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Cardiovascular risk profile of kidney transplant recipients at the Charlotte Maxeke Johannesburg Academic Hospital.

Muhammad, Aminu Sakajiki

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CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

Cardiovascular diseases (CVD) are more common in kidney transplant recipients (KTRs) than in the general population. Similar to the general population, the majority of deaths following renal transplantation are due to CVD, accounting for more than 50% mortality. A number of traditional risk factors such as smoking, hypertension, diabetes mellitus and dyslipidaemia, are known to be associated with an increased risk. Premature cardiovascular disease (CVD) is also common after kidney transplantation and young transplant recipients (aged 35–45 years) experience an almost 10-fold increase in cardiovascular disease-related mortality, and as kidney allograft survival improves, death with a functioning graft due to CVD has become an important cause of graft loss [1, 2]. Cardiovascular death rates underestimate the full impact of this disease process, given the large number of non fatal events including acute myocardial infarction (MI), cardiac arrhythmias, heart failure, and stroke that impacts on quality of life.

The high incidence of CVD in the KTRs can be attributed to three categories of risk factors. Firstly, there are the traditional risk factors such as age, gender, dyslipidaemia, diabetes mellitus, family history, smoking and hypertension. Secondly, there are additional risk factors associated with graft dysfunction, either due to recurrence of the original disease, genetic predisposition to progression of renal failure, or to abnormalities occurring secondary to graft dysfunction such as anaemia, proteinuria and hypertension. Thirdly, there are CV risk factors specifically related to immunosuppressive therapy, acute rejection and its treatment [1-3].

In a French study of post-transplant hyperlipidaemia, the incidence of MI in KTRs ranged from 8.1/1000 patient-years in men aged 35–44 years to 15.5/1000 patient-years in those aged 55–65 years, compared with 1.3/1000 and 7.5/1000 patient-years in the general population [2]. Similar results were observed in a Spanish study, in which the overall incidence of MI in KTRs was 7.4/1000 patient-years (prevalence: 3.4%), while the incidence and prevalence of ischaemic heart disease (IHD) were 10.5/1000 patients-years and 5.3%, respectively [3]. These prevalences are substantially higher than those of myocardial infarction (1.35%) and coronary event rates (2.1%) in the general male Spanish population [4–6].

Incident dialysis patients have a high burden of CVD before receiving a transplant. Even those patients without overt disease at transplantation often have been exposed for years to cardiovascular risk factors that place them at a higher risk for overt disease and subsequent events. Effective prevention and management of CVD in KTRs should begin during the pre-transplant period and extend throughout the life of the recipient [3, 7].

1.1 Epidemiology of cardiovascular disease in kidney transplant recipients

Cardiovascular diseases including congestive heart failure, coronary artery disease, cerebrovascular disease, and peripheral vascular disease are common in patients with end-stage renal disease (ESRD), and the risk of cardiac death in dialysis patients has been shown to be 10–20 times greater than that in the general population [8]. This increased risk is likely because of the presence of traditional cardiovascular risk factors such as: hypertension, hyperlipidaemia, diabetes, physical inactivity, smoking, and older age, and is compounded by the presence of

nontraditional risk factors related to poor kidney function ie: altered lipid and calcium-phosphate metabolism, hyperparathyroidism, homocysteinaemia, microalbuminuria, chronic inflammation, anaemia and volume overload [9, 10]. Kidney transplantation constitutes the standard of care for patients with ESRD, as it significantly prolongs patient life, largely by ameliorating the progression of cardiovascular disorders and improving renal function [11]. Kidney transplant recipients have up to a 10-fold reduced rate of cardiac death compared with dialysis patients [11, 12].

Although KTRs have a lower risk of fatal and non-fatal cardiovascular events compared to patients on dialysis [11], they still have a much higher risk in comparison with the general population [12]. Fifty to 60 percent of post-transplant deaths are directly attributable to CVD, with an incidence of IHD of approximately one per 100 person years at risk [3, 11]. The large number of diabetic patients in the end-stage renal disease (ESRD) population, that receive a kidney transplant are at a markedly increased cardiovascular risk compared with non-diabetic transplant recipients [13, 14]. However the cardiovascular risk among transplant recipients who do not have ESRD related to diabetes is still higher than in the general population [13]. Transplanted kidney recipients often have kidney function that remains lower than that of the normal population, and have up to 10 times the rate of cardiac death and 50 times the annual rate of fatal or nonfatal cardiovascular events compared to the general population [14]. Nearly 40% of patients have experienced a cardiovascular event at 36 months after renal transplantation [15, 16], with congestive heart failure and coronary artery disease (myocardial infarction) being the most common events. The prevalence of cerebrovascular events is still high in patients who have undergone kidney transplantation, and the risk of cerebral haemorrhage is higher than in the

general population [17, 18]. Although the incidence of peripheral arterial disease is lower in these patients, de novo peripheral arterial disease increases the relative risk for death by almost twofold [19]. The complex interplay among renal function, additional cardiac risk factors, and immunosuppressant drugs culminates in elevated cardiac risk in KTRs.

Traditional risk factors for coronary heart disease after renal transplantation were investigated in a report of 403 patients who received 464 kidney transplants during a ten-year period [20]. New atherosclerotic complications developed in 16% of patients [20]. After accounting for pre-transplant vascular disease, multivariate analysis revealed that increasing patient age, diabetes mellitus, male sex, cigarette smoking, elevated serum cholesterol and hypertension were independently associated with post-transplant atherosclerotic cardiovascular disease [20]. The nontraditional risk factors that have been associated with increased cardiovascular risk in various studies include reduced kidney function following transplantation, dialysis duration prior to transplantation, rejection, hyperhomocysteinemia, elevated levels of lipoprotein(a), elevated C-reactive protein and interleukin-6 levels, proteinuria, and low levels of physical activity [21].

Immunosuppressive regimens used to prevent graft rejection can actually undermine the benefits of the functioning organ [22]. Standard immunosuppressant agents used in transplantation, such as calcineurin inhibitors (CNIs) and corticosteroids, are nonselective drugs that affect signaling pathways in multiple cell types, leading to nephrotoxic, cardiovascular, and metabolic side effects that contribute to increased cardiac risk in KTRs [22]. Table 1 shows risk factors for the development of CVD after kidney transplant.

Table 1. Risk factors for the development of CVD after kidney transplantation

Traditional Risk Factors	Transplant-Associated Risk Factors	Emerging Risk Factors
Modifiable/potentially modifiable Obesity Diabetes Hypertension Hyperlipidaemia Smoking ***Non modifiable*** Gender Age Family history	Immunosuppression Graft dysfunction Proteinuria Rejection Anaemia	Inflammation Homocysteine C-reactive protein Advanced glycation end products

Modified from [Shirali AC, Bia MJ. Management of cardiovascular disease in renal transplant recipients. *Clin J Am Soc Nephrol* 2003: 491]

1.2 Framingham risk score

The Framingham risk score is a composite index designed to predict coronary heart disease in the general population based on traditional risk factors of age, gender, cholesterol levels, hypertension, and diabetes status [22, 23]. The FHS [2, 22 and 23] categorized patients into cat-1 and cat-2. Category 1 patients are those with very high risk defined as >30% 10-year CVD event risk. These are patients with established atherosclerosis and include IHD, heart failure (HF), cerebrovascular disease, peripheral vascular disease (PVD), type 2 diabetes mellitus (T2DM), type 1 diabetes mellitus (T1DM) with target organ damage (TOD), chronic kidney disease (GFR < 60ml/min/1.73m^2) and patients with genetic dyslipidaemias. In these patients no further risk scoring is required and they should be treated to a target LDL cholesterol of <1.8 mmol/l.

The second category is further subdivided into low risk (1-2.8% for men and 1-2.9% for women 10 year risk of CVD event), moderate risk (3.3-13.2 % for men and 3.4-13.5% for women 10 year risk of CVD event), high risk (15.6-29.4 % for men and 15.6-27.5% for women 10 year CVD event) and very high risk (>30 % risk for CVD event for both men and women). This last group of patients has an overall score that is equivalent to those with established atherosclerosis.

The Framingham risk assessment score has important limitations in that patients classified as very high risk do not require further classification for management decision. Patients with a markedly elevated single risk factor for example a severely elevated SBP>180 mmHg and or DBP>110 mmHg will be underestimated, or patients with high BP and associated target organ damage. Patients with severe dyslipidaemia also have risk underestimated as the FHS scoring is only accurate up to a total cholesterol of 7.25 mmol/l and triglycerides >5 mmol/l. Family history of premature atherosclerotic disease is not taken into consideration and this also leads to underestimation of overall CVD risk in this patients. Important CVD risk factors like abdominal obesity and glucose intolerance are not taken into account in this scoring system and overall risk is thus underestimated [22, 23].

Although the Framingham risk score performs well in the general population, it generally underestimates the risk of cardiovascular events among KTRs [23]. This prediction error is greatest among patients at highest risk, and is driven in part by an underestimate of diabetes-related risk [2, 22].

In a retrospective study of 1124 KTRs with stable graft function at one year, the observed increase in risk conferred by diabetes was greater in transplant recipients compared with the Framingham Heart Study population (hazard ratios of 2.8 versus 1.5 respectively, among men, and 5.4 versus 1.8, respectively, among women) [2]. The risks conferred by age and smoking were also underestimated by the Framingham risk score, though to a lesser extent [2, 23]. The risk score performed well among low risk patients and young KTRs [24].

In a prospective cohort study of 540 transplant recipients, the Framingham risk score underestimated cardiac events but not stroke, with ratios of observed-to-predicted cardiac events of 1.64 and 0.96, respectively [24]. As noted in the previous studies, the risk score performed least well among the highest risk patients; subgroup analysis showed higher observed-to-predicted cardiac event ratios among patients with pretransplant diabetes, prior cardiac disease or both (2.12; 2.00; 2.74 respectively) [23-24].

1.3 Spectrum of Cardiovascular Disease in Kidney Transplant Recipients

Three types of distinct cardiovascular diseases are highly prevalent in kidney transplant recipients (KTR) that impact on poor outcome. These include alterations in cardiac geometry, atherosclerosis and arteriosclerosis.

1.3.1 Alterations in cardiac geometry

Left ventricular hypertrophy is present in 40–60% of renal transplant recipients, and its persistence in the first year after renal transplantation is associated with reduced patient survival [25]. Left ventricular hypertrophy has also been shown to be the strongest predictor of all-cause mortality, together with diabetes. Left ventricular hypertrophy is inversely correlated with renal function [25, 26]. Improved renal function following renal transplantation ameliorates LVH; however, a degree of LVH is often still present in KTRs and may be exacerbated as graft function declines [26].

