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RISK FACTORS FOR ATHEROSCLEROSIS IN BLACK SOUTH AFRICAN PATIENTS ON HAEMODIALYSIS

Christiana Oluwatoyin Amira

A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the degree of Master of Science in Medicine

Johannesburg, 2005

DECLARATION

I, Christiana Oluwatoyin Amira, declare that this dissertation is my own work. It is being submitted for the degree of Master of Science in Medicine in the branch of Nephrology in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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CHRISTIANA OLUWATOYIN MIRA

……day of …… 2005.

DEDICATION

I dedicate this work to:

My mother, Mrs Victoria Olubukun Elewa without whose support this programme could not have been accomplished and to the memory of my late father Mr Perkins Ogunbiyi Elewa who started me in this career.

To my lovely children, Oluwatobi and Omolola Amira for bearing with my long absence from home.

To God who makes all things possible.

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS STUDY

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Amira CO, Naicker S, Sliwa K, Mia A, Manga P. Risk factors for atherosclerosis among chronic haemodialysis patients. 3rd World Congress of Nephrology, Singapore, June, 2005

ABSTRACT

INTRODUCTION

The risk of cardiovascular disease in patients with end stage renal disease (ESRD) is far greater than in the general population. Amongst patients with ESRD, the prevalence of coronary artery disease (CAD) and congestive heart failure is approximately 40% compared with 5-12% in the general population. The excess risk is caused by multiple traditional and non-traditional risk factors for ischaemic heart disease present in these patients. There is little information on CAD and its risk factors in black haemodialysis patients as most of these studies were carried out in the white population. This study is therefore aimed at determining the risk factors for atherosclerosis in Black and non-black (White and Indian) South African patients on haemodialysis.

METHODS

Fifty-eight black patients and twenty-six non-black patients on haemodialysis were recruited. Sixty-three age and sex matched controls (staff, students and kidney donors) were also recruited. Fasting venous blood samples were drawn for measurement of C-reactive protein, homocysteine, Lp (a), serum lipids and adiponectin. Carotid intima-media thickness and plaque occurrence was measured by B-mode ultrasonography. Echocardiography was used to determine LVH.

RESULTS

Haemodialysis (HD) patients had significantly lower total cholesterol, LDL cholesterol and triglycerides compared with controls ($p<0.001$; $p= 0.042$). Hs-CRP, adiponectin and homocysteine levels were significantly higher in patients compared with controls ($p< 0.001$). The prevalence of plaques was significantly higher among HD patients (32%) compared with controls (7%) $X^2 = 60.72$ $p< 0.001$. LVMI was significantly higher among HD patients ($194.25\pm 7.69 gm/m^2$) compared with controls (93.21 ± 3.27 gm/m^2) $p < 0.001$. No significant difference between patients (Black or Asian/White) and controls with respect to CIMT was found. CVD risk factors in black haemodialysis patients and black controls showed a similar pattern to the whole study population combined. Risk factors associated with CIMT on regression analysis were total cholesterol, LDL-cholesterol, age, Hs-CRP, family history of CKD. Risk factors associated with plaque occurrence on logistic regression analysis were age, systolic blood pressure, male gender, smoking, calcium phosphate product and serum phosphate.

CONCLUSION

HD patients have a high prevalence of traditional and non-traditional risk factors for atherosclerosis and this is independent of race. Traditional risk factors like lipids were much lower in ESRD patients. HD patients showed a high prevalence of atherosclerosis as measured by increased carotid intima-media thickness and plaque occurrence in carotid arteries. Hs-CRP correlated significantly with a surrogate marker of atherosclerosis (CIMT).

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LIST OF ABBREVIATIONS

ABC1gene	ATP-binding cassette transporter 1 gene
ACE	angiotensin converting enzyme
ADMA	asymmetric dimethylarginine
ADPN	Adiponectin
AGES	advanced glycation end products
AHA	American Heart Association
AMVL	anterior mitral valve leaflet
Ang II	angiotensin II
APKD	Adult polycystic kidney disease
Apo	apolipoprotein
apo(a)	apolipoprotein (a)
ARBs	angiotensin II receptor blockers
ARIC	Atherosclerosis Risk in Communities
ASCVD	Atherosclerotic cardiovascular disease
AT	acceleration time
BMI	body mass index
BP	blood pressure
BSA	body surface area
CAD	coronary artery disease
CDC	Centers for Disease Control and Prevention
CE	cholesterol ester
CHF	congestive heart failure

CHOICES	Choices for Healthy Outcomes in Caring for ESRD study
CIMT	carotid intima-media thickness
CKD	chronic kidney disease
CON ABN	Congenital anomalies
CP	Chronic pyelonephritis /reflux nephropathy
CREED	The Cardiovascular Risk Extended Evaluation in Dialysis
CRF	chronic renal failure
CRP	C-reactive protein
CV	cardiovascular
CVD	cardiovascular disease
DBP	diastolic blood pressure
DCMO	dilated cardiomyopathy
DDAH	dimethylarginine dimethylaminohydrolase
DNA	deoxyribonucleic acid
DT	deceleration time
EBCT	electron beam computed tomography
Echo	echocardiography
ESRD	end-stage renal disease
ESRF	end-stage renal failure
GFR	glomerular filtration rate
GN	glomerulonephritis
GSH	reduced gluthathione
H_2O_2	hydrogen peroxide
Hb	haemoglobin

Hct	haematocrit
HCT	haematocrit
HD	haemodialysis
HDL	high density lipoprotein
HEMO	The Haemodialysis study
HMG-CoA	hydroxy methylglutaryl coenzyme A
HOPE	Heart Outcomes Prevention Evaluation study
HOST	Homocysteinemia in Kidney and End Stage Renal Disease Study
Hs-CRP	high sensitivity C-reactive protein
HTN	hypertension
ICAM	soluble intercellular adhesion molecule 1
IDL	intermediate density lipoprotein
IHD	ischaemic heart disease
IL-2	interleukin-2
IL-6	interleukin-6
IMT	intima-media thickness
IVSTd	interventricular wall thickness during diastole
LCAT	lecithin- cholesterol acyl-transferase
LDL	low density lipoprotein
LMW	low molecular weight
Lp	lipoprotein
Lp(a)	lipoprotein (a)
LPL	lipoprotein lipase
Lupus	Lupus Nephritis

LV	left ventricular
LVEDD	left ventricular internal dimension measurements in end-diastole
LVESD	left ventricular internal dimension measurements in end-systole
LVH	left ventricular hypertrophy
LVM	left ventricular mass
LVMI	left ventricular mass index
MAP	mean arterial pressure
MCP-I	macrophage chemotactic protein-I
M-CSF	monocyte colony stimulating factor
MDRD	Modification of Diet in Renal Disease
MI	myocardial infarction
MISC	Miscellaneous causes
MNSA	muscle sympathetic nerve activity
NA	Not available.
NCEP	National Cholesterol Education Program
NHANES II	2nd National Health and Nutrition Examination Survey
NHLS	National Health Laboratory
NKF	National Kidney Foundation
NMA	N-monomethyl-L-arginine
NOS	nitric oxide synthase
O_2^-	superoxide anion
oxLDL	oxidized low density lipoprotein
PD	peritoneal dialysis
PL	phospholipids

PP	pulse pressure
PRMTI	protein arginine methyl transferase type I
PTH	parathyroid hormone
PVD	peripheral vascular disease
PWTd	posterior wall thickness during diastole
ROS	reactive oxygen species
RRT	renal replacement therapy
RWT	Relative wall thickness
SAA	serum amyloid protein A
SBP	systolic blood pressure
SCr	serum creatinine
sd-LDL	small dense LDL
SDMA`	symmetric dimethylarginine
SEM	standard error of mean
SOD	superoxide dismutase
SPACE	Secondary Prevention with Antioxidants of CVD in ESRD
TG	triglyceride
TNF α	tumour necrosis factor α
UKM	unknown
US	United States
USRDS	United States Renal Data System
VCAM-1	vascular adhesion molecule 1
VLDL	Very- low- density- lipoprotein
X^2	Chi-squared

CHAPTER 1

1.0 INTRODUCTION AND LITERATURE REVIEW

The risk of cardiovascular disease in patients with end stage renal disease (ESRD) is far greater than in the general population [1]. Several studies from the early 1970s to date have shown that the prevalence of cardiovascular diseases was significantly higher in uraemic patients treated by haemodialysis or peritoneal dialysis compared with other populations of a similar age [1-3]. Amongst patients with ESRD, the prevalence of coronary artery disease (CAD) and congestive heart failure (CHF) is approximately 40% compared with 5-12% in the general population [1]. Even after stratification by age, sex, race and diabetes, the cardiovascular mortality in dialysis patients is 10-20 times higher than in the general population [1, 3, 4]. Atherosclerotic cardiovascular disease (ASCVD) accounts for approximately half of deaths in end stage renal disease (ESRD) and contributes to the extraordinarily high annual mortality of 23% observed in such patients [1, 4]. The incidence of myocardial infarction (MI) and stroke in the dialysis population is 5 to15-folds higher in ESRD [1, 5]. Similarly, about one-third of hospitalizations in dialysis patients are due to cardiovascular diseases (CVD) [4]. Patients with ESRD should therefore be considered in the highest risk group for subsequent CVD. It therefore follows that this patient population should be carefully assessed and treated for CV risk factors early in the course of the disease. The Special Report from the National Kidney Foundation Task Force on Cardiovascular Disease [6] called for studies of ASCVD and its risk factors in ESRD patients.

Coronary artery disease was found to be rare in black subjects in the general population, but the incidence is increasing with changes in socio-economic status [7]. A study carried out in Soweto in the 1960s showed that coronary atherosclerosis accounted for less than 1% of deaths [7]. Cheung et al [8] using data from the Hemodialysis (HEMO) Study reported that black race was associated with a 36% reduction

in CAD and a 46% reduction in peripheral vascular disease (PVD) but was not associated with a reduction in cerebrovascular disease . Most studies of ASCVD risk factors in ESRD patients were carried out in the white population in the United States and Europe. There is limited data on ASCVD in South African patients with ESRD. Therefore this study is aimed at determining the prevalence of traditional and some non-traditional risk factors for ASCVD in the black South African patients on haemodialysis and to determine the relationship of these risk factors to sub-clinical atherosclerotic cardiovascular disease in this population. The study will provide baseline data base of CVD in this high risk population.

1.1 Epidemiology of cardiovascular disease in chronic renal disease

The initial enthusiasm for dialysis as a survival measure for patients with chronic renal failure (CRF) was tempered in 1974 when Linder and colleagues noted the extraordinarily high frequency of coronary heart disease and cardiac death in the first patients who underwent dialysis in Seattle at that time [2]. This observation lead to the hypothesis of accelerated atherosclerosis in CRF, which has remained disputed to date. Cardiovascular disease (CVD) is the leading cause of morbidity and mortality among patients with chronic kidney disease, accounting for 44% of overall mortality in patients receiving long-term dialysis [1, 4, 6]. The report of the Task Force on CVD convened by the National Kidney Foundation (NKF) showed that the annual mortality rates in dialysis patients (≈ 50 000 deaths) are much greater than in the general population (≈ 2 million deaths) despite stratification by age, sex, race and diabetes [1, 4, 6]. The high CVD mortality in ESRD is due to a high prevalence of CVD and also a high case fatality rate in those who already have CVD. Numerous data have shown that the prevalence of ischaemic heart disease and congestive heart failure is much higher in dialysis patients compared to the general population [1, 3, 6] table 1.1. Herzog et al [9] using data from the United States Renal Data System (USRDS), looked at

outcomes in 34,189 patients on long term dialysis who were hospitalized between 1977 and 1995 with acute MI and found that mortality rates at 2 years and 5 years after MI were 73% and 90% respectively. These figures are much higher than the rate after MI in the general population, even in patients with co morbid conditions. In the Worcester Heart Attack Study [10], mortality rates at 2 years after MI in diabetic men and women were 25% and 34% respectively. Cardiac failure is also associated with high case fatality in dialysis patients. In one study cardiac failure at the inception of dialysis was associated with a 2-fold increase in mortality rate [11]. Recent evidence has shown that the processes contributing to CVD commence early in the course of kidney disease and vascular and kidney diseases tend to progress together [1, 3 12]. The prevalence of CVD is increased in all the stages of CKD and also in renal transplant recipients who are also at high risk for CVD.

Table 1.1 Approximate prevalence (%) of CVD in the General Population and Dialysis Patients

Population	CAD (clinical)	LVH (echo)	CHF (clinical)
General population	5-12	20	5
Chronic renal insufficiency	NA	25-50 (varies with renal function)	NA
Haemodialysis	42	75	40
Peritoneal dialysis	40	75	40
Renal transplant recipient	15	50	NA

From [1]. Abbreviation: NA, Not available. Echo, echocardiography

1.2 Spectrum of CVD in patients with CKD

There are three distinct types of CVD that are highly prevalent in patients with CKD, all of which lead to poor outcome. These include alterations in cardiac geometry, atherosclerosis and arteriosclerosis.

1.2.1 Alterations in cardiac geometry

The alteration in cardiac geometry includes concentric left ventricular hypertrophy (LVH) and eccentric LVH (left ventricular dilatation plus hypertrophy). Concentric LVH is associated with pressure overload, as in hypertension, arteriosclerosis or occasionally aortic stenosis [1, 5, 12]. It is characterized by increased wall thickness (increased myocyte thickness) and normal or decreased left ventricular chamber size [5, 12]. Concentric LVH is common among patients with CKD with a prevalence of approximately 40% in those starting dialysis [1, 12]. The prevalence of LVH is inversely related to glomerular filtration rate (GFR). In a study by Levin et al [13], the prevalence of LVH as measured by echocardiography was 45%, 31%, and 27% in patients with creatinine clearance of < 25, 25 to 50, and >50ml/L respectively. Concentric LVH results in increased ventricular stiffness or diastolic dysfunction [5]. Eccentric LVH is characterized by an increase in wall thickness (increase in myocyte length) that is proportional to the increase in left ventricular (LV) diameter [1, 5, 12]. Risk factors for eccentric LVH include volume overload secondary to salt and water retention, anaemia and arteriovenous fistulae [1, 5, 12]. Eccentric LVH leads to systolic dysfunction. In a Canadian prospective cohort study of 432 patients who had echocardiography done within one year of starting dialysis, only 16% had normal cardiac function and dimensions, 42% had concentric LVH, 27% LV dilatation and 16% had systolic dysfunction [14]. LV mass index is strongly associated with cardiovascular mortality in the general population; this association is also found in dialysis patients [14].

1.2.2 Atherosclerosis

Atherosclerosis is the main cause of ischaemic heart disease in dialysis patients. Atherosclerosis is focal and primarily involves the intima causing narrowing or occlusion of the arteries with restriction of blood flow [12, 15]. In comparison with the general population, the atherosclerotic lesions in kidney failure tend to be more advanced and are frequently calcified [12, 15]. Surrogates of atherosclerosis include

carotid intima-media thickness (IMT) which is measured by high resolution B-mode ultrasonography and inducible myocardial ischaemia which is detectable by coronary stress test [5, 12, 15]. The gold standard for the diagnosis of CAD is coronary angiography. Stress imaging is an important modality for testing for myocardial ischaemia; however it may not be sensitive in dialysis patients [12, 16]. Dobutamine echocardiography and combined dypyridamole-exercise thallium imaging have been shown to be accurate in detecting CAD and predicting future coronary events [16]. Atherosclerosis presents clinically as IHD, MI, CHF, PVD, sudden death and stroke [1, 12, 15]. The prevalence of IHD and cardiac failure is approximately 50% among patients starting renal replacement therapy in the United States [1, 5].

1.2.3 Arteriosclerosis

Arteriosclerosis is a disease of large vessels, such as the carotid and the aorta, in which there is diffuse involvement of the media resulting in increased arterial stiffness and decreased distensibility or compliance [12, 15]. Arterial stiffness is measured by pulse wave velocity. Increased arterial stiffness often results in increased pulse pressure causing increased LV after load and concentric LVH [12, 15, 17]. Arteriosclerosis also predisposes to IHD by decreasing subendocardial coronary perfusion [12, 15]. Higher systolic BP and pulse pressure, lower diastolic BP and LVH have been identified as independent risk factors for CV morbidity and mortality in ESRD [15].

1.3 Risk factors for CVD in chronic kidney disease

Several studies have shown that the high prevalence of CVD in ESRD patients can only be partly explained by the traditional risk factors [3, 8, 18]. The excess risk of CVD in ESRD is caused by multiple traditional and non-traditional risk factors for ischaemic heart disease present in these patients. The traditional risk factors are those variables that were defined in the general population in the Framingham study [19]. The traditional risk factors include older age, male gender, white race, hypertension, elevated

low-density lipoprotein cholesterol level (LDL Cholesterol), low levels of high density lipoprotein cholesterol (HDL cholesterol), diabetes mellitus, obesity, family history of CVD, cigarette smoking and psychosocial stress [12,19]. ESRD patients often have a high prevalence of these traditional risk factors [1, 3, 12]. The non traditional risk factors that are frequently observed in ESRD include: hyperhomocysteinaemia, increased levels of lipoprotein (a), enhanced oxidative stress, inflammation, abnormal calcium phosphate metabolism, disturbances of nitric oxide system, anaemia, malnutrition, and alterations in the thrombotic system (table 1.2). This latter group of risk factors may be related to uraemia and the dialysis procedure, and can actively contribute to the accelerated development and progression of CVD. Colorado et al [18], using data from the Modification of Diet in Renal Disease (MDRD) study examined traditional risk factors in 1,795 patients with CKD. They computed the coronary point score, which is an aggregate of traditional risk factors derived from the Framingham study, which predicts the probability of developing CAD over 5 to 10 years in individuals who do not have CAD. The results showed that the coronary point score in patients with CKD was no different from those in the general population thus suggesting that the traditional risk factors could not sufficiently account for the burden of CVD in CKD.

Table 1.2 Risk factors for cardiovascular disease in CKD

Traditional	**Non-traditional**
Hypertension	Inflammation
Diabetes	Oxidant stress
Smoking	Hyperhomocysteinaemia
Older Age	Anaemia
Male sex	Abnormal calcium/phosphate metabolism
Higher LDL Cholesterol	Lipoprotein (a) and apolipoprotein(a) isoforms
Lower HDL Cholesterol	Advanced glycation end products (AGES)
Family History of CVD	Asymmetric dimethylarginine (ADMA)
Menopause	Malnutrition
Physical inactivity	Aetiology of Chronic Kidney Disease (CKD)
LVH	Proteinuria
	Extracellular fluid volume overload
	Renin angiotensin system activity
	Decrease in glomerular filtration rate (GFR)
	Endothelial dysfunction
	Thrombogenic factors

1.3.1 Traditional risk factors

1.3.1.1 Hypertension

The prevalence of hypertension in chronic renal disease (CRD) is approximately 60% to 100% depending on the target population, cause of renal disease and residual renal function [20]. In the Choices for Healthy Outcomes in Caring for ESRD (CHOICE) study, the prevalence of hypertension was 96% in the study participants [21]. Hypertension contributes to the development of left ventricular hypertrophy (LVH), coronary artery disease and congestive heart failure [20]. LVH, coronary artery disease and congestive heart failure are associated with subsequent mortality [14, 20]. Patients with ESRD have a high prevalence of non-dipping and are also likely to have isolated systolic hypertension with an increased pulse pressure, which in turn has been associated with CVD and early death [22]. The aetiology of hypertension in ESRD is multifactorial and includes expanded extracellular volume, sodium retention, over activity of the sympathetic nervous system, activation of the renin angiotensin aldosterone system, endothelial dysfunction, use of erythropoietin and secondary hyperparathyroidism [20, 22]. Apart from causing hypertension, many of these factors probably cause damage to the kidney and the cardiovascular system. The association between blood pressure (BP) and mortality in haemodialysis shows a U- shaped relationship with both high as well as low BP carrying a high mortality [23]. Port et al [24] analyzed data from 4839 chronic haemodialysis patients in the Case Mix Adequacy Study of the U.S. Renal Data System: they found that a higher systolic BP (from 150 to > 180 mmHg) was associated with a lower mortality risk. When the predialysis systolic BP decreased below 110mmHg, the relative mortality rate increased significantly. The association between low blood pressure and mortality reflects the high frequency of advanced cardiomyopathy in dialysis patients. The BP level that minimizes death risk in dialysis patients has not been determined. There are conflicting views about the level of BP recommended in haemodialysis patients. Schomig et al [25] believe that the same principles and goals established for the general population should apply to the haemodialysis population (BP<120/80); others

feel that these guidelines do not apply to the majority of haemodialysis patients. The National Kidney Foundation Task Force on Cardiovascular Disease recommend that a target predialysis BP of 140/90mmHg or less appears reasonable in haemodialysis population [6]. Antihypertensive medications are effective in decreasing BP in patients with ESRD and the commonly used drugs are calcium channel blockers, angiotensin converting enzyme (ACE) inhibitors, and alpha and beta-adrenergic blockers [20,22]. These drugs have been shown to be beneficial in high-risk patient groups [20, 22]. Angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) are recommended in patients with CKD prior to ESRD [20, 22]. This is based on evidence of renoprotection. ACE inhibitors may also have a role in CVD management and are recommended in patients with ESRD with increased left ventricular mass index [20, 22].

1.3.1.2 Dyslipidaemia

1.3.1.2.1 Metabolism of lipids

Lipids are transported in the blood stream as macromolecular complexes [26, 27]. These complexes are known as lipoproteins and are composed of a core of lipids (mainly triglyceride, cholesterol and cholesterol esters), which are surrounded by a stabilizing coat of phospholipids. Embedded on the surface of these lipoproteins are proteins called apoproteins (Apo) which allow these particles to be recognized by receptors in the liver and peripheral tissues. Five principal types of lipoprotein particles are found in the blood based on their density and electrophoretic mobility. Chylomicrons are synthesized in the small intestine postprandially and contain mainly triglyceride (TG) with small amounts of cholesterol. They are the main mechanism for transporting products of dietary fat digestion to the liver and the peripheral tissues [26, 27]. Chylomicrons contain apoproteins B-48, A-1, AII and acquire Apo C-II and Apo E by transfer from HDL particles in the blood stream. Apo C-II binds to specific receptors in the adipose tissue, skeletal muscle and the liver and allows the endothelial enzyme lipoprotein lipase to remove most

of the triglyceride leaving a remnant chylomicron particle that contains the bulk of the original dietary cholesterol. The remnant particle is taken up by the liver and metabolized. Very- low- density-lipoprotein (VLDL) contains endogenously synthesized TG and a small amount of cholesterol. VLDL contains Apo B-100 and acquires Apo C-II and E by transfer from HDL particles. TG is removed from VLDL by the enzyme lipoprotein lipase through binding with Apo C-II, leaving an intermediate density lipoprotein (IDL) that contains cholesterol. IDL have Apo B-100 and Apo E molecules on their particle surface. Most IDL particles bind to liver LDL receptors through Apo E molecule and are catabolised. Some IDL particles have further TG removed by the enzyme hepatic lipase producing low-density lipoprotein particles (LDL) [27].

LDL is the main carrier of cholesterol and delivers it to the liver and peripheral tissues. Peripheral cells utilize LDL cholesterol for cell membrane structure and also the production of hormones. LDL particles contain Apo B-100 and Apo E. Apo B-100 is the principal ligand for the LDL clearance receptor [27]. Once bound to the receptor, the coated pit invaginates and fuses with liposomes that destroy the LDL particle. The number of hepatic LDL clearance receptors and the activity of the rate-limiting enzyme in the cholesterol synthetic pathway, hydroxy methylglutaryl coenzyme A (HMG-CoA) reductase, regulate the concentration of LDL in the blood. LDL particles are heterogeneous with respect to size, density, and lipid compositions [28, 29]. LDL particles have been divided into 2 distinct phenotypes: pattern A, with a higher proportion of large buoyant LDL particles, and pattern B, with a predominance of small dense LDL (sd-LDL) particles [26, 29]. The subclass pattern B is frequently associated with a more atherogenic lipoprotein profile. LDL subclass pattern B consists of higher levels of triglyceride, apolipoprotein B, intermediate density lipoprotein (IDL), very low-density lipoprotein (VLDL) and a lower level of high-density lipoprotein (HDL) [26,27]. It has been suggested that sd-LDL are highly atherogenic as a result of their higher penetration into the arterial wall, decreased affinity for the LDL receptor, their prolonged

plasma half-life, and increased susceptibility to oxidative stress compared to buoyant LDL [28, 29]. LDL is an atherogenic lipoprotein particle, and it is established that higher levels of LDL are associated with increased cardiovascular disease risk [29, 30]. In addition, the heterogeneity of LDL particle composition, due to differences in the amount of cholesterol per particle, suggests that particle size is an important consideration in the atherogenic potential of the LDL. High-density lipoprotein particles are produced in the liver and the intestine. They contain Apo A-1 and are transformed into mature HDL particles by acquisition of phospholipids, Apo E and Apo C. Mature HDL particles take up cholesterol from cells in the peripheral tissues aided by cholesterol-efflux regulatory protein - a product of the ATP-binding cassette transporter 1 gene (*ABC1 gene*). Apo A-1 is important for the activation of the enzyme lecithin- cholesterol acyl-transferase (LCAT), which transfers acetyl group from phosphatidylcholine to cholesterol to form cholesterol esters [26, 30]. HDL Cholesterol has an important role to play in the mechanism against atherosclerosis. First, it is a component of the reverse cholesterol transport system that is, HDL particles transport cholesterol away from the periphery and may transfer it indirectly to other particles such as VLDL in the circulation or deliver its cholesterol directly to the liver (reverse cholesterol transport). The direct delivery takes place through scavenger-receptor B1. Second, it has some anti- oxidant effects thereby reducing the oxidation of LDL [30]. It also decreases the cytokine-induced expression of adhesion molecules by the vascular endothelium [30].

Table 1.3 Lipoprotein classification

LIPOPROTEIN	MAJOR LIPID COMPONENT	MAJOR APOLIPOPROTEINS	SOURCE
Chylomicrons	TG	Apo A-1, A-II, A-IV Apo C-I, C-II, C-III; ApoB-48, ApoE	Intestine
VLDL	TG	ApoB-100 ApoC-I, C-II, C-III; ApoE	Liver
IDL	CE	ApoB-100; ApoE, ApoC	Catabolism of VLDL
LDL	CE	ApoB-100	Catabolism of IDL
HDL	CE, PL	ApoA-I, A-II, A-IV ApoC-I, C-II, C-III; ApoE	Liver, intestine

TG = Triglyceride; CE = Cholesterol ester; PL = Phospholipid; [From 26].

Table 1.4 Enzymes in Lipoprotein metabolism

ENZYME	FUNCTION
Lipoprotein lipase	Hydrolyses TG in chylomicrons and VLDL
Lecithin-cholesterol acyltransferase	Esterifies free cholesterol on the HDL surface
Hepatic-triglyceride lipase	Hydrolyses TG in IDL and HDL particles
Cholesterol ester transfer protein	Facilitates transfer between lipoprotein cholesterol esters and TG

[From 26]

1.3.1.2.2 Dyslipidaemia in End-stage renal disease

Dyslipidaemia remains a prominent feature of ESRD. Lipid abnormalities include hypertriglyceridaemia, increased levels of very low-density lipoproteins (VLDL), increased levels of intermediate density lipoproteins (IDL), apo B and low levels of high-density lipoproteins (HDL) [29, 30, 31, 32]. The dyslipidaemia is caused by a delayed catabolism of triglyceride rich apoB containing lipoproteins due to decreased activity of lipolytic enzymes such as lipoprotein lipase, hepatic triglyceride lipase and lecithin cholesterol acyl transferase (LCAT) [32]. There is also the presence of increased levels of oxidized low-density lipoprotein (LDL) that provides a more atherogenic attack complex that damages endothelial cells. Oxidized LDL [33] can cause disruption of the endothelial cell surface, promote inflammatory and immune changes via cytokine release from macrophages and antibody production, and increase platelet aggregation. It may also play a role in plaque instability. In addition to ox-LDL, there is the predominance of small dense LDL (sd LDL) which in itself is highly atherogenic [28, 29, 32]. An increased prevalence of small dense LDL particles has been noted in patients with CAD [29]. Therefore, measurement of LDL particle density or diameter has been proposed as a technique for further risk stratification in patients with elevated LDL levels, or for patients with normal LDL levels who have other high risk factors for CAD, or to predict response to a particular therapy [29].

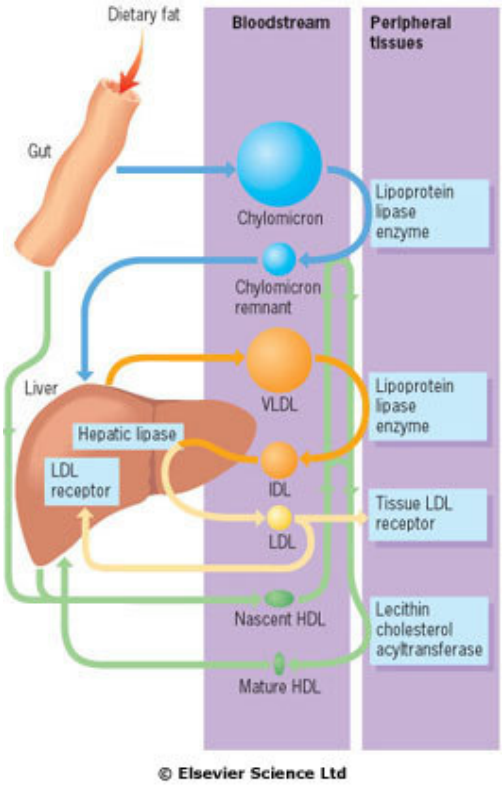


Figure 1.1 Schematic representation of sites of origin, interaction between and fate of the major lipoprotein particles. (From 27)

Although hypercholesterolaemia is associated with increased risk of CVD in the general population, in haemodialysis patients (with the exception of diabetic ESRD patients), low serum cholesterol is associated with a high mortality [34, 35]. Yeun et al [35], in their study involving 91 patients, showed that patients who died had lower cholesterol levels. Lowrie and Lew [34] reported an increase in overall mortality in chronic haemodialysis patients when the serum cholesterol decreased from the range of 5.20 to 6.50 mmol/l (200 to 250 mg/dl) to less than 2.60 mmol/l (100 mg/dl). The possible explanations for this reverse causality are: first, cholesterol may simply be a marker of inflammation or malnutrition both of which are powerful predictors of mortality in dialysis patients. Cytokines like tumour necrosis factor α

(TNF α), interleukin 2 (IL-2) have been reported to have inhibitory effects on lipoprotein lipase activity [30]. Bologa et al reported that high IL-6 levels predicted low serum cholesterol levels, suggesting that cholesterol may be suppressed in response to inflammation. [36]. Second, since most patients on dialysis have dyslipidaemia, it may not be a discriminating factor.

The role of hypertriglyceridaemia as a risk factor for atherosclerosis remains elusive. Although hypertriglyceridaemia is predictive of CVD risk in the general population, this association diminishes in multivariate analysis after adjusting for other risk factors like low HDL cholesterol and high LDL cholesterol. Triglycerides are physiologically linked to sdLDL and low HDL levels and it is postulated that the atherogenic risk for hypertriglyceridaemia is linked to the increased levels of sdLDL [28]. Serum triglyceride is used as a surrogate marker for sd-LDL and triglyceride levels of > 2mmol/l (180mg/dl) is associated with atherogenic levels of sd-LDL in ESRD patients [28, 29]. Apo C-III is a competitive inhibitor of lipoprotein lipase (LPL) and the levels are increased in renal failure [29, 30, 31]. LPL is the main enzyme necessary for the lipolysis of TG and for catabolism of VLDL and chylomicrons in the vascular endothelium. Increased Apo C III levels correlate with increased TG levels in renal failure [30]. This may be accompanied by an increase in IDL remnants of VLDL and chylomicrons [29, 30, 31, 32]. As renal function declines, HDL levels decrease due to many reasons. Firstly, there is decreased synthesis of HDL resulting from downregulation of the Apo A-1 gene expression by the liver [30]. Apo A-1 is an important activator of LCAT and levels are decreased. LCAT esterifies cholesterol and is necessary for the maturation of HDL; therefore HDL maturation is impaired [30]. Inflammation may alter the structure of HDL so that the Apo A-1 protein content of the particle is replaced by serum amyloid protein A (SAA). This new form of HDL has now acquired pro-inflammatory properties; it is chemoattractive to macrophages and has reduced capacity to reduce oxLDL and cannot suppress the effects of inflammatory cytokines on induction of adhesion molecules by the endothelial cells [30].

Renal failure is associated with increased levels of remnant particles (IDL). IDL particles stimulate endothelium-dependent contraction and also induce greater uptake of cholesterol by macrophages than does oxLDL [37]. IDL is an independent risk factor for CVD in patients with CKD [37]. LDL also activates the RAA [30], inducing Angiotensin II levels and upregulating the angiotensin type I (AT1) receptor. Through this mechanism, LDL augments the synthesis of superoxide anion (O^{-}_{2}) thus providing a link between lipids and oxidative stress [30]. Despite the absence of data consistently linking hyperlipidaemia to CAD in patients receiving dialysis, it is recommended that the lipid abnormality in patients with CKD be treated to the same target levels as patients with known CAD [29].

1.3.1.3 Diabetes mellitus

Diabetes mellitus is the most common cause of ESRD in many parts of the world [38, 39]. In the United States, 40% of patients starting dialysis have diabetes [39]. In the CHOICE Study, the prevalence of diabetes was 54% among the participants [21]. In Canada and Europe, almost one third of new patients starting renal replacement therapy are diabetic [39]. The majority of these patients have type-2 diabetes. Diabetes is a well-established and important risk factor for coronary artery disease. Diabetic dialysis patients have the highest cardiovascular mortality with 11% (haemodialysis) and 13% (peritoneal dialysis) per year [40]. Based on the high risk for CAD associated with diabetes and the attendant high CV mortality in these patients, the current recommendation is that all diabetic patients should be treated for risk factors such as hyperlipidaemia even in the absence of clinical signs and symptoms [3, 29]. It follows that all patients with CKD caused by diabetes should be considered for secondary preventive measures [6]. In South Africa, diabetic patients with significant co-morbidity (including cardiac disease) are excluded from the chronic dialysis programme [42].

1.3.1.4 Age

Patients entering renal replacement therapy (RRT) are increasingly over the age of 60years [39]. The average age of a patient starting dialysis in the US is almost 60years [38]. In South Africa, patients over the age of 60 years are excluded from the chronic dialysis programme due to limited resources [42]. CAD is generally a disease of the middle aged and older populations; the majority of patients receiving dialysis are in the age in which CAD is common in the general population.

Majority of the patients starting RRT are males, another known risk factor for CAD [39, 41]. Most women starting RRT are menopausal either by virtue of their age or because of their underlying disease or treatments [39]. Thus the demographics of patients in whom chronic renal dysfunction develops puts them at risk for CAD.

1.3.1.5 Smoking and lifestyle

Although incomplete data are available, a significant number of patients with CKD continue to smoke. Approximately 15-18% of ESRD patients are active smokers [43] and about 15-45% of them smoked before the onset of ESRD [43]. Smoking damages the kidneys and contributes to elevation of blood pressure. Data from USRDS showed that the relative risk for myocardial infarction in patients with type 1 diabetes who smoke was approximately 2.6 compared to patients who did not smoke [43]. Similar results were obtained for patients with type-2 diabetes [29]. Other life issues like exercise and dietary habits have not been well studied in patients with CRF with regards to modifying CV risk. Smoking is a modifiable risk factor for CVD and should be addressed in each patient.

1.3.2 Non-traditional risk factors

1.3.2.1 Inflammation

Inflammation is now considered to play an essential role in the initiation of atherosclerosis as well as plaque erosion and rupture [44]. Inflammatory stimulus leads to the release of a number of acute phase reactants, both negative and positive. The positive acute phase reactants include an elevation of C-reactive protein (CRP), fibrinogen, ferritin, interleukin-6 (IL-6) and lipoprotein (a). The negative acute phase reactants include cholesterol, albumin, prealbumin, transferrin and apolipoprotein B and apolipoprotein A1 and their levels fall during inflammation [45]. Patients receiving haemodialysis are exposed to a wide variety of events that may induce inflammation. These include their underlying diseases such as glomerulonephritis, repeated infection from dialysis access sites, use of bio-incompatible membranes, and water-borne toxins [46]. Many of these patients also suffer from intercurrent illnesses that would induce a state of inflammation. In view of the evidence implicating a key role to inflammation in atherosclerosis, several circulating markers of inflammation have been examined as potential tools for predicting the presence of vascular disease or the risk of vascular events. These markers include P-selectin, interleukin-6 (IL-6), tumour necrosis factor α (TNF α), soluble intercellular adhesion molecule 1 (ICAM) and C-reactive protein (CRP). CRP has emerged as the most powerful inflammatory marker of future cardiovascular risk in the general population and dialysis patients.

