shows the heart monitor, which can be kept in your pocket or tucked into your belt. This will be attached via 3 wires to 3 sticky pads on your chest

What will I have to do?

During the study,
1. You will be expected to keep the glucose monitoring system and heart monitor attached for the full period of study in that week.
2. You should continue to attend your dialysis sessions as usual and any other appointments with your GP or hospital.
3. You should carry on usual activity through this period.
4. You will need to take a finger prick blood sample for a glucose test once or twice a day in order to calibrate the sensor.

What are the possible disadvantages and risks of taking part?

You will have the continuous glucose monitoring system attached to you with a tiny needle under your skin, on your tummy for full week and a Holter (heart trace) monitor attached via three cables to sticky pads on your chest and tummy for 7 days.
The potential risks anticipated are;
1. Mild pain or discomfort due to the needle, inserted under the skin.
2. Minor inconvenience with some of the routine activities you undertake on a day to day basis.
3. This kind of monitoring system has been available for many years and used in different patient groups. The safety of these systems has been proven and there are no side effects other than minor discomfort.
5. You will be provided with a contact telephone number to ring anytime of the day, if you happen to suffer any untoward adverse effect due to the monitoring system.

What are the possible benefits of taking part?

A fall in glucose level is known to occur during dialysis sessions even in people without diabetes. This may not be significant to give you any symptoms, but if significant, it could mean that the glucose concentration of your dialysis fluid needs adjustment.
Your heart tracings will be studied in detail later for any changes in rate or rhythm, specifically looking for changes during periods of low glucose levels, as found on your week long glucose monitor readings. If any are found, results will be discussed with you and passed on to your doctor and a cardiologist (heart doctor) for further investigation or treatment.
If this monitoring is found helpful, we might suggest similar monitoring to be taken up as regular care.

This will also help us to understand the possible glucose changes before, during and after haemodialysis in patients. We expect to use this knowledge to improve the care of patients with diabetes undergoing haemodialysis, in terms of reducing time they spend with both high and low blood glucose levels.

What happens when you finish your study period?

When you finish your study period, you will continue with your dialysis as before. Your continuous glucose readings will be studied later and conveyed to you. Your ECG's and 7 day long heart tracing will be studied especially in relation to your salt and glucose levels. The results will be discussed with you and passed on to your doctor and also a cardiologist if required.

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Further information

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