Renal dysfunction may increase LVH through hypertension, volume expansion, hyperparathyroidism, and/or altered calcium-phosphate homeostasis [25-27]. Preliminary results from a clinical trial examining the effects of conversion from CNIs to sirolimus showed a significant regression of LVH in the majority of KTRs at 1 year after conversion, which suggests a mechanism of non haemodynamic effect of sirolimus on the left ventricular mass [27].

Eccentric LVH (LVH plus dilatation) occurs to a lesser degree than concentric LVH and is associated with systolic dysfunction. This form of LVH is characterized by an increase in wall thickness that is proportional to the increase in left ventricular diameter. Risk factors for eccentric LVH include volume overload, anaemia and AV fistula [26]. Increased left ventricular mass index is strongly associated with cardiovascular mortality in the general population, dialysis population and kidney transplant recipients [25-27].

1.3.2 Atherosclerosis

Kidney transplant recipients have an increased incidence of atherosclerotic plaques and other vascular alterations compared with the general population [20]. Atherosclerosis describes the association between fatty degeneration and vessel stiffness [20]. This process affects medium and large sized arteries and is characterized by patchy intramural thickening of the subintima that encroaches on the arterial lumen. The earliest visible lesion of atherosclerosis is the fatty streak, which is due to an accumulation of lipid-laden foam cells in the intimal layer of the artery which, with time, evolves into a fibrous plaque [20, 21]. Ultimately the lesion evolves to contain large amounts of lipids; if it becomes unstable, denudation of the overlying endothelium, or plaque rupture, may result in thrombotic occlusion of the overlying artery. Atherosclerotic lesions are composed of three major components [20, 21]. The first is the cellular component comprised predominantly of smooth muscle cells and macrophages. The second component is the connective tissue matrix and cellular lipids and the third is the intracellular lipid that accumulates within macrophages, thereby converting them to foam cells [21]. The development of these lesions then depends on inflammatory stimuli, subsequent release of cytokines, proliferation of smooth muscle cells, synthesis of connective tissue matrix and accumulation of macrophages and lipids [20, 21].

Surrogates of atherosclerosis include carotid intima-media thickness (IMT) which is measured by high resolution B-mode ultrasonography and inducible myocardial ischaemia which is measured by coronary stress test. The gold standard for the diagnosis of coronary atherosclerosis is angiography [20]. Dobutamine echocardiography and combined dypiridamole-exercise

thallium imaging have been shown to be accurate in detecting coronary artery disease (CAD) and predicting future cardiac events [20]. Carotid atherosclerosis can be measured with a B-mode ultrasound and it represents an accurate, direct, and noninvasive diagnostic method for selecting patients with the highest possibility of developing major cardiovascular events [6, 20].

1.3.3 Arteriosclerosis

Loss of elasticity and increased arterial stiffness of the large vessels such as the aorta and the carotids lead to decreased distensibility or compliance [28]. These arterial changes are characterized by thickening of the tunica adventitia, decreased elasticity of the tunica media and narrowing of the vessel lumen. Increased arterial stiffness often results in increased pulse pressure causing increased LV afterload and concentric LVH. Arteriosclerosis also predisposes to IHD by decreasing myocardial perfusion in diastole [28]. Arterial stiffness is measured by pulse wave velocity. High systolic blood pressure, pulse pressure, lower diastolic blood pressure and LVH, are all consequences of increased arterial stiffness. Arterial stiffness has been identified as an independent risk factor for cardiovascular morbidity and mortality in the general population, dialysis population and among KTRs [15].

1.4 Risk factors for CVD in KTRs

Most research on risk factors for CVD excludes patients with impaired kidney function. However, data available to date suggest that cardiovascular risk factors established in the general

population are also applicable in KTRs. Indeed, transplant recipients have been shown to have increased numbers of traditional risk factors [7–10], with studies showing that these risk factors can predict the incidence of IHD. In a retrospective study of 638 KTRs it was shown that older age, diabetes mellitus, male gender, anaemia and hypertension were dominant risk factors in the development of congestive heart failure and IHD [11], and an analysis of registry data in the USA showed that older age and comorbidities such as diabetes mellitus, angina or peripheral vascular disease were important risk factors for post-transplant myocardial infarction [12].

However, traditional risk factors do not alone fully predict the incidence of CVD in KTRs [10, 13]. In a retrospective analysis of 1500 transplant patients, calculations of risk based on equations from the Framingham Heart Study (FHS) were significant predictors of IHD ($P<0.001$), but tended to underestimate the actual risk [2]. This was largely related to differences in the risk associated with diabetes mellitus. In the FHS, diabetes increased the risk of IHD in men by 53%, compared with an almost 3-fold increase in KTRs ($P<0.05$), while in women the increase in the risk of IHD was 82% compared with a 5-fold increase in renal transplant recipients ($P<0.05$). Furthermore, having two or more episodes of acute rejection in the first year post-transplant was also associated with a subsequent risk of IHD [2, 20]. In addition, the analysis showed that cigarette smoking had a significantly greater association with IHD in male KTRs than observed in the FHS ($P<0.05$) [2]. Furthermore, in recent years, a number of non-traditional risk factors have been identified that could play an important role in cardiovascular risk in transplant patients.

In a prospective study in 344 renal transplant recipients, a high sensitivity C-reactive protein (hCRP) level of 0.52 mg/dl (relative risk 2.78; p=0.007) and a homocysteine level of 20.8 mmol/l (relative risk 4.19; p=0.008) were significantly predictive of increased risk [20, 21]. Overall, the FHS equation was a good predictor of cardiovascular events in low-risk patients (observed vs expected incidences, 0.6% vs 0.51%) but underestimated events in high-risk patients (6.4% vs 2.8%). In a second prospective study in 438 KTRs, an increased hCRP level (0.5 mg/dl) was associated with a 53% increase in mortality (P=0.04) compared with those with a lower level, and a body mass index >30 kg/m^2 was also associated with a significant increase in mortality (hazard ratio, 2.67; P=0.005) [2, 20 and 21].

1.4.1 Traditional cardiovascular risk factors

1.4.1.1 Hypertension

Hypertension after renal transplantation is defined according to criteria of the Seventh Report of the Joint National Committee on Prevention, Detection, and Evaluation of High Blood Pressure (JNC VII) as systolic BP (SBP) of 140 mmHg or diastolic BP (DBP) of 90 mmHg [29]. The JNC 7th Report recommends a target BP in patients with kidney disease (presumably including KTRs) of 130/80 mmHg [33, 34]. Given this definition, 75 to 90% of kidney transplant patients have hypertension [15], a figure that has changed little in recent years. In a large cohort (29,751 patients), the Collaborative Transplant Study, Opelz *et al* reported that up to 55% of kidney transplant patients did not reach the goal for BP control. Each 10-mmHg incremental rise in

SBP independently increases the risk for death and death censored graft failure in KTR by 18 and 17%, respectively [30].

K/DOQI guidelines suggest a goal BP of 130/80 mmHg for all KTRs with decreased targets considered appropriate for patients with proteinuria [31]. European Best Practice Guidelines specify a BP of 125/75 mmHg in patients with proteinuria [32]. High systolic blood pressure is independently associated with an increased risk of cardiovascular death in KTRs, whereas target blood pressure is associated with less cardiovascular death [30]. Hypertension at 1 year is a strong predictor of graft survival, even after controlling for renal function or previous acute rejections [15, 29]. Kasiske et al, found that each 10 mmHg increase in systolic blood pressure was independently associated with an increased risk of death-censored graft failure. The mean arterial pressure seems to be the most meaningful measurement and has shown an association with greater CV risk in KTRs; for every 10 mm Hg increase in MAP, the CV risk increases by 25% [33]. In addition, greater pulse pressure, (defined as the difference between systolic and diastolic BP) carries an independent direct association with all-cause mortality [33, 34]. Several studies have highlighted that as many as half of prevalent KTRs have BP that exceeds 140 mm Hg systolic and/or 90 mm Hg diastolic, despite prescriptions for at least 2 different BP medications [33, 34].

1.4.1.2 Dyslipidaemia

Similar to the normal population, elevated total cholesterol is associated with an increased risk of IHD in KTRs. Dyslipidaemia affects up to 74% of KTRs [35]. Kidney transplant recipients typically have increased levels of total cholesterol including LDL, VLDL and triglycerides. HDL often remains at normal levels, but can be elevated as well [35]. In these patients, dyslipidaemia correlates with the development of atherosclerosis in nontransplant vessels, as well as in the transplanted organ. Dyslipidaemia may further contribute to chronic allograft dysfunction. Posttransplant hypercholesterolaemia and hypertriglyceridaemia are independent risk factors for graft loss [36, 37].

Studies evaluating the associations between dyslipidaemias and adverse CV outcomes have demonstrated heterogeneous results. Roodnat studied 676 prevalent KTRs with a functioning graft at 1 year and found that higher serum cholesterol levels were associated with greater all-cause mortality [37]. Booth et al described a similar association between pretransplant total cholesterol concentrations and mortality [38]. In contrast, Schaeffner and colleagues found no associations between pretransplant cholesterol or triglyceride concentrations and patient survival [39].

The ALERT (Assessment of LEscol in Renal Transplantation)) trial -was conducted to settle this issue [40]. In several European centers, 2,102 KTRs with total cholesterol concentrations between 4-9 mmol/L were randomly allocated to receive 40 mg of fluvastatin or placebo.

Patients were followed for >5 years to primary endpoint of cardiac death, earliest non-fatal [MI], or coronary revascularization procedure). The trial failed to detect a difference between the randomized treatment and the primary study endpoint (p= 0.13). However, secondary analyses, revealed significant reductions in the rates of cardiac death (p= 0.03), definite MI (p= 0.05), and the combination of these (p= 0.005), while there was no effect on the rate of coronary revascularization. Due to the ambiguity of the study results, patients were offered to continue the study and patients in both randomization groups were offered open-label fluvastatin, 80 mg [40]. The results from this ALERT Extension study were significant; after 6.7 years of follow-up. On average, patients originally randomized to fluvastatin experienced a 21% reduction in the risk of the primary study endpoint compared with those in the original placebo arm (p= 0.036) [40, 41].

Statins do differ with regard to their safety, especially when considering drug interactions with certain immunosuppressant medications. Statins that are metabolized by the cytochrome P450 (CYP) 3A4 enzyme ie, lovastatin, simvastatin, and atorvastatin (but not fluvastatin and pravastatin), may be subject to decreased metabolism in the presence of calcineurin inhibitors [42]. The ALERT trial demonstrated the exquisite safety profile of fluvastatin specifically in KTRs [35] as a result, it is suggested that fluvastatin be regarded the standard statin in this specific population, or at least in those KTRs whose immunosuppression includes a calcineurin inhibitor.

Clinical guidelines recommend that all KTRs be treated to reach an LDL-cholesterol concentration <2.6mmol/L and no differentiation is made regarding primary or secondary prevention [42, 43]. It is also recommended that statin dosages be reduced in KTRs whose estimated glomerular filtration rate (GFR) is <30 or if cyclosporine is part of the concurrent regimen [42, 43].