1.3.2.2 C-reactive protein (CRP)

CRP is a member of the pentraxin family of proteins and is considered the protypical acute phase reactant in man. CRP is produced in the liver in response to IL-6. CRP levels rise rapidly within 6 hours after a cytokine-mediated response to most forms of tissue injury, infection and inflammation and peaks around 48 hours [45]. CRP levels increase with age and females tend to have slightly higher levels [45]. The circulating level of CRP tends to reflect more the on-going inflammation than do other biochemical

markers of inflammation. This is because the half-life of CRP (about 19 hours) is the same under all conditions and the sole determinant of its plasma concentration is an increased rate of synthesis, which in turn reflects the intensity of the pathological processes stimulating its production. The liver is the site of clearance of CRP, thus production of CRP is impaired in liver failure [45, 46, 47]. The biologic functions of CRP include the ability to recognize pathogens and all damaged cells of the host (including nuclear antigens, lipoproteins and apoptotic cells) and to mediate their elimination by recruiting the complement system and phagocytic cells [48, 49]. Initially CRP was thought to be a bystander marker of vascular inflammation without playing a direct role in the inflammatory process. However recent evidence suggests that CRP may contribute directly to the pro-inflammatory state. CRP stimulates monocytes to release inflammatory cytokines like IL-1, IL-6 and TNFα. It also induces the expression of adhesion molecules like E-selectin, ICAM-1, and VCAM-1 by the endothelial cells. CRP also opsonizes LDL and mediates LDL uptake by macrophages [48, 49]. CRP is produced in the liver, however recent data showed that arterial tissue can produce CRP as well as complement proteins [45, 47]. CRP has emerged as the most powerful inflammatory marker of future cardiovascular risk. In the general population, CRP has been shown to be predictive of cardiovascular events [50, 51]. CRP also carries important prognostic information in acute coronary syndromes. Subjects presenting with unstable angina or non ST- elevation myocardial infarction (MI) and elevated levels of high sensitivity CRP (Hs-CRP) are candidates for adverse events like recurrent angina, ST elevation, MI or coronary death [52]. In the Women Health Study [51], baseline levels of CRP were significantly higher among women who subsequently developed CV events compared with those who did not. CRP levels are known to be higher among patients with several traditional risk factors. Adipocytes are known to secrete IL-6 [47], the main hepatic stimulus for CRP production. Several studies in dialysis patients have shown an association between elevated CRP levels with the risk of cardiovascular events and death [35, 53, 54]. Zimmermann et al [53], in a prospective cohort study of 288 HD patients showed that all-cause and CV mortality was higher in

patients with elevated CRP being 31% and 16% respectively. Patients in the highest quartile of CRP had a 4.6 fold and 5.5 fold higher risks of all cause and CV mortality compared with patients in the lowest quartile [53]. In another study by Iseki et al [54] high CRP levels were associated with poorer survival compared with normal CRP levels. Five-year survival was 44.4% in the high CRP group compared with 82.5% in the normal CRP group. Yeun et al [35] have also identified CRP as the most powerful predictor of all-cause and CV mortality in 91 HD patients who were followed up for 34 months. Patients with CRP levels in the highest quartile had the lowest survival compared with those in the lowest quartile. CRP levels have also been found to be associated with various classic markers of CVD such as Lp (a), fibrinogen and low HDL in ESRD populations [53]. Elevated CRP levels have significant association with hypoalbuminaemia, malnutrition, erythropoietin resistance and morbidity and mortality in both haemodialysis (HD) and peritoneal dialysis (PD) patients [55]. Several studies have demonstrated that elevated CRP levels are associated with surrogate markers of atherosclerotic vascular disease in both HD [53] and predialysis patients [56]. Stenvinkel et al [56] also showed a significantly increased carotid intima media thickness of pre-dialysis patients with elevated CRP levels. The Cardiovascular Risk Extended Evaluation in Dialysis (CREED) did show that CRP was an independent predictor of the number of atherosclerotic plaques in the carotid artery of 112 chronic HD patients [57].

Hs CRP is usually ordered as one of several tests in a CV risk profile, often along with tests for cholesterol and triglycerides. Ridker et al [58] demonstrated that the combination of increased Hs-CRP (> 2.11mg/L) and increased total cholesterol is associated with a 5-fold increase risk of coronary events compared with a 1.5-fold and 2.3-fold increase respectively if only one parameter was elevated. The American Heart Association (AHA) and Centers for Disease Control and Prevention (CDC) have studied the effectiveness of inflammatory markers in clinical cardiac care and prevention. CRP appears to have the strongest association with cardiovascular disease, especially when the high-sensitivity CRP assay is

used [59]. The conclusion of the AHA/CDC report is that high-sensitivity CRP is probably the best supported inflammatory marker and should be measured twice (optimally, two weeks apart). CRP testing should not be used for widespread screening of the general adult population. The focus should be on the major risk factors such as high BP, high cholesterol, smoking and diabetes. Second, CRP is useful as an independent marker of risk and as a discretionary tool in the evaluation of those with moderate risk of CVD to help determine treatment course. Third point is that CRP is not for tracking treatment efficacy due to lack of evidence that reducing Hs-CRP levels improves outcomes such as survival [59].

The AHA/CDC [59] defined risk groups as follows:

- Low risk : < 1.0mg/L
- Average risk : 1.0 -3.0 mg/L
- High risk : > 3.0 mg/ L

Results greater than 10 mg/L may represent a chronic inflammatory or infectious process. Only high-sensitivity (Hs-CRP) or ultra-sensitive tests for CRP are useful for predicting heart attacks, since the elevation in the CRP level in those cases require CRP quantification in the sample in the concentrations below those traditionally measured by the majority of the commercially available assays. A recent publication by Danesh and colleagues [60] has questioned the usefulness of CRP as a predictor of coronary heart disease. In their study, CRP concentration was moderately predictive of coronary heart disease and added only marginally to the predictive value of established risk factors for coronary heart disease. As suggested by the CDC/AHA [59], further clarification of the utility of CRP in predicting future coronary heart disease in the general population is required.

1.3.2.3 Malnutrition

The prevalence of malnutrition in dialysis patients varies between 18-75% depending on dialysis modality, the nutritional assessment tool used and the origin of the patient population [61]. The causes of malnutrition in dialysis patients are multifactorial and include: inadequate nutrient intake, dietary restrictions, nutrient losses during dialysis, hypercatabolism caused by comorbid conditions [61]. Stenvinkel at al [56] in their study which involved 109 predialysis patients reported that 44% of their patients were malnourished. Low serum albumin concentrations are usually used as an index of malnutrition. Albumin is a negative acute phase protein. Its level drops with inflammation. Low serum albumin levels are correlated with high CRP and lipoprotein (a) levels in malnourished CRF patients [56].

Malnutrition and hypoalbuminaemia have been shown to be important predictors of mortality in patients undergoing haemodialysis or peritoneal dialysis [34, 55, 61]. Some recent studies have also shown an association of between hypoalbuminaemia and cardiac disease in haemodialysis patients [61]. The association between malnutrition and cardiac disease has been described in the malnutrition inflammation atherosclerosis syndrome [61]. It has been found that CRF patients with malnutrition have on-going acute phase response and /or carotid plaques. Salvage et al [62] reported serum albumin to be related to carotid plaques and IMT in 24 CRF patients. Stenvinkel at al [56] reported that malnourished patients had higher CRP levels and elevated calculated intima media area and a higher prevalence of carotid plaques.

1.3.2.4 Oxidative stress

Oxidative stress is defined as a disturbance in the balance between anti-oxidants and pro-oxidants, with increased levels of pro-oxidants leading to tissue damage. Oxidant by-products such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), are produced in the body as a consequence of normal metabolism.

These molecules are highly reactive with other biologic molecules, and are referred to as reactive oxygen species (ROS). Under normal physiologic conditions, the production of oxygen free radicals and peroxides is balanced by an efficient system of antioxidants, which are molecules capable of scavenging ROS thereby preventing oxidative damage. Phagocytic cells produce ROS as part of their defense against invading microorganisms [63]. Oxidative stress results in the oxidation of proteins, lipids, carbohydrates and DNA present in the environment [63, 64]. Renal failure is associated with increased oxidative stress and this has been shown to contribute to the pathogenesis and progression of renal failure [64, 65]. There are a number of anti-oxidants present in the body and they can be divided into intracellular or extracellular anti-oxidants. At the intracellular level, enzymatic anti-oxidants such as superoxide dismutase (SOD) or catalase and gluthathione peroxidase play an important role in the conversion of ROS to oxygen and water (figure1.2). Several non-enzymatic anti-oxidants also play important roles in scavenging free radicals. The main non-enzymatic intracellular anti oxidant is reduced gluthathione (GSH). There are several extracellular antioxidants, such as proteins like transferrin, lactoferrin, albumin, cerulopasmin and urate [66]. These compounds prevent free radical reaction by sequestering transition metal ions by chelation in plasma [66]. Albumin, bilirubin, urate and plasma ascorbic acid may also scavenge free radicals directly. Some anti-oxidants are located both intra and extracellularly, such as α tocopherol (vitamin E), β carotene and the water soluble anti-oxidants ascorbic acid (vitamin C) and gluthathione. Vitamin E is the major lipid soluble antioxidant and is present in the membranes and in plasma lipoproteins [66]. ROS themselves have short half lives of just a few seconds [65, 66] therefore oxidative stress is usually measured by the detection of various modified macromolecules that are generated from ROS in the body tissues, plasma and other body fluids. These marker molecules usually have longer half lives and include molecules derived from lipid peroxidation such as malondialdehyde and F2 isoprostanes. Oxidative stress has detrimental effects on cellular and tissue function, contributing to the pathogenesis of a wide variety of disease states, including CHF, atherosclerosis, cancer, and the

normal process of aging. Oxidative stress has emerged as an important cofactor for the development of endothelial dysfunction and possibly atherogenesis [64, 65, 66]. An excess production of ROS may injure endothelial and smooth muscle cell membranes by diminishing levels of nitric oxide (NO), through increased synthesis of asymmetric dimethylarginine (ADMA) and also inhibits guanylyl cyclase, which leads to a decreased production of guanosine monophosphate, and activation of platelet aggregation and adhesion. ROS also exert their toxic activity on the vasculature by oxidation of LDL particles to form oxidized LDL (oxLDL). Oxidized LDL is one of the key mediators of atherosclerosis. Several studies have demonstrated increased oxidative stress in patients with CRF compared to healthy controls [63, 64]. Since renal failure is associated with increased oxidative stress, the issue is whether antioxidant treatment will be of particular benefit in these patients. Two studies recently reported beneficial effects of vitamin E on lipid metabolism, atherosclerosis and CVD in haemodialysis patients. Mune et al [67] used vitamin E coated dialyzers in ESRD patients for 2 years and then measured oxLDL, LDL- malondialdehyde, and aortic calcification as an index of progression of atherosclerosis. This treatment resulted in a significant reduction in LDL oxidation and reduced the progression of aortic calcification. In the Secondary Prevention with Antioxidants of CVD in ESRD (SPACE) trial, the effect of high dose vitamin E supplementation at a dose of 800IU/ day was investigated in haemodialysis patients with pre-existing CVD. The results showed a significant reduction in CVD end points like myocardial infarction but no significant effects on all-cause or CV mortality [68].

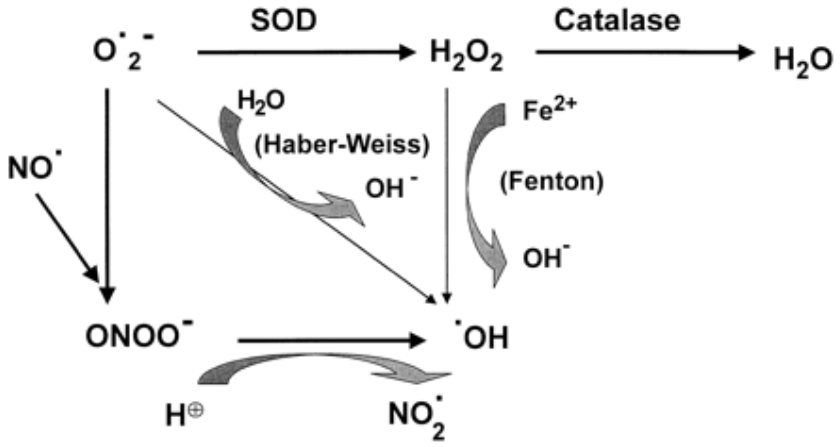


Figure 1.2 Formation of reactive oxygen species. [From 65]

1.3.2.5 Endothelial dysfunction

The endothelium is an active organ with endocrine and paracrine functions. It secretes relaxing and contractile factors and anti-aggregatory substances. The principal relaxing factors are NO and prostacyclin I, and those with contractile functions are thromboxane A_2 and prostaglandin E_2. Endothelial dysfunction in the context of vascular disease means a reduced vasodilatory capacity due to reduced NO activity. Accumulating evidence suggests that CRF is associated with impaired endothelial function [48, 69].

The mechanisms of endothelial dysfunction in CRF are possibly due to elevated levels of asymmetric dimethyl arginine (ADMA), a competitive inhibitor of NO, increased oxidative stress and accumulation of oxLDL. Nitric oxide (NO) is a very active molecule that is released by the endothelial cells into the circulation. It is synthesized from L-arginine by the action of the enzyme nitric oxide synthase (NOS). It is a potent vasodilator that regulates vascular resistance and tissue blood flow. NO also inhibits key processes of atherosclerosis such as monocyte endothelial adhesion, platelet aggregation and vascular smooth muscle proliferation [48]. The synthesis of NO can be blocked by inhibition of nitric oxide

synthase by ADMA and N-monomethyl-L-arginine (NMA) [70]. ADMA and NMA are derived from proteins that have been post-translationally methylated and subsequently hydrolyzed. These proteins are largely found in the nucleolus and appear to be involved in RNA processing and transcriptional control [70, 71]. ADMA and NMA are formed from the methylation of arginine by the enzyme protein arginine methyl transferase type I (PRMT I), while PRMT II forms symmetric dimethyl arginine (SDMA). SDMA is a stereo isomer of ADMA which has no inhibitory effect on NOS [71]. The major source of the methyl groups needed for the various methylating reactions is S-adenosylmethionine, an intermediate in the conversion of methionine to homocysteine (figure 1.3). Endothelial cells and other cells in the body form ADMA. ADMA is metabolized by the enzyme dimethylarginine dimethylaminohydrolase (DDAH) to dimethylamine and L citrulline and excreted by the kidneys to some extent (figure1.3).

Both animal and human studies have shown that elevated ADMA blood levels are associated with increased blood pressure and increased monocyte adhesiveness [48]. Evidence from published clinical studies has shown a strong correlation between increased ADMA blood levels and cardiovascular morbidity and mortality [70, 71]. Vallance et al [72] were the first to report high plasma concentrations of ADMA and SDMA in patients with ESRD. They proposed that the high prevalence of hypertension and atherosclerosis in patients with ESRD might be partly caused by dysfunction of the L-arginine/NO pathway secondary to the accumulation of ADMA due to declining renal excretion [72]. They noted that infusion of ADMA into the brachial artery of normal subjects caused vasoconstriction. Several other studies have confirmed markedly increased plasma ADMA concentrations in patients with ESRD [73, 74]. Decreased renal excretion and reduced degradation by DDAH are possible mechanisms responsible for the elevated plasma levels of ADMA in ESRD.

Evidence for the pathogenic role of ADMA in atherosclerosis includes the observation that ADMA levels are much higher in ESRD patients with atherosclerosis than in those with renal disease without atherosclerosis [73]. Zoccalli et al [74] showed that ADMA was a powerful independent predictor of CVD mortality in dialysis patients. In this study that involved 225 patients with ESRD, plasma ADMA levels were not only significantly related to the severity of carotid atherosclerosis but were the second strongest predictor of CV mortality after age [74]. Thus ADMA is an important factor for accelerated atherosclerosis through reduced NO availability.

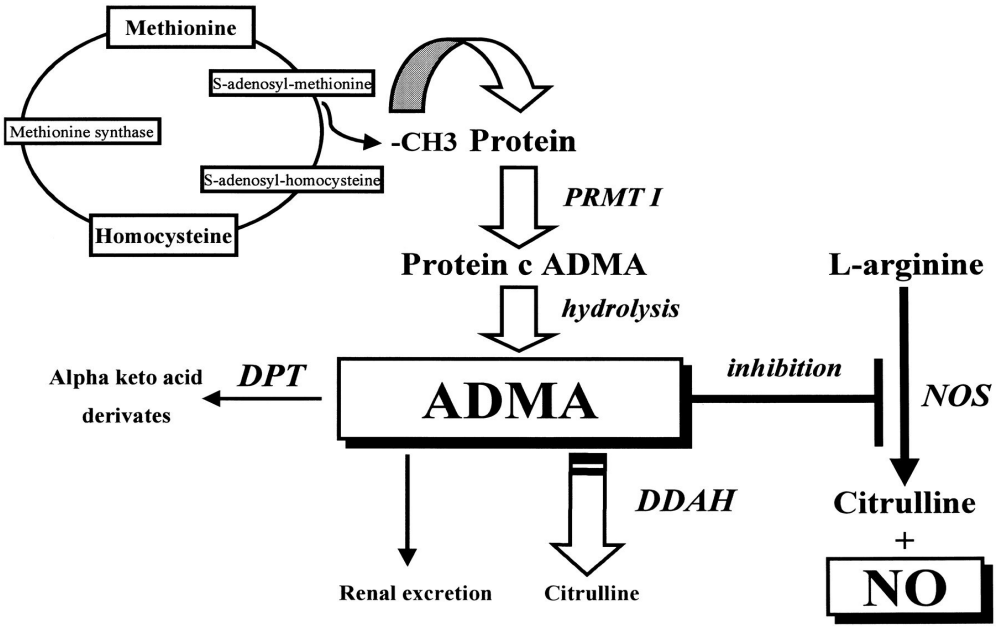


Figure 1.3 Biochemical pathways for the generation and degradation of asymmetric dimetylarginine (ADMA). The methionine/ homocysteine pathway is linked to the generation of ADMA [From 71]

1.3.2.6 Hyperhomocysteinaemia

Homocysteine is an intermediate amino acid formed during the metabolism of methionine, a sulphur-containing essential amino acid and is cleared by the kidneys. Homocysteine is metabolized either by remethylation to methionine or trans-sulfuration. Re-methylation is catalyzed by the enzyme methionine synthase, the methyl donor being 5-methyl–tetrahydrofolate or methylation can also occur by betaine-homocysteine methyl transferase, the methyl donor in this case being betaine [75]. Vitamin B_{12} is an essential co-factor for methionine synthase. The enzyme cystathionine β- synthase catalyzes the transsulfuration of homocysteine to cysteine (see figure 1.4). This process requires vitamin B_6 (pyridoxine) as a cofactor. Homocysteine is present in the plasma in four forms; as free homocysteine (1%), as homocysteine disulfide (10%), as homocysteine-cysteine mixed disulfide (10%) or as the protein bound form (80%) [75]. Total homocysteine refers to the sum of all the free and protein bound homocysteine species (t Hcy); normal value in the fasting state is 5 -15µmol/l. In renal failure, the concentration of both the free and protein bound homocysteine fractions increase but their ratio remains normal [76]. High levels of homocysteine occur in chronic renal failure and deficiency of folate, vitamin B12 or vitamin B6 [75, 76]. Homocysteine concentrations increase in a graded manner with the level of renal impairment [76]. Hyperhomocysteinaemia has been identified as an independent risk factor for cardiovascular morbidity and mortality in the general population [75, 77, 78] and in ESRD patients on dialysis [79, 80]. High homocysteine levels were reported in subjects with IHD and stroke in the general population [77]. The Homocysteine Studies Collaboration meta-analysis [77] was the results of prospective studies of apparently healthy populations which showed elevated homocysteine level to be an independent risk factor for cardiovascular disease. Delport et al [78] reported high levels of homocysteine among Caucasian men with vascular disease. The mechanisms by which homocysteine might contribute to atherogenesis include induction of endothelial dysfunction, promotion of platelet aggregation and enhanced coagulability, increased smooth muscle cell proliferation, cytotoxicity and stimulation of LDL

oxidation [75, 76]. In the general population, homocysteine levels can be readily lowered by treatment with folic acid and vitamin B_{12} [81]. Folic acid is converted to 5-methyl tetrahydrofolate, which donates a methyl group to homocysteine and reconstitutes methionine from which homocysteine has been derived. In most normal subjects or in patients with cardiovascular disease, a dose of 400 to 600 µg produces a fall in the plasma concentration by 20-30% [81]. In contrast, patients with ESRD are resistant to this homocysteine lowering action of folic acid, even when given in pharmacological doses. Other alternatives have been tried with varying outcomes; none of them could normalize homocysteine levels. In one study [82] vitamin B_{12} when given orally lowered homocysteine levels by 17% and by 30% when administered subcutaneously. There are recent reports on the use of acetylcysteine in the reduction of plasma homocysteine levels. Acetylcysteine is a powerful antioxidant. It has some beneficial effects on gluthathione stores and adhesion molecules, and improves peripheral and coronary arterial function [83]. Scholze et al [83] used a 5g dose of acetylcysteine intravenously at the time of dialysis. They noted a significant fall in plasma homocyteine level. Homocysteine concentration fell from a mean of 20µmol/L before haemodialysis to 2.2µmol/L after; this is in contrast to a fall to a mean value of 12µmol/L in the absence acetylcysteine. The mechanism of action is probably related to improved endothelial function. Results of trials on the usefulness of high doses of folic acid in patients with renal disease, eg. Homocysteinemia in Kidney and End Stage Renal Disease Study (HOST) are being awaited.

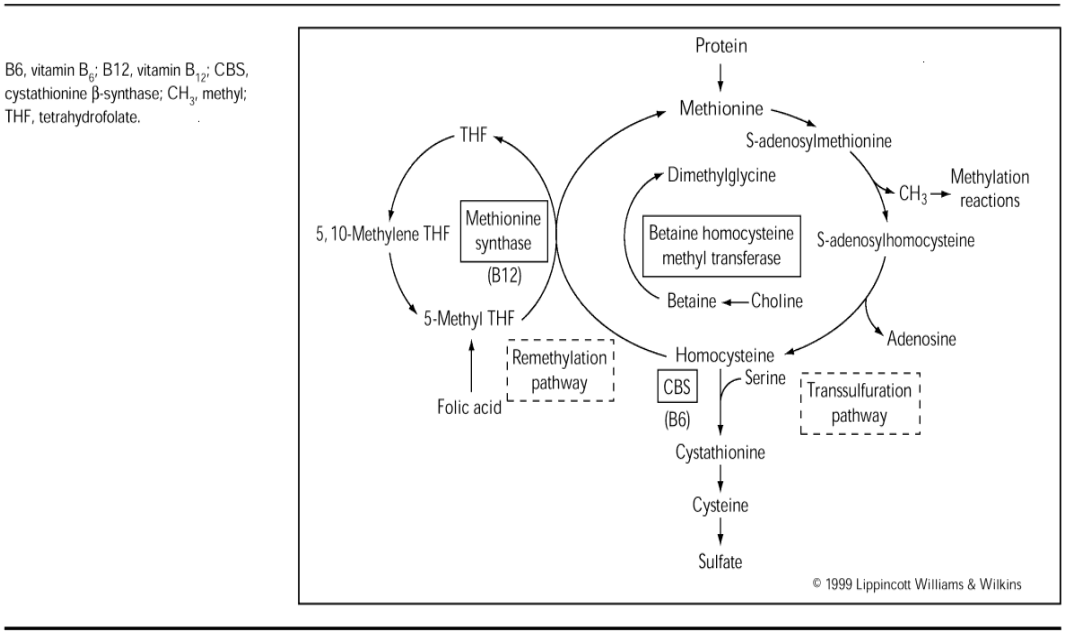


Figure 1.4 Metabolism of Homocysteine (From 75)

1.3.2.7 Anaemia

Anaemia has many deleterious effects, both directly and indirectly on the cardiovascular system [84]. Anaemia results in decreased erythrocyte count, systemic arterial dilatation that leads to a decrease in systemic vascular resistance and reduced after load, which in turn may increase stroke volume [84]. Anaemia results in decreased blood viscosity, which leads to an increase in venous return, and thus, augments preload. Anaemia also results in decreased haemoglobin level and oxygen delivery and increased sympathetic activity, which results in an increase in heart rate and venous tone. Increased venous return, increased heart rate and increased venous tone with decreased afterload all act to raise cardiac output [84]. Anaemia is a risk factor both for the development of de novo and recurrent CHF, LVH, LV dilatation, higher cardiac morbidity and mortality, and lower quality of life [11].

Many trials show that treatment of severe anaemia with recombinant human erythropoietin reduces left ventricular mass in patients on dialysis and in those with CKD. Hayashi et al [85] showed regression of LVH after anaemia was corrected to levels of 11-12g/dl. Accumulating evidence shows a variety of other beneficial effects such as improved quality of life, avoidance of the need for transfusion and cardiovascular benefits. The risks and benefits of normalizing haematocrit (Hct) in haemodialysis patients with cardiac disease have been recently evaluated. In the Normal Haematocrit Trial [86], in which 1200 patients with ischaemic heart disease and congestive heart failure were randomized to Hct goal of either 30% or 42%; the primary end points were time to first myocardial infarction or death. There was no evidence of benefit; instead there were more deaths and non-fatal myocardial infarction in the group with normal Hct. In addition patients with normalized Hct had increased arterio-venous access thrombosis, an apparently small decrease in dialytic urea clearance and increased use of iron [86]. In the Canadian Normalization of Haemoglobin Trial, [87] 146 patients with either asymptomatic concentric LVH or LV dilatation were randomly assigned to receive doses of recombinant human erythropoeitin designed to achieve a target haemoglobin level of either 10gm/dl or 13gm/dl. The result showed that there was no further decrease in LVMI when Hb increased from 10 to 13g/dl. However, patients in the higher haemoglobin group with concentric LVH were less likely to progress to dilatation. In addition the higher target haemoglobin group was associated with better quality of life. The results of these trials suggest that normalization of Hb may not work well when cardiac disease is established. Additional trials should be conducted to determine the patient groups that are likely to benefit from higher Hb levels and the timing for these interventions. The current standard practice is partial correction of anaemia using erythropoietin and iron with target Hb in the 11-12g/dl range [88].

1.3.2.8 Calcium and phosphorus metabolism

Alterations in calcium/phosphorus metabolism are directly related to decline in kidney function. As the level of kidney function declines, phosphate levels increase and calcium levels decrease and parathyroid hormone (PTH) levels increase. Using USRDS data, Block et al [89] have shown that an elevated serum phosphate and a calcium / phosphate product in excess of 4.5-5 mmol/l (72 when expressed in mg/dl) is associated with an increased risk of mortality in patients on haemodialysis. Serum phosphorus has been shown to be associated with increased IMT of the carotid artery [90]. Hyperphosphataemia is associated with increased blood pressure, hyperdynamic circulation, increased cardiac work and high arterial tensile stress [29]. Heavy calcification of the large vessels has long been observed as a feature of chronic uraemia. Goodman et al [91] demonstrated that coronary artery calcification occurred and was related to increased calcium/phosphate product in a group of young patients receiving dialysis. With the advent of electron beam computed tomography (EBCT), the calcium content of the heart, coronary vessels, the heart valves and the aorta can now be noninvasively assessed. Braun et al [92] used EBCT to assess calcification in the coronary arteries and valves of 49 patients on haemodialysis; they showed that patients receiving dialysis had calcification scores that are much higher than those of non uraemic patients with established CAD. The coronary artery calcification scores were 2.5 to 5- fold higher in haemodialysis patients compared with non-haemodialysis patients regardless of the age group [92]. Other factors associated with calcification include longer duration of dialysis, high calcium intake and elevated PTH levels [15]. The possible mechanism by which abnormal calcium/phosphate product increases cardiovascular risk is via PTH. PTH is a growth factor for smooth muscle cells and may contribute to sclerosis of the major peripheral vessels causing increased afterload and subsequent LV dysfunction.

1.3.2.9 Lipoprotein a [Lp (a)]

Lipoprotein (a) is similar to LDL cholesterol, with the addition of a large glycoprotein designated as apolipoprotein (a). The kidney catabolises Lp (a) therefore levels of Lp (a) increase as renal function progressively deteriorates [93]. Lp (a) levels are elevated in both haemodialysis and peritoneal dialysis patients although they are usually higher in the latter group [3]. Lipoprotein (a) is a strong risk factor for vascular disease in most populations [94]. Studies have shown that the apolipoprotein (a) [apo(a)] gene locus determines the risk for CVD through its allelic control of Lp (a). Subjects who express the low molecular weight (LMW) apo (a) phenotype show on average markedly higher Lp (a) concentrations than those with higher molecular weight apo (a) phenotypes and these have been shown to be associated with increased cardiovascular risk [29, 93]. Kronenberg et al [93], in a cross sectional study involving 167 patients found that the LMW phenotypes of apo (a) and higher serum Lp (a) levels were associated with carotid plaques as investigated by ultrasound. They demonstrated that haemodialysis patients with the LMW phenotypes of apo (a) had significantly more carotid sites affected by atherosclerotic plaques than those with other phenotypes. Delport et al [78] however reported that the high molecular weight apo (a) phenotype was associated with increased risk of vascular disease especially in the presence of hyperhomocysteinemia.

1.3.2.10 Activation of the renin angiotensin system and sympathetic hyperactivity

Studies have shown that patients with CRF have increased plasma catecholamine concentrations pointing to increased sympathetic activity [95, 96]. Sympathetic hyperactivity is very important because it may influence cardiovascular and renal prognosis. Renal ischaemia appears to be the main factor for causing sympathetic hyperactivity in patients with kidney disease. Experimental evidence shows that sympathetic activity contributes to the progression of renal failure. The exact mechanism by which sympathetic activity causes kidney damage is not fully known but the effects include vascular and glomerular injury.

Injury to the podocytes is postulated to be one of the mechanisms through which sympathetic activity causes kidney damage. The podocytes have adrenergic as well as angiotensin II (Ang II) receptors [97]. Cathecholamines also induce vasoconstriction, proliferation of smooth muscle cells and adventitial fibroblasts in the vascular wall. Sympathetic activity to the resistance vessels can be measured with microneurographic techniques in the sympathetic muscle nerve fibres i.e. muscle sympathetic nerve activity (MNSA). MNSA is increased in haemodialysis patients who still have their kidneys and also in hypertensive patients with moderate renal failure [95, 96]. Converse et al [98] reported that MNSA was increased in patients on haemodialysis. MSNA is also increased in many types of hypertension such as renovascular hypertension, malignant hypertension, pre-eclampsia, hypertension associated with obesity and hypercapnia, and also with age [95]. It is postulated that decreased NO availability may also contribute to sympathetic hyperactivity in CRF. NO levels are reduced in CRF either due to inhibition by asymmetric dimethylarginine (ADMA), or via NO scavenging by reactive oxygen species (ROS). The NO system is a natural inhibitor of catecholamines. Other factors that contribute to sympathetic hyperactivity include smoking, obesity and hypercapnia [96]. ACE inhibitors and Ang II receptor antagonist reduce MSNA whereas calcium channel blockers do not [99]. Beta blockers or a centrally acting sympatholytic agent might also be beneficial in reducing sympathetic hyperactivity [99]. Sympathetic hyperactivity contributes to hypertension and LVH in CKD patients [97]. Sympathetic activity also contributes to the development of organ damage independent of its effect on blood pressure [100]. It is associated with heart failure, arrhythmia and in experimental conditions, with atherogenesis.

1.3.2.11 Advanced glycation end products (AGES)

Advanced glycation end products (AGES) are formed by a non enzymatic reaction of reducing carbohydrates with amino groups in proteins and subsequent rearrangements and oxidation reactions [101]. Their accumulation may cause macro and microangiopathy [101] and may lead to progression of

atherosclerosis by interaction with receptors that induce production of ROS followed by release of inflammatory cytokines such as IL-6. [102].

1.3.2.12 Thrombogenesis

Fibrinogen is an acute-phase protein as well as a clotting factor and is synthesized by the liver in response to inflammatory cytokines [29]. Plasma fibrinogen levels increase with age and are greater in women than men [29]. Elevated fibrinogen levels are associated with CVD in patients with normal renal function. The odds ratio for CAD was 2.3 in subjects with the highest versus the lowest tertile of fibrinogen in a metanalysis [103]. Cross sectional studies have shown that fibrinogen levels are higher in haemodialysis and peritoneal dialysis patients compared with the general population [104]. Koch et al showed an association between fibrinogen levels and CAD in haemodialysis patients [104]. Fibrinogen has also been identified as a risk predictor for recurrent vascular events and myocardial infarction in longitudinal studies [105].

1.3.2.13 Extracellular fluid volume overload and electrolyte imbalance

Fluid and electrolyte abnormalities are common in patients on dialysis. However, little data is available on their role in the pathogenesis of CVD. Potassium shifts between the extracellular and intracellular compartments have been hypothesized to contribute to sudden deaths in dialysis patients [22]. Fluid overload is associated with eccentric LVH, non atherosclerotic hypertrophy of the large conduit arteries as well as occlusive atherosclerotic lesions of the small vessels. These changes are important in the pathogenesis of CVD in CKD [22].

1.3.2.14 Glomerular filtration rate

Recently, renal dysfunction has been included among the strongest predictors of cardiovascular risk [106]. The increase in cardiovascular risks starts early in the course of renal disease at levels of serum creatinine (or endogenous creatinine clearance) and albumin excretion rates not thought to be previously associated with CV risk. Reduced GFR is associated with a high prevalence of CV risk factors and this may be useful for risk stratification and clinical CVD in both low risk and high risk populations. The Framingham Heart Study [106] was the first longitudinal study to identify that minor renal dysfunction was associated with increased risk of cardiovascular disease. This study involved 6233 individuals drawn from the general population. The mean age was 54 years and subjects were followed up for a mean period of 15 years. Mild renal dysfunction was present in 8% of women and 9% of men at baseline. Mortality rates in men with serum creatinine levels of 124 to 265 μmol/l (1.4-3mg/dl) were significantly higher than controls but not in women. This observation was also confirmed in the 2nd National Health and Nutrition Examination Survey (NHANESII) in which renal insufficiency emerged as a significant predictor of subsequent deaths resulting from CVD [107]. The Hope study also showed that the cardiovascular risk and mortality was higher in individuals with serum creatinine (Scr) above 124 μmol/L (1.4mg/dl) than in those with lower values and there was a linear increase of risk with increasing quartiles of Scr [108]. Decreased GFR has been found to be an independent risk factor for CVD outcomes and all-cause mortality in subjects with hypertension [109]. There are a number of possible explanations for the independent association of reduced GFR and CVD outcomes. First, reduced GFR is associated with an increased number of non-traditional CVD risk factors [12]. Second, reduced GFR may be a measure of residual confounding from traditional risk factors. Subjects with reduced GFR may have more severe hypertension or dyslipidaemia and therefore are more likely to suffer more vascular damage from hypertension or dyslipidaemia [12]. Third, reduced GFR could be a marker of undiagnosed vascular

disease or a marker of the severity of vascular disease, especially in high or the highest risk population [12].

1.3.2.15 Microalbuminuria

Microalbuminuria can result from a specific renal abnormality such as altered haemodynamics or glomerular membrane abnormality or it may reflect a more generalized increase in vascular permeability secondary to endothelial dysfunction. The prevalence of microalbuminuria is raised among patients with type 1 and type 2 diabetes [110], and hypertension [111] and is a useful marker of the likelihood of progressive renal impairment. In type 2 diabetic patients, microalbuminuria at baseline is associated with an increased risk of CV events [110]. Microalbuminuria is also associated with an increased risk of CAD among non diabetic patients [112] and increased risk of LVH and myocardial ischaemia among patients with essential hypertension [111]. However since microalbuminuria is associated with risk factors for CAD, whether microalbuminuria is itself an independent cause of CVD remains uncertain.