1.4.1.3 Diabetes Mellitus

Patients with pretransplant diabetes have a greatly increased cardiac risk, which is estimated to be 2–5 times greater than the risk in nondiabetic KTRs. The risk is similar for patients who develop new-onset diabetes after transplantation (NODAT) [44]. Patients with pretransplant diabetes had a hazard ratio (HR) of 1.13 for post-transplant MI versus an HR of 1.60 for patients who developed NODAT [44]. In a retrospective analysis of 398 patients transplanted at the Charlotte Maxeke Johannesburg Academic Hospital over a ten year period, the incidence of NODAT was 15.6% with a mean time to onset of 22.9 months. Patients with NODAT had higher infection rates and overall mortality [45].

In a prospective study of 201 consecutive KTRs for 3 months after transplantation, it was found that patients with pretransplant diabetes mellitus and patients with NODAT had 5 times and a 3-fold increase in mortality and rate of major cardiac events respectively when compared to patients who were free from diabetes [46]. Despite similar patient survival at 12 years, graft survival was lower in patients with NODAT compared with nondiabetic patients (48% vs. 70%)

[46]. While the adequate control and monitoring of KTRs with preexisting diabetes is clearly relevant, prevention of a new occurrence of diabetes after kidney transplantation appears to be even more important [46].

NODAT appears predominantly related to 2 distinct factors: weight gain after transplantation and use of immunosuppressive drugs that impair glucose tolerance for eg, corticosteroids or CNIs. Cosio and colleagues further refined this observation by studying 490 patients who were free from diabetes at transplantation and stratifying them by the presence of posttransplant impaired glucose tolerance or overt NODAT. They found that there was a steady increase in cardiovascular risk with increasing fasting glucose impairment and the risk was greatest in patients who developed posttransplant overt diabetes mellitus [47].

Thus, even early indicators of impaired glucose tolerance are important risk factors of future CV risk and should be treated seriously. The risk of NODAT, particularly, is driven by the use of corticosteroids as part of the immunosuppressant regimen. As a result, efforts to reduce corticosteroid dosages or eliminate these medications altogether have been implemented.

1.4.1.4 Obesity

Weight gain after transplant is common, and may be related to improved appetite with reversal of uraemia and relatively high steroid doses in the peri-transplant period, but is also associated with physical inactivity [48]. The metabolic syndrome was identified among 63 percent of 606 prevalent KTRs assessed at a median of six years post-transplant at one center [49]. The relationship between body mass index (BMI) and CV events in KTRs is U-shaped ie, both underweight and obese patients experience greater CV event rates compared to those with normal BMI [50]. In a study of nearly 52,000 patients in the USRDS registry, the adjusted risk of cardiac death increased at both low BMI (adjusted hazard ratio (HR) 1.3 for BMI <20) and high BMI (adjusted HR 1.2 for BMI 30 to 32; adjusted HR 1.4 for BMI >36) compared with the reference group with BMI 22 to 24 [51]. High BMI was independently associated with increased risk of congestive heart failure (CHF) and atrial fibrillation (AF) in several USRDS registry studies [51, 52]. As an example, BMI greater than or equal to 30 predicted up to 59 percent relative risk increase compared with BMI less than 30 [51]. In other studies of USRDS data, BMI greater than 28 independently predicted a 57 and 79 percent relative increases in the risk of hospitalized CHF and AF, respectively, compared with lower BMI [52, 53].

High BMI correlates with diabetes, high BP and plasma lipids as well as proteinuria and graft dysfunction [50]. A combined assessment of BMI and waist circumference may enable differentiation of visceral adiposity from muscle and/or nonvisceral fat mass [50]. In a study by Kovesdy et al, higher BMI was associated with lower mortality after adjustment for waist circumference (0.48 [0.34, 0.69], $p < 0.001$) [54], and higher waist circumference was more

strongly associated with higher mortality after adjustment for BMI (2.18 [1.55–3.08], $p < 0.001$) [54]. The association of waist circumference with mortality remained significant after additional multivariable adjustments. Higher BMI and waist circumference display opposite associations with mortality in KTRs. Waist circumference appears to be a better prognostic marker for obesity than BMI [54].

1.4.1.5 Smoking

In a study by Kasiske et al, in 1334 patients; the 24.7% prevalence of smoking at the time of transplantation was similar to that in the general population. After adjusting for multiple predictors of graft failure, smoking more than 25 pack-years at transplantation (compared to smoking less than 25 pack-years or never having smoked) was associated with a 30% higher risk of graft failure (relative risk 1.30; 95% confidence interval [CI], 1.04 to 1.63; $p= 0.021$) [55]. The relative risk for major CVD events with smoking 11 to 25 pack-years at transplant was 1.56 (95% CI, 1.06 to 2.31; $p= 0.024$), whereas that of smoking more than 25 pack-years was 2.14 (95% CI, 1.49 to 3.08; $p< 0.001$).

In another study, the impact of smoking was determined as a major cardiovascular risk factor, on patient and renal graft survival. The study population included all adult recipients of first cadaveric kidney transplants from 1984 to 1991 [56]. By selection, all patients were alive and had a functioning graft for at least 1 yr after transplantation. Smoking history was gathered prior to transplantation. The follow-up period was 84.3 ± 41 months and during this time 28% of the patients died and 21% lost their graft [56]. By univariate and multivariate analysis, patient

survival, censored at the time of graft loss, correlated with these pre-transplant variables: age ($p<0.0001$); diabetes ($p= 0.0002$); history of cigarette smoking ($p= 0.004$); time on dialysis prior to the transplant ($p= 0.0005$); and cardiomegaly by chest X-ray ($p= 0.0005$) [56]. Cox regression showed that, patient survival time was significantly shorter in diabetics ($p<0.0001$), smokers ($p= 0.0005$), and recipients older than 40 years [56]. Patient death was the most common cause of kidney transplant failure in smokers, in patients older than 40 years, and in diabetics. The prevalence of different causes of death was not significantly different between smokers and non-smokers. In conclusion, a history of cigarette smoking correlates with decreased patient survival after transplantation, and the magnitude of the negative impact of smoking in KTRs were quantitatively similar to that of diabetes [56].

1.4.1.6 Age

Cardiovascular disease is generally a disease of middle age and the elderly in the general population [1, 2, and 5]. In the United States and Europe, patients are started on renal replacement therapy with an average age of 60 years [2, 3]. In South Africa, patients above the age of 60 years were excluded from renal replacement therapy in the public sector due to limited resources [57].

1.4.1.7 ***Physical inactivity***

In the KTRs, it has been shown that lack of physical exercise adds significantly to their increased cardiovascular risk [2, 3]. Low physical activity (PA) as a risk factor is largely unexplored in KTRs. Dorien et al found that independent of age, PA was inversely associated with the metabolic syndrome, history of CVD, fasting insulin, and triglyceride concentrations, and positively associated with kidney function and 24-hour urinary creatinine excretion (*i.e.*, muscle mass) [58]. In Cox regression analyses, adjustment for potential confounders including a history of CVD, muscle mass, and traditional risk factors for CVD did not materially change these associations [58]. Low PA is strongly associated with increased risk for cardiovascular and all-cause mortality in KTRs.

1.4.1.8 Gender

The higher risk for CV disease among men in the general population was also seen in the KTRs [2, 6 and 17]. However, when other confounders were eliminated with a multiple regression this significance was lost.

1.4.2 NON-TRADITIONAL CARDIOVASCULAR RISK FACTORS

1.4.2.1 Graft function and cardiovascular disease

A US study in 8786 individuals from a national database showed that mortality rates increase with increasing proteinuria and decreasing glomerular filtration rate (GFR) [59]. Cardiovascular disease-related mortality rates increased from 6.2 deaths/1000 person-years in patients with a

urinary protein level <0.030 g/L to 37.2 deaths/1000 person years among those with a urinary protein level of >0.3 g/L. Similar results were seen with regard to GFR, with mortality rates varying from 4.1 deaths/ 1000 person-years in those with a GFR of 90 ml/min to 20.5 deaths/1000 person-years among participants with a GFR <70 ml/min [29, 59]. After adjusting for confounding variables, there was a trend towards increased all-cause mortality and CVD-related mortality with increasing proteinuria ($p<0.001$ and $p<0.002$, respectively) and decreasing GFR (both $p<0.001$) [59]. Data from the ALERT study also shows an increase in cardiac death, non-cardiovascular death, all cause mortality and major adverse cardiac events with increasing serum creatinine, particularly in patients with a serum creatinine level >200 mmol/l (2.3 mg/dl; $p<0.0001$) [41]. However, it is important to remember that the patients in these studies had been on dialysis for a considerable period of time before receiving a kidney transplant, and thus the subsequent cardiovascular risk may have been influenced by the effects of CKD and dialysis.

Decreased renal function can cause and/or exacerbate hypertension, dyslipidaemia, anaemia, hyperglycaemia, and LVH, all of which are well established risk factors for CVD [7, 29, and 59]. It is difficult to separate the interrelated effects of renal dysfunction and hypertension. Renal dysfunction affects hypertension via volume expansion, sodium retention, increased circulating vasoactive substances, and effects on the sympathetic nervous and renin–angiotensin–aldosterone systems (RAAS) [7]. Increased blood pressure can then cause additional renal damage, which further reduces GFR, creating a vicious cycle.

Decreased renal function and the uraemic state can affect lipase function and increase insulin resistance, leading to hyperglycaemia [60]. This can lead to reductions in high-density lipoprotein (HDL) cholesterol levels with hypertriglyceridaemia, including an accumulation of partly metabolized triglyceride-rich particles [60]. Patients with ESRD can have a highly abnormal, pro atherogenic cholesterol sub fraction profile, typically of small, dense low-density lipoprotein (LDL) particles. Lipid and apolipoprotein abnormalities increase in severity as renal function deteriorates [60]. Table 2 shows kidney function and the risk of CVD from different studies.

Table 2. Kidney function and CVD risk.

Study	**Renal function measure**	**RR (95% CI)**	**P-value**
Meier-Kriesche et al [7].	Serum creatinine at 1 year	For cardiovascular death:	
	1.5-1.6 mg/dl (133-141 umol/l)	1.19 (1.02-1.39)	0.025
	1.7-1.8 mg/dl (150-160 umol/l)	1.37 (1.16-1.62)	<0.001
	1.9-2.1 mg/dl (168-186 umol/l)	1.49 (1.25-1.76)	<0.001
	2.2-2.5 mg/dl (195-221 umol/l)	1.67 (1.38-3.03)	<0.001
	2.6-4.0 mg/dl (230-354 umol/l)	2.26 (1.85-2.75)	<0.001
Fellstrom et al [60].	Creatinine increase per 100umol at any time	For MACE: 1.89 (1.42-2.55)	<0.0001
		For cardiac death: 2.94 (2.01-4.31)	<0.0001

CI, confidence interval; MACE, major adverse cardiac event; RR, relative risk

1.4.2.2 Proteinuria

Post transplant proteinuria at 1 year was detected in 15.3% of patients in the Spanish Chronic Allograft Nephropathy Study. The main cause of death was vascular disease and proteinuric patients had higher relative risk for both graft failure and death [61]. The risk of death from all cause and from CVD was increased with increasing amounts of proteinuria [61]. In another study by Suhail et al, glomerular proteinuria and not tubular proteinuria is associated with a significant increase in graft dysfunction and graft loss [62]. Proteinuria is a significant risk factor for graft loss, cardiovascular events and death [62]. Even minor proteinuria is associated with poorer outcomes; early proteinuria is indicative of kidney injury [62], and microalbuminuria is associated with inflammatory markers, such as C-reactive protein (CRP) and cardiovascular risk factors [62]. Blockers of the RAAS-system effectively lower proteinuria, although their effect on graft outcome was unclear in retrospective studies [63]. The use of mTOR inhibitors is associated with a higher frequency of proteinuria, it is unclear to what extent mTOR inhibitor-associated proteinuria is clinically relevant and further research on the mechanisms and treatment is warranted [64].

1.4.2.3 Anaemia

Anaemia is common after renal transplantation and is frequently under-treated [65]. Some studies, for example, have reported incidences of anaemia that approach 40% at one year post-transplant [66, 67]. Despite the belief that KTRs receive more care from nephrologists, iron status evaluation and appropriate erythropoietin therapy occurred in only 25 percent of the patients [67]. Left ventricular hypertrophy, an important risk factor for cardiovascular mortality among patients with CKD, may be, in part, a consequence of untreated anaemia [67]. Treatment

of anaemia using iron therapy and erythropoietic stimulating agents (ESA) such as erythropoietin or darbepoetin has been hypothesized to decrease the cardiovascular morbidity and mortality in KTRs [67, 68].

Rigatto et al assembled a retrospective cohort of 638 incident KTRs who were free from CV disease at 1 year after transplantation; the outcomes for the study were de novo CVD, de novo congestive heart disease, and mortality [66]. After multivariate analyses, lower haemoglobin concentrations were associated with an increased risk in all 3 outcomes, but due to the relatively small number of outcomes, only limited adjustments for confounding were possible and most CV risk factors mentioned in the present review were unavailable in that study [66]. The findings of increased CV risk in patients with CKD, who were treated to a relatively higher target haemoglobin concentration (13.3 g/dL) compared with patients treated towards a lower target (11.8 g/dL), were alarming and should promote a conservative approach to using ESA in KTRs as well, until solid trial evidence becomes available [68-70].

1.4.2.4 Carotid intima-media thickness (cIMT)

Increased IMT in the common carotid artery is related to generalized atherosclerosis and increase risk of future MI and cerebrovascular disease. Carotid artery lesions may represent a useful predictive marker of clinical events in KTRs and carotid artery evaluation by B-mode ultrasound has been shown to be a good non invasive marker of CAD and might be routinely included in the management of these patients to identify patients at high risk of CVD.

Over the years, clinical trials have provided outcomes that support the role of c-IMT measurements for predicting cardiovascular events (ie, the thicker the c-IMT, the higher the rate of MI or stroke) [6, 71]. In a metanalysis, Lorenz et al reviewed and summarized the data from more than 37,000 individuals and concluded that increments of 0.1 mm in c-IMT translated to an increased risk of 10% to 15% for having a MI and an increased risk of 13% to 18% for having a stroke [71]. The application of c-IMT has become an accepted, reliable surrogate marker for determination of atherosclerosis and is endorsed by the US Food and Drug Administration and by the European Agency for the Evaluation of Medicinal Products [71].

Carotid-IMT measurements represent the preferred technique for noninvasively assessing atherosclerosis in most clinical trial studies [71]. Holland et al found increased cIMT to be correlated with angiographically proven coronary artery disease in South African black population [72]. This unique and timely study utilized a true ultrasound-based comparative format in which the authors evaluated vessel wall thickness in the coronary arteries (by using intravascular ultrasound) and the carotid arteries (by using extravascular ultrasound). The authors reported a statistically significant correlation between carotid artery IMT and coronary atherosclerosis, providing additional support for the expanded clinical use of c-IMT for assessment of systemic atherosclerosis [72].

1.4.2.5 Measurement of Carotid Intima-media thickness

There are several important differences in the B-mode measurement of cIMT between laboratories. These include the sites of measurement, which are the segments of the artery, the near/far wall, and the angles of interrogation and automated versus manual measurement [73,

74]. 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 measurements for the bifurcation and internal segments have more missing values [73, 74]. The sensitivity of cIMT measurement has ranged from 86% to 100%, with sensitivity increasing with increasing thickness of the arteries, whereas the specificity has ranged from 65% to 100% [74].

1.4.2.6 Left Ventricular Hypertrophy

Left ventricular hypertrophy is present in 40–60% of KTRs, and its persistence in the first year after renal transplantation is associated with reduced patient survival [75]. Left ventricular hypertrophy has also been shown to be the strongest predictor of all-cause mortality, together with diabetes in KTRs [25-27, 75]. Left ventricular hypertrophy is inversely correlated with renal function [75]. Improved renal function following renal transplantation ameliorates LVH; however, a degree of LVH is often still present in renal transplant recipients and may be exacerbated as graft function declines [75]. Renal dysfunction may increase LVH through hypertension, volume expansion, hyperparathyroidism, and/or altered calcium-phosphate homeostasis [76, 77].

1.4.2.7 Rejection

Multiple acute rejection episodes have also been demonstrated to be a risk factor for CVD - a strong argument for the use of immunosuppressive drugs to reduce acute rejection [20]. Acute graft rejection also appears to be a cardiovascular risk factor in KTRs [20]. The use of high

doses of corticosteroids to manage acute graft rejection possibly accounts for this relationship [20, 36]. Poor kidney function from rejection also places the patient at risk for later graft failure and a return to dialysis [14, 20 and 29].

1.4.2.8 Donor type

The living donor is more likely to be better HLA matched than a cadaveric donor and therefore the use of intensive immunosuppression is less likely [11, 12 and 14]. Similarly there is less likelihood of rejection episodes [11]. Furthermore, infections with immune modulatory viruses are also reduced and cardiovascular risk factors are higher among KTRs of cadaver donor [11, 14].

1.4.3 Immunosuppression and Cardiovascular risk

Immunosuppressants have been used in transplantation to prevent rejection of the transplanted organ. They have contributed to the decrease in the frequency of rejection, increased graft survival and longevity of the recipients. They are of different classes with different mechanisms of action and side effect profiles. Higher doses are utilized in the early phases of transplantation, and over time, the immunosuppressive agents are gradually decreased in an effort to minimize side effects without compromising graft function.

1.4.3.1 Effects on renal function

The majority of KTRs are treated with calcineurin inhibitors (CNIs), either cyclosporine (CsA) or tacrolimus. The nephrotoxic effects of CNIs are well established and are known to reduce graft function after renal transplantation [78]. The long-term nephrotoxic effects of CNIs are further examplified by declining renal function in CNI-treated recipients of nonrenal grafts [79]. Following 10 years of treatment with CNIs after heart or liver transplantation, approximately 5–10% of patients develop ESRD, and almost one-third experience poor renal function (GFR < 30 ml/min) [79]. Calcineurin inhibitors potentially contribute to the risk of cardiovascular events through the development of new-onset diabetes mellitus, hypertension and hyperlipidaemia [11, 15]. The extent to which CNIs affect these risk factors is difficult to establish since pre-existing renal disease and concomitant immunosuppressive agents such as steroids also exact an effect [11, 51]. The risk of hypertension and hyperlipidaemia is reported to be 5% higher with cyclosporine than tacrolimus [11]. An approach to the choice of CNIs, by which cyclosporine or tacrolimus are selected, should be based on patient's particular risk profile. Tacrolimus and cyclosporin have similar overall nephrotoxic profiles. A study comparing patients treated with cyclosporine versus tacrolimus found a high prevalence of chronic allograft nephropathy in both groups and similar histopathologic changes [80]. Despite similar histologic changes, renal function in some studies was marginally better preserved with tacrolimus compared with cyclosporin, possibly owing to less vasoconstriction [80, 81].

1.4.3.2 Effects on diabetes

Several immunosuppressants exert pathogenic effects on the physiology of glucose metabolism, resulting in deleterious effects on insulin secretion (tacrolimus, cyclosporine, sirolimus) and

insulin sensitivity (corticosteroids, tacrolimus) [82-84]. In the FREEDOM study, three corticosteroid strategies were compared: complete steroid avoidance, early steroid withdrawal on Day 7, and continued standard steroid therapy, all in combination with other immunosuppressants, including cyclosporine [85]. A significant 7.9% reduction in the incidence of insulin-requiring (but not overall) NODAT was observed after steroid withdrawal versus low-dose steroid therapy. Calcineurin inhibitors are also associated with impaired glucose metabolism and NODAT [81, 82]. Calcineurin signaling is critical for pancreatic cell growth and function. Cyclosporin reduces pancreatic beta cell volume, decreases insulin synthesis and secretion, and may alter glucagon production of pancreatic alpha cells [82]. Tacrolimus may cause insulin resistance, hyperinsulinaemia, morphologic damage to beta cells, enhanced glucagon production, and impaired insulin synthesis and secretion [82].

It remains unclear whether sirolimus has diabetogenic properties and to what extent. There are reports that sirolimus is associated with diabetogenic risk [86], and mammalian target of rapamycin (mTOR) inhibitors have been shown to increase insulin resistance in vitro and preclinical models [86]. However, data from prospective studies have not identified a strong association between mTOR inhibitor therapy and the development of NODAT, calling into question the overall diabetogenic effects of these agents [87].

1.4.3.3 Effects on hypertension

Immunosuppressive therapies are implicated in the induction and persistence of hypertension post-transplant [88]. The incidence of hypertension increases with CNI therapy, from 42–60% with azathioprine to 63–78% with cyclosporin at 1 year post-transplant [88]. At 5 years post-

transplant, 70–85% of cyclosporin treated patients have been reported to be hypertensive and tacrolimus appears to exert a slightly lesser hypertensive effect than cyclosporin [88]. A meta-analysis shows that tacrolimus causes similar rates of hypertension to cyclosporin [89],

1.4.3.4 Effects on dyslipidemia

Alterations in the lipid levels of KTRs typically occur early post-transplant and are likely a consequence of immunosuppressant effects on lipid metabolism [90]. Steroids, especially in combination with cyclosporin, are associated with increased total cholesterol and a dose-dependent correlation between cholesterol levels and steroid dose has been observed [90]. Steroids may affect lipid levels by altering the activity of acetyl-coenzyme A carboxylase, free fatty acid synthetase 3-hydroxy-3-methylglutaryl coenzyme A reductase, and lipoprotein lipase, resulting in increased levels of VLDL, total cholesterol, and triglycerides [84, 90].