1.4 Adipocyte cytokines

1.4.1 Adiponectin

There is growing interest in adipose tissue as an endocrine organ. In addition to leptin, fat cells secrete a variety of biologically active molecules, which may influence the function, as well as the structural integrity of the cardiovascular system. Adiponectin (ADPN), a recently discovered collagen-like protein, is secreted exclusively by adipocytes and circulates in the blood. Adiponectin stimulates fatty acid oxidation, decreases plasma triglycerides, and improves glucose metabolism by increasing glucose sensitivity [113]. Interest in adiponectin derives from its potential protective role for the cardiovascular system. Adiponectin inhibits the inflammatory process and possibly atherogenesis by suppressing the migration of monocytes / macrophages and their transformation to foam cells [113]. ADPN also

modulates the endothelial response to inflammatory stimuli [114]. ADPN is inversely related to creatinine clearance [114,115]. ADPN is markedly increased in patients with ESRD [114, 115]. However, what is not known is whether this increase is just due to accumulation or whether it represents a counter-regulatory response to several metabolic and haemodynamic risk factors of renal insufficiency. The multiple links of adiponectin to several metabolic risk factors like glucose, TG, insulin, and HDL cholesterol in uraemic patients are all consistently in line with the hypothesis that adiponectin is a protective factor [115]. Plasma adiponectin concentrations are reduced in patients with obesity, type 2 diabetes mellitus and coronary artery disease [116]. ADPN in patients with ESRD is increased compared with healthy subjects, but within the uraemic milieu, low adiponectin is still a marker of high risk because it predicts a higher rate CV event [115]. Plasma adiponectin is an inverse predictor of CV events in dialysis patients, and this is independent of traditional and nontraditional risk factors [115]. However, adiponectin levels were found to be increased in both patients with low incidence of cardiovascular events (20.8 ± 6.8 µg/mL) and those with high CV events (9.3 ± 2.7µg/ mL) compared with healthy subjects (5.9 ± 2.6µg/mL) [115]. This observation suggests that the biological phenomena underlying the cardioprotective role of adiponectin must be down-regulated in ESRD, thus resetting the relationship between this protein and cardiovascular damage and clinical complications in these patients at a higher level [115]. Adiponectin levels inversely correlate with CRP and interleukin-6 levels [115].

1.4.2 Leptin

Leptin plays an important role in regulation of food intake and energy expenditure. Serum leptin concentrations in normal humans have been reported to correlate with body mass index (BMI) as well as body fat mass [117]. Leptin levels increase in renal failure due to reduced clearance and there is some suggestion that inflammation may also contribute to hyperleptinaemia [117]. Leptin is an independent predictor of adverse events in the general population.

1.5 Carotid intima-media thickness

Epidemiological and clinical studies have shown that damage to large arteries is a major factor in the high cardiovascular morbidity and mortality in patients with ESRD [118]. There are two distinct types of large artery damage that occur in ESRD. The first is atherosclerosis, which is focal and primarily involves the intima causing narrowing or occlusion of the arteries with restriction of blood flow [12, 15]. This condition leads to IHD, CHF, PVD, sudden death and stroke. The second is arteriosclerosis in which there is diffuse involvement of the media resulting in increased arterial stiffness and decreased distensibility or compliance [12, 15]. The consequences of arterial stiffening are higher systolic blood pressure (SBP) and lower diastolic blood pressure (DBP), thereby causing increased left ventricular afterload and alteration of coronary perfusion [17]. The principal outcomes of these changes are LVH and aggravation of coronary ischaemia. Higher SBP and pulse pressure, lower DBP, and LVH have been identified as independent factors of cardiovascular morbidity and mortality in ESRD patients [118]. Subtle structural changes, such as increase in the intima media thickness (IMT), occur early in the course of atherosclerosis. Carotid intima-media thickness (CIMT) is usually measured by B-mode ultrasonography in which two echogenic lines representing the lumen-intima interface and the media-adventitia interface can be identified [119]. Studies comparing ultrasound measurements of CIMT with histology showed that ultrasound far wall CIMT truly and accurately represents intima-media thickness (IMT), whereas the best estimate of the near wall CIMT measurement tends to underestimate the true IMT [119]. Increased IMT in the common carotid artery is related to generalized atherosclerosis and increase risk of future myocardial infarction and cerebrovascular disease [119,120]. Several studies in the general population have shown that increased CIMT was associated with increased risk of cardiovascular events like fatal and non fatal MI and strokes [120, 121]. In the Atherosclerosis Risk in Communities (ARIC) Study, 7289 women and 5552 men aged 45- 64 years were followed up for 4 to 7 years [121]. Analysis was based on 290 coronary heart disease events that occurred during the period. CIMT was

calculated as the mean of the far wall of the common carotid artery, the bifurcation and the internal carotid artery. The age adjusted risk of coronary heart disease increased gradually with increasing CIMT: the risk of coronary heart disease increased by 69% (95% CI 50-90%) in middle-aged women and 36% (23-51%) in middle-aged men per 0.19mm increase in mean maximum CIMT. The IMT has also been shown to be directly associated with most of the risk factors for atherosclerosis [119]. Kawagishi et al showed that IMT was significantly higher in dialysis patients than age- and gender matched controls [90]. London et al did show that IMT was strongly associated with left ventricular mass index [122]. Studies have shown that the frequency of atherosclerotic plaques is far larger in the haemodialysis patients when compared to healthy subjects or patients without renal disease when matched for traditional risk factors [62, 90]. Calcified plaques are more common in dialysis patients [15, 62]. Benedetto et al in their study of 138 patients who were receiving chronic dialysis showed that IMT was associated with concentric LVH and also an independent predictor of cardiovascular death [123]. IMT is non invasive, cheap, simple and reproducible and could be used in early diagnosis of coronary artery disease. Stenvinkel et al demonstrated that coronary ultrasonography is a useful diagnostic tool and the results obtained were comparable to exercise test and variance electrocardiogram for the detection of coronary heart disease in a high prevalence population [56].

1.5.1 Methods of measurement of carotid intima-media thickness

There are several important differences in the B-mode measurement of carotid IMT between laboratories. These include the sites of measurement, which are the segments of the artery, the near/far wall, and the angles of interrogation, automated versus manual measurement [119, 124]. The argument in favour of the use of common carotid IMT rather than the mean maximum IMT is that the common carotid IMT can be assessed in a more reproducible manner than mean IMT, the data collection is nearly

always complete whereas the measurements for the bifurcation and internal segments have more missing values [119, 124]

1.6 Pathogenesis of atherosclerosis

In the past, atherosclerosis was mainly understood to be a consequence of cholesterol accumulation together with infiltration of macrophages and smooth muscle cells covered by a fibrous cap. However despite changes in life style and the use of drugs to lower cholesterol, many people still die from CVD in the US, Europe and Asia. New insights into disease pathology have shown it to be a disease of many ramifications. Inflammation as well as impaired NO production are now considered to play an essential role in each of the stages of atherogenesis from the initial recruitment of leucocytes to eventual rupture of the unstable atherosclerotic plaque [44].

The vascular endothelium plays a critical role in maintaining vascular integrity and homeostasis. Atherosclerosis starts with endothelial dysfunction which can result from mechanical shear stresses from morbid hypertension, biochemical abnormalities from elevated and modified LDL, diabetes, elevated plasma homocysteine, immunological factors like free radicals from cigarette smoking, inflammation (e.g. infections such as herpes virus, Chlamydia pneumonia and Helicobacter pylori) and genetic alterations [44]; figure 1.5. Endothelial dysfunction leads to alterations in the normal homeostatic properties of the endothelium [44, 48], thereby leading to increased adhesiveness of the endothelium to leucocytes and platelets as well as increased permeability to oxidized lipoproteins (ox LDL) and decreased NO production [44, 48]. The injured endothelium also releases vasoactive molecules, cytokines and growth factors [44] which lead to further recruitment of leucocytes and smooth muscle cell proliferation. The adherence and subsequent migration of leucocytes across the vascular endothelium are mediated by the expression of cellular adhesion molecules on the endothelial surface [125]. The selectins

are adhesion molecules that mediate the initial rolling of inflammatory cells along the endothelial surface. P-selectin is stored in the α granules of platelets and Weibel Palade bodies, while E-selectin is synthesized de-novo by endothelial cells when activated by interleukin-1 (IL-1) or TNF α. Intercellular adhesion molecule 1 (ICAM-1) and vascular adhesion molecule 1 (VCAM-1) belong to the immunoglobulin superfamily of cellular adhesion molecules [125]. These adhesion molecules are thought to regulate attachment and transendothelial migration of leucocytes [125]. ICAM I is produced by macrophages and endothelial cells in response to inflammatory cytokines such as IL-1, TNF α and interferon γ, whereas VCAM-1 production is mainly restricted to endothelial cells.

LDL, which may be modified by oxidation, glycation (in diabetes), aggregation, association with proteoglycans, or incorporation into immune complexes, is a major cause of injury to the endothelium and underlying smooth muscle [48, 125]. When LDL particles become trapped in an artery they undergo progressive oxidation. The oxidized LDL cannot be internalized by cells via the LDL receptor pathway. The oxLDL has to utilize the scavenger receptor pathway expressed only on tissue macrophages [125]. The internalization leads to the formation of lipid peroxides and facilitates the accumulation of cholesterol esters resulting in foam cells. Cholesterol accumulation in the foam cells leads to mitochondrial dysfunction, apoptosis and necrosis, with resultant release of cellular proteases, inflammatory cytokines and prothrombotic molecules [125]. In addition to causing disruption of the endothelial surface, oxLDL stimulates endothelial cells to produce pro-inflammatory molecules such as macrophage chemotactic protein-I (MCP-I) and monocyte colony stimulating factor (M-CSF). Mediators of inflammation such as TNFα, IL-1, M-CSF increase the binding of LDL to endothelium and smooth muscle and also increase the transcription of the LDL receptor gene [44]. Thus a vicious circle of chronic inflammation, modification of LDL and further inflammation can be maintained in the artery by the presence of these lipids.

As the inflammatory process continues, lymphocytes, macrophages, foam cells and the resident vascular wall cells secrete cytokines and growth factors that can promote smooth muscle cell migration and proliferation [48]. Smooth muscle cell growth is a key event in the development of atherosclerosis. The smooth muscle cells before migrating undergo a change leading to the acquisition of a synthetic phenotype profile [48]. Collagen is produced in larger and larger quantities by the smooth muscle cells and the whole sequence of events cumulates as an 'advanced' or raised fibro-lipid plaque (intermediate lesion). With continued inflammatory response, there is thickening of the arterial wall which compensates by gradual dilation (remodeling) [44, 48]. Continued inflammation results in increased number of macrophages and lymphocytes which emigrate from the blood and multiply within the lesion. Activation of these cells leads to the release of hydrolytic enzymes, cytokines, chemokines and growth factors [44], which can induce further damage and eventually lead to focal necrosis. Thus the cycle of accumulation of mononuclear cells and formation of fibrous tissue leads to further enlargement and restructuring of the lesion so that it becomes covered by a fibrous cap that overlies a core of lipid and necrotic tissue, a so-called advanced complicated lesion. The 'advanced plaque' may grow slowly and encroach on the lumen or become unstable, undergo thrombosis and produce an obstruction (complicated plaque). Inflammation plays a key role in plaque instability [44]. Acute events depend on the composition of the plaque. Vulnerable lesions have an increased number of activated inflammatory cells with scarce amounts of smooth muscle cell content. The physical disruptions of the fibrous cap, together with the exposure of the plaque necrotic core are key events that lead to thrombotic complications. The integrity of the fibrous cap depends on the balance of matrix production (mainly due to smooth muscle cells) and enzymatic degradation [48]. These processes are influenced by inflammatory cytokines.

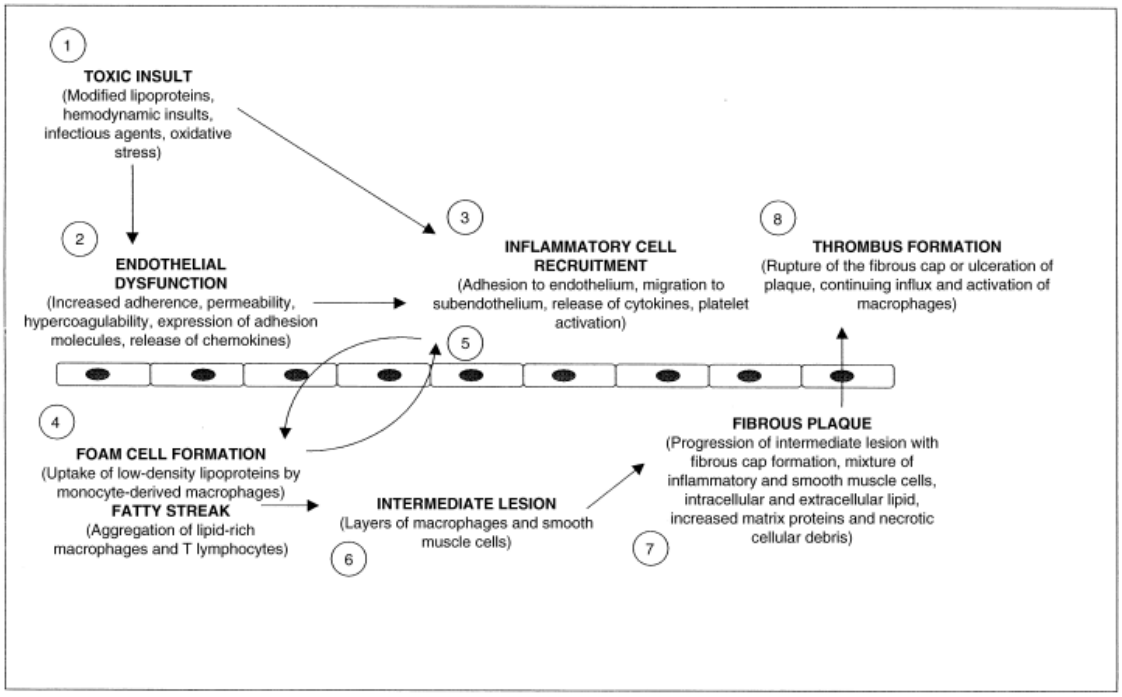


Figure 1.5 Development of atherosclerotic lesions [From 49].

1.7 Primary and secondary prevention of CVD in chronic kidney disease

Most patients with CKD are excluded from randomized clinical trials that test for the efficacy of treatment interventions for the prevention of CVD. Some of the recommendations given are based on extrapolation from the general population [126]. Strategies for risk factor reduction in CKD should take into account both traditional and uraemia related risk factors. Measures to prevent CVD in this group of patients should not be based on the presence or absence of previous CVD or other risk factors but rather the highest risk nature of these patients.

1.7.1 Hypertension

The preferred therapy is control of extracellular fluid volume and maintenance of dry weight through salt reduction, diuretics in patients with mild to moderate CKD, reduction in fluid intake and ultrafiltration in haemodialysis and peritoneal dialysis patients. It is reasonable to use the current guidelines of the Seventh Joint National Committee for Prevention, Detection, Evaluation, and Treatment of High Blood Pressure to define optimal BP control in haemodialysis patients. Target BP for antihypertensive therapy is <140/90. Persistent low BP is indicative of CVD. All classes of antihypertensive agents are effective in ESRD patients except diuretics. ACE inhibitors, angiotensin receptor blockers, beta blockers are suggested because of their effects in reducing sympathetic overactivity. ACEI also improve endothelial function and LVH [126].

1.7.2 Hyperlipidaemia

It may be reasonable to use the guidelines of the National Cholesterol Education Program (NCEP) Adult Treatment Panel for the initial classification, treatment initiation, and target cholesterol level. The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors are effective in lowering cholesterol and are safe [126].

1.7.3 Hyperglycaemia

The recommendation is for intensive glycaemic control in patients with type1 and type 2 diabetes with CKD to reduce adverse CVD outcomes [29,126].

1.7.4 Tobacco use

The recommendation is counseling on smoking cessation. Nicotine replacement can be used and is safe in CKD [126].

1.7.5 Physical inactivity

The American Heart Association recommends a moderate level of physical activity for 30 minutes per day for most days of the week in the general population. This level of activity is feasible in patients with CKD and they should be encouraged to start exercise programmes [126].

1.7.6 Menopause

Hormone replacement therapy may help with post menopausal hormone deficiency but there is need to monitor serum lipids [126].

1.7.7 Homocysteine

Vitamin B supplements (Folate, Vitamin B6) have been administered; however the levels do not drop to normal and further research is required [126].

1.7.8 Thrombogenic factors

Patients with CKD have decreased platelet function and increased procoagulant activity, and aspirin may worsen platelet function. It seems reasonable to prescribe low dose aspirin (75 to 150mg/d) to reduce the risk of CVD outcomes in patients with CAD or those at high risk for developing CAD [126].

1.7.9 Anaemia

Anaemia is a risk factor for LVH and LVH is associated with worse CVD outcomes. Anaemia should be treated with iron supplementation to achieve serum ferritin concentration ≥100μg/l and transferrin saturation > 20%. Epoietin should be used to maintain haemoglobin concentrations > 11g/dl (usual target is 11-12.5g/dl) [88]. Treatment of anaemia causes LVH to regress in CKD [88].

1.7.10 Calcium and phosphate

Non- aluminium phosphate binders can be given if serum phosphate is > 1.5 mmol/l. Active vitamin D supplements should be used when parathyroid hormone is > 2.5 times normal and phosphate concentration is < 1.7 mmol/l. Dietary counseling and improving on dialyisis treatment is helpful. Aim for a normal calcium phosphate product (< 5mmol/l) [29, 126].

1.7.11 Left ventricular hypertrophy

Treatment of hypertension and anaemia can cause regression of LVH in CKD 20, 22, 126].

1.8 Rationale for the study

Myocardial infarction is less frequent in black South Africans [7, 127]. Study of risk factors for CVD may identify the reasons for the difference. Patients with ESRD on dialysis are considered to be a high risk group for CVD and there have been several studies done in the white population. There is limited data on ASCVD in South African patients with ESRD. Therefore a study of this nature is necessary to determine the prevalence of risk factors for ASCVD in the South African patients on haemodialysis and to determine the relationship of these risk factors to sub-clinical atherosclerotic cardiovascular disease in this population. To determine if race has any effect on the differences in ASCVD prevalence we also studied a group with a high prevalence of ASCVD (whites and Indians) and compared risks factors in both groups. The study will provide baseline data base of CVD in this high risk dialysis population.

1.9 Aim of study

The aim of this study is to determine the risk factors for atherosclerosis in Black (low risk), and non-black (high risk) South African patients on haemodialysis.

1.9.1 Objectives

1. To assess the levels of CRP, homocysteine, lipoprotein (a) and adiponectin in patients with ESRD on haemodialysis

2. To measure carotid intima-media thickness (CIMT) as a non-invasive indicator of CAD in these patients.

3. To determine the relationship of these cardiovascular risk factors to the degree of sub clinical atherosclerosis as determined by CIMT in the two dialysis groups, as well as a matched normal control group.

4. Choice of cytokines

Hs-CRP, homocysteine and Lp (a) have been extensively studied in both the general population and ESRD and are associated with increased risk of CVD. There is very little data in our population hence we decided to include these molecules in this study. ADPN is a recently discovered adipocyte cytokine with little information of its effect in ESRD patients hence we decided to include this in our study.

CHAPTER 2

MATERIALS AND METHOD

The protocol for this study was approved by the Human Ethics Committee of the University of the Witwatersrand; ethics clearance certificate number M03-08-02.

2.1 Study population

2.1.1 Sample size

The sample size was calculated based on a 2 sample t-test to detect a difference of 4.32 in the level of inflammatory markers using data from a previous study [128] with a standard deviation of 0.99. A minimum sample size of 44 per group is adequate to detect this difference at 0.05 significance level with a power of 0.9.

2.1.2 Inclusion criteria

- Patients who have been on maintenance chronic haemodialysis for three months or more at the Johannesburg, Helen Joseph and Chris Hani Baragwanath dialysis units who agree to participate in the study. Consecutive patients who agreed to participate in the study were recruited.
- Age between 18years and 60years

There were fifty-eight black and twenty-six non black patients (from a population with high prevalence of CAD i.e. Indians and Whites). All patients were dialyzed thrice weekly with standard bicarbonate buffer. Fifty-one patients (60.7%) dialyzed through arteriovenous fistulae, 19 had temporary catheters in the internal jugular vein, 10 had permcaths and 4 had grafts.

2.1.3 Exclusion Criteria

- Patients with active or chronic infections including vascular access related sepsis.
- Patients with diabetes mellitus.
- Patients who are seropositive for hepatitis B, C and the human immunodeficiency virus
- Patients with active inflammatory disease.

2.1.4 Controls

Thirty-five black and twenty-eight non-black, age and sex-matched "healthy" volunteers were recruited from:

- Staff and students of Johannesburg and Chris Hani Baragwanath hospitals.
- Donors from the transplant work-up clinic (prior to kidney donation).

2.2 Methods

This was a cross-sectional study. Enrollment of all eligible patients was initiated in January 2004 to October 2004. Using a structured interview form, information on age, race, gender, physical activity, and tobacco use was obtained. Current physical activity was evaluated by asking if the patients engaged in any regular exercises such as brisk walking, jogging, bicycling or swimming. Patients were classified into three groups based on smoking history. They were categorized as current smokers if they smoke, former smokers if they stopped smoking six months previously and non- smokers if they never smoked. ASCVD was defined as a history of angina, MI, coronary artery bypass graft surgery or angioplasty, stroke, peripheral by-pass, peripheral angioplasty, or amputation. The use of antihypertensive medications, statins, and calcium containing phosphate binders was noted. Height and weight were measured using the

Detecto scale (New York) and body mass index (BMI) was calculated as weight to height squared. The dry weight was used to calculate BMI in haemodialysis patients.

2.2.1 Blood Pressure

Blood pressure (BP) was recorded non-invasively in the arm without the A-V fistula with an acusson mercury syphygmomanometer in the sitting position. Blood pressure was estimated by averaging all pre dialysis and post dialysis blood pressure recordings taken during the month before the study (total of 12 measurements; that is 3/wk). For the controls blood pressure was taken in the sitting position after resting for 5 minutes. The average of three readings taken 5 minutes apart was taken as the blood pressure. Pulse pressure was calculated as systolic blood pressure minus diastolic blood pressure. Mean arterial pressure (MAP) was calculated as diastolic blood pressure plus one third pulse pressure.

2.2.1.1 Pre-dialysis or post-dialysis measurements?

Most studies of blood pressure in haemodialysis patients have used pre-dialysis blood pressure measurements[23, 24]. Predialysis pressure may not be the most appropriate to study. Two recent large studies showed no increase in cardiovascular mortality with increasing pre-dialysis blood pressure, but did find increasing mortality at higher levels of post-dialysis blood pressure [24, 25]. There is a rapid rise in blood pressure a few hours before each haemodialysis session and several ambulatory blood pressure monitoring studies have shown a closer relationship between mean ambulatory blood pressure and post-dialysis blood pressure than with pre-dialysis blood pressure. The closest estimate of the ambulatory blood pressure may be obtained by measuring blood pressure 20 minutes after completion of dialysis but this is impractical in routine practice, and a measurement shortly after completion of dialysis has to be

acceptable, despite the concern that this may be influenced by haemodynamic instability caused by continued equilibration between the blood volume and the interstitial compartment. Mean ambulatory systolic pressure may be more closely related to the pre-dialysis measurement and diastolic pressure to the post–dialysis measurement.

2.2.2 Dialysis treatment

Patients were asked questions about their dialysis treatment including date of commencement of treatment, previous treatments received including previous kidney transplants. The type of access and dialyzers used were also noted. The dose of erythropoietin received was noted. The monthly Kt/V was calculated using single pool urea kinetic modeling. The post dialysis blood urea sample was obtained by the slow stop technique. A Kt/V of 1.20 was considered adequate. Most patients were virtually anuric (24-hour urine volume <200 mL/day) and were being treated three times a week with standard bicarbonate dialysis (sodium 138 mmol/L, HCO_3 35 mmol/L, potassium 1.5 mmol/L, calcium 1.50 mmol/L, magnesium 0.75 mmol/L). Sixty-seven patients (79.76%) patients dialyzed with the Fresenius hollow fibre polysulfone dialyzers (F5 to F10HPS, surface area 1.0 to 2.2m^2 (Fresenius Medical Care Bad Hamburg Germany) while 17 (20.24%) dialyzed with the Gambro hemophan dialyzers (GFS 16 surface area 1,2m^2 Gambro Germany).

2.2.3 Carotid intima- media thickness

Carotid artery intima-media thickness (IMT) was measured by high resolution B-mode ultrasonography with a 7.5-MHz transducer (Toshiba Nemio 30, Toshiba Corporation Otawara-Shi Japan). Patients were examined in the supine position with the neck hyperextended and the head turned 45^o from the side being scanned. Reference point for the measurement of intima-media thickness (IMT) was the beginning of the

dilatation of the carotid bulb (flow tip divider). IMT was taken as the distance from the leading edge of the first echogenic line to the leading edge of the second echogenic line. Where possible, measurements were taken at the near and far falls of all carotid segments. Measurement of the near wall of the bifurcation and the internal carotid artery was difficult especially some dialysis patients due to presence of temporary catheters in the neck. Hence in this study we focused on measurement at the far walls of the three segments on both sides.

Measurements were taken on the longitudinal views of the far walls of the common carotid artery 1cm proximal to the dilatation of the carotid bulb, carotid bifurcation (the 1cm-segment proximal to the flow divider), and internal carotid artery at about 1cm segment distal to the flow divider (figure 2.1). The maximum IMT in the far wall of the various carotid segments was recorded. For statistical analysis the mean of right and left common carotid IMT was considered. The presence of plaques was defined as localized echo structures encroaching into the vessel lumen for which the distance between the media-adventitia interface and the internal side of the lesion was ≥ 1.2 mm [63, 119]. Plaques were classified as soft, mixed or calcified plaques according to their calcification. Soft plaques appeared as faint grey echoes which protruded into the lumen. Mixed plaques included small areas of calcification incorporated in a soft plaque. Highly echogenic lesions, often causing shadowing, represented calcified plaques [63]. The number and the thickness of carotid plaques were measured from both carotid systems. Plaque thickness was measured in a suitable longitudinal view. Plaque score was calculated by summing up the thickness of all the plaques for both carotid systems [129]. IMT was measured in the plaque free areas and measurements were carried out on both sides by two trained Ultrasonographers from the Vascular Unit of the Johannesburg hospital. The presence of atherosclerosis in this study was thus determined by measuring CIMT and occurrence of plaques.

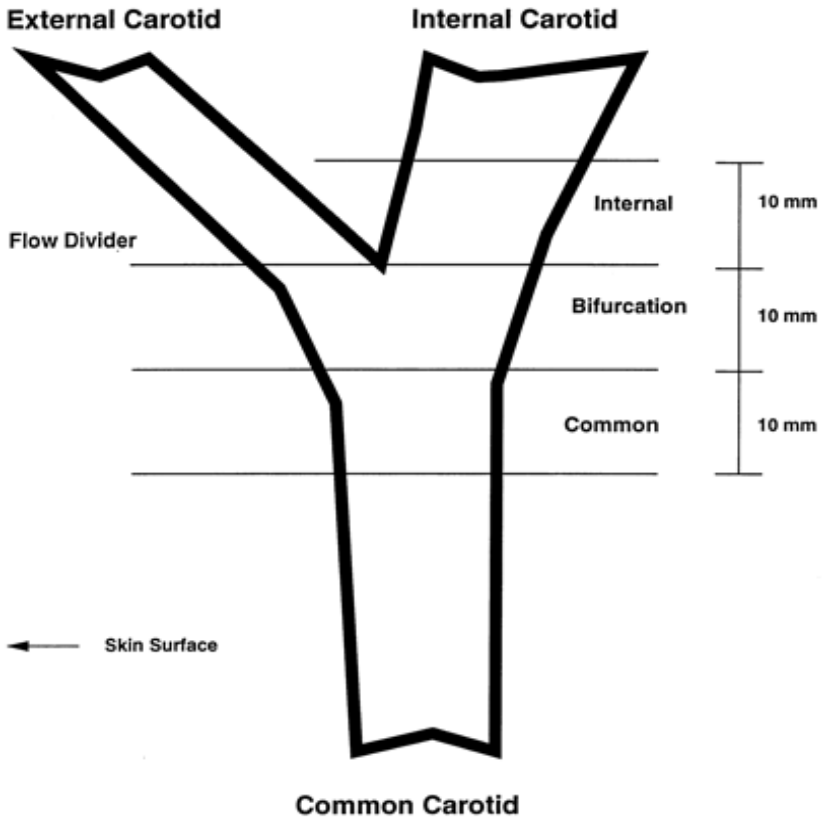


Figure 2.1 Carotid intima-media thickness measurement sites [From 121]

2.2.4 Echocardiography

Echocardiography is a non invasive test that gives information about cardiac dimensions of the heart assessing for cardiac geometry and wall motion abnormalities. Echocardiography was done in the Cardiology Unit using the Acuson Sequoia C256 machine (Acuson Corporation California USA) equipped with 2 to 4-MHz probes allowing M-mode, two dimensional, and pulsed doppler measurements. Echocardiography was performed by 3 Consultant Cardiologists under the supervision of a Professor. These studies were performed on a non-dialysis day where possible. Echocardiography was performed according to the standard guidelines [130]. All scans were performed with the head of the table inclined at

an angle of 15 degrees and the participant rotated 30–45 degrees in the left lateral decubitus position at end-expiration. Left ventricular internal dimension measurements in end-diastole (LVEDD), and end-systole (LVESD) were made at the level of the mitral valve leaflet tips in the parasternal long-axis view. The normal range for LVEDD is 3.5- 5.6cm, and 2.0-4.0cm for LVESD [130]. The interventricular wall thickness during diastole ($IVST_d$) and posterior wall thickness during diastole (PWT_d) were measured by M-mode. From these measurements, the left ventricular mass (LVM) and left ventricular mass index (LVMI) were calculated according to Devereux and Reichek [131]. LV mass was divided by body surface area (BSA) to calculate the LV mass index (LVMI) [130, 131]. The cutoff points for LV hypertrophy using the LVM/BSA ratio were 136 g/m^2 for males and 100 g/m^2 for females [131].

$$LVM = ([LVEDD/10 + IVST_d/10 + PWT_d/10]^3 \times 1.04) - ([LVEDD/10]^3 - 13.6)$$

$$LVMI = LVM/ \sqrt{\frac{(weight \times length}{3600}}$$

$$RWT = (2 \times PWTd)/ LVEDD$$

Relative wall thickness (RWT) was used to characterize LVH into eccentric and concentric LVH. [132]. RWT greater than 0.45 in the presence of LVH is indicative of concentric hypertrophy and eccentric hypertrophy if less than 0.45 in the presence of LVH. LV diastolic function is complex and dependent on factors like age, preload, afterload, heart rate and the co-existence of other abnormalities like mitral valve disease [130]. Diastolic function was assessed using M-mode. The anterior mitral valve leaflet (AMVL) during diastole has a characteristic M- shaped (E-A) pattern assuming the individual is in sinus rhythm and no mitral stenosis. The E-wave is the result of passive early diastolic left ventricular filling. The A-wave represents active late diastolic LV filling due to atrial contraction. The acceleration time (AT) and deceleration time (DT) of the E-wave can be measured. AT is the time from the onset of diastolic flow to

the peak of the E-wave. DT is the time from the E-wave peak to the point where the deceleration slope hits the baseline. The E-wave is often greater than the A-wave. If the left ventricle is stiffer than usual, there may be diminished AMVL excursion (E-wave) and an increase in A-wave size (as the atrial contraction contributes to a greater extent to ventricular filling of the left ventricle); the E: A ratio is thus reduced. The E-wave, E: A ratio and E-wave deceleration times tend to fall with increasing age [130]. The normal ranges are E:A ratio of 1.04 ± 0.38 for men and 1.03 ± 0.34 for women [130]. Systolic function was assessed by measuring the left ventricular ejection fraction and fractional shortening. Systolic dysfunction was defined as a shortening fraction less than 28% or ejection fraction less than 50% [130].

2.2.5 Biochemistry

Blood samples for CRP, lipids, lipoprotein (a), and adiponectin were collected in anti-coagulant free tubes after an overnight fast on the mid-week day, predialysis. Samples for homocysteine were collected in EDTA tubes after overnight fast. Samples were kept on ice until the plasma was separated within 30 minutes of blood collection. Samples were centrifuged at 3000 rpm for 10 minutes at room temperature and frozen at -70^{o}C till ready for assay.

2.2.5.1 Routine Tests

Serum albumin, cholesterol, triglyceride, HDL-Cholesterol, urea, creatinine, albumin, calcium, phosphate, and parathyroid hormone measurements were done in the National Health Laboratory (NHLS) as part of the routine monthly tests in the dialysis patients. All samples were analyzed using the Roche/Hitachi Modular ISE 900 automated analyzer (Roche Diagnostics Corporation, Mannheim Germany). The mean of three measurements taken over 3-months from the time of recruitment including

the month of entry into the study were used for analysis. All routine tests were measured with commercially available kits and carried out according to manufacturers instructions.

2.2.5.1.1 Serum lipids

Serum total cholesterol and triglycerides were determined using enzymatic colometric test High density lipoprotein (HDL) cholesterol was measured after precipitation of the non-HDL fraction with phosphotungstate-magnesium. The concentration of LDL cholesterol was estimated indirectly by use of the Friedewald formula [133].See appendix A for details.

Friedewald LDL cholesterol = total cholesterol – (HDL cholesterol + triglyceride/5).

2.2.5.1.2 Serum calcium

Serum calcium was determined using the complexometric methods in addition to atomic absorption spectrometry. This method is based on the reaction of calcium with o-cresol-phthalein complexone in alkaline solution to form calcium-o-cresolphthalein complex. Magnesium is masked with 8-hydroxyquinoline. The colour intensity of the purple complex formed is directly proportional to the calcium concentration and was measured spectrophotometrically. Normal values are 2.15 – 2.55mmol/L.

2.2.5.1.3 Serum phosphate

Serum phosphate was measured based on the reaction of phosphate with ammonium molybdate to form ammonium phosphomolybdate. Inorganic phosphate forms an ammonium phosphomolybdate complex with ammonium molybdate in the presence of sulphuric acid. The complex was determined spectrophotometrically in the ultraviolet region (340nm). See appendix B for details. Normal values are 0.87 – 1.45mmol/L.

2.2.5.1.4 Serum parathyroid hormone

The intact parathyroid hormone (PTH) peptide consists of 84 amino acids sequenced and designated according to reactivity. The N-terminal or amino terminal 1-34 region of the intact PTH molecule is biologically active. This region of the PTH molecule contains the amino acid sequence that enables the PTH to bind to the parathyroid hormone receptors in the target tissues and regulate the extracellular calcium. The middle and the carboxyl-terminal 35-84 region of the intact PTH molecule are biologically inert but possess immunological reactivity. PTH circulates in the blood as both intact PTH and PTH fragments. Intact PTH has a half-life of less than 4 minutes and is cleared by the kidney and the liver. The kupffer cells in the liver are responsible for cleaving the intact molecule into fragments and releasing them into circulation. The middle and the carboxyl- terminal PTH fragments vary in size and have longer half-lives and are cleared in the kidney by glomerular filtration. In renal insufficiency, the concentration of the middle and carboxyl- terminal PTH fragments is increased. The ratio of the circulating concentrations of intact PTH to middle and carbonyl-terminal PTH can vary between individuals, particularly in patients with CRF. Quantification of circulating intact PTH in conjunction with the measurement of ionized calcium assists in the differential diagnosis of hyperparathyroidism, hypoparathyroidism and hypercalcaemia. The measurement of intact PTH using two-site immunoassays provides a more accurate assessment of parathyroid secretory status especially with renal impairment (ADVIA Centaur Assay manual). Intact PTH was measured using the ADVIA Centaur Intact PTH assay (Bayer Diagnostics, United Kingdom). The ADVIA Centaur Intact PTH assay is a two-site sandwich immunoassay using direct chemiluminometric technology, which uses constant amounts of two anti-human PTH antibodies. The first is a polyclonal goat anti-human PTH antibody labeled with acridinium ester directed at the N-terminal (N-terminal 1-34). The second antibody is a biotinylated polyclonal goat anti-human PTH (39-84 regions) antibody. Streptavidin in the Solid Phase is covalently coupled to the paramagnetic latex particles. The system performs a series of steps (for details of the methods see

appendix C). The amount of PTH in the patient's sample is equivalent to the amount of relative light units (RLU) detected by the system. Normal values are 7– 53ng/L

2.2.5.1.5 Serum creatinine

Serum creatinine was measured by the modified Jaffe method. Creatinine in alkaline solution forms a yellow-orange complex with picrate. The colour intensity is directly proportional to the creatinine concentration and was measured spectrophotometrically at 510nm. Normal range in men is 62 –106 μmol/L and 44 – 80μmol/L in women.