Early studies showed that cyclosporin increased cholesterol levels by up to 45% by 3 months post-transplant [91]. Cyclosporin may affect cholesterol levels by reducing bile acid synthesis from cholesterol, which, in turn, may limit the clearance of circulating cholesterol via reduced transport to intestines [91]. Cyclosporin increases circulating LDL cholesterol levels by reducing synthesis of the LDL receptor, and increases oxidation of LDL cholesterol, leading to larger circulating LDL particles and increased cardiac risk [84, 90]. The effects of cyclosporin on hepatic lipase and lipoprotein lipase activities may cause impaired clearance of VLDL and LDL cholesterol [91]. Tacrolimus appears to exert a lesser effect on lipid levels than Cyclosporin [88, 89]. Hyperlipidaemia is a significant side-effect of mTOR inhibitors and appears to be dose-dependent [92]. A review of multiple studies showed that approximately

twice as many patients receiving mTOR inhibitors required lipid-lowering drugs than those patients not receiving these inhibitors [92].

Furthermore, sirolimus and everolimus exacerbated lipid levels when used in conjunction with cyclosporin and steroids. As such, mTOR inhibitors may affect dyslipidaemia differently depending on whether they are used alone, with steroids or cyclosporin, or with tacrolimus [92]. The mechanisms underlying the effect of mTOR therapies on lipids remain unclear, but may include reduced catabolism of lipoproteins and/or increased production of triglycerides and secretion of VLDL [92]. Also, sirolimus may interfere with insulin signaling, possibly disturbing lipid metabolism [92]. It remains unclear whether the mTOR inhibitor-induced dyslipidaemia is associated with an increased cardiovascular risk compared with other immunosuppressants.

1.4.3.5 Effects on anaemia

Anaemia is a well-known side-effect of both azathioprine and mycophenolate mofetil (MMF), resulting from impaired bone marrow function. Anemia is also associated with mTOR inhibitor use. In a direct comparison between sirolimus and MMF, anaemia was significantly more prevalent with sirolimus use (57% vs. 31%) [93]. The potential mechanisms driving sirolimus-associated anemia include interference with erythropoietin receptor signaling, impaired erythroid cell proliferation, altered iron homeostasis, induction of an 'erythropoietin-resistant' state, and persistent inflammatory response [93].

1.4.3.6 Duration and type of dialysis

Dialysis for more than 1 year before transplantation was found to be an independent risk factor for cardiovascular events after transplantation and peritoneal dialysis was associated with even higher cardiac events than haemodialysis(p=0.0002) [59, 65]. Increasing dialysis duration prior to transplantation is associated with a graded increase in the risk of cardiovascular death after transplant [65, 92]. A likely explanation for this is that accelerated atherogenesis is observed as part of the uraemic syndrome. Pro-atherogenic factors that contribute to the progression of vascular disease prior to transplantation may include hyperhomocysteinaemia, hyperfibrinogenaemia, and increased calcium ingestion, abnormalities of mineral metabolism, dyslipidaemia, and modification of low-density lipoproteins (LDL) by advanced glycosylation end-products (AGE), particularly in diabetic patients [65].

1.4.3.7 C - reactive protein

In the general population, CRP is regarded as a risk factor for cardiovascular events and all-cause mortality [2, 21]. The inflammation markers IL-6 and hsCRP are independently associated with major cardiovascular events and all-cause mortality in KTRs [21]. In a prospective registry-based study, Winkelmayer and colleagues showed that KTRs with a CRP above 5 mg/dl had an increased mortality compared with patients below that threshold, and in a subsequent follow-up paper, the authors demonstrated a J-shaped association between hsCRP (below 5 mg/L) and mortality [94]. In a small study, Ducloux et al reported that those KTRs who have CRP in the highest quartile (>5.2 mg/L) had 2.6-times the risk compared to those in the lowest quartile (<2.1

mg/L) [23]. While CRP is a useful indicator of CV risk in KTRs, no interventions targeting elevated CRP have been studied in this specific population. It is possible that the beneficial effect of aspirin on CV risk is attributable to its anti-inflammatory properties, which are also reflected in lower CRP concentrations [23, 94].

1.4.3.8 Homocysteine

Homocysteine is an amino acid and hyperhomocysteinaemia is present in the majority of KTRs. In one study, three-quarters of prevalent KTRs had an elevated fasting total homocysteine (>12 mmol/L) [20,]. Homocysteine concentrations are directly associated with increased CV risk in the general population as well as in patients with diabetes or in the elderly [2, 21]. Similar associations were recently demonstrated in KTRs, both for CV outcomes and for all-cause mortality [2, 23]. To date, it is unclear whether these associations are actually causal or whether elevated homocysteine is a casual bystander in patients with higher CV risk [20]. Although vitamin supplementation has been shown to reduce homocysteine concentrations in several patient groups, including KTRs, it is unclear whether this intervention would actually reduce CV risk [2, 20, and 21].

Effective reduction in serum homocysteine levels in KTRs may be obtained with the administration of folic acid and vitamins B6 and B12 [95]. However, although hyperhomocysteinaemia is an independent risk factor for cerebrovascular, peripheral vascular and CAD, lowering homocysteine levels has not been shown to decrease cardiovascular risk in

renal transplant recipients [95, 96]. As an example, an NIH-sponsored multicentre randomized trial showed no reduction in all-cause mortality, ESRD, or in a composite outcome including cardiovascular death, MI, resuscitated sudden death, stroke, revascularization, amputation or aortic aneurysm repair, among 4110 KTRs treated with vitamin B6 and vitamin B12 and with either high or low dose folic acid, despite the fact that homocysteine was effectively lowered with high dose folic acid [96].

1.5 Management of Cardiovascular Disease in Kidney Transplant Recipients

1.5.1 Hypertension

1.5.1.1 Lifestyle Modification for Blood Pressure Control

Lifestyle modification for hypertension control includes diet and exercise. Non transplant patients who followed the Dietary Approaches to Stop Hypertension (DASH) diet were demonstrated to have a decrease in SBP (11.4 mmHg) and DBP (5.5mmHg) [97]. This diet, which features a high intake of fruits and vegetables and a low intake of fats, is now recommended for all patients with hypertension by national guidelines, including JNC VII [97]. The new DASH diet recommends a sodium intake of 1600 mg/d [97]. Unfortunately, despite its proven efficacy, evidence suggests that the DASH diet is seldom followed. In a study of 4386 hypertensive patients, Mitka et al reported that only 22% of patients adhered to this diet [98]. Smoking cessation and physical exercise have all been associated with improved CVD outcome among KTRs [55, 58].

1.5.1.2 Drug Therapy

No single class of antihypertensive agents has proved to be superior for all KTRs, and the choice depends on the particular patient [99]. The use of calcium channel blockers (CCB) is popular as first-line therapy [33], because they are often used to counteract the vasoconstrictive effects of cyclosporine as well as posttransplant hyperuricaemia [33]. In studies in which improved allograft function has been reported with the use of dihydropyridine (DHP) CCB compared with angiotensin-converting enzyme inhibitors (ACEI), the results are likely explained by the haemodynamic effects of ACEI to decrease GFR and may not represent an advantage for long-term survival [99]. There are some concerns about the use of CCBs. In a retrospective, single-centre analysis, Kasiske *et al.* found that DHP CCBs imposed a relative risk of 2.26 for major IHD events, independent of other variables, including BP [2]. Furthermore, there is a greater risk for proteinuria with these agents compared with ACEIs in nontransplant patients with CKD [33, 99]. Oedema can be a major problem with the use of DHP CCBs in KTRs, and the non-DHP CCBs can delay metabolism and elevate the levels of cyclosporine and tacrolimus [33]. The use of ACEIs and angiotensin II receptor blockers (ARB) in KTRs is more widespread now than in the past. The cardioprotective and renoprotective effects of renin-angiotensin system blockade in the general population and in patients with CKD make the use of these agents attractive in the KTR population; however, there is limited prospective data in the transplant population to confirm these benefits. Regarding cardioprotection, Midtvedt *et al* found equal benefits of lisinopril and Nifedipine in reducing left ventricular mass index after transplantation [100].

In a meta-analysis of the use of ACEIs/ARBs in 1500 KTRs who were enrolled in a randomized controlled trial (RCT), Hiremath *et al* reported a 0.47 g/day reduction in proteinuria [64]. Although caution has been advised in using these agents too early after transplantation, studies have reported no deleterious effect of these drugs on GFR when used in the early posttransplant period [64, 101]. Whether these agents are renoprotective aside from improving proteinuria, as they are in patients with CKD, is controversial. Adverse effects such as anaemia, hyperkalaemia, and decrease in GFR must be monitored with patients who are taking these agents [101]. In KTRs, multiple drugs are often used to meet BP goals [102]. The addition of beta blockers (labetalol or carvedilol) or centrally acting drugs (clonidine) is often needed to achieve control. Selective alpha 1 blockers like doxazosin may be helpful in men who also have prostatism, but monotherapy is associated with a higher incidence of heart failure [102, 103]. Beta blockers are used for patients with IHD [102]. In addition, diuretics are often used and are associated with increases in episodes of prerenal azotaemia [102]. The inhibitory effects of many of these drugs on sexual function, especially thiazides, beta blockers, and clonidine [102, 103], must be considered and discussed with patients. Table 3 shows the different classes of antihypertensive drugs used post transplant, indication, possible drug interactions and side effects.

Table 3. Classes of antihypertensive agents commonly used in KTRs

Parameter	Diuretics	CCB	ACEI/ARB	B-Blockers
Selected examples	Loop: frusemide Thiazide: HCTZ	Non-DHP: Verapamil DHP: Nifedipine	ACEI: Lisinopril ARB: Losartan	Selective: metoprolol Nonselective: carvedilol
Consideration for use in KTRs	Volume overload	CNI induced vasoconstriction	Proteinuria, CHF, LVH	IHD
Adverse effects	Volume depletion	ED, oedema with DHP.	Elevated K+ and creatinine, anaemia	Bradycardia, ED
Interactions with immunosuppressive medication		Non-DHP CCB increase CNI levels		

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; CHF, congestive heart failure; DHP, dihydropyridine; ED, erectile dysfunction; HCTZ, hydrochlorothiazide; IHD, ischemic heart disease; LVH, left ventricular hypertrophy; KTRs, kidney transplant recipients.
Modified from [Shirali AC, Bia MJ. Management of cardiovascular disease in renal transplant recipients. *Clin J Am Soc Nephrol* 2003: 491]

1.5.2 Diabetes Mellitus

The recommendations for glycaemic control in established diabetes were based on glycosylated haemoglobin (HbA1c) <6.5% and fasting plasma glucose 5 mmol/L to 7.2 mmol/L [32]. K/DOQI guidelines are similar, except that HbA1c <7% is considered acceptable [16, 47]. Testing for HbA1c is not recommended in first 3 months after transplantation [16, 47]. Glycaemic control may be difficult for patients to achieve but plays an essential part in reducing adverse outcomes. In non-KTRs, a 1% drop in HbA1c decreases CV mortality by 15 to 20% [104].

1.5.2.1 Lifestyle Modification for Prevention and Treatment of NODAT

There is strong evidence in non-KTRs that lifestyle modification through weight loss and exercise for overweight individuals can prevent diabetes more dramatically and in a more sustained manner than any pharmacologic agent [105]. In a study of patients with overt diabetes, the combination of weight resistance training and aerobic exercise (30 min three times weekly) was more effective than either alone in leading to a 1% decrease in HbA1c [106]. Although there is no direct evidence in KTRs, it seems logical to assume that lifestyle modification would play an important role in the treatment and prevention of diabetes in this population as well [106].

In addition to lifestyle modification, stepwise initiation of oral monotherapy, oral combination therapy, and/or insulin therapy may be required to maintain targets for glycaemic control [105]. The choice of initial oral therapy depends on patient characteristics and physician preference, because no RCTs are available to compare drug classes in KTRs. The K/DOQI guidelines [107] addressed drug therapy for diabetes in patients with CKD, which are also appropriate for KTRs. The risk for hypoglycaemia is highest with insulin and insulin secretagogues such as sulfonylureas and is potentiated with renal insufficiency. Thus, drugs that have hepatic clearance are preferred over those that have renal clearance [105-107]. Meglitinides are a reasonable option. They also enhance insulin secretion but are less hypoglycemic. In many cases, combination therapy is needed, and it is logical to use drugs that increase both insulin availability and insulin sensitivity [107].

Recent data from nontransplant patients attributed increased incidence of CV-related death to use of one of the thiazolidinediones, rosiglitazone [108]. Recently, however, a metanalysis demonstrated that this is not a class effect, because patients who were taking another thiazolidinedione, pioglitazone, had decreased CV-related events and mortality [109]. Although the pioglitazone-treated patients had an increased incidence of heart failure, this did not result in increased mortality [109]. In the light of these findings, it seems safe to continue therapy with pioglitazone. Metformin belongs to the biguanide class of drugs, but its use in renal transplantation has been limited because of its association with lactic acidosis, especially in renal insufficiency and CHF [110]. The literature, however, suggests that the incidence of metformin-associated lactic acidosis, even with renal insufficiency, is rare [110]. Although this drug is usually avoided in KTRs, it may be reasonable to challenge this belief in KTRs with preserved renal function. There are other agents, currently in use and on the horizon, which may also be of use in transplant patients. Alpha-Glucosidase inhibitors reduce hyperglycaemia by blocking intestinal uptake of carbohydrates [111]. To date, no interactions with immunosuppressive drugs have been reported, although renal impairment with serum creatinine of 170umol/l and gastrointestinal adverse effects limit its use [111]. Newer drugs include the glucagon-like peptide-1 receptor agonists such as exenatide (Byetta; Eli Lilly, Indianapolis, IN) and the dipeptidyl peptidase IV inhibitors such sitagliptin (Januvia; Merck, Whitehouse Station, NJ), which may be promising agents for the future [111].

Insulin therapy is required in up to 40% of KTRs [111], and referral to an endocrinologist is recommended [107], although earlier referral should be considered for all patients who are not

achieving target glucose levels. Different preparations of insulin are available, and they are chosen according to the desired effect and half-life. Insulin glargine is relatively newer among the agents currently in use, and because it does not reach peak levels in serum, it has the advantage of providing basal insulin coverage [111]. Statins improve insulin sensitivity and have also been reported to decrease the prevalence of diabetes [21]. In a retrospective study of 300 KTR, Prasad *et al* reported a 70% reduction in the incidence of diabetes with statin use. These data suggest additional benefit with the use of these agents in KTRs [112].

1.5.3 Smoking Cessation

The most successful approaches to smoking cessation involve both pharmacologic therapy (usually nicotine replacement therapy [NRT]) and sustained behavioral therapy (counseling) [2, 113]. The important role of the physician in this process needs to be emphasized. Unfortunately, evidence reveals that only 20% of physicians reportedly provide antismoking advice during an office visit [114]. Physicians should intervene during the pretransplant evaluation visit by providing resources for tobacco cessation. Long-term smoking is a sign of chemical dependence on nicotine, providing the rationale for NRT as pharmacologic therapy [113-115]. Several modalities exist, including transdermal patches, gum, and inhalers, with all displaying nearly equal efficacy [115]. Published studies in the nontransplant literature report that tobacco users are 1.5 to 2.5 times more likely to quit with NRT compared with placebo, but success rates depend on a supportive environment [115]. Although no data are yet available in KTRs with the use of varenicline (Chantix; Pfizer, New York, NY), a partial agonist that selectively binds nicotinic acetylcholine receptors [115], it has been used safely in several

patients. Varenicline is cleared by the kidney, and prescribing information suggests dosage reduction for patients with creatinine clearance <30 ml/min. It has been associated with suicides and carries a FDA blackbox warning since 2009 [116].

1.5.4 Dyslipidaemia Treatment

1.5.4.1 Lifestyle Modification

Lifestyle modifications presented in the National Cholesterol Education Project Plan III (NCEP III) are appropriate goals for transplant patients and include diet, weight reduction, and increased physical activity [117]. Diet composition should contain <200 mg/d cholesterol, <7% saturated fat, plant sterols (2g/d), and increased soluble fiber (10 to 25 g/d) [117]. Although benefits in lipid profile in KTRs have been demonstrated in small studies most transplant patients need drug therapy to reach lipid profile goals [118].

1.5.4.2 Drug Therapy

Benefit of statins use may extend beyond lipid control, including decreased proteinuria [42], decreased CRP, and a decrease in interstitial fibrosis in transplant protocol biopsies [42]. Among different statins, atorvastatin is the most potent and along with pravastatin and fluvastatin does not need dosage adjustment for renal insufficiency. Most statins are metabolized by the same cytochrome P450 system (CP3A4) as cyclosporine, leading to an accumulation of the lipids in plasma and resulting in a greater frequency of rhabdomyolysis [41, 42]. Recent FDA warning

of risk of rhabdomyolysis with statins and calcium channel blockers, drugs commonly used post transplant was issued [42]. Among statins in current use, simvastatin and lovastatin are the most liphophilic compounds, whereas atorvastatin and fluvastatin are less so and pravastatin is hydrophilic [119, 120]. In considering these differences among statins, fluvastatin, pravastatin, and atorvastatin seem to have a more favorable safety profile over simvastatin and lovastatin [120]. K/DOQI guidelines do not exclude any statin from use after renal transplantation but suggest decreasing maximum dosing of all statins in patients who are on cyclosporine or tacrolimus [16]. Choice of statin is also influenced by insurance restrictions and cost [16]. If maximal dosages of statins are not sufficient, then combination therapy with ezetimibe has been shown to be safe and effective in lowering LDL levels in a limited number of KTRs with no adverse effect on kidney function or drug interaction reported [121]. Ezetimibe is available as monotherapy or in combination with simvastatin [Vytorin; Merck (Whitehouse Station, NJ)/Schering-Plough (Kenilworth, NJ)] vytorin is known in South Africa as ezetrol; however, as detailed previously, given the greater potential for rhabdomyolysis as a result of the interaction between cyclosporine and simvastatin, cautious use and close monitoring are especially advised in KTRs on this combination therapy [121]. Managing triglycerides can be a challenge, especially for some patients who are taking sirolimus. Increased risk for rhabdomyolysis has been associated with fibrate therapy for hypertriglyceridaemia, especially with concurrent use of statins [121]. Fibrates may also cause elevations in serum creatinine, especially in cyclosporine-treated patients [42]. Of the fibrates available, gemfibrozil has less of an effect on kidney function in such patients and is preferred [42]. Nicotinic acid (niacin) can cause glucose intolerance and may not be suitable for certain patients [42]. Bile acid sequestrants are not recommended for KTRs because they can interfere with the absorption of immunosuppressants

[42]. Fish oil therapy may be used for patients who are intolerant of conventional therapies but is not likely to be effective monotherapy. A recent meta-analysis of omega-3 use after renal transplantation demonstrated only modest triglyceride lowering and no decrease in CV mortality [122]. Table 4 shows recommendations of NKF working group guidelines for managing dyslipidaemia in KTRs.

Table 4. Management of dyslipidaemia in KTRs: National kidney foundation working group guidelines

Dyslipidaemia	Goal	Initiate	Increase	Alternate
Triglycerides 5.65 mmol/L	Triglycerides <5.65 mmol/L	Therapeutic lifestyle changes	Therapeutic lifestyle changes + fibrate or niacin	Fibrate or niacin
LDL 2.6-3.3 mmol/L	LDL <2.6 mmol/L	Therapeutic lifestyle changes	Therapeutic lifestyle changes + low dose statin	Bile acid sequestrants or niacin
LDL 3.4 mmol/L	LDL <2.6 mmol/L	Therapeutic lifestyle changes + low dose statin	Therapeutic lifestyle changes + maximum dose statin	Bile acid sequestrants or niacin
Triglycerides 2.3 mmol/L and non-HDL 3.4 mmol/L	Non-HDL <3.4mmol/L	Therapeutic lifestyle changes + low dose statin	Therapeutic lifestyle changes + maximum dose statin	Fibrate or niacin

1. HDL=high density lipoprotein; LDL=low density lipoprotein.
Modified from [Kasiske B, Cosio FG, Beto J, *et al*. Clinical practice guidelines for managing dyslipidemias in kidney transplant patients: a report from the Managing Dyslipidemias in Chronic Kidney Disease Work Group of the National Kidney Foundation Kidney Disease Outcomes Quality Initiative. *Am J Transplant* 2004; 4 (Suppl 7):13-53]

1.5.5 Treatment of Anaemia

Anaemia occurs in 20 to 60% of KTRs and contributes to LVH and CHF [123, 124]. Factors that contribute to post transplant anaemia, such as iron deficiency, erythropoietin deficiency, infections, and drugs (ACEI, ARB, sirolimus, and mycophenolate mofetil), must be evaluated and treated accordingly. Preventing worsening of renal function, which increases risk for CVD, must also be addressed in the care of KTRs [123, 124].