2.2.5.1.6 Serum albumin

Serum albumin was measured by a colorimetric assay with end point method. Albumin binds with bromocresol green (an ionic dye stuff) at a pH value of 4.1 to form a blue-green complex. The colour intensity of the blue-green colour is directly proportional to the albumin concentration and was determined spectrophotometrically at 510nm. Normal values are 34– 48g/L.

2.2.5.2 Special biochemical tests

2.2.5.2.1 Hs-CRP

CRP is a member of the pentraxin family of protein and is considered the protypical acute phase reactant in man. CRP is produced in the liver in response to IL-6 its concentration increases rapidly during inflammatory reactions [45]. CRP was measured using an immunoturbidimetric assay (CRP latex high sensitive assay, Roche Diagnostics, Manmheim Germany). The Roche CRP assay is based on the principle of particle-enhanced immunological agglutination. The kit was used on the Roche/Hitachi 911 Modular machine and assay performed according to the manufacturer's instructions. The test principle is based on the reaction of anti-CRP antibodies coated with latex reacting with antigen in the sample to

form an antigen-antibody complex. The agglutination was measured by immunoturbidimetry. Briefly, 10μL of serum was mixed with 125μL of R1 (buffer).

After which 125μL of R2 solution which is an anti-CRP antibody-latex solution was added. Following the reaction, agglutination was measured turbidimetrically. The measurement ranged is from 0.1-20.0mg/L with an expected value of < 5mg/L.

2.2.5.2.2 Adiponectin

Serum adiponectin was measured by ELISA immunoassay technique using the Quantikine human adiponectin kit by R and D systems (Abingdon Oxon United Kingdom). The assay uses the quantitative sandwich enzyme immunoassay technique with a microplate pre-coated with a monoclonal antibody specific for adiponectin. Standards and samples were pipetted into the wells and any adiponectin present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for adiponectin was added to the wells. Following washing to remove any unbound antibody – an enzyme reagent (adiponectin conjugated to horseradish peroxidase), and a substrate solution (tetramethylbenzidine) were added to the wells for colour development (in proportion to the amount of adiponectin bound in the initial step). Colour development was stopped by the addition of sulfuric acid and the intensity of the colour was measured at 450nm.

Table 2.1 Assay procedure for adiponectin

Sample and Reagents	Volume	Remarks
Assay Diluent RD1 W	100µl	
Diluted Sample / Standard/ Saline diluent (blank)	50µl	*Plate was incubated at room temperature for 2 hrs*
Washing solution	400µl	*Wells were washed four (4) times*
Adiponectin conjugate	200µl	Plate was covered with adhesive tape and incubated for 2hrs at room temperature
Washing solution	400µl	*Wells were washed four (4) times*
Substrate solution	200µl	Plate was protected from bright light and incubated for 30min at room temperature
Stop solution	50µl	The absorbance was read at 450nm.

The lowest level of human adiponectin detected by this assay by is 1ng/ml in a 100µL sample size. Adiponectin measurements were done in the laboratory of the Nephrology Unit. The expected values are 5 – 30µg/mL.

2.2.5.2.3 Homocysteine

Homocysteine (Hcy) was measured by Fluorescence Polarization Immunoassay (FPIA) technique using the AXSYM kit (Abbott Laboratories, USA) on the Abbott IMx autoanalyzer. Homocysteine assay was done according to manufacturers instructions. Briefly, bound homocysteine (oxidized form) is reduced to free homocysteine that is enzymatically converted to S-adenosyl-L-homocysteine (SAH). Homocysteine, mixed disulfide, and protein bound forms of Hcy in the sample are reduced to free Hcy by the use of dithiothreltol (DDT). Free Hcy is converted to SAH by the use of SAH hydrolase and excess adenosine. Under physiological conditions, SAH hydrolase converts SAH to homocysteine. Excess adenosine in the pretreatment solution drives the conversion of Hcy to SAH by the bovine SAH Hydrolase.

The AXSYM Kit was made-up of the following:

- Pretreatment solution containing dithiothreitol (DTT)
- 8-adenosyl-L-homocystein Hydrolase (bovine) in phosphate buffer with protein (bovine) stabilizer
- Anti-S-adenosyl-L-homocystein Antibody (mouse monoclonal) in phosphate buffer with protein (porcine) stabilizer
- 8-adenosyl-L-cystein Fluorescein Tracer in phosphate buffer protein (bovine) stabilizer.

Samples and all AxSYM reagents required for test were pipetted by the sampling probe into the various wells of the reaction vessel (RV). The sample, pretreatment solution, the line diluent, and SAH were pipetted into the one of the wells to make up the predilution mixture. The RV was immediately transferred into the processing centre by the processing probe. An aliquot of the predilution mixture, antibody, and solution 4 (line diluent) were transferred into the cuvette of the RV. Tracer, solution 4 and a second aliquot of the predilution mixture were transferred to the cuvette. SAH and labeled fluorescein

tracer compete for the sites on the monoclonal antibody molecule. The intensity of polarized fluorescent light was measured by the FPIA optical assembly. Further information can be obtained from the AxSYM System Operation Manual Section 3.

2.2.5.2.4 Lipoprotein (a)

Lipoprotein (a) was measured by a monoclonal anti-Lp (a) antibody technique using the human Gesellschak kit (Biochemica and Diagnostica Wiesbaden Germany). Assay was performed according to the manufacturer's instructions. Lp (a) in the serum sample causes agglutination of latex particles coated with anti-Lp (a) antibodies. The agglutination is proportional to the Lp (a) concentration in the sample and can be measured by turbidimetry. The samples and controls were diluted 1:10 with normal saline. Dilutions of the Lp (a) standards were prepared using normal saline as the diluent and these were used directly for the assay. Normal saline was used as reagent blank. The concentration of Lp (a) standard was multiplied by the corresponding factor indicated in the table below to obtain the Lp (a) concentration of the dilution. The factor allows for the 1:10 dilution of the sample.

Table 2.2 Dilution factor for Lp (a) standards

Dilution	1	2	3	4	5	6
STD (µl)	0	10	10	10	20	40
Saline (µl)	200	790	390	190	180	160
Factor	0.0	0.125	0.25	0.5	1	2

Table 2.3 Lp (a) assay procedure

Sample and Reagents	Volume	Remarks
Diluted Sample / Standard/ Saline diluent (blank)	30µl	
Buffer Solution	800µl	Solution were mixed well and initial absorbance A_1 (at 37°C) read after 10sec at 570nm
Latex reagent	60µl	
This was well mixed, and incubated at 37°C for 10 min before final absorbance A_2 was read at 570nm.		

The absorbance difference ($\Delta A = A_2 - A_1$) of each standard dilution was calculated and the values plotted (Y-axis) against the respective Lp (a) concentrations (X- axis) lin-lin paper. The Lp (a) concentration in the undiluted sample was calculated by interpolation of its absorbance (ΔA) on the calibration curve. If the absorbance of the sample exceeded the absorbance of the highest standard, the sample was diluted 1:20 with normal saline and the assay repeated using this diluted sample. The result obtained was multiplied by the dilution factor of 2. The normal range is up to 30 mg/L.

2.3 Statistics

Data are reported as mean ± SEM. Differences in means for continuous variables were compared using Student t-test while for categorical variables the Chi-squared (X^2) test was used. Results are reported at 95% Confidence Interval. The relationship between CIMT and CVD risk factors was tested by the generalized linear regression model using stepwise selection method. The generalized linear regression model was used because the variables entered into the models were a combination of both continuous and categorical variables.

The generalized linear model can be used to predict responses both for dependent variables with discrete distributions and for dependent variables which are nonlinearly related to the predictors. This analysis was based on established risk factors for atherosclerosis in patients on dialysis like age, gender, smoking, systolic pressure, diastolic blood pressure, cholesterol, TG, HDL- Cholesterol, LDL- cholesterol, calcium phosphate product, albumin, Hs- CRP, Lp (a), and homocysteine.

Significant independent variables were ordered according to their standardized effect, defined as regression coefficient (β). Logistic regression analysis was used to determine risk factors associated with plaque occurrence. All analysis was done using Stata version 8.0 statistical software (Stata Corporation 2003).

CHAPTER 3

RESULTS

3.1 Demographic data of the study group

In this cross-sectional study, 90 patients who had been on haemodialyisis for at least three months were recruited. Six died during the course of the study, two out of the six patients that died completed the study. Two patients were transplanted and two were transferred to another dialysis facility. Only 84 patients were able to complete all aspects of the study and were thus included in the analysis. There were 58 (69%) black patients and 26 (31%) non-black patients. There were 44 (52.4%) male and 40 (47.6%) female patients. The mean age for the male patients was 40.3 ± 1.68 years and 39.8 ± 1.53 years for the female patients. Table 3.1 shows the age distribution by gender and race. The black patients were older than the non-black patients, mean age for the black patients was 41.6 ± 1.25 years and 36.6 ± 2.27 years for the non-black patients $p = 0.042$. The mean age for the black female patients was 42.3 ± 1.63 years, and 35.7 ± 2.83 for the non-black female patients while for the black male patients mean age was 41.1 ± 1.84 and 37.8 ± 3.88 years for non-black male patients. The mean duration of dialysis was 45.95 ± 4.92 months (range 3 months to 192 months). Seventy-nine (94.05%) patients received erythropoietin therapy. The dose ranged from 2000IU weekly to 16,000IU per week. Twenty-seven patients (31.1%) had received a previous kidney transplant and 44.1% had peritoneal dialysis before being moved to haemodialysis.

The controls were matched for age, sex and race. There were 30 (47.62%) male and 33 (52.38%) female subjects. The mean age for male controls was 40.5 ± 2.02 years; while for female controls was 39.4 ± 1.84 years.

Table 3.1 Age distribution by gender and race

Age (years)	Patients Mean ±SEM	Controls Mean ± SEM	t value	p value
N	**84**	**63**		
Black females	42.28 ± 1.63	42.00 ±2.30	0.10	NS
Non-black females	35.73 ± 2.83	36.56 ± 2.80	–0.21	NS
Black males	41.09± 1.84	41.67 ± 2.41	–0.19	NS
Non-black males	37.82± 3.88	38.75 ± 3.61	–0.18	NS

3.2 Aetiology of End-stage renal disease

The causes of end-stage renal failure were hypertension in 43 (51.2%) patients, glomerulonephritis in 17 (20.2%), chronic pyelonephritis/reflux nephropathy in 7 (8.33%), Lupus nephritis in 4(4.76%) patients, congenital abnormalities of the kidneys in 4 (4.76%) patients, polycystic kidney disease in 2 (2.38%), miscellaneous causes like trauma in 1(1.19%), obstructive uropathy in 1 (1.19%), and unknown in 5 patients (5.96%) Figure3.1. The congenital anomalies of the kidneys varied from horse-shoe shaped kidneys to dysplastic kidneys. When aetiology was analysed by race, 37 (63.8%) black patients had essential hypertension while only 6 (23.1%) non-black patients had essential hypertension.

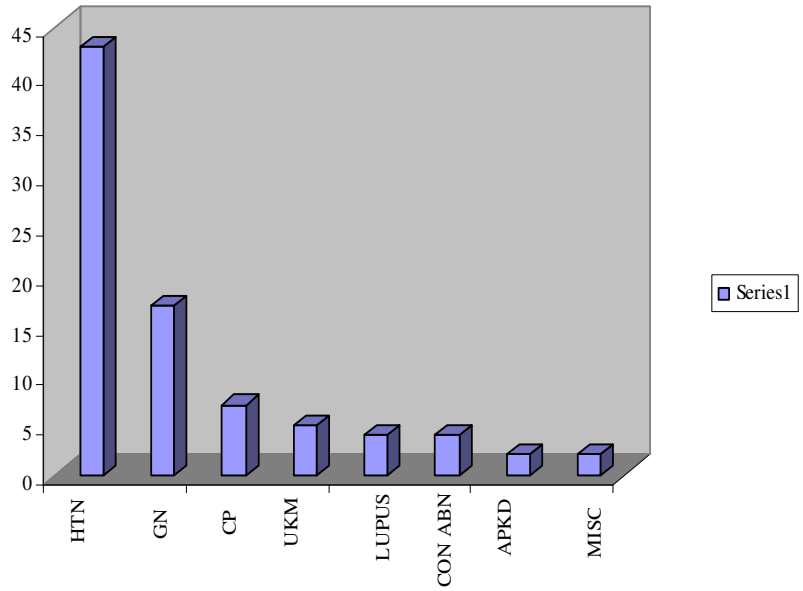


Figure 3.1 Aetiology of ESRD in the study patients

Abbreviations are: HTN, hypertension; GN, Glomerulonephritis; CP, Chronic pyelonephritis /reflux nephropathy; UKM, unknown; Lupus, Lupus Nephritis; CON ABN, Congenital anomalies; APKD, Adult polycystic kidney disease; MISC, Miscellaneous causes.

3.3 Traditional Cardiovascular disease risk factors

3.3.1 History of Cardiovascular disease

Three white patients had a history of ischaemic heart disease. There was no history of cerebrovascular disease or peripheral vascular disease in the study population. Family history of myocardial infarction, diabetes mellitus, hypertension and chronic kidney disease were similar in both patients and controls (table 3.2). Almost half of the study participants (45.58%) reported a family history of hypertension in the first degree relatives, 21.77% reported history of diabetes, 16.33% reported history of myocardial infarction, 16.33% reported family history of CKD and only 6.8% did not report any history of these CVD risk factors in the family.

Table 3.2 Family history of CVD in the study population

Positive family history	Patients N=84	Controls N=63	Pearson X^2	p value
Myocardial infarction	11 (13.1%)	13 (20.63%)	1.50	0.221
Hypertension	41 (48.81%)	26 (41.27%)	0.83	0.364
Diabetes Mellitus	18 (21.43%)	14 (22.22%)	0.01	0.908
Chronic kidney disease	5 (5.95%)	9 (14.29%)	2.90	0.089

3.3.2 Hypertension

Systolic blood pressure, diastolic blood pressure, pulse pressure and mean arterial pressure were significantly higher among patients compared to controls. Mean systolic blood pressure for the patients was 147.1 ± 1.98 mmHg versus 120 ± 1.68 mmHg for controls $p< 0.001$. Diastolic blood pressure was 83.9 ± 1.44 mmHg in patients and 74.3 ± 1.30 mmHg in controls $p< 0.001$. Pulse pressure was 63.12 ± 1.25 mmHg in patients and 45.79 ± 1.28 mmHg in controls $p < 0.001$ and the mean arterial pressure (MAP) was 105 ± 1.53 mmHg in patients compared with 89.6 ± 1.28 mmHg in controls $p <0.001$ (table 3.3). Fifty-seven patients (79.76%) were being treated with various combinations of antihypertensive medications: angiotensin-converting enzyme (ACE) inhibitors N=46; calcium channel blockers N= 61; β blockers N= 28; α blockers 14; [15 on monotherapy with either angiotensin-converting enzyme (ACE) inhibitors, calcium channel blockers, and beta blockers, 24 on double, 19 on triple, 8 on quadruple therapy and 1 patient on 5 drug-therapy with various combinations of these drugs). In addition most of the patients were on commonly used medications in ESRD such as phosphate binders, vitamin B, and D supplementation. Only one patient was on HMG-Co A – reductase inhibitor.

Table 3.3 Cardiovascular disease risk factors of the study population

Risk factors	Patients Mean ± SEM or % N = 84	Controls Mean ± SEM or % N = 63	t value or X^2	p value
Current smokers	18 (21.43%)	12 (19.05%)	4.48	NS
Exercise	24 (28.57%)	30 (47.62%)	5.62	0.018
BMI (kg/m2	24.45 ± 0.55	25.48 ± 0.58	– 1.28	NS
Systolic BP (mmHg)	147.1 ± 1.98	119.9 ± 1.69	10.01	< 0.001
Diastolic BP (mmHg)	83.9 ± 1.45	74.3 ± 1.26	4.84	<0.001
Pulse pressure (mmHg)	63.12 ± 1.25	45.79 ± 1.28	9.50	<0.001
MAP (mmHg)	105 ± 1.53	89.6 ± 1.28	7.37	<0.001
Total Cholesterol (mmol/L)	3.73 ± 0.08	4.97 ± 0.16	–7.25	< 0.001
Triglyceride (mmol/L)	1.25 ± 0.08	1.57 ± 0.13	–2.11	0.04
HDL-Cholesterol (mmol/L	1.18 ± 0.04	1.28 ± 0.05	–1.72	NS
LDL- Cholesterol (mmol/L)	1.98 ± 0.07	2.94 ± 0.15	– 6.39	< 0.001
Carotid IMT (mm)	0.65± 0.02	0.61 ± 0.02	1.50	NS
Hs-CRP (mg/L)	8.06 ± 1.31	2.27 ± 0.34	3.73	< 0.001
Homocysteine μmol/L	20.22± 0.70	9.16 ± 0.48	12.38	< 0.001
Adiponectin (μg/L)	22.19 ± 0.98	9.93 ± 0.68	9.63	< 0.001
Lipoprotein(a) (mg/dl)	43.31 ± 4.13	42.1 ± 4.77	0.19	NS
LVH	69(82.14%)	11 (17.5%)	60.72	<0.001
Plaques	32 (38.1%)	5 (7.93%)	17.39	<0.001

3.3.3 Body Mass Index

BMI was similar in both patients and controls with a mean BMI of 24.45 ± 0.55 kg/m^2 in patients compared with 25.48 ± 0.58 kg/m^2 in controls; p = 0. 204. (Table 3.3)

3.3.4 Smoking and Physical activity

There was no difference in the number of smokers between patients and controls. Eighteen patients (21.43%) reported as current smokers while 25 (29.76%) had smoked before. Among the controls, 12 subjects (19.05%) are current smokers while 35 (23.81%) had smoked before. In terms of physical

activity, more controls did exercise compared with patients, 30 (47.62%), versus 24 (28.57%) Pearson X^2 5.62; p =0.018.

3.3.5 Serum Lipids

The patients had significantly lower serum total cholesterol, LDL cholesterol level and triglyceride levels compared with controls $p < 0.001$ and $p= 0.04$ respectively (table 3.3). Although the haemodialyisis patients had lower HDL–cholesterol compared to controls, this was not statistically significant.

3.3.6 Left Ventricular Hypertrophy

The prevalence of left ventricular hypertrophy was significantly higher in the patients compared with the controls (82.14 % versus 17.5% $X^2 = 60.72$, $P < 0.001$) table 3.3 and figure 4.2. Of these patients, 86.5% (64 patients) had concentric LVH, 6.75% eccentric LVH and 6.75% had dilated cardiomyopathy (DCMO) figure 4.3. Seventeen patients (20.24%) had systolic dysfunction, 54 (65.06%) diastolic dysfunction; while none of the control subjects had systolic dysfunction and only 7 (11.48%) had diastolic dysfunction. This difference was statistically significant Pearson $X^2 = 14.42$; $p< 0.001$: $X^2 = 41.35$; $p <0.001$ respectively). The LVMI was significantly higher in haemodialyisis patients compared to controls mean LVMI was 194.25 ± 7.69 gm/m^2 in patients versus 93.21± 3.27 gm/ m^2 in the controls $p< 0.001$. Ejection fraction was significantly lower in haemodialyisis patients compared with controls (60.88 ± 1.28 versus 67.36 ± 0.81 $p< 0.001$ (table 3.4). Risk factors associated with LVMI in the study population were male gender, diastolic blood pressure (BP), presence of CKD, smoking, adiponectin and presence of plaques $R^2= 0.67$ (table3.5). Male subjects had higher LVMI by 25.58gm/m^2 while adjusting for the other risk factors. Haemodialyisis patients had higher LVMI by 56.14gm/m^2 compared with controls when all other risk factors are adjusted for. Patients with plaques also had higher LVMI by 45.64 gm/m^2. Smoking was also associated with a higher LVMI. When the control subjects were taken out of

the model (that is looking at the haemodialyisis patients alone), the risk factors retained their significance. However in a model which looked at only the control subjects, the factors associated with LVMI were age and male gender. Seven patients (8.33%) had calcification of their heart valves.

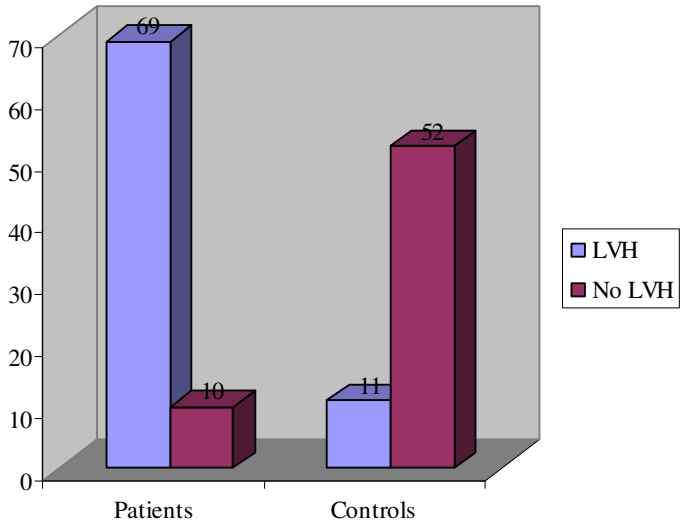


Figure 3.2 Prevalence of LVH in the study population

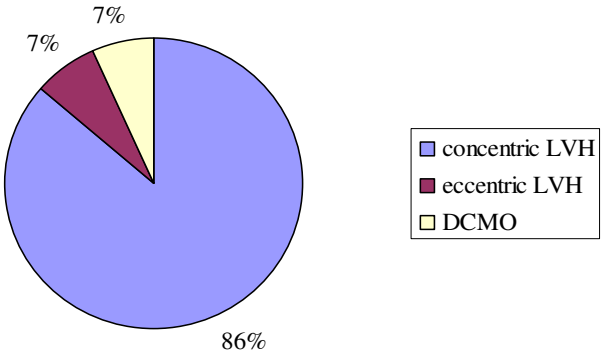


Figure 3.3 Distribution of the different types of LVH in haemodialysis patients

Abbreviation: DCMO, Dilated cardiomyopathy**.**

Table 3.4 Echocardiographic values for the study population

Variables	Patients Mean ± SEM or % N = 84	Controls Mean ± SEM or % N = 63	t value or X^2	p value
IVS_d (cm)	1.41 ± 0.33	1.07 ± 0.20	4.475	<0.001
PWT_d (cm)	1.28 ±0.03	0.92 ± 0.02	9.49	<0.001
$LVID_d$ (cm)	4.90 ± 0.09	4.31 ± 0.06	5.12	<0.001
LVM (gm)	334.48 ± 14.57	168.15 ± 7.11	9.28	< 0.001
LVMI (gm/m2)	194.25 ± 7.69	93.21± 3.27	10.93	<0.001
FS (%)	33.09 ± 0.87	37.45 ± 0.68	−3.73	<0.001
EF (%)	60.87± 1.28	67.36 ± 0.81	− 3.96	<0.001
Diastolic dysfunction	54 (65.06%)	7 (11.48%)	41.35	< 0.001
Systolic dysfunction	17 (20.24%)	0 (0%)	14.42	<0.001

Table 3.5 Risk factors associated with LVMI in the study population (Patients and controls) (R^2 = 0.67)

LVMI	Coefficient β	t value	p value	95% Confidence interval	
Plaque/no plaques	45.642	4.96	<0.001	25.433	63.851
Male/Female	− 25.577	−3.22	0.002	− 41.302	− 9.852
Diastolic BP	1.640	5.28	<0.001	1.025	2.254
Patients/controls	−56.137	−5.54	<0.001	−76.176	−36.099
Adiponectin	1.468	3.01	0.003	0.504	2.433
Smoker/non smoker	− 34.421	−3.73	<0.001	− 52.668	−16.174

Table 3.6 Risk factors associated with LVMI in haemodialysis patients ($R^2 = 0.48$)

LVMI	Coefficient β	t value	p value	95% Confidence interval	
Smoker/Non smoker	−50.140	−3.57	0.001	−78.100	−22.180
Male/female	− 39.951	−3.05	0.003	− 66.067	− 13.836
Plaque/No plaques	50.72	4.09	<0.001	25.997	75.444
Adiponectin	1.463	2.21	0.030	0.146	2.780
Diastolic BP	1.835	4.08	<0.001	0.941	2.730

Table 3.7 Regression model of risk factors for LVMI in the control group ($R^2 = 0.30$)

LVMI	Coefficient β	t value	p value	95% Confidence interval	
Age	1.146	4.38	<0.001	0.622	1.669
Male/female	−13.104	−2.35	0.022	−24.259	−1.949

3.4 Non-traditional Cardiovascular disease risk factors

3.4.1 Inflammatory markers

The serum Hs-CRP levels were much higher in haemodialysis patients compared to controls with a mean level of 8.06 ± 1.31 mg/L in patients and 2.27 ± 0.34 mg/L in controls. Patients were divided into two groups according to Hs-CRP levels: CRP < 5 mg/L (N = 49), CRP ≥ 5 mg/L (N= 35). The patients who had abnormal Hs- CRP levels had significantly higher total cholesterol and LDL-Cholesterol levels; and lower serum albumin and haemoglobin levels (table 3.8). Seventeen patients (48.57%) with Hs-CRP > 5 mg/L had plaques while 15 (30.61%) of patients with Hs-CRP < 5mg/L had plaques this difference was of borderline significance X^2 =3.71, p = 0.054. CIMT was similar in both groups. Only 8 controls had

Hs-CRP levels > 5mg/L. None of them had plaques and 4 of these patients had LVH. In a linear regression model, Hs-CRP was associated with higher CIMT and high homocysteine levels.

Table 3.8 CVD risk factors in patients with normal and abnormal Hs-CRP

Risk factors	**Patients with N CRP Mean ± SEM or % N = 49**	**Patients with Abn CRP Mean ± SEM or % N = 35**	**t value or X^2**	**p value**
Gender M/F	26/23	22/13	0.80	NS
Age (years)	40.14 ± 1.47	39.94 ± 1.81	0.87	NS
BMI (kg/m2	23.88 ± 0.59	25.24 ± 0.89	−1.22	NS
S BP (mmHg)	146.43 ± 2.89	147.94 ± 2.51	0.38	NS
DBP (mmHg)	83.86 ± 1.98	84.06 ± 2.12	−0.07	NS
Total Cholesterol (mmol/L)	3.52 ± 0.11	4.03 ± 0.65	−3.13	0.002
Triglyceride (mmol/L)	1.24 ±0.12	1.27 ± 0.11	− 0.17	NS
HDL-Cholesterol (mmol/L	1.16± 0.04	1.20 ± 0.06	−0.37	NS
LDL- Cholesterol (mmol/L)	1.79 ± 0.09	2.25 ± 0.10	− 3.13	0.002
Albumin gm/L	40.67±0.51	38.37± 0.69	2.68	0.009
Calcium mmol/L	2.26 ± 0.03	2.39 ± 0.05	− 2.37	0.020
Phosphate mmol/L	1.50±0.07	1.62 ± 0.09	−1.04	NS
Calcium x Phosphate	3.39± 0.16	3.88 ± 0.24	−1.79	NS
Parathyroid hormone	579.31 ± 65.49	523.67 ± 87.70	−0.54	NS
Haemoglobin	9.89 ± 0.19	8.89± 0.26	3.18	0.002
Carotid IMT (mm)	0.65± 0.12	0.66 ± 0.03	−0.37	NS
Homocysteine µmol/L	19.44 ± 0.73	21.29 ± 1.30	−1.21	NS
Adiponectin (µmol/L)	22.63 ± 1.29	21.57 ± 1.52	0.53	NS
Lipoprotein(a) (mg/dl)	43.26 ± 5.30	43.37 ± 6.65	−0.01	NS

Abbreviation: N CRP, normal CRP. Abn CRP, abnormal CRP.

3.4.2 Adiponectin

Serum adiponectin levels were significantly higher in patients compared with controls. Mean level of 22.19 ± 0.98 μg/mL in patients and 9.93 ± 0.68 μg/mL in control subjects p< 0.001. Patients who were completely anuric had the highest levels of serum adiponectin. On multivariate analysis, risk factors found to be associated with serum adiponectin levels in the study population were total cholesterol, triglycerides, and LDL- cholesterol (table 3.9). Adiponectin had a significant inverse relationship with serum triglyceride (figure 3.4) and LDL levels. Adjusting for these, adiponectin was significantly higher in patients by an average of 13.1μg/mL P< 0.001. Among the haemodialysis patients, risk factors found to be associated with adiponectin were total cholesterol, triglycerides, LDL-Cholesterol and LV dysfunction. Serum triglyceride and LDL-Cholesterol were inversely associated with adiponectin table 3.10. When race was fixed into the model, these factors still remained significant with no evidence of a race effect. Serum adiponectin was higher in female subjects compared with the male subjects but this difference was not significant. Mean level was 23.09 ± 1.37μg/ml for female patients and 21.37 ± 1.39 μg/mL for male patients p = 0.383. Among the controls, the female controls had a mean level of 11.15 ± 0.86 μg/ml while for the male controls the mean was 8.60 ± 1.02 μg/ml p = 0.059.

Table 3.9 Risk factors found to be significantly associated with Adiponectin in the study population (generalized linear regression model) ($R^2 = 0.47$)

Adiponectin	Coefficient β	t value	p value	95% Confidence interval	
Triglyceride	−4.872	−4.68	<0.001	−6.929	− 2.815
LDL cholesterol	−4.617	−2.68	0.008	−8.020	− 1.214
Patients/controls	−13.145	−9.21	<0.001	−15.967	−10.323
Total cholesterol	5.186	3.06	0.003	1.838	8.534

Table 3.10 Factors associated with adiponectin in haemodialysis patients

($R^2 = 0.32$)

Adiponectin	Coefficient β	t value	p value	95% Confidence interval	
Total cholesterol	7.564	2.9	0.005	2.359	12.768
LDL cholesterol	−8.939	−3.09	0.003	−14.697	− 3.181
Triglyceride	−7.107	−4.93	<0.001	−9.978	− 4.235
Diastolic dysfunction	5.357	2.8	0.007	1.543	9.171
Systolic dysfunction	5.781	2.5	0.001	1.165	10.396

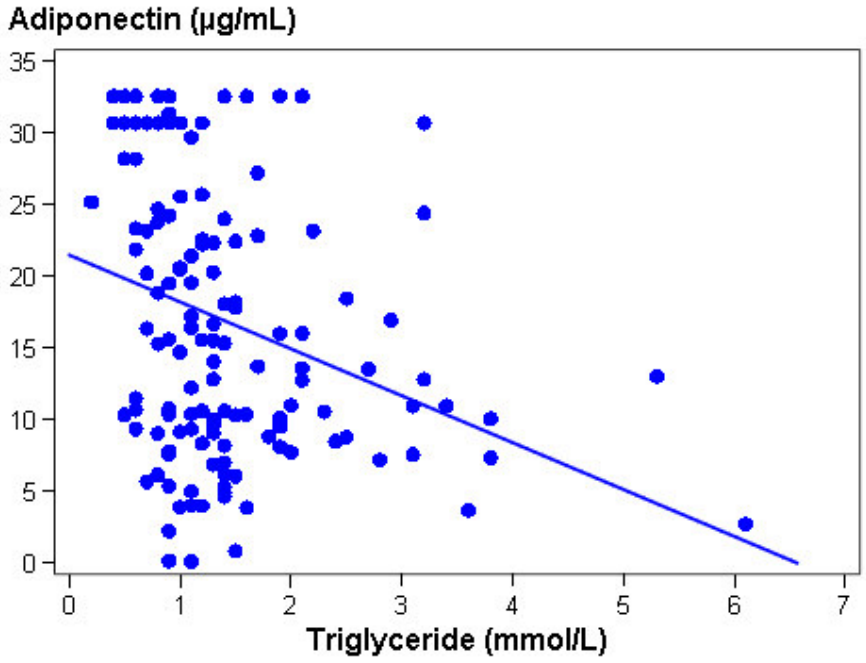


Figure 3.4 Regression of serum adiponectin and triglyceride in the study population.

3.4.3 Homocysteine

Plasma homocysteine levels were higher in patients compared to controls $P < 0.001$ Table 3.3. Using stepwise regression model, risk factors found to be associated with plasma homocysteine levels in both patients and controls were: high levels of Hs-CRP, increasing age, and increase in total cholesterol. Adjusting for these, homocysteine was higher in haemodialysis patients ($P< 0.001$) by an average of 11.6 μmol/L table 3.11. In another regression model, looking at haemodialyisis patients alone, homocysteine was associated with Hs-CRP and age and inversely associated with adiponectin levels however this was a poor model as adjudged by the R^2 of 0.19. When race was fixed into the model, these factors remained significant with no evidence of a race effect table 3.12.

Table 3.11 Risk factors found to be significantly associated with plasma homocysteine levels in the study population ($R^2 = 0.57$)

Homocysteine	Coefficient β	t value	p value	95% Confidence interval
Hs-CRP	0.117	2.53	0.013	0.026 0.209
patients/controls	−11.629	−10.98	<0.001	− 13.725 − 9.534
Age	0.898	2.2	0.029	0.009 0.170
Total cholesterol	1.096	2.45	0.015	0.213 1.978

Table 3.12 Factors associated with homocysteine in haemodialyisis patients ($R^2 = 0.19$)

Homocysteine	Coefficient β	t value	p value	95% Confidence interval
Blacks/non Blacks	0.626	0.41	0.684	−2.429 3.682
Hs-CRP	0.119	2.21	0.030	0.012 0.226
Adiponectin	− 0.195	−2.56	0.012	−0.346 −0.043
Age	0.160	5.21	0.021	−0.025 0.295

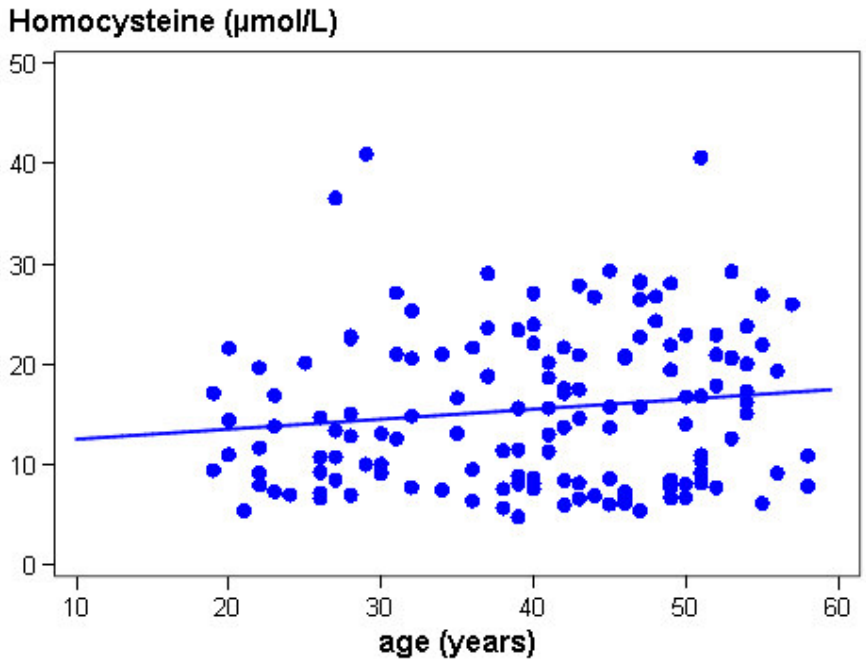


Figure 3.5 Regression of homocysteine with age

3.4.4 Lipoprotein (a)

Serum lipoprotein (a) levels showed marked variability among patients and controls. Mean levels were similar in both haemodialyisis patients and controls p= 0.848 (Table 3.3). Elevated Lp (a) concentrations (>30mg/dl) were observed in 52.4% of patients and 49.2% of controls. On regression analysis, Lp (a) concentration was significantly associated with serum adiponectin levels, total cholesterol and inversely associated with age. The R^2 indicates that only 19% of the variability in lipoprotein can be explained by this model. Black subjects had significantly higher Lp (a) by an average of 26.01mg/dl.