1.5.6 Aspirin Therapy in the Management of CVD

Aspirin therapy has been found to be beneficial after MI even in patients who have CKD and may have uraemic platelet dysfunction [125]. In a single retrospective study in KTRs, aspirin therapy was demonstrated by multivariate analysis to be associated with improved allograft function and survival [126, 127]. Current guidelines recommend the use of aspirin, 65 to 325 mg/d, for primary and secondary prevention in KTRs with IHD, diabetes, or other high risk factors for CVD [128]. It remains to be proved whether all KTRs should take aspirin for CVD prevention [127, 128]. The cardio protective effect of aspirin is limited mainly by the risk for serious gastrointestinal bleeding, a risk that may be higher in KTRs (128). If aspirin is to be used in all KTRs, then it would seem prudent to recommend the use of low-dosage aspirin [127-128].

AIMS AND OBJECTIVES

This study was aimed at determining the cardiovascular risk profile of kidney transplant recipients at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) and to determine the correlation of cardiovascular risk with carotid artery intima media thickness.

1.6 **STUDY JUSTIFICATION**

Cardiovascular diseases cause significant morbidity and mortality among KTRs and are an important cause of death with a functioning graft. Risk stratification of these patients is important in instituting appropriate therapeutic interventions and lifestyle modification, so as to stem this important complication of kidney transplantation and prolong both patients and graft survival.

1.7 STUDY OBJECTIVES

1. To determine the prevalence and predictors of cardiovascular risk among KTRs at CMJAH
2. To examine the correlation of cardiovascular risk factors with carotid intima media Thickness

CHAPTER TWO

2.0 MATERIALS AND METHODS

The protocol for this study was approved by the Human Ethics Committee of the University of the Witwatersrand; ethics clearance certificate number M120596

2.1 Study population

Patients aged 18 years and above who received a kidney transplant at the Charlotte Maxeke Johannesburg Academic Hospital between Jan 2005 and Dec 2009. A hundred patients were consecutively selected by convenience sampling.

2.1.1 Inclusion criteria

Adult transplant recipients aged 18 years and above

Able to give informed consent

2.1.2 Exclusion Criteria

Patients whose data is incomplete from the records

Patients who withheld consent

2.2 METHODS

This was a cross-sectional study. Enrollment was started in June 2012 and completed in October 2012. Using a structured interview form, information on age, gender, race, physical activity,

tobacco use, age at diagnosis of renal failure, cause of renal failure, prior cardiovascular event, family history of cardiovascular disease, duration on dialysis before transplant, type of dialysis, and number of transplants was collected. Physical activity was evaluated by asking patients if they engage in any regular exercises such as brisk walking, jogging, swimming or bicycling. Patients were classified into three group based on their smoking history. They were categorized as smokers if they were current smokers, former smokers if they stopped smoking for at least six months and non smokers if they never smoked. Previous CVD event was defined as history of angina, MI, CABG, percutaneous coronary intervention, stroke, peripheral by-pass, peripheral angioplasty or leg amputation. The type of immunosuppression, use of BP medications, statins, ARVs, number of HLA mismatches, biopsy proven rejection were all recorded. Height and weight were measured with the Detecto scale (New York) and body mass index (BMI) was calculated as ratio of weight to height squared. Waist circumference was measured using a tape measure and the point of measurement was the anterior superior iliac spine [55].

2.2.1 Blood Pressure

Blood pressure was recorded at the time of clinic visit using an accusson mercury sphygmomanometer in the sitting position. Blood pressure average of four clinic visits was taken as the patient's actual BP (averaged over 12 months). Pulse pressure was calculated as the difference between the SBP and DBP whereas mean arterial pressure (MAP) was the sum of DBP and one third of the pulse pressure [30]. Hypertension was defined as the finding of any of; systolic blood pressure of more than 140 mmHg, diastolic blood pressure of more than 90 mmHg or the use of antihypertensive medication.

2.2.2 Echocardiography

Echocardiography was done at the cardiology unit of CMJAH using the Philips iE33 machine equipped with a S5-1 1-5 MHz transducer, allowing for M-mode, two dimensional and colour doppler measurements (Philips Corporation USA). Echocardiography was performed by the investigator under the supervision of a cardiologist. These studies were performed on a clinic visit day. All scans were performed with the head of the table inclined at an angle of 15 degrees and the participant rotated 30 to 45 degrees in the left lateral position. Left ventricular internal dimension measurements, in end-diastole (LVEDD), and endsystole (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 [129]. 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 [130]. Left ventricular mass was divided by body surface area (BSA) to calculate the LVMI [129, 130]. 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 [130].

$$LVM = ([LVEDD/10 + IVST_d/10 + LVPWT_d/10]^3 \times 1.04) - ([LVEDD/10]^3 - 13.6)$$
$$LVMI = LVM/BSA$$
$$RWT = (2 \times LVPWTd)/ LVEDD$$

Relative wall thickness (RWT) was used to characterize LVH into eccentric and concentric LVH [130]. Relative wall thickness greater than 0.45 in the presence of LVH was indicative of concentric hypertrophy and eccentric hypertrophy if it was less than 0.45 in the presence of LVH. 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 has 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. Acceleration time is the time from the onset of diastolic flow to the peak of the E-wave. Deceleration time 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 [131]. The normal ranges are E: A ratio of 1.04 ± 0.38 for men and 1.03 ± 0.34 for women [131]. 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%, diastolic dysfunction was defined as abnormal left ventricular relaxation pattern evidenced by the doppler transmitral E:A ratio of less than 1.0 (impaired LV relaxation pattern) or more than 1.5 (restrictive LV filling pattern) [131].

2.2.3 **Carotid intima-media thickness**

Carotid artery IMT was measured using high resolution B-mode ultrasonography with a L3-11MHz linear array transducer (Philips Corporation USA). Patients were examined in the supine position with the neck hyper extended and the head turned 45 degrees from the side being examined. Reference point for the measurement of IMT was the beginning of the dilatation of the carotid bulb. Intima media thickness was taken as the distance from the leading edge of the

first echogenic line to the leading edge of the second echogenic line. Carotid IMT was done by the investigator under the guidance of a cardiologist; this was done at the time of echocardiographic examinations. 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. The argument in favour of this reference point is that it can be assessed in a more reproducible manner and data collection is almost always complete [74]. The linear array transducer generates a measurement of the IMT and displays it on the screen with a percentage success of the procedure, ranging from < 50% success to 100%. For this study a percentage success of >95% was used. The same procedure was done for each side and 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 [74]. Intima media thickness was measured in the plaque-free areas and measurements were carried out on both sides by the researcher and a cardiologist at the Cardiology Unit of the Johannesburg hospital.

2.2.4 Laboratory tests

Serum creatinine, urea, lipids, complete blood count and urinary protein quantification were done at the National Health Laboratory (NHLS) as part of the routine tests for the follow up of patients at the kidney transplant clinic. All samples for serum chemistry were analyzed using ADVIA^{R} Chemistry Systems (Siemens Healthcare Diagnostics Inc). Blood samples were taken

on the morning of a clinic visit, before the patients were seen at the clinic and the imaging studies were done same day after the clinic.

2.2.4.1 Serum creatinine

Serum creatinine was measured using the modified Jaffe method. Creatinine reacts with picric acid in an alkaline medium to produce a red-colored-picrate complex. The rate of complex formation is measured at 505/571 nm and is proportional to the creatinine concentration. This method uses rate blanking and intercepts correction. Rate blanking is used to minimize bilirubin interference. Also, because non-specific serum/plasma protein interactions with this reagent have been found to produce a positive bias of approximately 27umol/l, serum/plasma proteins measurements are automatically corrected by subtracting this value from the results [132].

Reaction equation

$$Creatinine + Picric\ acid \xrightarrow{OH-} Creatinine\text{-}Picrate$$

The normal range for males is 62-106 umol/l and for females is 44-80 umol/l.

2.2.4.2 Serum lipids

Serum cholesterol and triglycerides were determined using enzymatic colometric methods. The cholesterol esters are hydrolyzed by the cholesterol esterase to cholesterol and free fatty acids. The cholesterol is converted to cholest-4-en-3-one by cholesterol oxidase in the presence of oxygen to form hydrogen peroxide. A colored complex is formed from hydrogen peroxide, 4-aminoantipyrine and phenol under the catalytic influence of peroxidase. The absorbance of the complex is measured as an endpoint reaction at 505/694 nm. The triglycerides concentration was measured based on the Fossati three-step enzymatic reaction with a trinder endpoint. The

single-reagent procedure quantitates the total triglycerides including the mono and diglycerides and the free glycerol fractions. The triglycerides are converted to glycerol and free fatty acids by lipase. The glycerol is then converted to glycerol-3-phosphate by glycerol kinase followed by its conversion by glycerol-3-phosphate-oxidase to hydrogen peroxide. A colored complex is formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase. The absorbance of the complex is measured at 505/694 nm. High density lipoprotein (HDL) cholesterol was measured after precipitation of the non-HDL fraction with phosphotungstate-magnesium, while LDL cholesterol was estimated indirectly by the use of Friedewald formular [133, 134] LDL cholesterol = total cholesterol - (HDL cholesterol + triglycerides/5).

2.2.4.3 Complete blood count

Complete blood count was done using automated haematology analyser (ADVIA 2120^{R} PHILIPS CORPORATION USA). This also formed part of routine tests done at every clinic visit [135].

2.2.4.4 Urine protein

Urine protein concentration was measured using a shift in absorption that occurs when pyrogallol red-molybdate complex binds with pronated basic amino groups of proteins at an acidic pH. The increase in absorbance at 596/694 nm is proportional to the concentration of protein in the urine sample.

Reaction equation

$$Pyrogallol\ red + Molybdate + Protein \xrightarrow{H+} Pyrogallol\ red\text{-}molybdate\text{-}protein\ complex$$

Urine creatinine concentration was measured in the same way as serum creatinine using the modified Jaffe equation but without the correction for bilirubin, the result obtained in micromole is converted to millmole per liter. For 24 hour urine protein excretion (UPCR), the ratio of the urinary protein excretion in g/l to that of urinary creatinine in millimole/l from a spot urine sample gives the urine protein excretion per day. Spot urine protein to creatinine ratio (UPCR) has been validated as a better test than 24 hour urinary protein quantification, it is not affected by urine volume or concentration, whereas the latter is prone to errors of collection and it is inconvenient to the patient and laboratory staff [136].

2.2.5 Cardiovascular disease risk score

Cardiovascular disease risk score was calculated using the Framingham scoring system for calculating the 10-year risk of major coronary events in adults without diabetes [24].

2.2.6 STATISTICS

All of the data obtained were analysed using the statistical package for social science (SPSS) for windows software version 17. Data are reported as mean ± SD. Differences in means for continuous variables were compared using Student's t-test, while categorical variables were compared using Chi-square or Fisher's exact tests as appropriate. The relationship between cIMT and CVD risk factors was tested by the generalized linear regression model. This model was used because the variables are both continuous and categorical. Multiple regression analysis was done in addition to univariate analysis. Established risk factors for CVD were analysed. P-value of 0.05 or less was taken as statistically significant and confidence intervals reported were at 95% intervals.