Table 3.13 Risk factors associated with serum Lp (a) levels ($R^2 = 0.19$)

Lipoprotein (a)	Coefficient β	t value	p value	95% Confidence interval	
Total cholesterol	9.733	3.44	0.001	4.141	15.326
Age	−0.795	−2.79	0.006	−1.359	− 0.231
Blacks/non blacks	−26.011	−3.98	<0.001	−38.933	− 13.090
Adiponectin	1.137	3.61	<0.001	0.515	1.759

3.5 Carotid-intima media thickness

The mean carotid intima media thickness (CIMT) was higher in haemodialysis patients compared with controls. Mean CIMT was 0.65 ± 0.02 mm in patients and 0.61± 0.02mm in controls, however this difference was not statistically significant (p = 0.137) Table 3.3. Complete measurements of the far wall for all the segments were available. Measurements were not complete for the near wall in 34.69% of the study population. The highest CIMT measurements were at the birfurcation in both groups. The haemodialysis patients had significantly higher CIMT measurements at this segment on both sides (table 3.14). Risk factors found to be associated with CIMT in a regression model in both subjects and controls were total cholesterol, LDL cholesterol, Hs-CRP, age, and family history of CKD (table 3.15). CIMT was positively associated with age, serum LDL-Cholesterol, Hs-CRP and family history of CKD. Adjusting for all these, controls had slightly lower CIMT (0.02mm) but insignificant (p=0.42). There was an inverse relationship between total cholesterol and CIMT.

On univariate analysis looking at some dialysis related factors in haemodialysis patients alone, there was no relationship between CIMT and the length of time patient has been on dialysis, serum calcium or calcium/phosphate product (r = 0.05; p = 0.542). On multivariate analysis risk factors found to be

associated with CIMT in haemodialyisis patients were Hs-CRP and family history of CKD. After adjusting for these, there was no significant difference between the races Table 3.16**.**

Table 3.14 The IMT measurements at the various carotid segments

Carotid segment (far wall only)	**Patients Mean ± SEM or % N = 84**	**Controls Mean ± SEM or % N = 63**	**t value or X^2**	**p value**
CIMT (mm)	0.65 ± 0.02	0.61 ± 0.02	1.50	NS
LCCA (mm)	0.65 ± 0.02	0.61 ± 0.02	1.50	NS
RCCA (mm)	0.65 ± 0.02	0.61 ± 0.02	1.50	NS
LBIF (mm)	0.78 ± 0.04	0.67 ± 0.03	2.26	0.025
RBIF (mm)	0.77 ± 0.03	0.68 ± 0.03	1.94	0.053
RICA (mm)	0.61 ± 0.02	0.57 ± 0.02	1.42	NS
LICA (mm)	0.58 ± 0.02	0.60 ± 0.03	– 0.56	NS

Table 3.15 Risk factors associated with CIMT in both patients and controls

CIMT	**Coefficient β**	**t value**	**p value**	**95% Confidence interval**	
Cases/controls	–0.023	–0.80	0.424	–0.079	0.033
Total cholesterol	0.050	–1.97	0.051	–0.101	0.000
LDL cholesterol	0.053	1.92	0.057	–0.002	0.107
Age	0.006	5.46	0.001	0.004	0.008
Hs-CRP	0.003	2.41	0.017	0.000	0.005
Family history of CKD	0.131	3.37	0.001	0.054	0.209

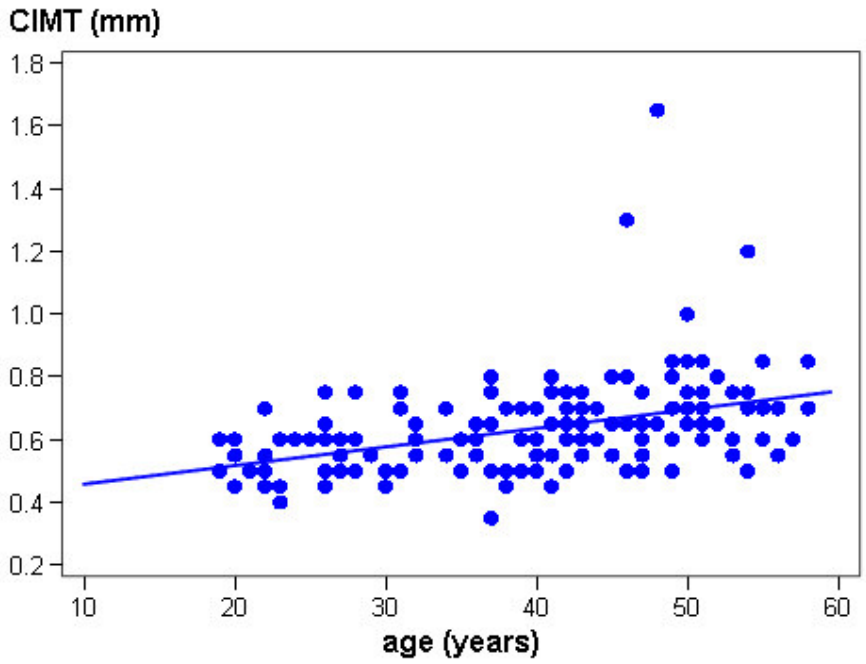


Figure 3.6 Regression of CIMT and age in the study population

Table 3.16 Risk factors associated with CIMT in haemodialysis patients only

CIMT	Coefficient β	t value	p value	95% Confidence interval	
Blacks/non-blacks	−0.380	−1.11	0.273	−0.106	0.030
Hs-CRP	0.003	2.44	0.017	0.000	0.006
Family history of CKD	0.231	3.62	0.001	0.104	0.358

3.6 Carotid artery plaques

The prevalence of plaques in the carotid arteries was significantly higher in haemodialysis patients (38.1%) compared with controls (7.93%). Majority of the patients (90.48%) had plaques at more than one site. Most of the plaques (> 85%) were found at the bifurcation and internal carotid arteries. As expected most of the dialysis patients, (29) had calcified plaques, 2 had soft plaques and 1 had mixed plaque while all control subjects had soft plaques and all occurring at only 1 site. The plaque score ranged from 1.1mm to 18.8mm with a median score of 5.13mm in patients and median score of 1.3 mm in controls (1.2-2.0mm). When we compared patients who had plaques with those who did not have plaques, haemodialyisis (HD) patients who had plaques were older, there were more male patients, and they had been on haemodialyisis for a much longer duration. They had significantly higher systolic BP (154 .38 ± 2.86 mmHg versus 142.56 ± 2.49 mmHg p= 0.003), and MAP but diastolic blood pressure was similar (DBP) (85.97 ± 2.19 versus 82.69 ± 1.90 mmHg; p = 0.274) table 3.17. The lipid parameters were similar in the two groups. As expected the CIMT in those patients with plaques was significantly higher than in those patients with out plaques. There were more smokers among patients with plaques compared with those without plaques. The prevalence of LVH was similar in both groups. The HD patients with plaques had higher Hs-CRP levels although this was not statistically significant (p= 0.381). The mean Kt/V was similar in both groups mean level of 1.33 ± 0.04 in patients without plaques and 1.28 ± 0.04 in patients with plaques. Logistic regression analysis showed that age, male gender, smoking, systolic blood pressure, serum phosphate and calcium phosphate product were significantly associated with plaque occurrence table 3.18.

Table 3.17 CVD risk factors in haemodialyisis patients with and without plaques

Risk factors	Patients with plaques Mean ± SEM or % N =32	Patients without plaques Mean ± SEM or % N = 52	t value or X^2	p value
Gender M/F	22/10 (68.75%)	22/30 (42.31%)	5.55	0.018
Current smokers	11 (34.38%)	7 (13.46%)	5.15	0.023
Exercise	8 (25%)	16 (30.77%)	0.57	NS
Age (years)	45.8 ± 1.56	36.6 ± 1.36	4.33	<0.001
BMI (kg/m2	24.24± 0.59	24.58 ± 0.81	–0. 29	NS
HD Duration (months)	59.38± 7.85	37.69± 6.08	2.19	0.031
S BP (mmHg)	154.4±2.86	116.1 ± 2.66	3.04	0.003
DBP (mmHg)	86 ± 2.19	71.8 ± 1.68	1.1	NS
PP (mmHg)	68.41±2.08	59.87 ± 1.38	3.56	<0.001
MAP (mmHg)	108.8 ± 2.23	102.7± 2.01	1.97	0.052
Cholesterol (mmol/L)	3.72 ± 0.17	3.74 ± 0.09	–0.11	NS
Triglyceride (mmol/L)	1.15 ±0.14	1.32 ± 0.10	– 0.96	NS
HDL (mmol/L)	1.18 ± 0.06	1.18 ± 0.05	0.07	NS
LDL (mmol/L)	2.01 ± 0.14	1.98 ± 0.08	0.21	NS
Albumin gm/L	39.22±0.55	39.98± 0.62	– 0.85	NS
Calcium mmol/L	2.37± 0.05	2.28 ±0.03	1.44	NS
Phosphate mmol/L	1.54±0.10	1.55 ± 0.07	–0.06	NS
Calcium x Phosphate	3.67 ± 0.25	3.55 ± 0.17	0.43	NS
Parathyroid hormone	457.85 ±79.49	616.6 ± 65.36	–1.53	NS
Haemoglobin	9.40±0.25	9.54 ± 0.22	– 0.42	NS
Carotid IMT (mm)	0.70± 0.04	0.62 ± 0.01	2.42	0.018
Hs-CRP (mg/L)	9.53 ± 1.90	7.15 ± 1.76	0.88	NS
Homocysteine µmol/L	20.43± 1.02	20.09 ± 0.94	0.14	NS
Adiponectin (µmol/L)	22.86 ± 1.61	21.78 ± 1.24	0.54	NS
Lipoprotein(a) (mg/dl)	39.88 ± 6.01	45.42 ± 5.57	0.65	NS
LVH	22(68.75%)	42 (80.77%)	2.79	NS

Abbreviation: SBP is systolic blood pressure, DBP is diastolic blood pressure, PP is pulse pressure, MAP is mean arterial pressure, and HD duration is duration on dialysis,

Table 3.18 Risk factors associated with plaque occurrence

Plaque	Odds Ratio	z	p value	95% Confidence interval	
Age	1.167	3.64	0	1.073	1.267
Systolic BP	1.036	1.98	0.048	1.000	1.072
Male/female	0.259	−1.95	0.051	0.067	1.005
Phosphate	0.009	−1.92	0.055	0.000	1.116
Calcium x phosphate	8.248	2.17	0.03	1.224	55.568
Smoker/non smoker	8.097	2.53	0.011	1.606	40.805

3.7 Cardiovascular risk factors by race

When the black patients were compared with the non-black patients with respect to cardiovascular risk factors, the black patients were older and had significantly lower total cholesterol and triglyceride levels compared with the non-black patients. Mean cholesterol was 3.61 ± 0. 10mmol/L in blacks and 4.00 ± 0.14 mmol/L in non-blacks, p= 0.030; mean triglyceride of 1.04 ± 0.08mmol/L in black patients and 1.74 ± 0.17 mmol/L in non-blacks p <0.001 (table 3.19).

The serum lipoprotein was significantly higher in the black patients compared with the non-black patients. Mean lipoprotein level (a) was 49.25 ± 5.04 mg/dl in the blacks versus 30.05 ± 6.57 mg/dl in the non-black patients p = 0.031. Black patients had significantly higher adiponectin levels when compared with the non-black patients (23.55 ± 1.14 µg/mL versus 19.15 ± 1.77 µg/mL p=0.037).

Systolic blood pressure, diastolic blood pressure, HDL-cholesterol, LDL cholesterol, plasma homocysteine level was similar in both groups. The non-black patients had slightly higher Hs-CRP levels but this difference was not significant table 3.19. The black patients did less exercise compared with the

non-black patients. Prevalence of smoking was higher among the non-black patients (26.9%) compared with black patients (18%) but this difference was not statistically significant X^2 =0.29 p= 0.592.

In a sub analysis comparing CIMT by race in all the groups, the black subjects (patients and controls) had higher CIMT compared with the non-black subjects but this difference was not significant. Mean CIMT in black patients was 0.66 ± 0.17mm vs. 0.63 ± 0.14mm in the non-black patients table 3.19; 0.64mm ± 0.13 in black controls vs. 0.59 ± 0.17mm in non-black controls table 3.20.

The prevalence of atherosclerotic plaques was slightly higher in the non-black patients (42.3%) than in the black patients (36.2%) although not significant table 3.19. There was no difference in occurrence of LVH in both groups.

Among the control groups, the black controls had significantly higher systolic BP (p=0.042) than the non-black subjects but no significant difference in the diastolic BP. The black controls had significantly lower total cholesterol, HDL cholesterol and homocysteine levels (p = 0.003, p = 0.006, p = 0.035 respectively) but had higher lipoprotein (a) p = 0.056 (Table 3.20). More black controls (25.7%) had LVH than whites (7.14%), this difference was of borderline significance X^2 3.72 p = 0.054. Prevalence of plaques was similar in both groups.

Table 3.19 Comparison of CVD risk factors in black and non-black haemodialyisis patients

Risk factors	**Black Patients Mean ± SEM or % N = 58**	**Non-black patients Mean ± SEM or % N = 26**	**t value or X^2**	**p value**
Gender M/F	33/25 (56.9%)	11/15 (42.31%)	1.53	NS
Age (years)	41.6± 1.25	36.6± 2.27	2.07	0.042
Current smokers	11 (18.97%)	7 (26.92%)	0.29	NS
Exercise	13 (22.41%)	11 (42.31%)	3.48	0.062
BMI (kg/m2	24.73 ± 0.55	25.48 ± 0.58	– 1.28	NS
Systolic BP (mmHg)	148.9 ± 2.36	143.0 ± 3.56	1.37	NS
Diastolic BP (mmHg)	85.7 ± 1.70	80.1 ± 2.63	1.81	NS
Pulse pressure (mmHg)	63.19 ± 1.53	62.96 ± 2.17	0.08	NS
MAP (mmHg)	106.74 ± 1.80	101.06 ± 2.80	1.67	NS
Total Cholesterol (mmol/L)	3.61 ± 0.10	4.00 ± 0.14	–2.21	0.03
Triglyceride (mmol/L)	1.04 ± 0.08	1.74 ± 0.17	– 4.32	<0.001
HDL-Cholesterol (mmol/L	1.21 ± 0.04	1.10 ± 0.07	1.31	NS
LDL- Cholesterol (mmol/L)	1.93 ± 0.09	2.09 ± 0.13	– 1.01	NS
Carotid IMT (mm)	0.66± 0.02	0.63 ± 0.03	0.68	NS
Hs-CRP (mg/L)	7.26 ± 1.29	9.85 ± 3.13	–0.92	NS
Homocysteine µmol/L	20.20 ± 0.72	20.26 ± 1.58	–0.04	NS
Adiponectin (µmol/L)	23.55 ± 1.14	19.15 ± 1.77	2.12	0.037
Lipoprotein(a) (mg/dl)	49.25 ± 5.04	30.05 ± 6.57	2.20	0.031
LVH	49 (84.48%)	20 (76.92%)	0.70	NS
Plaques	21 (36.21%)	11 (42.31%)	0.28	NS

Table 3.20 CVD characteristics in black and non-black controls

Risk factors	Black controls Mean ± SEM or % N =35	Non-black controls Mean ± SEM or % N = 28	t value or X^2	p value
Gender M/F	18/17 (51.43%)	12/16 (42.88%)	0.46	NS
Current smokers	5 (14.29%)	7 (25%)	1.16	NS
Exercise	12 (34.29%)	18 (64.29%)	5.61	0.018
Age (years)	41.8± 1.65	37.5 ± 2.19	1.61	NS
BMI (kg/m2	25.98 ± 0.85	24.84 ± 0.74	0.984	NS
Systolic BP (mmHg)	123 ± 2.05	116.1 ± 2.66	2.076	0.042
Diastolic BP (mmHg)	76.3 ± 1.78	71.8 ± 1.68	1.807	NS
Pulse pressure (mmHg)	46.97 ± 1.61	44.32 ± 2.07	1.026	NS
MAP (mmHg)	91.94 ± 1.72	86.56 ± 1.81	2.143	0.036
Total Cholesterol (mmol/L)	4.55 ± 1.72	5.49 ± 0.22	–3.046	0.003
Triglyceride (mmol/L)	1.58 ±0.18	1.55 ± 0.19	0.137	NS
HDL-Cholesterol (mmol/L	1.16 ± 0.04	1.44 ± 0.09	– 2.854	0.006
LDL- Cholesterol (mmol/L)	2.71 ± 0.20	3.24 ± 0.20	– 1.832	NS
Carotid IMT (mm)	0.64± 0.02	0.59 ± 0.03	1.315	NS
Hs-CRP (mg/L)	2.30 ± 0.49	2.23 ± 0.47	0.112	NS
Homocysteine µmol/L	8.26 ± 0.46	10.28 ± 0.88	–2.160	0.035
Adiponectin (µmol/L)	9.09 ± 0.97	10.99 ± 0.91	–1.401	NS
Lipoprotein(a) (mg/dl)	50.21 ± 6.45	31.96 ± 6.71	0.192	0.056
LVH	9 (25.7%)	2 (7.14%)	3.723	0.054
Plaques	3 (8.57%)	2 (7.14%)	0.043	NS

3.8 Cardiovascular risk factors based on previous kidney transplant status

Table 3.21 shows the comparison of cardiovascular risk factors in haemodialysis patients who have had a failed transplant and have returned to dialysis. The only significant findings were a higher systolic and diastolic blood pressure among patients who have never been transplanted. The patients who have had a previous failed transplant had significantly higher LDL cholesterol levels compared with those who did

not (2.16 ± 0.14mmol/L versus 1.90 ± 0.09mmol/L, t = 7.87 p <0.000). CIMT, LVMI and prevalence of plaques were similar in both groups.

Table 3.21 Comparison of CVD risk factors among patients with and without previous failed kidney transplant

CVD risk factors	**Previous kidney transplant**	**No kidney transplant**	**t-value/X^2**	**p value**
	Mean ± SEM or %	**Mean ± SEM or %**		
	N = 27	**N = 57**		
CIMT (mm)	0.62 ± 0.02	0.67 ± 0.02	– 1.12	NS
LVMI gm/m2	194.5 ± 13.1	194.1 ± 9.27	0.02	NS
Plaques	13 (48.2%)	19 (33.3%)	1.68	NS
Total cholesterol (mmol/L)	3.89 ± 0.15	3.65 ± 0.10	1.32	NS
Triglyceride (mmol/L)	1.48 ± 0.18	1.15 ± 0.09	1.92	0.058
HDL cholesterol (mmol/L)	1.05 ± 0.04	1.24 ± 0.05	– 0.74	NS
LDL cholesterol (mmol/L)	2.16 ± 0.14	1.90 ± 0.09	7.87	<0.001
Systolic BP (mmHg)	139.5 ± 3.07	150.6 ± 2.40	– 2.72	0.008
Diastolic BP (mmHg)	79.6± 2.30	86 ± 1.78	– 2.09	0.04
Hs-CRP (mg/L)	9.18 ± 2.72	7.53 ± 1.45	0.59	NS
Lipoprotein (a) (mg/dl)	37.12 ± 6.76	46.24 ± 5.16	– 1.03	NS
Homocysteine (µmol/L	21.44 ± 1.40	19.76 ± 0.77	1.14	NS
Adiponectin (µmol/L)	20.83 ± 1.67	22.83 ± 1.21	– 0.95	NS

3.9 CVD risk factors in black patients; comparison of essential hypertensive black patients with non-essential hypertensive black patients

The black patients were divided into two groups based on aetiology of ESRF. There were 37 patients with essential hypertension as the cause of ESRD and 21 patients with other causes of ESRF (non-essential hypertension. Table 3.22 shows a comparison of the CVD risk factors in the groups. The patients with essential hypertension were older (mean age of 43.8 ± 1.4years vs 37.8 ± 2.2yrs), had significantly higher total cholesterol, LDL-cholesterol and higher homocysteine levels compared with non-essential hypertensive group. The essential hypertensive group also had significantly lower adiponectin levels. The prevalence of plaques was significantly higher in the essential hypertensive group (46% vs 19.1% uncorrected X^2 = 4.12, p = 0.042) however when this was corrected for the small numbers, there was no significant difference. Hs-CRP, LVMI and BMI were similar in both groups.

Table 3.22 Comparing CVD risk factors by aetiology in black patients

CVD risk factors	Essential hypertension n = 37 Mean ± SEM or %	Non-essential hypertension n = 21 Mean ± SEM or %	t value or X 2	p value
age (years)	43.8 ± 1.43	37.8 ± 2.17	2.37	0.021
BMI (kg/m2)	25.10 ± 0.85	24.09 ± 1.19	0.7	NS
Total cholesterol (mmol/L)	3.79 ± 0.4	3.30 ± 0.10	2.42	0.019
Triglyceride (mmol/L)	1.06 ± 0.10	0.99 ± 0.14	0.45	NS
HDL-cholesterol (mmol/L)	1.25 ± 0.06	1.15 ± 0.05	1.06	NS
LDL-cholesterol (mmol/L)	2.06 ± 0.12	1.70 ± 0.10	1.98	0.053
CIMT (mm)	0.66 ± 0.03	0.66 ± 0.02	0.03	NS
Adiponectin (µmol/L)	21.49 ±1.51	27.18 ± 1.38	-2.51	0.015
Lipoprotein (a) (mg/dl)	44.44 ± 5.45	57.73 ± 10.02	-1.27	NS
Homocysteine (µmol/L)	21.51 ± 0.85	18.21 ± 1.19	2.28	0.026
Hs-CRP (mg/L)	7.16 ± 1.78	7.42 ± 1.73	-0.09	NS
LVMI (kg/m2)	192.82 ± 11.98	24.09 ± 1.19	-0.59	NS
Plaques **	17 (46)	4 (19.1)	4.12	0.042

** Yates corrected X^2 value = 3.11, p = 0.078

CHAPTER 4

DISCUSSION

Cardiovascular disease is a leading cause of death in haemodialyisis patients. One of the reasons for this high CVD mortality in haemodialyisis patients is due to a high prevalence of both traditional and non-traditional risk factors for CVD present in this patient population. There are three distinct types of CVD that are highly prevalent in patients with CKD, all of which lead to poor outcomes. These include alterations in cardiac geometry (LVH), atherosclerosis and arteriosclerosis. This study was carried out to evaluate the prevalence of traditional and some non-traditional risk factors for atherosclerosis in our haemodialyisis patients and to examine the relationship between these risk factors and the presence of sub-clinical atherosclerosis. In this study, common carotid intima-media thickness and occurrence of plaques were measured as surrogate markers of sub-clinical atherosclerosis, while LVMI was used to determine cardiac geometry. The cohort in this study includes patients with diverse causes of renal disease except diabetic nephropathy. Diabetic patients were excluded because diabetes accelerates atherosclerosis.

4.1 Demographic data

There was a preponderance of black patients in this study; this is similar to reports from the HEMO Study [8] but differs from the CHOICE Study [21] and the USRDS data [4] in which there was a predominance of white patients. This difference is due to the different population demographics. Our general population is predominantly black, while in the US the whites are the predominant racial group. The gender distribution was similar to that reported by these studies [4, 21], however differs from the HEMO study in which there was a slight female preponderance. The demographic data of the HEMO study appears to be different from other reported studies from the US because of selection bias. There was a preponderance

of urban dialysis units in the HEMO Study. Our patients were much younger than the patients reported in other studies [4, 8, 21, 129, 136] but similar to the study subjects reported by Salvage et al [62]. In South Africa [42], patients who are older than 60 years, patients with advanced cardiovascular disease, and diabetic patients with cardiovascular disease are not accepted onto the chronic dialysis programme because of limited resources. This would explain the relatively younger population in our study compared to the other studies. The predominant cause of CKD in the CHOICE Study [21] and USRDS [4, 39] data was diabetes while in our study we found hypertension to be the commonest cause of CKD. Again this may be due to a predominance of black subjects in our study and also the exclusion of diabetics from our study. Hypertensive nephrosclerosis is the commonest cause of CKD amongst the blacks [134] whereas in the white and Indian population it is diabetes mellitus [39].

4.2 Traditional risk factors

4.2.1 Hypertension

We found a high prevalence of hypertension in our study population. This is in keeping with reports from other studies in which the prevalence ranged between 75 and 100% depending on the target population, cause of renal disease and residual renal function [20, 21].

Hypertension is more prevalent among the blacks and also in patients with glomerular diseases than interstitial diseases [127, 134]. In the CHOICE study [21] and according to the USRDS data [4], the prevalence of hypertension was 96% and 74% respectively. The high prevalence of hypertension in our population could be due to the predominance of black patients. The HD patients had significantly higher systolic and diastolic pressures compared with controls. This result is similar to that reported in the CHOICE study [21], however differs from the report by Salvage et al [62], in which they found no

difference in blood pressure measurements between the dialysis patients and controls. The problem with this study is the very small number of patients and controls; there were only 24 subjects in each group.

4.2.2 Serum Lipids

The haemodialyisis patients had lower total cholesterol, triglyceride and LDL levels. This is not surprising, since the concept of reverse epidemiology has been addressed recently [135]. Numerous studies have shown that in contrast to the general population, where indices of over-nutrition are associated with increased risk of cardiovascular disease, markers of malnutrition like low BMI, low serum albumin and low cholesterol levels are associated with increased morbidity and mortality [35, 36]. The finding of lower serum cholesterol in the dialysis patients is not surprising for the following reasons: many are on dietary restrictions and some may also be malnourished although this appears not to be the case in our patients because the mean BMI of our patients was similar to that of the controls. The low cholesterol could be due to higher number of black patients in our study population. Studies carried out in the general population in South Africa have shown that the black population has lower serum cholesterol, LDL-cholesterol levels compared with other races [127, 138]. Although lately with increased urbanization and the attendant changes in life style and dietary habit, a gradual transition towards increased lipids has been observed in the black South Africans. Oosthuizen et al [138] reported higher serum total cholesterol and LDL-cholesterol in their subjects living in the urban and suburban township compared with rural dwellers. However they noted that these values were still within the recommended levels. This finding was confirmed in our study where the non-black patients had higher cholesterol, TG and HDL-cholesterol compared with the black patients. Third, cholesterol could be lowered in our patients because of inflammation. Our patients had significantly higher Hs-CRP compared with the controls.

4.2.3 Left ventricular hypertrophy

Echocardiographic abnormalities are common among HD dialysis patients and are associated with poor outcomes. These have been characterized into LVH, dilated cardiomyopathy and LV dysfunction by Parfrey [12]. Left ventricular hypertrophy includes concentric LVH and eccentric LVH (left ventricular dilatation plus hypertrophy). In this study we found a high prevalence of concentric LVH in our HD patients compared with controls. Only 12% of our HD patients had normal cardiac size. In a Canadian prospective cohort study of 432 patients who had echocardiography done within one year of starting dialysis, only 16% had normal cardiac function and dimensions, 42% had concentric LVH, 23% had eccentric LVH, 4% isolated LV dilatation and 16% had systolic dysfunction [14].

Our HD patients had a higher prevalence of concentric LVH and diastolic dysfunction compared with the Canadian cohort [14]. LV dilatation, systolic dysfunction and percentage of patients with normal echocardiograms were similar in both groups. Stewart and colleagues [132] reported a higher prevalence of concentric LVH in their HD patients and also a significantly higher LVMI in their patients compared with controls. Concentric LVH is associated with pressure overload, as in hypertension, arteriosclerosis or occasionally, aortic stenosis [1, 5, 12]. The higher prevalence of concentric LVH in our patients could be due to a higher prevalence of hypertension in our population compared with the Canadian cohort. Concentric LVH results in increased ventricular stiffness or diastolic dysfunction [5], hence the higher prevalence of diastolic dysfunction in our study population. Eccentric LVH is characterized by an increase in wall thickness that is proportional to the increase in left ventricular (LV) diameter [1, 5, 12]. Risk factors for eccentric LVH include volume overload secondary to salt and water retention, anaemia and arteriovenous fistulae [1, 5, 12]. Eccentric LVH leads to systolic dysfunction. It is surprising that despite the low haemoglobin levels reported in our study population, the prevalence of eccentric LVH was low.

LV mass index is strongly associated with cardiovascular mortality in the general population; this association is also found in dialysis patients [14]. The LVMI was significantly higher in our dialysis patients compared with controls. LVMI was predictably determined by male gender, diastolic BP, presence of CKD, smoking, plaque occurrence and adiponectin levels. Stewart et al [132] reported an association between LVMI with age, gender, BMI, blood pressure and CKD status. In our study, male gender was associated with significantly higher LVMI in keeping with their findings. Also the presence of ESRD was associated with a higher LVMI confirming results from previous studies showing an inverse relationship between prevalence of LVH and GFR. In a study by Levin and colleagues [13], the prevalence of LVH as measured by echocardiography was 45%, 31%, and 27% in patients with creatinine clearances of < 25, 25 to 50, and >50ml/L respectively.

4.3 Carotid artery plaques

In our study, we found an increased plaque prevalence and plaque score which is consistent with the previous reports on carotid atherosclerosis in CRF [56, 62, 136, 133). Approximately 38% of the study population had sub- atherosclerosis as determined by the presence of plaques in the carotid arteries. Salvage et al [62] reported a prevalence of 71% in their patients while Hoys et al reported prevalence of 64% [136]. Most of the patients had more than one plaque; this is in contrast to the control subjects who had plaques at only one location. Similar results have been reported by several authors [56, 63, 136, 139]. Foley et al [1] reported prevalence of CAD to be 40% in haemodialysis (HD) patients. This prevalence is higher than that in the general population which has been reported to be 5-12% depending on age and gender [1]. The prevalence of plaques in our HD population is higher than that reported in the general population (134) but much lower than reports from studies carried out in the white population [62, 136, 139]. This confirms that patients with ESRD are in the highest risk group for CVD irrespective of their

race or gender. Risk factors associated with plaques in our patients include age, systolic blood pressure, male gender, serum phosphate, calcium phosphate product and smoking. This finding is similar to reports by Salvage [62], Hoys [136] and Leskinen [139]. The association of plaques with age is not surprising since CAD is a disease of middle age and the elderly. Our findings also support the hypothesis that apart from the known traditional risk factors, some uraemia-related risk factors may also play a part in ASCVD in ESRD patients. Our study showed that plaque occurrence was related to serum phosphate and calcium phosphate product. Calcification of plaques was more common in HD patients than in the control group in our study. This result is in agreement with previous findings of carotid atherosclerosis in CRF [62]. Increased coronary artery calcification in CKD has been shown in a study using electron-beam computed tomography (EBCT) [92]. Thus, arterial calcification is another characteristic feature of the arterial disease in CKD and our study confirms this. In view of the increased vascular calcification, it is hypothesized that atherosclerosis in CKD patients is different from the general population and is more related to deposits of calcified products in the arterial wall. The following predisposing factors specific to CKD may contribute to the development of vascular calcification in patients with CKD: secondary hyperparathyroidism, disordered calcium and phosphorus homeostasis, and the use of vitamin D and high-dose calcium preparations [29]. Alterations in calcium/phosphorus metabolism are directly related to decline in kidney function. As the level of kidney function declines, phosphate levels increase and calcium levels decrease and parathyroid hormone (PTH) levels increase. Block et al [89] have shown that an elevated serum phosphate and a calcium / phosphate product in excess of 4.5-5 mmol/l (72 when expressed in mg/dl) is associated with an increased risk of mortality in patients on haemodialysis. Serum phosphate has been shown to be associated with increased IMT of the carotid artery [89]. In our study we did not find a relationship between serum phosphate IMT; rather the relationship was with plaque occurrence. Hyperphosphataemia has been shown to be associated with increased blood pressure, hyperdynamic circulation, increased cardiac work and high arterial tensile stress [29]. Other factors

associated with calcification include longer duration of dialysis [15]. In our study, patients who had plaques had been on dialysis for a much longer duration. This is similar to other published studies in the literature [62, 139]. The relationship between presence of plaques and duration on dialysis will suggest that haemodialysis may contribute to accelerated atherosclerosis. The possible mechanism by which abnormal calcium/phosphate product increases cardiovascular risk is via PTH. PTH is a growth factor for smooth muscle cells and may contribute to sclerosis of the major peripheral vessels causing increased after-load and subsequent LV dysfunction [29]. As expected the IMT measurements were significantly higher among the patients who had plaques compared with those without plaques. The significance of increased plaque burden has been shown to exist in the general population. In the study by Ebrahim et al [140] plaque status explained prevalent cardiovascular disease rather than IMT.

Carotid IMT and plaque burden may prove to be useful markers of atherosclerosis in CKD, because they are associated with risk factors for atherosclerosis in the present study. Our results suggest that increased plaque burden is more characteristic of atherosclerosis in CKD because when the CIMT measurements in the patients were compared with controls there was no significant difference; however adding plaque occurrence and plaque score improved the diagnostic yield.

4.4 Carotid-intima media thickness

Atherosclerotic changes in the carotid artery mirror generalized atherosclerosis in the other arterial districts including the coronary tree. Carotid intima-media thickness (CIMT) is usually measured by high resolution B-mode ultrasonography which is a non invasive, cheap, simple and reproducible method used in early diagnosis of coronary artery disease. Several studies in the general population have shown that increased CIMT was associated with increased risk of cardiovascular events like fatal and non fatal MI and strokes [120, 121].

In our study the CIMT in the haemodialysis patients was higher than in controls although this was not statistically significant. Similar results were reported by Salvage et al [62] and Konings et al [141]. However, Kawagishi et al [90] and Hoys et al [136] in their studies reported significantly higher CIMT in HD patients compared with age matched controls. The large number of patients in these studies compared with small numbers studied by Salvage [62] and Konings may account for this difference. Although we studied 84 patients, this number still falls below that of Kawagishi [90] and Hoys [136]. The mean difference in CIMT values was small hence very large study population is required to detect this difference. The other possible reasons may be due to differences in the methodology of the carotid artery ultrasound examination or in the selection of the control group.

In some studies, the extent of plaques may have an effect on the IMT measurement, since the exclusion of the measurement at the site of a plaque has not been consistently described. There is also variation in the descriptions of CIMT and plaques in previous studies; some studies used IMT measurement of 1mm [62] others have used measurements > 1.2mm [139]. In addition some studies [141] have included plaque measurements as part of the carotid intima media thickness. Furthermore, there is high variation in the site of the IMT measurement: bifurcation, carotid bulb, common carotid artery, internal carotid artery, and a combination of the above. The IMT measurement of the common carotid artery alone has been considered to be more reliable and reproducible than measurements from external or internal carotid arteries [124]. However, the IMT measurement of the common carotid artery may not be equally associated with prevalent cardiovascular disease as an IMT measurement of the bifurcation or the carotid bulb [119, 124]. In our study we tried to take measurements of the far wall and near wall in all three segments where possible, but had problems because the near wall could not be seen distinctly in some of our patients. Some of them still had temporary catheters in the internal jugular vein which made

examination difficult and others had suffered previous injury to the carotid arteries in the process of temporary catheter insertion, hence we resolved to use the mean of the far wall of the common carotid which has good reproducibility rather the mean of all measurements. It is however argued that atherosclerotic changes occur more frequently in the bifurcation and the internal carotid arteries than in the common carotid arteries because of the turbulence of blood flow at these sites. Our study confirms this as most of the plaques were seen in these regions and we found a significant difference in the IMT measurements taken at the bifurcation between our cases and controls.

From this we propose that CIMT measurements could be useful diagnostic tools in HD patients if measurements are taken at the different carotid segments (common carotid, internal carotid and birfurcation) and also assessment of plaque burden is added to it. In our study we found that black subjects (patients and controls) had higher CIMT compared with white subjects. Several studies [142, 143] have shown that the black population has a higher carotid IMT compared with the whites. A genetic inheritance pattern is postulated to be responsible for this differences.The selection of the control group could also affect the differences between the groups. In our study, the control group represents the healthy general population or subjects with similar cardiovascular risk factor profiles with the exception of renal disease. Except for previously known chronic diseases, our control group was not selected based on other cardiovascular risk factors. In the study by Kawagishi [90], the control group, were subjects without major coronary risk factors and this could have exaggerated the differences between their patients and controls. The confounding influence of risk factors associated with obesity and smoking was diminished in our study because the controls were matched for body mass index, and prevalence of smoking was similar in both groups.

CIMT has also been shown to be directly associated with most of the risk factors for atherosclerosis [119]. In our study population, CIMT was associated with Hs-CRP, total cholesterol, LDL cholesterol, age, and a family history of chronic kidney disease. This is consistent with previous reports that demonstrated significant correlation between CIMT and age both in the general population [119] and in HD patients [62, 90, 136]. Kawagishi et al [90] found a relationship between carotid and femoral IMT and age. In our study we found a relationship between Hs-CRP and CIMT. Zoccalli et al [129] reported a significant relationship between CIMT and Hs-CRP on univariate analysis, but this relationship lost statistical strength on multivariate analysis. We found a relationship between CIMT and LDL-cholesterol and this was similar to the findings of Hoy et al [136] but differs from the findings of Kawagishi et al [90]. This finding confirms the finding that abnormally elevated LDL-cholesterol contributes to the pathogenesis of atherosclerosis. The lack of association of lipids in the study by Kawagishi [90] could be due to selection bias of their control group. The controls were matched with the HD patients for age, gender, BMI and total cholesterol. This may not be reflective of the true distribution of cholesterol in the general population in their setting. Cholesterol had an inverse relationship with CIMT in our study. This is not surprising because although hypercholesterolaemia is associated with increased risk of CVD in the general population, in haemodialysis patients (with the exception of diabetic ESRD patients), low serum cholesterol is associated with a high mortality [34, 35]. The possible explanations for this reverse causality are: first, cholesterol may simply be a marker of inflammation or malnutrition both of which are powerful predictors of mortality in dialysis patients [34, 35, 36]. Cytokines like tumour necrosis factor α (TNF α), interleukin 2 (IL-2) have been reported to have inhibitory effects on lipoprotein lipase activity [30]. Bologa et al reported that high IL-6 levels predicted low serum cholesterol levels, suggesting that cholesterol may be suppressed in response to inflammation. [36]. Hs-CRP which is also a marker of inflammation was found to be increased in our patients; thus inflammation could partly explain the low

cholesterol level in our HD patients. Second, since most patients on dialysis have dyslipidaemia, it may not be a discriminating factor.

Third, the total cholesterol-HDL cholesterol ratio is a better indicator of atherogenic risk than total cholesterol [29]. Analysis of risk factors in HD patients alone showed CIMT to be associated with Hs-CRP and family history of CKD. This confirms that inflammation plays a very important role in the atherosclerosis of CKD.

We found a significant relationship between family history of CKD and CIMT. Chronic kidney disease has recently been included among the strongest predictors of cardiovascular risk [106]. It is therefore not surprising that a family history of CKD is associated with increased CIMT; possible explanation is that CKD is associated with a high prevalence of both traditional and non-traditional risk factors for CVD; risk factors like hypertension, LVH, dyslipidaemia, microalbuminuria, endothelial dysfunction, and oxidant stress. Some of these risk factors like hypertension; dyslipidaemias are known to have some complex inheritance patterns.

We found no relationship between CIMT and duration on dialysis, calcium phosphate product, phosphate and PTH on regression analysis. This finding is similar to that of Hoy [136] et al but differs from that reported by Kawagishi [90] and Salvage [62]. Kawagishi et al [90] reported an independent relationship between CIMT and serum phosphorus level, while Salvage et al [62] reported a relationship with calcium phosphate product. London et al did show that IMT was strongly associated with left ventricular mass index [122]. Benedetto et al [123] in their study of 138 patients who were receiving chronic dialysis

showed that IMT was associated with concentric LVH and was also an independent predictor of cardiovascular death [123]. We did not find any relationship between LVMI and CIMT.

4.5 Non-traditional risk factors

4.5.1 Hs-CRP

One of the major findings in this study is that HD patients had significantly higher Hs-CRP levels compared with controls. This finding is in keeping with results from other studies [53, 54, 56, 57]. Iseki et al [54] looked at baseline CRP levels in 163 HD patients. They divided the patients into two groups based on normal and abnormal CRP levels. Those patients with higher CRP levels had significantly lower albumin levels and a higher mortality rate. Our patients with increased CRP levels had lower serum albumin levels. CRP is an acute phase reactant whose serum levels tend to reflect on-going inflammation. Albumin is a negative acute phase reactant. In the presence of on-going inflammation, proinflammatory cytokines induce an acute phase response in the liver resulting in the increased degradation of albumin. Stenvinkel [56] also reported a higher CIMT in patients with low serum albumin. His study was carried out on patients with advanced chronic renal prior to starting dialysis. Our patients with higher Hs-CRP levels also had unfavourable lipid profiles compared with those with normal CRP. They had a higher total cholesterol and LDL-cholesterol levels. The prevalence of plaques was also higher among this group of patients. These findings are in keeping with results from other studies [56] and also demonstrate that atherosclerosis, in addition to being a disease of lipid accumulation, also represent an inflammatory process [56, 57].

The Cardiovascular Risk Extended Evaluation in Dialysis (CREED) study [57] did show that CRP was an independent predictor of the number of atherosclerotic plaques in the carotid artery of 112 chronic HD patients. Stenvinkel et al [56] also showed a significantly increased carotid intima media thickness of pre-

dialysis patients with elevated CRP levels. Generalized linear model regression analysis in HD patients did show a strong relationship between CIMT and Hs-CRP levels. Our patients with high Hs- CRP levels also had lower haemoglobin levels. Anaemia is a known risk factor for eccentric LVH. Hs-CRP was found to be associated with plasma homocysteine, another risk factor for CVD. These findings suggest that Hs-CRP may be an important factor for CVD risk assessment in view of its association with other CVD risk factors. Several studies, both in the general population and haemodialysis patients, have pointed to the strong predictive value of Hs-CRP. In the Women Health Study [51], baseline levels of CRP were significantly higher among women who subsequently developed CV events compared with those who did not. In the MONICA (monitoring trends and determinants in cardiovascular disease) Study, men who subsequently developed coronary heart disease had higher baseline CRP levels compared with those who did not. [50]. All these factors are in support of the role of inflammation in atherosclerosis and that Hs- CRP is a sensitive indicator for atherosclerosis. Zimmermann et al [53], in a prospective cohort study of 288 HD patients showed that all-cause and CV mortality was higher in patients with elevated CRP, being 31% and 16% respectively. Patients in the highest quartile of CRP had a 4.6 fold and 5.5 fold higher risks of all cause and CV mortality compared with patients in the lowest quartile. Yeun et al [35] have also identified CRP as the most powerful predictor of all-cause and CV mortality in 91 HD patients who were followed up for 34months. Patients with CRP levels in the highest quartile had the lowest survival compared with those in the lowest quartile. CRP levels have also been found to be associated with various classic markers of CVD such as Lp (a), fibrinogen and low HDL in ESRD populations [53]. Because of the cross sectional nature of our study, it will be difficult to make any statements about the predictive value of Hs-CRP for future CV events. Hs-CRP was significantly associated with CIMT on regression analysis therefore the addition of Hs-CRP testing to the routine lipid tests will improve the predictive value of this test for CVD. Ridker et al [58] demonstrated that the combination of increased Hs-CRP (> 2,11mg/L) and increased total cholesterol is associated with a 5-fold

increased risk of coronary events compared with a 1.5-fold and 2.3-fold increase respectively if only one parameter was elevated. These data lend support to the hypothesis that inflammation plays a role in the pathogenesis of atherosclerosis in these patients.

4.5.2 Lipoprotein (a)

Numerous studies have shown that patients with ESRD treated by haemodialysis, peritoneal dialysis and patients with proteinuria have elevated plasma Lp (a) levels. In this study, the mean serum lipoprotein (a) levels were similar in both patients and controls and also among patients with and without plaques. This finding is similar to the results of Stenvinkel et al [56] in which there was no difference in the serum Lp(a) levels among patients with low versus high CRP levels and among patients with plaques and without plaques. The black subjects had significantly higher Lp (a) levels compared with the non-black subjects in keeping with findings in the literature. Even though black patients have much higher Lp(a) levels compared with white subjects, the heterogeneity of apo (a) iso form sizes could account for differences in prevalence of CAD in the two populations [144]. Lipoprotein (a) is a strong risk factor for vascular disease in the general populations [78, 94] as well as in HD patients [93]. Studies have shown that it is the apolipoprotein (a) [apo(a)] gene locus that determines the risk for CVD through its allelic control of Lp (a). Subjects who express the low molecular weight (LMW) apo (a) phenotype show on average markedly higher Lp (a) concentrations than those with higher molecular weight apo (a) phenotypes and these have been shown to be associated with increased cardiovascular risk [29, 93]. Delport et al [78] carried out a study in 91 male Caucasians with vascular disease in Pretoria; they reported an increased risk in patients with high-molecular apo (a) isoforms especially in the presence of hyperhomocysteinaemia. The lack of correlation between Lp (a) and the surrogate markers of atherosclerosis in our study could be attributed to the fact that we did not measure the apo (a) iso forms. Kronenberg et al [93], in a cross sectional study involving 167 patients found that the LMW phenotypes

of apo (a) and higher serum Lp (a) levels were associated with carotid plaques as investigated by ultrasound. They demonstrated that haemodialysis patients with the LMW phenotypes of apo (a) had significantly more carotid sites affected by atherosclerotic plaques than those with other phenotypes.

4.5.3 Adiponectin

Adiponectin (ADPN), a recently discovered collagen-like protein, is secreted exclusively by adipocytes and circulates in the blood. Interest in adiponectin derives from its potential protective role for the cardiovascular system. Adiponectin has anti-atherogenic and anti -inflammatory properties [113]. ADPN is inversely related to creatinine clearance and serum levels are markedly increased in patients with ESRD [114, 115]. This was confirmed in our study. Our haemodialysis patients had significantly higher serum ADPN levels compared with controls and even among the HD patients, those who were completely anuric had the highest adiponectin levels. Zoccali and colleagues [115] reported that HD patients had ADPN levels about 2.5 times that of controls, while Huang et al [145] reported that patients on peritoneal dialysis (PD) and haemodialysis had significantly higher ADPN levels compared with controls. ADPN concentrations seem to be gender-dependent, being higher among women than men. In our study women had higher adiponectin levels than the men and this was particularly significant among the controls. However, what is not known is whether this increase is just due to accumulation or whether it represents a counter-regulatory response to several metabolic and haemodynamic risk factors of renal insufficiency. Adiponectin has been linked to several metabolic risk factors like glucose, TG, insulin, and HDL cholesterol in uraemic patients and these are all consistently in line with the hypothesis that adiponectin is a protective factor [115]. Adiponectin stimulates fatty acid oxidation, decreases plasma triglycerides, and improves glucose metabolism by increasing glucose sensitivity [113]. Our study confirms the association of adiponectin with lipid metabolism; adiponectin level was inversely related to serum triglyceride and LDL levels. Among HD patients, these factors remained significant in addition;

adiponectin was related to LV dysfunction and serum cholesterol. Huang et al [145] did not find any relationship between left ventricular ejection fraction, LVM and ADPN levels. There are many reasons for the observed differences between Huang's report and ours. There are methodological differences with regards to echocardigraphic examination in their study. First the LVM was corrected for body weight in their study not body surface area as in our study. Second, the cut-off level of ejection used in their study was much higher than the recommended level of <50% for determining systolic dysfunction [130]. Third LVH was defined as posterior wall thickness > 1.2cm and there was no distinction into the various types of LVH. Fourth, the study was carried out in PD patients. The mechanism underlying the association of ADPN with LV dysfunction is not clear, further studies are needed for clarification. Adiponectin was positively associated with cholesterol in our study. Most of our dialysis patients had low to normal cholesterol levels. In HD patients, low cholesterol is not only a marker of nutrition but also a marker of inflammation. The observed low cholesterol in our HD patients could be a marker of inflammation and inflammatory processes may play a role in the increased plasma levels of ADPN; although in our study we did not find any association between ADPN and Hs-CRP levels. One limitation of this study is that we did not measure insulin resistance and this could have effects on the association of cholesterol with ADPN. Plasma adiponectin concentrations are reduced in patients with obesity, type 2 diabetes mellitus and coronary artery disease [116]. ADPN in patients with ESRD is increased compared with healthy subjects, but within the uraemic milieu, low adiponectin is still a marker of high risk because it predicts a higher rate CV event [115]. Our study did not show any difference in the serum adiponectin levels between patients with carotid plaques and those without plaques. Zoccali et al [115], reported that adiponectin levels were increased in both HD patients with low incidence of cardiovascular events (20.8 ± 6.8 µg/mL) and those with high CV events (9.3 ± 2.7µg/ mL) compared with healthy subjects (5.9 ± 2.6µg/ mL) [115]. Although it is speculated that ADPN is a protective factor for CVD in uraemic patients, however there are some questions that need clarification. What is the level of ADPN that

prevents CVD in uraemic patients since levels are higher in ESRD compared with controls irrespective of CVD status? The exact metabolism and role of ADPN in ESRD also needs further clarification. Zoccali and colleagues [115] hypothesized that in ESRD, the cardioprotective role of ADPN may be downregulated, perhaps at the receptor level, thus resetting the relationship between ADPN, cardiovascular damage and clinical complications at a higher plasma concentration. This hypothesis must be fully tested in vitro and in vivo experiments, to better elucidate the role of this cytokine in human diseases.

4.5.4 Homocysteine

Homocysteine is an intermediate amino acid formed during the metabolism of methionine, a sulphur-containing essential amino acid and is cleared by the kidneys. Hyperhomocysteinaemia has been identified as an independent risk factor for cardiovascular morbidity and mortality in the general population [75, 77, 78,] and in ESRD patients on dialysis [79, 80]. High homocysteine levels were reported in subjects with IHD and stroke in the general population [77, 78]. Delport et al [78] reported that hyperhomocysteinaemia was associated with a 7.20 fold increased risk for vascular disease among Caucasian South African males. In our study, homocysteine levels were significantly higher in patients compared with controls. This finding is similar to reports from previous studies [80]. Risk factors associated with plasma homocysteine levels in our study include Hs-CRP, age, and total cholesterol. Among HD patients, homocysteine was independently associated with age, Hs-CRP and inversely related to serum adiponectin levels. The relationship between plasma homocysteine levels and CVD in HD patients remains controversial; some studies found that plasma homocysteine was an independent CV morbidity and mortality predictor in uremic patients [80, 146] while another study did not [79]. Robinson et al [80] reported that high total plasma homocysteine concentration is an independent risk factor for atherosclerotic complications of end-stage renal disease. On the other hand, Suliman et al [79]

found a negative relationship between CVD and plasma homocysteine levels, but the prevalence of hypoalbuminemia, malnutrition and inflammation was high in their patients and more prevalent in those without CVD. In our study the association of homocysteine with other risk factors for CVD like inflammation (Hs-CRP), increasing age hypercholesterolaemia and the inverse relationship with ADPN, support the fact that homocysteine itself is an important risk factor for CVD in ESRD. The mechanisms by which homocysteine might contribute to atherogenesis include induction of endothelial dysfunction, promotion of platelet aggregation and enhanced coagulability, increased smooth muscle cell proliferation, cytotoxicity and stimulation of LDL oxidation [75, 76].

4.6 CVD risk factors based on previous kidney transplantation

It is well known that cyclosporine and steroids used as immunosuppressive therapy in kidney transplant patients do cause hypertension and atherosclerotic vascular changes [147].

We compared the CVD risk factors in patients who have had a previous kidney transplant with those who have never been transplanted. The significant differences were higher LDL-cholesterol and triglycerides in patients who received kidney transplant. This is not surprising as steroids and calcineurin inhibitors are known to cause dyslipidaemia [147]. Patients who had previously been transplanted had significantly lower blood pressure compared with those who had not been transplanted, CIMT, LVMI and plaque occurrence was similar in both groups. This suggests that prior kidney transplantation does not contribute significantly to the vascular changes seen in ESRF patients.

4.7 Cardiovascular risk factors by aetiology

Hypertension is the most common cause ESRF in the black population [127]. Hypertension is a known risk factor for CVD and it is suggested that the presence of hypertension could contribute to accelerated atherosclerosis in CRF and these changes occur well before the commencement of dialysis [1, 3, 12]. We analyzed CVD risk factors by aetiology especially in our black patients since hypertension is more prevalent in these patients. Our results suggest that hypertension could contribute to accelerated atherosclerosis in these patients as evidenced by significantly higher levels of some CVD risk factors and higher prevalence of plaques in the essential hypertensive group.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The findings from this study, demonstrate that:

1. Prevalence of sub-clinical atherosclerosis as determined by increased CIMT and carotid plaques was higher in HD patients than the controls, in keeping with previous studies and also confirming the statement by the NKF that patients with CKD are in the highest risk for CVD. No evidence of any differences in the prevalence of sub-clinical atherosclerosis (plaque occurrence) between the racial groups was found.
2. HD patients have a prevalence of traditional (hypertension, LVH, physical inactivity) and non-traditional risk factors (hyperhomocysteinaemia, inflammation) for atherosclerosis, in keeping with reports in the literature on this subject.
3. Some traditional risk factors like lipids appear to have opposite effects in ESRD patients (reverse epidemiology)
4. Inflammation appears to play an important role in the pathogenesis of atherosclerosis in ESRD, as demonstrated by the association of Hs-CRP to CIMT a surrogate marker of sub-clinical atherosclerosis.
5. Some racial differences in risk factors like smoking, exercise, lipids, blood pressure, adiponectin and Lp(a), LVH were noted, with, the black race having lower serum cholesterol, triglycerides, LDL-cholesterol compared with the non-black race. The black subjects in this study were older, had higher Lp (a) and adiponectin levels, higher LVMI and prevalence of LVH. More non-black

subjects currently smoked cigarettes and exercised compared with black subjects. There was no significant difference in CIMT between the different racial groups.

6. CIMT showed significant correlation with some major risk factors for atherosclerosis like age, Hs-CRP, LDL cholesterol and family history of CKD
7. Plaque occurrence showed significant correlation with some major risk factors for atherosclerosis like age, systolic BP, male gender, smoking, serum phosphate and calcium phosphate product.
8. HD patients had higher ADPN levels than controls but the exact role of this adipocyte cytokine in ESRD needs to be defined.

5.2 Recommendations for clinical practice

1. Ultrasound assessment of the carotid artery is a useful tool for the early diagnosis of atherosclerosis especially if measurements of IMT are done in all carotid segments and plaque occurrence is also incorporated. It is simple, highly reproducible and cheap and therefore recommended as a screening tool in high risk subjects.
2. The addition of Hs-CRP to routine screening of HD patients could help identify those at risk of CVD
3. Patients with ESRD are considered to be in the highest risk group for CVD. It is therefore recommended that this patient population should be carefully assessed and treated for CV risk factors early in the course of the disease.

5.3 Recommendations for future work

The cross-sectional nature of this study is a limitation; a longitudinal study will provide more substantial data.

1. A longitudinal study will help determine the relationship between these non-traditional risk factors and CVD outcomes in our environment.
2. It is desirable that a similar study be carried out involving patients with early CKD, patients on peritoneal dialysis and kidney transplant patients to determine if there are differences in prevalence of these risk factors and atherosclerosis in these conditions and whether treatment modality influences their occurrence.

Appendix A

- **Test principle for cholesterol, HDL-Cholesterol and triglyceride**
- Enzymatic colorimetric test for cholesterol
- Sample plus addition of R1 (cholesterol reagent) start of reaction:
- Cholesterol is determined enzymatically using cholesterol esterase and cholesterol oxidase.
- $\text{Cholesterol esters} + H_2O \xrightarrow[\text{esterase}]{\text{cholesterol}} \text{cholesterol} + \text{RCOOH}$
- Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids.
- $\text{Cholesterol} + O_2 \xrightarrow[\text{Oxidase}]{\text{Cholesterol}} \text{cholest-4-en-3-one} + H_2O_2$
- Cholesterol is converted by oxygen with the aid of cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide.
- $2\ H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{4-(p-benzoquinone-monoimino)} - \text{phenazone} + 4\ H_2O$
- Hydroen peroxide created forms a red dyestuff by reacting with 4-aminophenazone and phenol under the catalytic action of peroxidase. The color intensity is directly proportional to the concentration of cholesterol and can be determined spectrophotometrically.

Test principle for HDL

HDL was determined directly by the use of magnetically responsive particles such as polyanion-metal combinations and the use of Polyethyleneglycol (PEG) with anti-apoprotein B and anti-apoprotein CIII antibodies. The automated method for direct determination of HDL-cholesterol in serum uses PEG-modified enzymes and dextran sulphate. When the cholesterol esterase and cholesterol oxidase enzymes are modified by PEG, they show selective catalytic activities toward lipoprotein fractions, with the reactivity increasing in the order: LDL, VLDL ≈ chylomicrons < HDL. Homogeneous enzymatic colorimetric test.

- Sample and addition of R1 (buffer).
- In the presence of magnesium sulphate, dextran sulphate selectively forms water-soluble complexes with LDL, VLDL and chylomicrons which are resistant to PEG-modified enzymes.
- Addition of R2 (PEG-modified enzymes/4-amino-antipyrine/buffer) and start of reaction:
- The cholesterol concentration of HDL-cholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups (approx.40%).
- HDL-cholesterol esters + H_2O $\xrightarrow[\text{Esterase}]{\text{PEG-cholesterol}}$ Cholesterol + RCOOH
- Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.
- HDL-cholesterol + O_2 $\xrightarrow[\text{Oxidase}]{\text{PEG-cholesterol}}$ Δ^4-cholestenone + H_2O_2
- In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ^4-cholestenone and hydrogen peroxide.

- $2H_2O_2$ + 4-amino-antipyrine + HSDA* + H^{*} + H2O $\xrightarrow{\text{Peroxidase}}$ purple-blue pigment + 5H2O
- HSDA=N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline
- In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-amino-antipyrine and HSDA to form a purple-blue dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured spectrophotometrically.

Test principle for triglyceride

- Enzymatic colorimetric test
- Sample and addition of R1 (buffer/4-chlorophenol/enzymes) and start of reaction:
- triglycerides + 3 H_2O $\xrightarrow{\text{LPL}}$ glycerol + 3 RCOOH
- glycerol + ATP $\xrightarrow[Mg^2]{\text{GK}}$ glycerol-3-phosphate + ADP
- glycerol-3-phosphate + O_2 $\xrightarrow{\text{GPO}}$ dihydroxyacetone phosphate + H_2O_2
- H_2O_2 + 4-aminophenazone + 4-chlorophenol $\xrightarrow{\text{peroxidase}}$
    - 4-(p-benzoquinone-monoimino)-phenazone + 2 H_2O + HC

Appendix B

Assay principle for PTH

The ADVIA Centaur Intact PTH assay is a two-site sandwich immunoassay which uses directed chemiluminometric technology which uses constant amounts of two anti-human PTH antibodies in the Lite reagent. The first antibody is a polyclonal goat anti-human PTH antibody labeled with acridinium ester. The second is a biotinylated polyclonal goat anti-human PTH (39–84) antibody. Streptavidin in the solid Phase is covalently coupled to the paramagnetic latex particles.

- The system automatically performs the following steps:
- dispenses 200µL of sample into a cuvette
- dispenses 50µL of Lite Reagent and incubates for 5.0 minutes at 37°C
- dispenses 200µL of Solid Phase and incubates for 2.5 minutes at 37°C
- separates, aspirates, and washes the cuvettes with reagent water[13]
- dispenses 300 µL each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction
- reports results according to the selected option, as described in the system operating instructions or in the online help system
- A direct relationship exists between the amount of PTH present in the patient sample and the amount of relative light units (RLUs) detected by the system.

Appendix C

Pat No	Age	sex	Race	SUBJECT	FHx MI	FHx HTN	FHx DM	FHx CKD	height	weight	HT SQ	BMI	smoking	Quantity
1	24	1	1	1	0	1	0	0	1.66	63	2.76	22.86	2	0
2	28	1	1	1	0	0	0	0	1.48	33	2.19	15.07	1	1
3	50	1	1	1	0	1	1	0	1.75	59.6	3.06	19.46	2	0
4	31	1	1	1	0	0	0	0	1.65	55	2.72	20.20	2	0
5	19	1	1	1	0	0	0	0	1.62	56.4	2.62	21.49	2	0
6	45	1	1	1	0	0	0	0	1.77	91.3	3.13	29.14	0	0
7	52	1	1	1	0	1	0	0	1.67	70.5	2.79	25.28	0	0
8	47	1	1	1	0	1	0	0	1.71	66.3	2.92	22.67	0	0
9	41	1	1	1	0	1	0	0	1.77	77.9	3.13	24.87	2	0
10	50	1	1	1	0	0	0	0	1.59	57	2.53	22.55	2	0
11	26	1	1	1	0	1	0	1	1.65	56.9	2.72	20.90	0	0
12	28	1	1	1	1	1	0	0	1.59	51	2.53	20.17	0	0
13	39	1	1	1	0	0	0	0	1.62	56	2.62	21.34	1	1
14	19	1	1	1	0	1	1	0	1.73	56.5	2.99	18.88	2	1
15	41	1	1	1	0	0	0	0	1.69	47.2	2.86	16.53	2	1
16	46	1	1	1	0	0	0	0	1.76	62.7	3.10	20.24	0	0
17	37	1	1	1	0	1	0	1	1.71	73	2.92	24.96	2	1
18	42	1	1	1	0	1	0	0	1.74	80	3.03	26.42	1	1
19	43	1	1	1	0	1	0	0	1.72	75.7	2.96	25.59	2	0
20	43	1	1	1	0	0	0	0	1.62	57.7	2.62	21.99	2	0
21	50	1	1	1	0	1	1	0	1.72	76	2.96	25.69	1	1
22	47	1	1	1	0	0	0	0	1.68	64.3	2.82	22.78	2	0
23	46	1	1	1	0	1	0	0	1.83	82	3.35	24.49	1	1
24	42	1	1	1	0	1	0	1	1.66	60.3	2.76	21.88	1	1
25	26	1	1	1	0	0	1	0	1.5	47.2	2.25	20.98	1	1
26	45	1	1	1	0	0	0	0	1.69	59.8	2.86	20.94	1	1
27	34	1	1	1	0	1	0	0	1.78	57	3.17	17.99	1	1
28	52	1	1	1	0	0	0	0	1.61	60	2.59	23.15	0	0
29	55	1	1	1	0	1	0	0	1.7	84.5	2.89	29.24	2	2
30	55	1	1	1	0	0	1	0	1.62	67.9	2.62	25.87	2	1
31	53	1	1	1	0	1	1	1	1.67	77.9	2.79	27.93	2	1
32	53	1	1	1	0	1	0	0	1.71	72.2	2.92	24.69	2	1

33	47	1	1	1	0	0	0	0	1.62	69	2.62	26.29	2	0
34	37	2	1	1	0	0	0	0	1.57	54.1	2.46	21.95	0	0
35	39	2	1	1	0	1	0	0	1.61	63	2.59	24.30	0	0
36	40	2	1	1	0	0	0	0	1.62	69.4	2.62	26.44	0	0
37	40	2	1	1	0	0	0	0	1.62	78.2	2.62	29.80	0	0
38	38	2	1	1	0	1	1	0	1.52	63.5	2.31	27.48	0	0
39	45	2	1	1	1	0	0	0	1.5	53	2.25	23.56	0	0
40	49	2	1	1	0	0	0	0	1.54	93.6	2.37	39.47	0	0
41	47	2	1	1	0	1	1	0	1.65	106	2.72	38.93	0	0
42	44	2	1	1	0	1	1	0	1.64	66.7	2.69	24.80	0	0
43	28	2	1	1	0	1	0	0	1.5	50.4	2.25	22.40	0	0
44	40	2	1	1	0	0	0	0	1.5	52.8	2.25	23.47	0	0
45	37	2	1	1	0	0	0	0	1.55	53.5	2.40	22.27	1	1
46	43	2	1	1	0	0	0	0	1.64	59	2.69	21.94	0	0
47	49	2	1	1	0	1	0	0	1.52	90	2.31	38.95	2	1
48	36	2	1	1	0	0	0	0	1.49	58	2.22	26.12	0	0
49	35	2	1	1	0	1	1	0	1.54	88	2.37	37.11	1	1
50	48	2	1	1	0	1	0	0	1.59	88	2.53	34.81	0	0
51	37	2	1	1	0	0	0	0	1.49	56.4	2.22	25.40	0	0
52	20	2	1	1	0	0	0	0	1.66	52	2.76	18.87	0	0
53	51	2	1	1	0	1	0	0	1.64	63.4	2.69	23.57	0	0
54	53	2	1	1	0	0	0	0	1.66	70.3	2.76	25.51	0	0
55	57	2	1	1	0	1	0	0	1.55	68.5	2.40	28.51	0	0
56	49	2	1	1	0	0	0	0	1.55	71	2.40	29.55	2	0
57	47	2	1	1	1	1	1	0	1.64	48.5	2.69	18.03	0	0
58	48	2	1	1	0	1	0	1	1.56	69.8	2.43	28.68	0	0
59	25	1	2	1	1	1	1	0	1.57	51.7	2.46	20.97	0	0
60	29	1	2	1	0	0	0	0	1.8	105.5	3.24	32.56	2	0
61	23	1	2	1	0	0	1	0	1.57	60.5	2.46	24.54	2	0
62	58	1	2	1	0	0	0	0	1.67	75.3	2.79	27.00	2	5
63	31	1	2	1	0	1	0	0	1.79	87	3.20	27.15	1	1
64	22	1	2	1	0	1	0	0	1.73	56.4	2.99	18.84	1	1
65	52	1	2	1	1	1	0	0	1.76	70.1	3.10	22.63	2	0
66	54	1	2	1	0	0	0	0	1.72	70	2.96	23.66	1	3
67	39	1	2	1	0	0	0	0	1.67	62	2.79	22.23	1	1
68	42	1	2	1	1	0	0	0	1.63	59	2.66	22.21	1	1
69	41	1	2	1	0	1	1	0	1.78	82.5	3.17	26.04	0	0

70	32	2	2	1	0	1	0	0	1.55	58.4	2.40	24.31	0	0
71	27	2	2	1	0	0	0	0	1.66	55.4	2.76	20.10	1	1
72	32	2	2	1	0	1	1	0	1.66	100.3	2.76	36.40	0	0
73	32	2	2	1	1	1	0	0	1.58	48	2.50	19.23	0	0
74	37	2	2	1	1	0	0	0	1.57	52.5	2.46	21.30	0	0
75	35	2	2	1	1	1	1	0	1.58	37	2.50	14.82	2	1
76	23	2	2	1	0	0	0	0	1.55	48	2.40	19.98	0	0
77	28	2	2	1	1	0	1	0	1.55	60.5	2.40	25.18	1	1
78	43	2	2	1	0	0	0	0	1.48	52.4	2.19	23.92	0	0
79	20	2	2	1	0	0	0	0	1.5	50	2.25	22.22	2	1
80	26	2	2	1	0	0	0	0	1.68	50	2.82	17.72	0	0
81	54	2	2	1	0	0	0	0	1.64	67.8	2.69	25.21	0	0
82	51	2	2	1	0	1	0	0	1.65	78	2.72	28.65	0	0
83	54	2	2	1	1	1	1	0	1.45	57	2.10	27.11	0	0
84	42	2	2	1	0	0	0	0	1.65	68.7	2.72	25.23	0	0

Exercise	**Ex FREQ**	**SBP**	**DBP**	**Pulse P**	**PP/3**	**MAP**	**total chol**	**Triglyceride (mmol/L)**	**HDL**	**LDL**	**LCCA**	**RCCA**	s CCA	**CIMT (mm)**
0	0	118	67	51	17.00	84.0	2.9	0.6	1	1.5	0.5	0.7	1.2	0.6
1	3	141	92	49	16.33	108.3	2.9	0.6	1	1.6	0.6	0.6	1.2	0.6
0	0	138	87	51	17.00	104.0	3.8	0.8	1.3	2.2	0.9	0.8	1.7	0.85
0	0	133	60	73	24.33	84.3	4.5	0.8	1.1	3	0.7	0.8	1.5	0.75
0	0	152	88	64	21.33	109.3	3.5	0.5	1	2.3	0.5	0.5	1	0.5
0	0	118	71	47	15.67	86.7	4.8	0.5	0.9	3.7	0.5	0.6	1.1	0.55
1	3	114	61	53	17.67	78.7	3.4	1.4	1	1.8	0.6	0.7	1.3	0.65
0	0	141	70	71	23.67	93.7	4	1.4	1	2.4	0.4	0.7	1.1	0.55
0	0	148	93	55	18.33	111.3	4.5	0.6	1.5	2.7	0.6	0.5	1.1	0.55
0	0	169	98	71	23.67	121.7	3.6	0.9	1.4	1.9	0.6	0.7	1.3	0.65
1	3	145	86	59	19.67	105.7	3.7	1.7	1	1.9	0.6	0.7	1.3	0.65
0	0	160	95	65	21.67	116.7	3.4	0.6	1.5	1.6	0.9	0.6	1.5	0.75
0	0	178	106	72	24.00	130.0	2.6	0.2	1.5	1	0.6	0.6	1.2	0.6
1	2	159	99	60	20.00	119.0	2.4	0.8	1	1	0.6	0.6	1.2	0.6
1	3	153	82	71	23.67	105.7	4.1	1.3	1.9	1.6	0.9	0.7	1.6	0.8
0	0	143	91	52	17.33	108.3	3.5	3.2	1	1	0.9	0.7	1.6	0.8
1	2	150	100	50	16.67	116.7	4.3	2.5	1	2.2	0.8	0.7	1.5	0.75
0	0	152	95	57	19.00	114.0	3.8	1.3	1.1	2.1	0.6	0.4	1	0.5

1	3	141	92	49	16.33	108.3	3	0.6	1.1	1.6	0.6	0.7	1.3	0.65
1	2	150	77	73	24.33	101.3	3.6	1.3	1	2	0.8	0.7	1.5	0.75
0	0	137	87	50	16.67	103.7	3.1	0.6	1.2	1.6	0.7	0.7	1.4	0.7
0	0	158	86	72	24.00	110.0	4	0.4	1.3	2.6	0.8	0.7	1.5	0.75
0	0	186	100	86	28.67	128.7	2.1	0.6	0.7	1.1	0.8	0.8	1.6	0.8
0	0	138	84	54	18.00	102.0	2.2	1.1	1.4	0.3	0.6	0.9	1.5	0.75
1	3	155	101	54	18.00	119.0	3	0.9	0.9	1.7	0.7	0.8	1.5	0.75
0	0	150	85	65	21.67	106.7	3.7	0.9	1.2	2.1	0.7	0.6	1.3	0.65
0	0	163	109	54	18.00	127.0	3	0.5	0.5	2.3	0.7	0.7	1.4	0.7
0	0	148	76	72	24.00	100.0	4	0.4	1.4	2.4	0.6	0.7	1.3	0.65
0	0	170	91	79	26.33	117.3	2.9	0.9	1.3	1.3	0.5	0.9	1.4	0.7
0	0	155	79	76	25.33	104.3	3.2	0.8	1	1.8	0.6	0.6	1.2	0.6
0	0	145	77	68	22.67	99.7	4.3	1.9	1.3	2.1	0.7	0.5	1.2	0.6
0	0	165	77	88	29.33	106.3	3.3	0.6	1.3	1.7	0.7	0.8	1.5	0.75
0	0	184	115	69	23.00	138.0	3.1	0.7	0.7	2.1	0.6	0.6	1.2	0.6
0	0	123	58	65	21.7	79.7	4	0.9	2.6	1	0.3	0.4	0.7	0.35
0	0	130	89	41	13.7	102.7	3.8	1.4	1.3	1.8	0.5	0.7	1.2	0.6
1	3	130	76	54	18.0	94.0	4	1.2	1.5	2	0.4	0.6	1	0.5
0	0	140	80	60	20.0	100.0	3.1	1.3	1.1	1.5	0.7	0.7	1.4	0.7
0	0	147	78	69	23.0	101.0	2.5	0.7	1.2	1	0.7	0.7	1.4	0.7
0	0	145	90	55	18.3	108.3	3.2	1.1	1.1	1.6	0.6	0.5	1.1	0.55
0	0	141	70	71	23.7	93.7	3.9	1.5	1.1	2.1	0.8	0.8	1.6	0.8
0	0	115	63	52	17.3	80.3	4.9	3.2	1.2	2.2	0.5	0.6	1.1	0.55
1	2	140	93	47	15.7	108.7	3.1	0.8	1.1	1.6	0.6	0.6	1.2	0.6
0	0	147	82	65	21.7	103.7	4	0.4	1.6	2.2	0.4	0.6	1	0.5
0	0	160	90	70	23.3	113.3	3.1	1.2	1.1	1.5	0.5	0.6	1.1	0.55
0	0	129	82	47	15.7	97.7	4.5	1.7	1.3	2.5	0.5	0.5	1	0.5
1	2	168	89	79	26.3	115.3	3.4	1	1.1	1.8	0.7	0.7	1.4	0.7
0	0	142	81	61	20.3	101.3	4.7	1.9	0.9	3	0.9	0.8	1.7	0.85
0	0	188	114	74	24.7	138.7	3	0.4	1.3	1.5	0.7	0.6	1.3	0.65
1	3	140	85	55	18.3	103.3	3	1.1	0.7	1.8	0.5	0.5	1	0.5
0	0	127	76	51	17.0	93.0	3.4	0.8	0.7	2.3	0.7	0.6	1.3	0.65
0	0	142	93	49	16.3	109.3	3.6	0.8	1.3	1.9	0.7	0.9	1.6	0.8
0	0	149	78	71	23.7	101.7	3.8	1.3	1.6	1.6	0.5	0.6	1.1	0.55
0	0	143	75	68	22.7	97.7	3.8	1	1.4	2.1	0.6	0.6	1.2	0.6
0	0	170	100	70	23.3	123.3	3.4	0.9	1.3	1.7	0.5	0.6	1.1	0.55
0	0	140	78	62	20.7	98.7	6.1	0.8	2.1	3.6	0.6	0.6	1.2	0.6

0	0	162	82	80	26.7	108.7	5.9	1.4	1.2	4.1	0.7	0.7	1.4	0.7
0	0	199	110	89	29.7	139.7	3.1	0.5	1.5	1.4	0.4	0.6	1	0.5
0	0	160	80	80	26.7	106.7	3.8	1	1.3	2	1.8	1.5	3.3	1.65
1	3	157	88	69	23.00	111.00	3.7	3.1	0.8	1.5	0.6	0.6	1.2	0.6
0	0	168	100	68	22.67	122.67	4.1	1.9	0.7	2.5	0.5	0.6	1.1	0.55
0	0	130	70	60	20.00	90.00	3.8	0.7	1.2	2.2	0.7	0.5	1.2	0.6
0	0	167	94	73	24.33	118.33	4	2.5	1	1.8	0.7	0.7	1.4	0.7
0	0	165	90	75	25.00	115.00	3.8	2	0.7	2.2	0.7	0.7	1.4	0.7
0	0	142	80	62	20.67	100.67	3.8	0.9	1.1	2.3	0.7	0.7	1.4	0.7
1	2	144	80	64	21.33	101.33	2.5	1.9	0.8	0.9	0.7	0.6	1.3	0.65
0	0	162	74	88	29.33	103.33	3.3	0.6	1.9	1.1	1.7	0.7	2.4	1.2
1	3	156	81	75	25.00	106.00	4.7	3.2	0.9	2.3	0.7	0.7	1.4	0.7
1	3	122	65	57	19.00	84.00	5.7	1.2	1	4.1	0.6	0.8	1.4	0.7
0	0	130	80	50	16.67	96.67	3.5	3.8	0.6	1.2	0.6	0.7	1.3	0.65
1	3	117	52	65	21.67	73.67	5.2	2.9	0.9	3	0.6	0.5	1.1	0.55
1	3	166	110	56	18.67	128.67	4.3	0.8	1.2	2.8	0.5	0.5	1	0.5
1	2	130	65	65	21.67	86.67	3.3	1.9	0.8	1.7	0.7	0.6	1.3	0.65
0	0	144	88	56	18.67	106.67	4.4	2.2	1.3	2	0.6	0.6	1.2	0.6
1	2	114	69	45	15.00	84.00	3.9	1.6	1	2.1	0.7	0.6	1.3	0.65
0	0	155	92	63	21.00	113.00	4.8	2.1	1.8	2	0.6	0.6	1.2	0.6
0	0	142	80	62	20.67	100.67	3.7	0.9	1	2.3	0.4	0.4	0.8	0.4
1	2	122	72	50	16.67	88.67	2.3	1.5	0.8	0.8	0.7	0.5	1.2	0.6
0	0	110	62	48	16.00	78.00	4.1	1.2	1.1	2.4	0.5	0.6	1.1	0.55
0	0	120	80	40	13.33	93.33	4.3	1.1	1.3	2.5	0.6	0.6	1.2	0.6
0	0	163	100	63	21.00	121.00	3.9	0.9	1.3	2.2	0.6	0.6	1.2	0.6
0	0	144	65	79	26.33	91.33	3.9	0.9	1.4	2.1	0.6	0.8	1.4	0.7
1	2	147	73	74	24.67	97.67	4.5	2.1	1.2	2.3	0.6	0.6	1.2	0.6
1	3	144	83	61	20.33	103.33	4.3	1.6	1.2	2.3	0.6	0.4	1	0.5
0	0	158	89	69	23.00	112.00	4.3	1.7	1.7	1.8	0.6	0.7	1.3	0.65

LBIF	LICA	RBIF	RICA	PLAQUE	Plq No	PLQ Score	Plq type	nwLCCA	nwLBIF	nwLICA	nwRCCA	nwRBIF	nwRICA
0.5	0.6	0.4	0.6	0	0	0		0.6	0	0	0.6	0	0
0.5	0.5	0.7	0.6	0	0	0		0.7	0	0	0.7	0	0
0.8	0.6	0.8	0.7	0	0	0		0.6	0	0	0.7	0	0
0.6	0.7	0.7	0.7	0	0	0		0.7	0	0	0.7	0	0

0.5	0.5	0.6	0.5	0	0	0		0.5	0	0	0.5	0	0
0.5	0.7	0.6	0.5	0	0	0		0.5	0	0	0.6	0	0
0.8	0.7	0.9	0.7	0	0	0		0.7	0.8	0.7	0.9	0.6	0.5
1	0.8	0.5	0.3	0	0	0		0.7	0	0	0.5	0	0.5
0.8	0.4	1.5	0.7	0	0	0		0.6	0	0	0.6	0	0
0.7	0.7	0.7	0.4	0	0	0		0.7	0	0	0.5	0	0
0.4	0.4	0.5	0.4	0	0	0		0.5	0.4	0.4	0.6	0.5	0.5
0.9	0.6	0.7	0.6	0	0	0		0.7	0.9	0.6	0.6	0.7	0.6
0.9	0.5	0.7	0.6	0	0	0		0.6	0.6	0.5	0.6	0.6	0.6
0.7	0.6	0.5	0.5	0	0	0		0.7	0.5	0.5	0.5	0.5	0.6
1.1	0.4	0.6	0.5	0	0	0		0.6	1.1	0.5	0.7	0.4	0.7
1.3	0.6	1.1	0.6	0	0	0		1.5	0	0.6	1.1	0	1.3
1.3	0.9	0.7	0.7	0	0	0		0.7	0.5	0	0.8	0	0.9
0.8	0.7	0.9	0.9	1	1	1.3	C	0.6	0	0.7	0.6	0	0
0.7	0.5	0.7	0.6	1	1	1.4	C	0.6	0	0	0.6	0	0
1.4	0.6	0.9	0.9	1	2	3.1	M	1.1	0	0	0.9	0	0
0.6	0.6	0.8	0.7	1	1	1.1	S	0.7	0	0	0.6	0	0
0.9	0.7	1.1	0.8	1	3	6.7	C	0.7	0	0	0.7	0	0
0.9	0.8	1.2	0.8	1	2	3.5	C	0.7	1	0.6	0.8	1.1	0.6
1.3	0.6	1.5	0.5	1	2	3.7	C	0.9	0	0	0.9	0	1.5
0.8	0.9	1.5	1.3	1	2	2.9	C	0.8	0.7	1	1	1.5	1
0.5	0.4	0.5	0.5	1	3	4.1	C	0.6	0.6	0.4	0.6	0.4	0.5
0.7	0.4	0.4	0.8	1	1	1.3	C	0.6	0.7	0.4	0.6	0.9	0.7
1	0.5	0.7	0.4	1	2	2.2	C	0.6	0.7	0.5	0.5	0.4	0.6
2.5	0.6	0.6	0.7	1	3	6.2	C	0.7	0	0	0.5	0	0.8
0.9	1.7	1.7	0.6	1	6	8.7	C	0.6	0	0	0.7	0	0
0.7	0.8	0.6	0.9	1	1	2.2	C	1.3	1.4	0.7	0.7	0.9	0.6
1	0.7	1.1	0.8	1	2	4.9	C	0.9	0	0.8	0.9	0.6	1
1.7	1.1	0.5	0.6	1	4	7.8	C	0.6	1	1.1	0.5	0	1.1
0.5	0.5	0.6	0.5	0	0	0		0.4	0.6	0.5	0.5	0.6	0.5
0.6	0.4	0.6	0.6	0	0	0		0.4	0	0	0.6	0	0
0.6	0.5	0.6	0.6	0	0	0		0.7	0	0	0.6	0	0.4
0.7	0.6	0.6	0.6	0	0	0		0.8	0	0	0.8	0	0
0.9	0.7	0.8	0.7	0	0	0		0.7	0	0	0.7	0	0
0.6	0.5	0.6	0.6	0	0	0		0.6	0	0	0.5	0	0
0.6	0.7	0.8	0.6	0	0	0		0.7	0	0	0.7	0	0
0.8	0.5	0.6	0.5	0	0	0		0.5	0	0	0.7	0	0

2.3	1	0.8	0.8	0	0	0		0.6	0	0	0.6	0	0
0.5	0.5	0.6	0.4	0	0	0		0.4	0	0	0.6	0	0
0	0	0.7	0.4	0	0	0		0.5	0.8	0	0.5	0.7	0
0.8	0.5	0.5	0.4	0	0	0		0.5	0.8	0.4	0.5	0.7	0.6
0.7	0.5	1	0.8	0	0	0		0.7	0.7	0.5	0.7	0.7	0.5
0.7	0.6	0.7	0.6	0	0	0		0.9	0.8	0.6	0.8	0.6	0.5
0.6	0.5	0.5	0.4	0	0	0		0.7	0.8	0.5	0.6	0.5	0.4
0.6	0.5	0.6	0.5	0	0	0		0.6	0.7	0.5	0.5	0.5	0.6
0.9	0.5	1.1	0.4	0	0	0		0.7	0	0	0.6	0	0
1	0.5	1.7	0.7	0	0	0		0.4	0.7	0.5	0.5	0.7	0.7
0.6	0.6	0.5	0.5	0	0	0		0.6	0.5	0.5	0.6	0.5	0.5
0.8	0.5	0.7	0.5	0	0	0		0.8	0	0	0.5	1.1	0
0.8	0.6	0.7	0.5	1	3	4.8	C	0.7	0	0	0.5	0	0
0.6	0.4	0.5	0.4	1	4	7.6	M	0.6	1	0.4	0.6	0.6	0.5
1	0.5	1.5	0.6	1	2	3.7	C	0.7	0.7	0.5	0.7	0.7	0.6
0.5	0.4	0.6	0.4	1	2	4.8	C	0.5	0	0.4	0.4	0.9	0.4
1.2	0	2	0.6	1	3	5	C	0.7	0	0	0.6	0	0
0.6	0.4	0.8	0.7	0	0	0		0.5	0.5	0	0.6	0.7	0
0.6	0.5	0.5	0.7	0	0	0		0.6	0.4	0.4	0.6	0.4	0.6
0.4	0.4	0.5	0.6	0	0	0		0.6	0.4	0.4	0.5	0.6	0.6
0.8	0.5	0.6	0.6	0	0	0		0.7	0.6	0.5	0.7	0.6	0.6
0.5	0.5	0.7	0.5	0	0	0		0.5	0	0	0.7	0	0
1.1	0.9	0.9	0.5	1	1	1.5	S	0.7	1.1	1.1	0.7	1	0.7
1	0.7	1	0.7	1	6	14.8	C	0.7	2	0.6	0.9	0.9	0.7
0.7	0.9	0.9	0.8	1	7	18.8	C	0.8	0.7	0.7	0.9	0.9	0
0.6	0.4	0.6	0.4	1	3	4	C	0.4	0	0	0.5	1	0
0.8	0.6	0.8	0.8	1	4	8.2	C	0.6	0.8	0.8	0.7	0.8	0.8
0.9	0.7	0.9	0.6	1	3	8	C	0.7	1	0.5	0.9	1.3	0.6
0.4	0.6	0.7	0.5	0	0	0		0.7	0	0	0.6	0	0
0.6	0.5	0.5	0.5	0	0	0		0.6	0.6	0.6	0.5	0.6	0.6
0.6	0.6	0.7	0.9	0	0	0		0.7	0.6	0	0.6	0.7	0
0.6	0.5	0.6	0.6	0	0	0		0.6	0.9	0.6	0.6	0.8	0.6
0.5	0.6	0.7	0.6	0	0	0		0.7	0	0	0.5	0	0
0.5	0.5	0.7	0.6	0	0	0		0.7	0	0	0.7	0	0
0.7	0.4	0.5	0.5	0	0	0		0.5	0.6	0.5	0.6	0.5	0.5
0.4	0.5	0.6	0.6	0	0	0		0.6	0.8	0.4	0.6	0.9	0.5
0.6	0.6	0.7	0.4	0	0	0		0.5	0	0.8	0.6	0.8	0.3

0.6	0.5	0.6	0.4	0	0	0		0.6	0.6	0	0.6	0.6	0
0.8	0.6	0.8	0.5	1	1	1.1	S	0.7	0.8	0	0.7	0.5	0
1	0.4	0.7	1	1	2	4.1	C	0.8	0.8	1.1	0.8	0.9	0.8
0.7	0.6	0.5	1.1	1	2	3.6	C	0.5	1.2	0.6	0.7	1	0.7
0.6	0.7	0.7	0.5	1	4	7	C	0.6	0.5	0	0.5	0.7	0
0.6	0.6	0.6	0.8	1	1	1.9	C	0.6	0.8	0.6	0.6	0.6	0.8

Syst dysf	dias dysf	EF	FS	LVH	LVEDD	IVSd	PWd	sum	Sum^3	LVID^3			LVM
0	1	64.92	35.63	1	4.94	1.32	0.9	7.16	367.06	120.55	246.51	256.37	242.77
0	1	67.1	36.8	1	4.21	1.6	1.18	6.99	341.53	74.62	266.91	277.59	263.99
0	0	55	29	0	4.03	0.75	1.15	5.93	208.53	65.45	143.08	148.80	135.20
0	0	65.5	36	0	4.88	1.41	1.27	7.56	432.08	116.21	315.87	328.50	314.90
0	1	57.7	30.4	1	4.93	1.66	1.27	7.86	485.59	119.82	365.76	380.40	366.80
1	1	43	26	1	5.45	1.86	1.19	8.5	614.13	161.88	452.25	470.34	456.74
0	1	52	28.8	1	5.8	1	1	7.8	474.55	195.11	279.44	290.62	277.02
0	1	63.1	34.2	1	4.65	1.48	1.26	7.39	403.58	100.54	303.04	315.16	301.56
0	1	66	37	1	5.62	0.96	1.48	8.06	523.61	177.50	346.10	359.95	346.35
1	0	43	21	1	4.66	1.27	1.67	7.6	438.98	101.19	337.78	351.29	337.69
0	0	66.8	37	1	4.94	1.2	1.28	7.42	408.52	120.55	287.96	299.48	285.88
0	1	72.4	41.5	1	4.65	2.08	1.72	8.45	603.35	100.54	502.81	522.92	509.32
0	1	68	34	1	4.3	1.5	1.68	7.48	418.51	79.51	339.00	352.56	338.96
1	0	40	20	1	6.31	1.26	1.19	8.76	672.22	251.24	420.98	437.82	424.22
0	0	55	23	2	4.4	1.1	1.03	6.53	278.45	85.18	193.26	200.99	187.39
1	1	48	24	1	4.65	1.48	1.25	7.38	401.95	100.54	301.40	313.46	299.86
1	0	46	23.7	1	5.6	1	1.09	7.69	454.76	175.62	279.14	290.31	276.71
0	1	62.06	34.21	2	6.47	1.77	1.51	9.75	926.86	270.84	656.02	682.26	668.66
0	1	54.87	28.4	1	5.01	1.48	1.15	7.64	445.94	125.75	320.19	333.00	319.40
0	1	71.6	40.7	1	4.6	1.27	1.04	6.91	329.94	97.34	232.60	241.91	228.31
0	1	75.5	44.68	1	5	1.7	1.7	8.4	592.70	125.00	467.70	486.41	472.81
1	1	48	24.3	1	6.2	1.76	1.49	9.45	843.91	238.33	605.58	629.80	616.20
0	1	61.61	33.65	1	5.9	2.36	2.13	10.39	1121.62	205.38	916.24	952.89	939.29
0	1	78.6	47.3	1	4.85	1.53	1.6	7.98	508.17	114.08	394.09	409.85	396.25
0	1	68	38	1	4.26	1.32	2.13	7.71	458.31	77.31	381.01	396.25	382.65

1	0	46	23	3	6.4	1.4	1.2	9	729.00	262.14	466.86	485.53	471.93
0	1	59	27	1	5.84	1.4	1.5	8.74	667.63	199.18	468.45	487.19	473.59
0	1	50	25	1	4.43	1.62	1.59	7.64	445.94	86.94	359.01	373.37	359.77
0	1	62	33.7	1	4.88	1.7	1.13	7.71	458.31	116.21	342.10	355.78	342.18
0	0	66	36	0	4.9	1.2	0.8	6.9	328.51	117.65	210.86	219.29	205.69
0	0	58	31	0	5.2	1.7	1.2	8.1	531.44	140.61	390.83	406.47	392.87
0	1	76	45	1	4.24	1.5	1.26	7	343.00	76.23	266.77	277.45	263.85
1	0	32	15	3	6.5	1	1	8.5	614.13	274.63	339.50	353.08	339.48
0	0	66	36	0	3.88	1.08	1.01	5.97	212.78	58.41	154.37	160.54	146.94
0	0	71.7	41.2	3	5.28	1.12	0.88	7.28	385.83	147.20	238.63	248.18	234.58
0	0	68.49	38.37	1	4.88	1.34	1.25	7.47	416.83	116.21	300.62	312.64	299.04
0	1	59	31.25	1	4.54	1.42	1.36	7.32	392.22	93.58	298.65	310.59	296.99
0	1	54	28	1	4.31	1.27	1.21	6.79	313.05	80.06	232.98	242.30	228.70
0	1	58	30.5	1	4.52	1.3	1.39	7.21	374.81	92.35	282.46	293.76	280.16
0	0	72.4	41.4	1	4.54	1.63	1.29	7.46	415.16	93.58	321.58	334.45	320.85
0	1	64.13	34.3	1	3.8	1.51	1.39	6.7	300.76	54.87	245.89	255.73	242.13
0	1	67.33	36.99	1	4.14	1.59	1.08	6.81	315.82	70.96	244.86	254.66	241.06
1	0	40	20	1	3.73	1.51	1.16	6.4	262.14	51.90	210.25	218.66	205.06
0	1	71	41.1	1	5.19	1.19	1.08	7.46	415.16	139.80	275.36	286.38	272.78
0	1	70	39	1	4.49	1.55	1.38	7.42	408.52	90.52	318.00	330.72	317.12
1	0	47.9	32	1	5.77	1.53	1.24	8.54	622.84	192.10	430.74	447.97	434.37
0	1	74	38	1	4.82	1.64	1.38	7.84	481.89	111.98	369.91	384.71	371.11
0	1	52	26.1	1	5	1.1	1.24	7.34	395.45	125.00	270.45	281.26	267.66
0	0	66.3	36.1	0	4.07	0.96	1.3	6.33	253.64	67.42	186.22	193.67	180.07
0	1	54.7	27.8	1	4.32	1.21	1.18	6.71	302.11	80.62	221.49	230.35	216.75
1	0	36	18	1	4.9	1.21	1.18	7.29	387.42	117.65	269.77	280.56	266.96
0	0	52	26	1	3.79	1.3	1.22	6.31	251.24	54.44	196.80	204.67	191.07
0	1	76	45	1	5	1.2	1.2	7.4	405.22	125.00	280.22	291.43	277.83
0	1	79.1	47.5	1	4.32	2.38	1.61	8.31	573.86	80.62	493.23	512.96	499.36
0	1	60	32.5	1	4.78	1.98	1.42	8.18	547.34	109.22	438.13	455.65	442.05
0	1	73	42	1	5.2	1.75	1.2	8.15	541.34	140.61	400.74	416.76	403.16
0	1	76	36	1	4.88	1.31	1.43	7.62	442.45	116.21	326.24	339.29	325.69
0	0	76.8	45.7	1	4.98	1.64	1.48	8.1	531.44	123.51	407.94	424.25	410.65
0	0	62.8	34.1	0	5.04	1.11	0.89	7.04	348.91	128.02	220.89	229.73	216.13
0	1	58.5	31.3	1	5.43	0.79	1.99	8.21	553.39	160.10	393.28	409.02	395.42
0	1	72.1	41.9	1	5.94	1.09	1.34	8.37	586.38	209.58	376.79	391.86	378.26
0	1	76	44.8	1	4.94	1.43	1.1	7.47	416.83	120.55	296.28	308.13	294.53

0	1	54.2	28.3	1	5.22	1.9	1.63	8.75	669.92	142.24	527.69	548.79	535.19
0	0	63.8	34.7	1	4.7	1.68	1.39	7.77	469.10	103.82	365.27	379.89	366.29
0	1	66.5	36.9	1	5.09	2.27	1.44	8.8	681.47	131.87	549.60	571.58	557.98
1	1	48	24	1	6	1.4	0.98	8.38	588.48	216.00	372.48	387.38	373.78
0	1	63.2	34.2	1	4.9	1.48	1.4	7.78	470.91	117.65	353.26	367.39	353.79
1	1	35	17	3	6.09	0.99	1.1	8.18	547.34	225.87	321.48	334.34	320.74
0	1	58	32	1	6.9	1.04	1.27	9.21	781.23	328.51	452.72	470.83	457.23
0	1	65.9	36	0	4.26	1.11	1.05	6.42	264.61	77.31	187.30	194.79	181.19
0	1	54.4	28.4	1	5.39	1.99	1.53	8.91	707.35	156.59	550.76	572.79	559.19
0	0	57	28.9	1	4.71	1.28	1.1	7.09	356.40	104.49	251.91	261.99	248.39
1	1	44.3	22.2	1	4.09	1.37	1.39	6.85	321.42	68.42	253.00	263.12	249.52
0	0	69.1	38.2	0	3.9	0.89	0.82	5.61	176.56	59.32	117.24	121.93	108.33
0	1	71.7	39.4	1	2.8	1.57	0.89	5.26	145.53	21.95	123.58	128.52	114.92
0	1	74.4	42.1	1	3.23	1.36	1.08	5.67	182.28	33.70	148.59	154.53	140.93
0	0	63	34	1	4.61	1.43	1	7.04	348.91	97.97	250.94	260.98	247.38
0	1	78.2	45.5	0	3.12	1.02	1.48	5.62	177.50	30.37	147.13	153.02	139.42
0	1	60.3	32	1	4.41	1.31	1.16	6.88	325.66	85.77	239.89	249.49	235.89
1	1	48.6	24.4	2	5.09	1.64	1.3	8.03	517.78	131.87	385.91	401.35	387.75
1	0	36	17.5	3	5.85	1.4	1.4	8.65	647.21	200.20	447.01	464.89	451.29
0	0	80	49.1	2	5.41	1.55	1.15	8.11	533.41	158.34	375.07	390.07	376.47
0	1	73.3	41.9	1	4.2	1.39	1.26	6.85	321.42	74.09	247.33	257.22	243.62
1	0	50	25	2	6.5	1.3	1.32	9.12	758.55	274.63	483.93	503.28	489.68

HT	Ht	wt	wt x Ht		BSA	LVMI	RWT	VALVES	Hs CRP	LP(a)	HCY	ADPN	Ca	Phos	Ca x PO4	PTH	HB
1.66	166	63	10458.00	2.91	1.70	142.44	0.36	0	5	35.2	7	21.85					
1.48	148	33	4884.00	1.36	1.16	226.65	0.56	2	4	118	12.85	32.55					
1.75	175	59.6	10430.00	2.90	1.70	79.43	0.57	0	2	101.6	22.92	24.67					
1.65	165	55	9075.00	2.52	1.59	198.34	0.52	0	2	77.6	27.11	18.83					
1.62	162	56.4	9136.80	2.54	1.59	230.24	0.52	0	2	167.6	17.12	32.55					
1.77	177	91.3	16160.10	4.49	2.12	215.57	0.44	0	7	13.6	29.33	10.33					
1.67	167	70.5	11773.50	3.27	1.81	153.18	0.34	0	5	37.7	22.89	32.55					
1.71	171	66.3	11337.30	3.15	1.77	169.93	0.54	2	6	44.2	26.47	6.97					
1.77	177	77.9	13788.30	3.83	1.96	176.97	0.53	0	1	8.5	15.64	28.16					
1.59	159	57	9063.00	2.52	1.59	212.83	0.72	0	3	50.6	14.03	15.59					
1.65	165	56.9	9388.50	2.61	1.61	177.03	0.52	0	8	71.3	9.2	13.7					

1.59	159	51	8109.00	2.25	1.50	339.36	0.74	2	7	56.4	22.55	32.55
1.62	162	56	9072.00	2.52	1.59	213.53	0.78	2	2	6.4	15.6	25.16
1.73	173	56.5	9774.50	2.72	1.65	257.45	0.38	1; 2	3	26.2	9.44	32.55
1.69	169	47.2	7976.80	2.22	1.49	125.89	0.47	0	0.1	40.9	18.67	20.26
1.76	176	62.7	11035.20	3.07	1.75	171.27	0.54	1,3	4.8	112	20.83	30.68
1.71	171	73	12483.00	3.47	1.86	148.60	0.39	0	1.7	14.4	29.04	8.77
1.74	174	80	13920.00	3.87	1.97	340.05	0.47	0	16	38.8	21.68	12.82
1.72	172	75.7	13020.40	3.62	1.90	167.95	0.46	0	5	30.8	27.83	11.5
1.62	162	57.7	9347.40	2.60	1.61	141.69	0.45	0	6	32.5	20.91	16.66
1.72	172	76	13072.00	3.63	1.91	248.12	0.68	0	2	25.6	16.75	9.34
1.68	168	64.3	10802.40	3.00	1.73	355.73	0.48	0	10	135.2	22.72	32.55
1.83	183	82	15006.00	4.17	2.04	460.07	0.72	0	3	26.2	20.63	32.55
1.66	166	60.3	10009.80	2.78	1.67	237.63	0.66	0	1	56.5	17.59	16.43
1.5	150	47.2	7080.00	1.97	1.40	272.85	1.00	0	14	15.4	14.69	24.21
1.69	169	59.8	10106.20	2.81	1.68	281.67	0.38	1	20	119.3	15.73	32.55
1.78	178	57	10146.00	2.82	1.68	282.10	0.51	0	2	81.1	21.01	28.2
1.61	161	60	9660.00	2.68	1.64	219.63	0.72	0	8.9	51.3	20.95	30.68
1.7	170	84.5	14365.00	3.99	2.00	171.30	0.46	0	3	57.8	21.94	32.55
1.62	162	67.9	10999.80	3.06	1.75	117.67	0.33	1	25.6	11.5	26.92	23.77
1.67	167	77.9	13009.30	3.61	1.90	206.67	0.46	0	2	18.1	20.59	10.14
1.71	171	72.2	12346.20	3.43	1.85	142.47	0.59	0	4.8	28.1	20.65	30.68
1.62	162	69	11178.00	3.11	1.76	192.66	0.31	1,2	8	26.3	15.76	30.68
1.57	157	54.1	8493.70	2.36	1.54	95.66	0.52	0	7	142.6	23.6	30.68
1.61	161	63	10143.00	2.82	1.68	139.75	0.33	2	1	32.9	23.44	15.34
1.62	162	69.4	11242.80	3.12	1.77	169.22	0.51	0	6	58.2	22.11	30.68
1.62	162	78.2	12668.40	3.52	1.88	158.32	0.60	0	3	60.6	27.04	14.04
1.52	152	63.5	9652.00	2.68	1.64	139.67	0.56	0	1	3.6	11.4	20.2
1.5	150	53	7950.00	2.21	1.49	188.53	0.62	0	31	21.5	13.71	29.64
1.54	154	93.6	14414.40	4.00	2.00	160.34	0.57	0	22	69.1	21.9	22.43
1.65	165	106	17490.00	4.86	2.20	109.85	0.73	0	3	22.2	28.1	24.4
1.64	164	66.7	10938.80	3.04	1.74	138.29	0.52	2	3	1.7	26.71	15.29
1.5	150	50.4	7560.00	2.10	1.45	141.50	0.62	2	11	93.9	15.03	30.68
1.5	150	52.8	7920.00	2.20	1.48	183.91	0.42	0	3	13.5	23.96	8.35
1.55	155	53.5	8292.50	2.30	1.52	208.94	0.61	0	1.2	48.1	18.77	27.19
1.64	164	59	9676.00	2.69	1.64	264.95	0.43	1	5	46.5	14.61	30.68
1.52	152	90	13680.00	3.80	1.95	190.37	0.57	0	8	54.1	28.06	15.98
1.49	149	58	8642.00	2.40	1.55	172.76	0.50	0	3	31.5	21.66	30.68

1.54	154	88	13552.00	3.76	1.94	92.81	0.64	0	1	75.5	16.63	0.09
1.59	159	88	13992.00	3.89	1.97	109.94	0.55	0	1	26.5	24.3	30.68
1.49	149	56.4	8403.60	2.33	1.53	174.73	0.48	0	52	23.6	18.87	30.68
1.66	166	52	8632.00	2.40	1.55	123.39	0.64	0	0.1	69.9	21.62	22.34
1.64	164	63.4	10397.60	2.89	1.70	163.48	0.48	1	4	5.2	16.85	30.68
1.66	166	70.3	11669.80	3.24	1.80	277.36	0.75	2	4	32.5	12.59	31.31
1.55	155	68.5	10617.50	2.95	1.72	257.40	0.59	1	7	10.7	25.97	30.68
1.55	155	71	11005.00	3.06	1.75	230.59	0.46	0	3.9	102.3	19.44	24
1.64	164	48.5	7954.00	2.21	1.49	219.11	0.59	1	3	71.5	28.26	30.68
1.56	156	69.8	10888.80	3.02	1.74	236.12	0.59	1,2	41.8	2	26.74	25.55
1.57	157	51.7	8116.90	2.25	1.50	143.93	0.35	2	1	24.5	20.13	7.57
1.8	180	105.5	18990.00	5.28	2.30	172.16	0.73	0	75	4.1	40.93	8.1
1.57	157	60.5	9498.50	2.64	1.62	232.87	0.45	0	4.1	47.3	13.81	23.15
1.67	167	75.3	12575.10	3.49	1.87	157.59	0.45	0	11	1.7	10.9	18.44
1.79	179	87	15573.00	4.33	2.08	257.32	0.62	3,4	6	7.7	21.01	10.98
1.73	173	56.4	9757.20	2.71	1.65	222.49	0.59	0	11	5.2	19.65	10.77
1.76	176	70.1	12337.60	3.43	1.85	301.41	0.57	1,2,3	3	13.3	17.83	32.55
1.72	172	70	12040.00	3.34	1.83	204.39	0.33	0	4	24.9	23.8	23.34
1.67	167	62	10354.00	2.88	1.70	208.62	0.57	0	8	24.3	15.66	12.82
1.63	163	59	9617.00	2.67	1.63	196.24	0.36	0	6	88.8	13.72	22.23
1.78	178	82.5	14685.00	4.08	2.02	226.39	0.37	0	5	47.8	20.19	7.33
1.55	155	58.4	9052.00	2.51	1.59	114.27	0.49	0	5	29.1	14.86	16.93
1.66	166	55.4	9196.40	2.55	1.60	349.86	0.57	3	6	98.5	36.55	30.68
1.66	166	100.3	16649.80	4.62	2.15	115.50	0.47	0	8	26.3	25.34	9.51
1.58	158	48	7584.00	2.11	1.45	171.91	0.68	0	7	76.6	20.58	23.15
1.57	157	52.5	8242.50	2.29	1.51	71.59	0.42	0	2	6.3	23.61	10.37
1.58	158	37	5846.00	1.62	1.27	90.18	0.64	0	3	3.2	13.13	32.55
1.55	155	48	7440.00	2.07	1.44	98.03	0.67	0	1	128.2	16.9	32.55
1.55	155	60.5	9377.50	2.60	1.61	153.27	0.43	2	4	2.3	22.81	17.86
1.48	148	52.4	7755.20	2.15	1.47	94.99	0.95	0	1	15.5	17.48	22.56
1.5	150	50	7500.00	2.08	1.44	163.43	0.53	2	8	7.5	14.42	19.53
1.68	168	50	8400.00	2.33	1.53	253.84	0.51	0	8	5.8	10.69	5.36
1.64	164	67.8	11119.20	3.09	1.76	256.79	0.48	1	4	12.6	20.04	30.68
1.65	165	78	12870.00	3.58	1.89	199.11	0.43	1,2,3,4	10	3.2	40.56	13.57
1.45	145	57	8265.00	2.30	1.52	160.79	0.60	1,2,3	7	39.5	15.1	32.55
1.65	165	68.7	11335.50	3.15	1.77	275.96	0.41	1,3	48	37.2	17.11	22.82

Appendix D

Controls	age	sex	Race	SUBJECT	FHx MI	FHx HTN	FHx DM	FHx CKD	height	weight	HT SQ	BMI	Controls	Smoking
1	30	2	2	2	0	0	0	0	1.68	67.0	2.82	23.74	1	1
2	38	2	2	2	0	1	1	0	1.59	52.8	2.53	20.89	2	0
3	30	2	2	2	0	0	0	0	1.70	75.6	2.89	26.16	3	2
4	42	2	2	2	1	0	0	0	1.63	57.0	2.66	21.45	4	2
5	32	2	2	2	0	0	1	0	1.56	70.2	2.43	28.85	5	0
6	27	2	2	2	0	0	0	0	1.63	58.0	2.66	21.83	6	0
7	56	2	2	2	0	0	0	0	1.65	69.6	2.72	25.56	7	0
8	41	2	2	2	0	0	0	0	1.50	52.0	2.25	23.11	8	0
9	36	2	2	2	0	0	0	0	1.64	64.5	2.69	23.98	9	0
10	22	2	2	2	0	0	0	0	1.64	53.5	2.69	19.89	10	0
11	26	2	2	2	1	0	1	0	1.68	64.4	2.82	22.82	11	0
12	29	2	2	2	0	0	0	0	1.72	58.0	2.96	19.61	12	0
13	51	2	2	2	1	1	0	0	1.57	64.5	2.46	26.17	13	0
14	22	2	2	2	1	1	1	0	1.56	45.2	2.43	18.57	14	2
15	23	1	2	2	0	0	0	0	1.75	66.0	3.06	21.55	15	0
16	26	1	2	2	1	1	0	0	1.64	78.0	2.69	29.00	16	0
17	39	1	2	2	1	1	1	0	1.67	60.0	2.79	21.51	17	1
18	30	1	2	2	1	1	0	0	1.68	80.0	2.82	28.34	18	2
19	54	1	2	2	0	0	0	0	1.72	94.0	2.96	31.77	19	0
20	53	1	2	2	0	0	0	0	1.73	82.5	2.99	27.57	20	1
21	23	1	2	2	1	1	1	1	1.75	71.5	3.06	23.35	21	1
22	27	1	2	2	0	0	0	1	1.71	72.0	2.92	24.62	22	2
23	54	1	2	2	0	0	0	1	1.77	82.0	3.13	26.17	23	2
24	40	1	2	2	0	0	0	0	1.65	65.7	2.72	24.13	24	1
25	46	1	2	2	1	0	1	0	1.84	97.2	3.39	28.71	25	1
26	34	2	1	2	0	1	0	0	1.49	68.3	2.22	30.76	26	0
27	38	2	1	2	0	0	0	0	1.66	47.0	2.76	17.06	27	0
28	44	2	1	2	0	1	0	0	1.59	81.2	2.53	32.12	28	0
29	47	2	1	2	1	1	1	1	1.58	87.1	2.50	34.89	29	0
30	45	2	1	2	0	0	0	0	1.65	92.0	2.72	33.79	30	0
31	49	2	1	2	0	1	0	0	1.65	61.6	2.72	22.63	31	0
32	36	2	1	2	0	0	0	0	1.72	61.2	2.96	20.69	32	0

33	40	2	1	2	0	1	1	0	1.62	67.0	2.62	25.53	33	0
34	27	2	1	2	0	1	0	0	1.54	59.2	2.37	24.96	34	0
35	56	2	1	2	0	1	0	0	1.62	68.0	2.62	25.91	35	0
36	49	2	1	2	0	1	1	0	1.60	78.5	2.56	30.66	36	0
37	39	2	1	2	0	0	0	0	1.57	47.4	2.46	19.23	37	0
38	49	2	1	2	0	1	0	0	1.58	86.0	2.50	34.45	38	0
39	21	2	1	2	0	1	0	0	1.65	48.5	2.72	17.81	39	0
40	40	1	1	2	0	0	0	0	1.72	68.2	2.96	23.05	40	2
41	31	1	1	2	1	1	0	1	1.70	60.2	2.89	20.83	41	0
42	41	1	1	2	1	0	1	0	1.79	85.5	3.20	26.68	42	0
43	49	1	1	2	0	0	0	0	1.70	70.0	2.89	24.22	43	1
44	22	1	1	2	0	1	0	0	1.64	60.6	2.69	22.53	44	0
45	20	1	1	2	0	0	1	0	1.73	72.5	2.99	24.22	45	0
46	28	1	1	2	0	0	0	0	1.80	84.0	3.24	25.93	46	0
47	51	1	1	2	1	1	0	1	1.61	67.5	2.59	26.04	47	0
48	43	1	1	2	0	1	0	0	1.83	85.0	3.35	25.38	48	0
49	51	1	1	2	0	0	0	0	1.64	72.0	2.69	26.77	49	2
50	46	1	1	2	0	0	0	0	1.64	77.9	2.69	28.96	50	2
51	45	1	1	2	0	0	0	0	1.60	53.5	2.56	20.90	51	1
52	50	1	1	2	0	0	0	1	1.77	55.6	3.13	17.75	52	1
53	46	1	1	2	0	1	0	1	1.62	62.7	2.62	23.89	53	0
54	58	2	1	2	0	1	1	0	1.68	103.0	2.82	36.49	54	0
55	51	2	2	2	0	0	0	0	1.65	84.1	2.72	30.89	55	2
56	43	2	1	2	0	1	0	0	1.50	72.8	2.25	32.36	56	0
57	39	2	1	2	0	1	0	0	1.49	62.4	2.22	28.11	57	0
58	39	1	1	2	0	0	0	0	1.78	93.3	3.17	29.45	58	0
59	52	2	2	2	0	0	0	1	1.52	50.0	2.31	21.64	59	1
60	55	1	1	2	0	0	0	0	1.60	67.3	2.56	26.29	60	0
61	51	1	1	2	0	0	0	0	1.60	67.2	2.56	26.25	61	1
62	42	1	1	2	0	0	0	0	1.75	70.0	3.06	22.86	62	1
63	50	1	2	2	0	1	1	0	1.83	113.0	3.35	33.74	63	0

Quantity	exercise	Ex FREQ	SBP	DBP	Pulse P	PP/3	MAP	total chol	Controls	TG	HDL	LDL	LCCA	RCCA
1	1	1	126	66	60	20.00	86.00	4.8	1	1.1	1.5	2.8	0.5	0.5
0	1	1	90	60	30	10.00	70.00	6.7	2	1.3	1.1	4.9	0.6	0.4
0	0	0	123	74	49	16.33	90.33	6.2	3	1.0	1.4	4.3	0.4	0.5
0	0	0	128	72	56	18.67	90.67	5.4	4	1.4	1.9	2.9	0.6	0.7
0	1	3	117	74	43	14.33	88.33	5.3	5	0.9	1.0	3.9	0.7	0.5
0	0	0	110	60	50	16.67	76.67	4.5	6	0.8	1.6	2.5	0.6	0.6
0	0	0	122	80	42	14.00	94.00	5.5	7	1.2	1.9	3.0	0.5	0.6
0	1	3	100	56	44	14.67	70.67	6.6	8	1.4	1.4	4.5	0.4	0.5
0	1	3	90	70	20	6.67	76.67	4.7	9	1.5	1.6	2.4	0.5	0.6
0	1	2	100	60	40	13.33	73.33	5.4	10	1.3	2.3	2.5	0.5	0.5
0	0	0	100	70	30	10.00	80.00	4.6	11	1.0	1.5	2.6	0.5	0.5
0	1	2	110	76	34	11.33	87.33	4.6	12	0.9	1.4	2.7	0.6	0.5
0	0	0	120	70	50	16.67	86.67	5.9	13	1.3	1.7	3.6	0.7	0.7
1	0	0	90	60	30	10.00	70.00	3.8	14	0.6	1.4	2.1	0.4	0.5
0	1	3	110	70	40	13.33	83.33	5.7	15	2.1	1.2	3.5	0.5	0.4
0	1	2	128	65	63	21.00	86.00	5.6	16	1.3	1.3	3.8	0.5	0.4
1	1	3	110	70	40	13.33	83.33	4.6	17	1.8	1.1	2.7	0.5	0.5
0	1	2	131	76	55	18.33	94.33	4.9	18	1.1	1.0	3.4	0.5	0.5
0	0	0	134	80	54	18.00	98.00	6.4	19	3.4	1.3	3.5	0.7	0.7
1	1	3	130	80	50	16.67	96.67	5	20	2.3	3.1	0.8	0.6	0.5
1	1	1	127	65	62	20.67	85.67	6.6	21	3.1	1.3	3.9	0.4	0.5
2	1	3	120	80	40	13.33	93.33	7.3	22	2.0	1.1	5.3	0.5	0.5
1	1	3	130	80	50	16.67	96.67	6.2	23	0.9	1.4	4.4	0.7	0.8
2	0	0	120	70	50	16.67	86.67	3.6	24	1.1	1.1	2.0	0.5	0.6
2	1	3	130	90	40	13.33	103.33	6.4	25	1.4	1.1	4.7	1.2	1.4
0	0	0	128	81	47	15.67	96.67	4.6	26	1.4	1.0	3.0	0.6	0.5
0	0	0	100	60	40	13.33	73.33	3.7	27	0.8	1.7	1.7	0.4	0.5
0	0	0	133	81	52	17.33	98.33	3.6	28	1.1	1.6	1.5	0.7	0.7
0	0	0	136	91	45	15.00	106.00	4.9	29	3.8	1.0	2.2	0.7	0.6
0	0	0	140	88	52	17.33	105.33	4.7	30	1.4	1.4	2.7	0.8	0.8
0	1	3	122	71	51	17.00	88.00	4.3	31	1.0	1.2	2.6	0.6	0.6
0	0	0	100	70	30	10.00	80.00	3.5	32	1.0	0.9	2.1	0.5	0.7
0	0	0	120	80	40	13.33	93.33	6.3	33	1.5	1.4	4.2	0.6	0.6

0	1	1	112	61	51	17.00	78.00	5.5	34	1.1	0.9	4.1	0.5	0.6
0	0	0	134	76	58	19.33	95.33	6.7	35	2.1	1.4	4.3	0.6	0.8
0	1	1	111	67	44	14.67	81.67	4.6	36	1.3	1.1	2.9	0.6	0.8
0	0	0	97	73	24	8.00	81.00	2.8	37	1.1	1.5	0.8	0.5	0.5
0	0	0	128	78	50	16.67	94.67	5.4	38	1.2	2.1	2.7	0.6	0.6
0	0	0	128	74	54	18.00	92.00	4.7	39	0.8	1.3	3.0	0.5	0.5
1	1	3	120	70	50	16.67	86.67	3.7	40	6.1	0.6	0*	0.7	0.7
0	0	0	132	67	65	21.67	88.67	4.9	41	1.0	1.1	3.3	0.5	0.5
0	0	0	136	90	46	15.33	105.33	5.1	42	1.1	1.2	3.4	0.7	0.8
1	1	1	120	80	50	16.67	96.67	4.2	43	3.6	1.1	1.4	0.5	0.5
0	1	1	135	76	59	19.67	95.67	5.7	44	0.9	1.2	4.2	0.5	0.6
0	0	0	118	57	61	20.33	77.33	2.8	45	1.5	1.1	1.0	0.4	0.5
0	1	3	120	80	40	13.33	93.33	4.3	46	1.4	1.4	2.3	0.5	0.5
0	0	0	110	70	40	13.33	83.33	4	47	0.9	1.1	2.4	0.8	0.9
0	1	1	120	75	45	15.00	90.00	3.8	48	1.5	0.9	2.2	0.6	0.5
1	1	3	130	90	40	13.33	103.33	4.9	49	1.2	1.1	3.2	0.8	0.7
0	0	0	120	90	30	10.00	100.00	2.6	50	1.2	0.3	0.7	0.5	0.5
2	1	1	120	80	40	13.33	93.33	2.8	51	0.7	1.1	1.4	0.6	0.7
1	0	0	114	65	49	16.33	81.33	4.7	52	1.3	0.9	3.2	0.8	1.2
0	0	0	130	92	38	12.67	104.67	3.2	53	0.9	0.9	1.9	0.7	0.6
0	0	0	137	80	57	19.00	99.00	4.1	54	0.9	1.0	2.7	0.8	0.9
0	1	2	135	81	54	18.00	99.00	9	55	5.3	0.9	0*	0.7	0.5
0	0	0	128	75	53	17.67	92.67	8.8	56	2.4	1.1	6.5	0.6	0.6
0	0	0	150	100	50	16.67	116.67	4.3	57	0.7	1.0	3.0	0.6	0.6
0	1	1	110	80	30	10.00	90.00	5.8	58	2.7	1.1	3.5	0.7	0.7
1	0	0	100	65	35	11.67	76.67	4.4	59	0.9	1.7	2.3	0.8	0.8
0	1	3	135	82	53	17.67	99.67	4.6	60	2.8	1.1	2.3	0.9	0.8
1	0	0	120	60	60	20.00	80.00	4.6	61	1.4	1.7	2.3	0.7	0.6
1	0	0	110	60	50	16.67	76.67	5.2	62	1.6	1.0	3.5	0.7	0.5
0	1	2	120	90	30	10.00	100.00	3.9	63	0.9	1.0	2.4	0.8	0.7

s CCA	CIMT	LBIF	LICA	RBIF	RICA	PLAQUE	Plq No	PLQ Score	Plq type	nwLCCA	nwLBIF	nwLICA	nwRCCA	nwRBIF
1.0	0.50	0.6	0.4	0.5	0.5	0	0	0	0	0.4	0.4	0.4	0.5	0.4
1.0	0.50	0.5	0.5	0.9	0.4	0	0	0	0	0.6	0.5	0.4	0.5	0.5
0.9	0.45	0.6	0.6	0.7	0.7	0	0	0	0	0.6	0.6	0.6	0.7	0.5
1.3	0.65	0.8	0.5	0.8	0.4	0	0	0	0	0.8	0.8	0.8	0.9	0.9
1.2	0.60	0.5	0.5	0.5	0.5	0	0	0	0	0.7	0.4	0.5	0.7	0.6
1.2	0.60	0.6	0.5	0.5	0.5	0	0	0	0	0.5	0.5	0.5	0.6	0.7
1.1	0.55	0.9	0.7	0.6	0.6	0	0	0	0	0.8	0.0	0.0	0.7	0.0
0.9	0.45	0.5	0.5	0.5	0.5	0	0	0	0	0.5	0.6	0.6	0.4	0.5
1.1	0.55	0.7	0.5	0.6	0.6	0	0	0	0	0.5	0.7	0.5	0.5	0.6
1.0	0.50	0.5	0.4	0.6	0.5	0	0	0	0	0.5	0.5	0.4	0.6	0.6
1.0	0.50	0.6	0.6	0.6	0.5	0	0	0	0	0.5	0.8	0.6	0.5	0.8
1.1	0.55	0.7	0.5	0.5	0.6	0	0	0	0	0.5	0.4	0.6	0.4	0.5
1.4	0.70	0.6	0.5	0.7	0.6	0	0	0	0	0.7	0.6	0.6	0.7	0.5
0.9	0.45	0.6	0.5	0.5	0.5	0	0	0	0	0.4	0.5	0.5	0.6	0.5
0.9	0.45	0.4	0.5	0.6	0.5	0	0	0	0	0.5	0.4	0.5	0.4	0.6
0.9	0.45	0.7	0.7	0.7	0.7	0	0	0	0	0.7	0.6	0.7	0.4	0.7
1.0	0.50	0.5	0.6	0.6	0.5	0	0	0	0	0.5	0.4	0.5	0.5	0.5
1.0	0.50	0.6	0.6	0.8	0.8	0	0	0	0	0.5	0.6	0.6	0.7	0.8
1.4	0.70	0.8	0.9	0.7	0.6	0	0	0	0	0.8	0.8	0.7	0.8	0.9
1.1	0.55	0.7	0.4	0.6	0.4	0	0	0	0	0.6	0.5	0.0	0.5	0.6
0.9	0.45	0.4	0.5	0.4	0.4	0	0	0	0	0.4	0.4	0.5	0.5	0.6
1.0	0.50	0.7	0.5	0.8	0.6	0	0	0	0	0.6	0.7	0.5	0.7	0.9
1.5	0.75	1.1	0.7	0.8	0.7	0	0	0	0	0.7	0.0	0.0	0.7	1.1
1.1	0.55	0.8	0.4	0.6	0.6	0	0	0	0	0.5	0.7	0.5	0.4	0.6
2.6	1.30	0.8	0.5	1	0.5	0	0	0	0	0.6	0.8	0.6	1.5	1.0
1.1	0.55	0.6	0.6	0.6	0.5	0	0	0	0	0.7	0.5	0.6	0.7	0.6
0.9	0.45	0.5	0.7	0.6	0.4	0	0	0	0	0.4	0.6	0.7	0.6	0.4
1.4	0.70	0.8	0.5	0.5	0.5	0	0	0	0	0.7	0.7	0.5	0.8	0.5
1.3	0.65	0.8	1	0.8	0.7	0	0	0	0	0.8	1.1	0.9	0.8	0.7
1.6	0.80	1	0.9	1.1	0.8	0	0	0	0	1.1	1.0	0.5	0.8	0.5
1.2	0.60	0.5	0.5	0.8	0.4	0	0	0	0	0.4	0.0	0.0	0.5	0.0
1.2	0.60	0.8	0.5	0.7	0.5	0	0	0	0	0.5	0.7	0.7	0.8	0.7

1.2	0.60	0.8	0.7	0.7	0.7	0	0	0	0	0.6	0.8	0.6	0.7	0.8
1.1	0.55	0.7	0.6	0.7	0.6	0	0	0	0	0.5	0.0	0.6	0.4	0.7
1.4	0.70	0.5	0.4	0.8	0.4	0	0	0	0	0.6	0.5	0.4	0.5	0.0
1.4	0.70	0.5	0.5	0.7	0.6	0	0	0	0	0.7	0.5	0.5	0.8	0.8
1.0	0.50	0.4	0.4	0.6	0.7	0	0	0	0	0.5	0.6	0.4	0.5	0.5
1.2	0.60	0.6	0.6	0.6	0.5	0	0	0	0	0.6	0.6	0.6	0.6	0.6
1.0	0.50	0.5	0.5	0.6	0.6	0	0	0	0	0.4	0.6	0.4	0.5	0.6
1.4	0.70	0.7	0.7	1	0.7	0	0	0	0	0.7	0.0	0.5	0.4	0.0
1.0	0.50	0.5	0.5	0.6	0.6	0	0	0	0	0.5	0.6	0.5	0.5	0.7
1.5	0.75	0.8	0.7	0.8	0.5	0	0	0	0	0.6	0.5	0.8	0.7	0.7
1.0	0.50	0.5	0.7	0.7	0.6	0	0	0	0	0.6	0.5	0.7	0.8	0.7
1.1	0.55	0.4	0.6	0.7	0.6	0	0	0	0	0.3	0.6	0.6	0.3	0.4
0.9	0.45	0.4	0.3	0.4	0.4	0	0	0	0	0.6	0.4	0.5	0.5	0.4
1.0	0.50	0.6	0.6	0.5	0.5	0	0	0	0	0.4	0.7	0.6	0.5	0.5
1.7	0.85	1.2	0.6	0.7	0.5	0	0	0	0	1.0	0.6	0.6	0.8	0.4
1.1	0.55	0.8	0.6	0.7	0.7	0	0	0	0	0.6	0.8	0.0	0.4	0.7
1.5	0.75	0.8	0.5	0.8	0.6	0	0	0	0	0.6	0.7	0.0	0.5	0.7
1.0	0.50	0.5	0.5	0.6	0.6	0	0	0	0	0.6	0.6	0.5	0.6	0.6
1.3	0.65	0.9	0.6	0.8	0.6	0	0	0	0	0.7	0.0	0.0	0.6	0.8
2.0	1.00	0.9	1.2	0.8	0	0	0	0	0	0.8	0.0	0.0	0.6	0.0
1.3	0.65	0.6	0.7	0.6	0.6	0	0	0	0	0.6	0.0	0.7	0.5	0.5
1.7	0.85	1.4	0.8	0.5	0.8	0	0	0	0	0.7	1.1	0.8	0.9	1.1
1.2	0.60	0.6	0.7	0.6	0.6	0	0	0	0	0.7	0.7	0.7	0.7	0.6
1.2	0.60	0.7	0.6	0.9	0.7	0	0	0	0	0.8	0.7	0.6	0.7	0.6
1.2	0.60	0.5	0.5	0.6	0.6	0	0	0	0	0.5	0.5	0.5	0.4	0.6
1.4	0.70	0	0	0	0.5	0	0	0	0	0.8	0.0	0.0	0.8	1.3
1.6	0.80	0.8	1.4	1.5	1.2	1	1	2	S	0.8	2.0	1.4	0.9	1.4
1.7	0.85	0.9	0.8	1	0.6	1	1	1.3	S	0.9	1.3	0.8	1.0	1.9
1.3	0.65	0.5	1	0.8	0.7	1	1	1.2	S	0.7	0.5	0.3	0.7	0.5
1.2	0.60	0.8	0.5	0.6	0.6	1	1	1.1	S	0.6	0.0	0.0	0.4	0.0
1.5	0.75	1	1	0.8	0.7	1	1	1.5	S	0.8	1.0	1.2	0.6	1.2

nwRICA	Syst dysf	dias dysf	EF	FS	Controls	LVH	LVEDD	IVSd	PWd	sum	Sum^3	LVID^3			LVM
0.4	0	0	65.0	35.1	1	0	3.82	0.92	0.84	5.58	173.74	55.74	118.00	122.72	109.12
0.5	0	0	59.2	31.3	2	0	4.60	0.66	0.72	5.98	213.85	97.34	116.51	121.17	107.57
0.5	0	0	69.0	39.6	3	0	4.00	1.00	1.00	6.00	216.00	64.00	152.00	158.08	144.48
0.5	0	0	73.6	41.7	4	0	3.57	0.95	0.67	5.19	139.80	45.50	94.30	98.07	84.47
0.5	0	0	65.4	35.7	5	0	4.48	0.92	0.77	6.17	234.89	89.92	144.97	150.77	137.17
0.5	0	0	68.0	38.2	6	0	3.37	0.90	0.83	5.10	132.65	38.27	94.38	98.15	84.55
0.0	0	0	60.9	32.0	7	0	3.78	0.99	0.70	5.47	163.67	54.01	109.66	114.04	100.44
0.5	0	0	66.0	36.0	8	0	4.54	0.85	0.73	6.12	229.22	93.58	135.64	141.07	127.47
0.6	0	0	63.5	34.2	9	0	4.31	1.08	0.91	6.30	250.05	80.06	169.98	176.78	163.18
0.5	0	0	70.5	39.2	10	0	3.67	0.84	0.79	5.30	148.88	49.43	99.45	103.42	89.82
0.6	0	0	64.2	34.7	11	0	4.26	0.96	0.91	6.13	230.35	77.31	153.04	159.16	145.56
0.5	0	0	65.0	34.8	12	0	4.02	0.92	0.88	5.82	197.14	64.96	132.17	137.46	123.86
0.6	0	0	65.5	36.0	13	0	4.86	0.79	1.05	6.70	300.76	114.79	185.97	193.41	179.81
0.5	0	0	67.0	36.0	14	0	3.51	0.92	0.86	5.29	148.04	43.24	104.79	108.98	95.38
0.4	0	0	72.0	40.5	15	0	3.98	0.98	0.85	5.81	196.12	63.04	133.08	138.40	124.80
0.6	0	0	63.2	34.2	16	0	4.24	1.00	0.96	6.20	238.33	76.23	162.10	168.59	154.99
0.4	0	0	58.8	30.8	17	0	4.15	1.28	1.09	6.52	277.17	71.47	205.69	213.92	200.32
0.8	0	0	63.2	34.2	18	0	4.71	1.08	0.85	6.64	292.75	104.49	188.27	195.80	182.20
0.8	0	0	79.7	48.0	19	1	4.26	1.22	1.10	6.58	284.89	77.31	207.58	215.88	202.28
0.0	0	0	67.0	37.0	20	0	4.93	1.01	0.86	6.80	314.43	119.82	194.61	202.39	188.79
0.4	0	0	65.4	35.7	21	0	4.47	1.12	0.74	6.33	253.64	89.31	164.32	170.89	157.29
0.6	0	0	62.3	33.3	22	0	4.32	0.94	0.72	5.98	213.85	80.62	133.23	138.55	124.95
0.0	0	1	73.9	42.5	23	1	4.14	1.36	1.19	6.69	299.42	70.96	228.46	237.60	224.00
0.5	0	0	70.0	40.5	24	0	4.45	1.20	1.02	6.67	296.74	88.12	208.62	216.96	203.36
0.5	0	0	73.5	43.0	25	0	5.67	1.25	0.85	7.77	469.10	182.28	286.81	298.29	284.69
0.5	0	0	60.6	31.9	26	0	3.91	1.08	0.68	5.67	182.28	59.78	122.51	127.41	113.81
0.4	0	0	51.8	26.4	27	0	4.60	1.40	0.77	6.77	310.29	97.34	212.95	221.47	207.87
0.5	0	0	67.2	37.1	28	0	4.52	1.14	0.74	6.40	262.14	92.35	169.80	176.59	162.99
0.8	0	0	75.0	44.0	29	1	4.99	1.15	1.15	7.29	387.42	124.25	263.17	273.70	260.10
1.1	0	0	78.0	47.0	30	0	4.66	0.98	0.98	6.62	290.12	101.19	188.92	196.48	182.88
0.0	0	0	67.6	37.7	31	0	4.82	0.96	1.13	6.91	329.94	111.98	217.96	226.68	213.08
0.5	0	0	72.6	41.3	32	0	4.26	0.96	0.85	6.07	223.65	77.31	146.34	152.19	138.59
0.7	0	0	75.0	43.2	33	0	4.02	0.91	0.88	5.81	196.12	64.96	131.16	136.40	122.80
0.6	0	0	60.0	29.0	34	0	4.21	0.88	0.78	5.87	202.26	74.62	127.64	132.75	119.15
0.4	0	1	63.4	33.3	35	1	4.62	1.18	1.22	7.02	345.95	98.61	247.34	257.23	243.63

0.6	0	0	70.7	40.2	36	0	4.60	1.02	0.88	6.50	274.63	97.34	177.29	184.38	170.78
0.6	0	0	58.0	30.0	37	0	4.73	0.88	0.55	6.16	233.74	105.82	127.92	133.04	119.44
0.5	0	0	60.2	32.2	38	0	4.99	0.85	0.85	6.69	299.42	124.25	175.17	182.17	168.57
0.5	0	0	61.6	32.2	39	0	3.35	0.91	0.85	5.11	133.43	37.60	95.84	99.67	86.07
0.0	0	0	61.2	32.6	40	0	4.42	1.14	0.94	6.50	274.63	86.35	188.27	195.81	182.21
0.6	0	0	64.7	35.0	41	0	4.17	0.91	0.83	5.91	206.43	72.51	133.91	139.27	125.67
0.5	0	0	79.7	48.0	42	0	4.15	1.18	1.10	6.43	265.85	71.47	194.37	202.15	188.55
0.6	0	0	70.3	40.0	43	0	5.43	1.11	0.63	7.17	368.60	160.10	208.50	216.84	203.24
0.6	0	0	65.4	42.0	44	1	3.86	0.96	1.36	6.18	236.03	57.51	178.52	185.66	172.06
0.4	0	0	68.0	34.5	45	0	3.90	1.01	0.86	5.77	192.10	59.32	132.78	138.09	124.49
0.5	0	0	70.7	40.0	46	0	4.60	1.12	0.88	6.60	287.50	97.34	190.16	197.77	184.17
0.5	0	0	60.8	32.6	47	0	4.88	1.08	0.74	6.70	300.76	116.21	184.55	191.93	178.33
0.4	0	0	68.7	38.2	48	0	4.31	1.04	1.03	6.38	259.69	80.06	179.63	186.82	173.22
0.0	0	0	68.0	38.0	49	1	4.72	1.20	0.88	6.80	314.43	105.15	209.28	217.65	204.05
0.0	0	0	65.0	36.0	50	0	4.54	1.00	1.01	6.55	281.01	93.58	187.43	194.93	181.33
0.0	0	0	61.9	33.3	51	1	4.74	1.33	1.12	7.19	371.69	106.50	265.20	275.81	262.21
0.0	0	0	57.9	30.3	52	0	4.31	1.11	0.69	6.11	228.10	80.06	148.04	153.96	140.36
0.0	0	1	65.5	35.5	53	1	4.04	1.44	0.90	6.38	259.69	65.94	193.75	201.51	187.91
0.0	0	1	83.8	52.2	54	1	4.90	1.40	1.20	7.50	421.88	117.65	304.23	316.40	302.80
0.6	0		74.8	43.3	55	0	4.36	0.99	0.95	6.30	250.05	82.88	167.17	173.85	160.25
0.7	0	1	79.0	46.0	56	0	2.75	1.43	1.04	5.22	142.24	20.80	121.44	126.30	112.70
0.6	0	1	69.3	38.1	57	1	3.57	1.25	1.19	6.01	217.08	45.50	171.58	178.45	164.85
0.0	0	1	69.7	39.3	58	1	4.88	1.30	1.42	7.60	438.98	116.21	322.76	335.67	322.07
1.2	0	0	76.0	46.0	59	0	4.26	1.04	0.98	6.28	247.67	77.31	170.36	177.18	163.58
1.0	0	0	80.7	48.7	60	0	3.88	1.05	0.98	5.91	206.43	58.41	148.01	153.93	140.33
0.4	0	0	65.0	35.2	61	0	4.42	1.07	0.99	6.48	272.10	86.35	185.75	193.18	179.58
0.0	0	0	60.0	30.0	62	0	4.10	1.04	0.92	6.06	222.55	68.92	153.62	159.77	146.17
0.7	0		64.0	34.6	63	0	4.26	1.93	1.30	7.49	420.19	77.31	342.88	356.60	343.00

HT	Ht	Controls	wt	wt XHt		BSA	LVMI	RWT	VALVES	Hs CRP	LP(a)	Homocysteine (µmol/L)	ADPN
1.68	168	1	67.0	11256.0	3.13	1.77	61.71	0.44	0	1.0	5.3	10.05	21.40
1.59	159	2	52.8	8395.2	2.33	1.53	70.44	0.31	0	1.0	47.9	7.58	9.80
1.70	170	3	75.6	12852.0	3.57	1.89	76.47	0.50	0	1.0	140.5	13.08	14.71
1.63	163	4	57.0	9291.0	2.58	1.61	52.58	0.38	2	0.1	112.5	5.98	18.07
1.56	156	5	70.2	10951.2	3.04	1.74	78.65	0.34	0	3.0	19.2	7.74	7.61
1.63	163	6	58.0	9454.0	2.63	1.62	52.18	0.49	0	1.0	54.3	13.37	9.03
1.65	165	7	69.6	11484.0	3.19	1.79	56.24	0.37	0	2.0	17.8	9.14	15.56
1.50	150	8	52.0	7800.0	2.17	1.47	86.60	0.32	0	2.0	42.8	11.27	10.61
1.64	164	9	64.5	10578.0	2.94	1.71	95.20	0.42	0	1.0	2.9	9.51	18.21
1.64	164	10	53.5	8774.0	2.44	1.56	57.54	0.43	0	10.0	43.5	11.68	15.52
1.68	168	11	64.4	10819.2	3.01	1.73	83.96	0.43	2	4.0	5.4	6.70	9.13
1.72	172	12	58.0	9976.0	2.77	1.66	74.41	0.44	0	1.0	43.8	10.01	7.57
1.57	157	13	64.5	10126.5	2.81	1.68	107.21	0.43	1	1.0	45.8	10.95	9.08
1.56	156	14	45.2	7051.2	1.96	1.40	68.15	0.49	0	1.0	38.3	7.94	10.68
1.75	175	15	66.0	11550.0	3.21	1.79	69.68	0.43	0	1.0	18.0	7.32	12.75
1.64	164	16	78.0	12792.0	3.55	1.89	82.22	0.45	0	1.0	10.7	7.17	6.86
1.67	167	17	60.0	10020.0	2.78	1.67	120.07	0.53	0	0.1	38.2	8.80	8.83
1.68	168	18	80.0	13440.0	3.73	1.93	94.30	0.36	0	1.0	2.2	9.10	12.23
1.72	172	19	94.0	16168.0	4.49	2.12	95.45	0.52	0	8.0	10.0	17.29	10.97
1.73	173	20	82.5	14272.5	3.96	1.99	94.82	0.35	0	8.0	1.4	29.26	10.54
1.75	175	21	71.5	12512.5	3.48	1.86	84.37	0.33	0	1.0	18.3	7.34	10.93
1.71	171	22	72.0	12312.0	3.42	1.85	67.57	0.33	1,2	0.1	104.4	10.75	7.71
1.77	177	23	82.0	14514.0	4.03	2.01	111.56	0.57	2	1.0	1.8	16.26	7.80
1.65	165	24	65.7	10840.5	3.01	1.74	117.19	0.46	0	2.0	22.7	8.61	4.01
1.84	184	25	97.2	17884.8	4.97	2.23	127.72	0.30	0	4.0	1.7	7.37	5.32
1.49	149	26	68.3	10176.7	2.83	1.68	67.69	0.35	2	0.1	15.4	7.47	8.18
1.66	166	27	47.0	7802.0	2.17	1.47	141.20	0.33	0	0.1	4.0	5.68	6.15
1.59	159	28	81.2	12910.8	3.59	1.89	86.07	0.33	0	1.0	62.7	6.90	5.02
1.58	158	29	87.1	13761.8	3.82	1.96	133.03	0.46	0	1.0	97.4	5.42	10.06
1.65	165	30	92.0	15180.0	4.22	2.05	89.06	0.42	1,2	1.0	16.8	8.58	4.63
1.65	165	31	61.6	10164.0	2.82	1.68	126.81	0.47	0	0.1	76.2	6.68	20.59
1.72	172	32	61.2	10526.4	2.92	1.71	81.05	0.40	0	1.0	39.3	6.39	3.88
1.62	162	33	67.0	10854.0	3.02	1.74	70.72	0.44	0	4.0	80.8	7.62	6.05
1.54	154	34	59.2	9116.8	2.53	1.59	74.87	0.37	0	1.0	113.5	8.43	17.25

1.62	162	35	68.0	11016.0	3.06	1.75	139.27	0.53	1, 2	3.0	72.9	19.34	16.01
1.60	160	36	78.5	12560.0	3.49	1.87	91.43	0.38	0	1.0	38.4	8.49	10.03
1.57	157	37	47.4	7441.8	2.07	1.44	83.07	0.23	0	0.1	21.6	8.09	9.36
1.58	158	38	86.0	13588.0	3.77	1.94	86.77	0.34	0	9.0	16.5	7.75	10.60
1.65	165	39	48.5	8002.5	2.22	1.49	57.73	0.51	0	1.0	25.3	5.39	6.11
1.72	172	40	68.2	11730.4	3.26	1.81	100.94	0.43	0	0.1	44.3	8.10	2.71
1.70	170	41	60.2	10234.0	2.84	1.69	74.53	0.40	0	0.1	95.3	12.58	20.45
1.79	179	42	85.5	15304.5	4.25	2.06	91.45	0.53	0	1.0	9.4	12.97	10.39
1.70	170	43	70.0	11900.0	3.31	1.82	111.79	0.23	0	6.0	16.0	8.07	3.69
1.64	164	44	60.6	9938.4	2.76	1.66	103.55	0.70	0	1.0	113.4	9.19	7.58
1.73	173	45	72.5	12542.5	3.48	1.87	66.70	0.44	0	1.0	47.0	10.97	10.31
1.80	180	46	84.0	15120.0	4.20	2.05	89.86	0.38	2	5.0	3.1	6.93	4.88
1.61	161	47	67.5	10867.5	3.02	1.74	102.64	0.30	0	1.0	60.1	8.16	10.39
1.83	183	48	85.0	15555.0	4.32	2.08	83.33	0.48	2	1.0	68.1	6.60	0.85
1.64	164	49	72.0	11808.0	3.28	1.81	112.67	0.37	0	2.0	25.5	9.03	25.69
1.64	164	50	77.9	12775.6	3.55	1.88	96.26	0.44	0	1.0	15.2	6.78	3.98
1.60	160	51	53.5	8560.0	2.38	1.54	170.04	0.47	0	8.0	51.6	6.05	16.35
1.77	177	52	55.6	9841.2	2.73	1.65	84.89	0.32	0	1.0	153.3	8.04	9.57
1.62	162	53	62.7	10157.4	2.82	1.68	111.87	0.45	0	1.0	15.7	6.08	2.23
1.68	168	54	103.0	17304.0	4.81	2.19	138.11	0.49	0	8.0	126.5	7.83	10.35
1.65	165	55	84.1	13876.5	3.85	1.96	81.62	0.44	3	3.0	9.2	9.21	13.03
1.50	150	56	72.8	10920.0	3.03	1.74	64.71	0.76	0	3.0	41.6	8.17	8.50
1.49	149	57	62.4	9297.6	2.58	1.61	102.58	0.67	1,2	12.0	21.5	4.80	5.65
1.78	178	58	93.3	16607.4	4.61	2.15	149.95	0.58	0	1.0	67.3	11.45	13.53
1.52	152	59	50.0	7600.0	2.11	1.45	112.58	0.46	0	1.0	2.1	7.71	19.50
1.60	160	60	67.3	10768.0	2.99	1.73	81.14	0.51	0	2.0	54.3	6.14	7.17
1.60	160	61	67.2	10752.0	2.99	1.73	103.91	0.45	0	1.0	29.6	10.40	6.19
1.75	175	62	70.0	12250.0	3.40	1.84	79.24	0.45	0	1.0	17.9	8.42	3.87
1.83	183	63	113.0	20679.0	5.74	2.40	143.11	0.61	0	2.0	34.1	6.70	0.14

APPENDIX E

Code for data entry

Sex:

Male	= 1
Female	= 2

Smoking:

Current smoker	= 1
Former	= 2
Non smoker	= 3

Exercise

Yes	= 1
No	= 0

Exercise frequency

>3times/week	= 3
1-3 times/week	= 2
<1 /week	= 1
Never	= 0

Antihypertensive

yes	= 1
No	= 0

Family history

Yes = 1

No = 0

Aetiology

Hypertension = 1

Glomerulonephritis = 2

APKD = 3

Lupus nephritis = 4

Unknown = 5

Reflux nephropathy = 6

Miscellaneous = 7

Echo report

Concentric LVH = 1

Eccentric LVH = 2

LV dilation = 3

Systolic dysfunction

Yes = 1

No = 0

Diastolic dysfunction

Yes = 1

No = 0

Valvular abnormality

Mitral regurgitation = 1

Tricuspid regurgitation = 2

Aortic regurgitation	= 3
Aortic stenosis	= 4

Vascular access

Arteriovenous fistula	= 1
Temporary catheter	= 2
Perm cath	= 3
Graft	= 4

Race

Non-blacks (i.e. Whites and Indians)	= 2
Blacks	= 1

Subjects

Controls	= 2
Patients	= 1

Family history

Fhx MI	family history of myocardial infarction
Fhx HTN	family history of hypertension
Fhx DM	family history of diabetes
Fhx CKDd	family history of CKD
LCCA	far wall left common carotid
RCCA	far wall right common carotid
SCCA	sum of the right and left common carotid
LBIF	far wall left bifurcation
RBIF	far wall right bifurcation

LICA	left internal carotid artery
RICA	right internal carotid
nwLCCA	near wall left common carotid
nwRCCA	near wall right common carotid
nwLICA	near wall left internal carotid
nwRICA	near wall right internal carotid artery
nwRBIF	near wall right bifurcation
nwLBIF	near wall left bifurcation
PLQ Score	plaque score
syst dysf	systolic dysfunction
diast dysf	diastolic dysfunction
HT SQ	height squared
HD dur	haemodialysis duration

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