CHAPTER THREE

3.0 RESULTS

3.1 Demographic data of the study population

In this cross sectional study, 100 patients who were transplanted between January 2005 and December 2009 were recruited. There were 63 males (63%) and 37 females (37%). Ninety five (95%) had primary transplant while 5 (5%) were second transplants. The mean age of the study population was 42.2 ± 12.42 with a range of 19 to 70 years. The mean age of male patients was 43.59 ± 11.32 and for female was 39.84 ± 13.93; Table 3.1.

Table 3.1 Mean ages and gender of the different racial group of the study population

	Black (N=77)	**White (7)**	**Asian (5)**	**Coloured (11)**
Race				
Male	51	3	4	5 63
Female	26	4	1	6 37
Mean age (years)				
Male	43.53 ± 11.19	36.67 ± 15.14	40.25 ± 11.03	51.00 ± 10.00
Female	40.58 ± 14.67	39.50 ± 15.20	30.00 ± 0.00	38.50 ± 12.50

p = 0.677 (Anova)

There was statistically significant difference between the age and gender of the study population (p= 0.022). Comparison of race and age showed no significant difference (p= 0.677).

Mean duration on dialysis before transplant was 4. 04 ± 2.41 years; 64% were on haemodialysis and 36% were on peritoneal dialysis at the time of transplantation. Five patients had received a kidney transplant previously.

The distribution of HLA matches of the study population is demonstrated in figure 1.

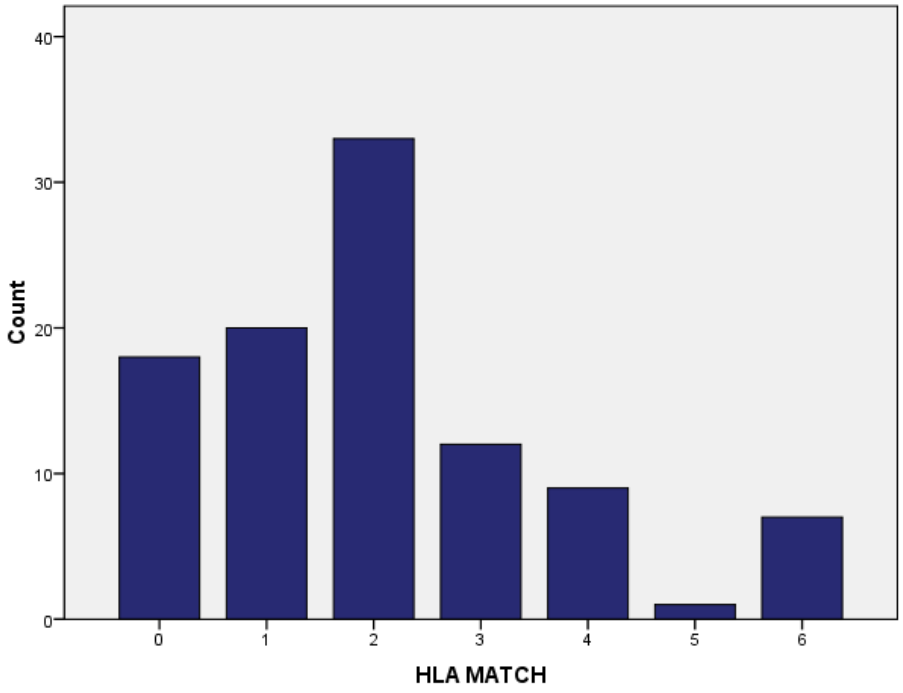

Figure 1 HLA Matching of study population

Thirty three patients (33%) had a HLA match of two out of six while 18 (18%) had complete mismatch. Higher HLA match correlated negatively with SBP (p= 0.027), MAP (p= 0.021) and LVMI (p= 0.001).

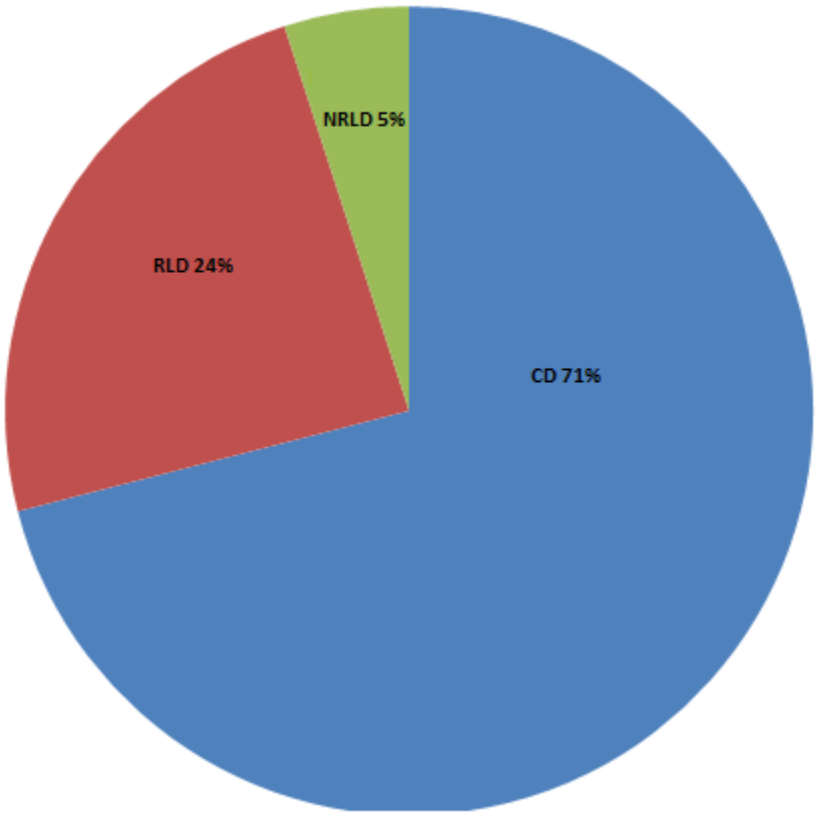

Figure 2. Source of Donor Kidneys

Donor type: RLD; related living donor, NRLD; non-related living donor, CD; cadaver donor

The majority of the study population (71%) received a kidney from a cadaver donor, while related living donors accounted for 24%; 5% was accounted for by non-related living donors and these were mostly spouses; Figure 2. There was a significant association between cadaver donor and higher LVMI ($X^2 = 10.127$, df = 2 and p= 0.006). Rejection episodes and graft function were similar among the different donor types (p= 0.672 and 0.058) respectively.

3.2 Aetiology of End-stage renal disease

Hypertension was the presumed cause of ESRD in 53% of the patients. The cause was unknown in 18% of the study patients (figure 3).

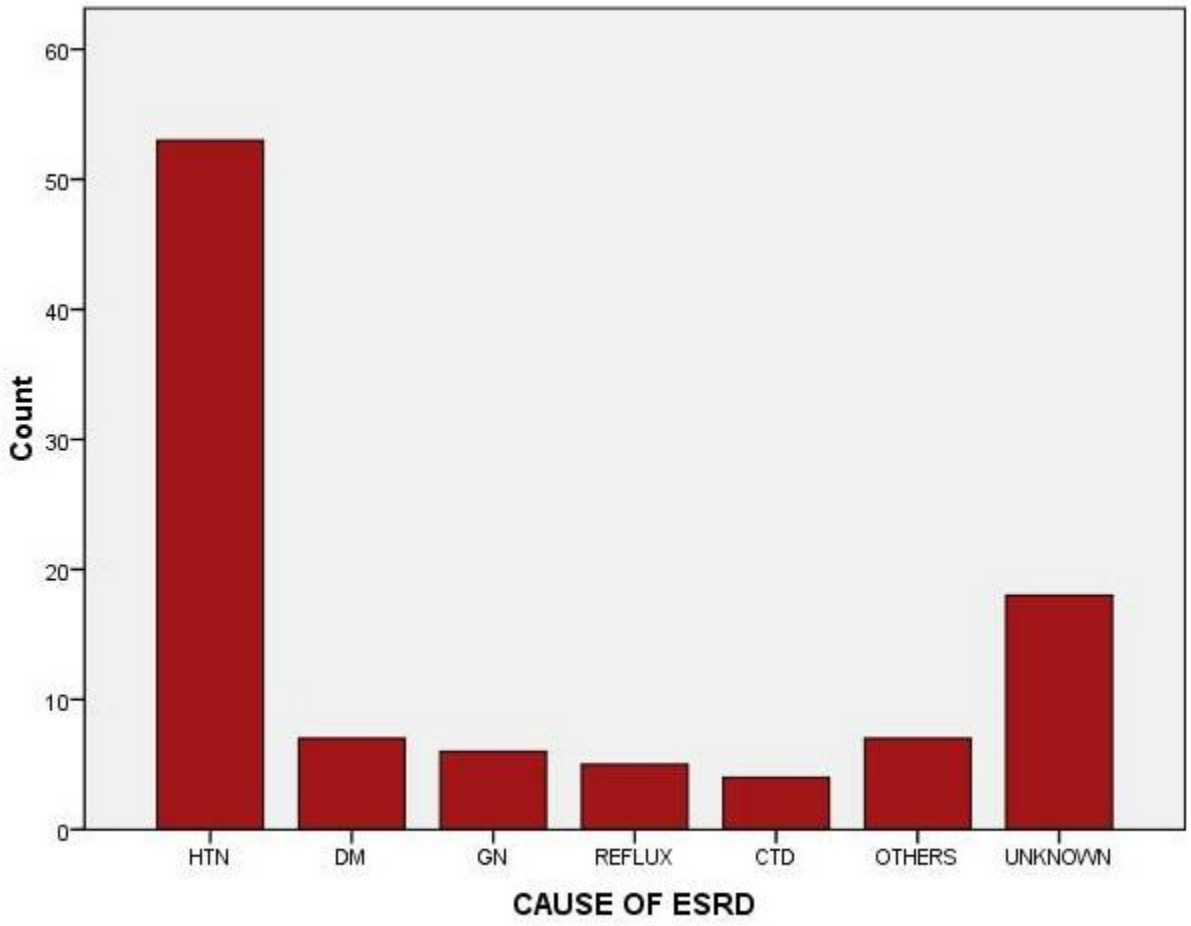

Figure 3. Causes of ESRD in the study population

Abbreviations are: HTN: hypertension; DM: diabetes mellitus; GN: glomerulonephritis; CTD: connective tissue disease; others: congenital anomalies, trauma.

Cardiovascular risk score of the study population:

Seventeen patients were high CVD risk and nineteen were very high CVD risk. Figure 4 shows the 10 year CVD risk of the study population.

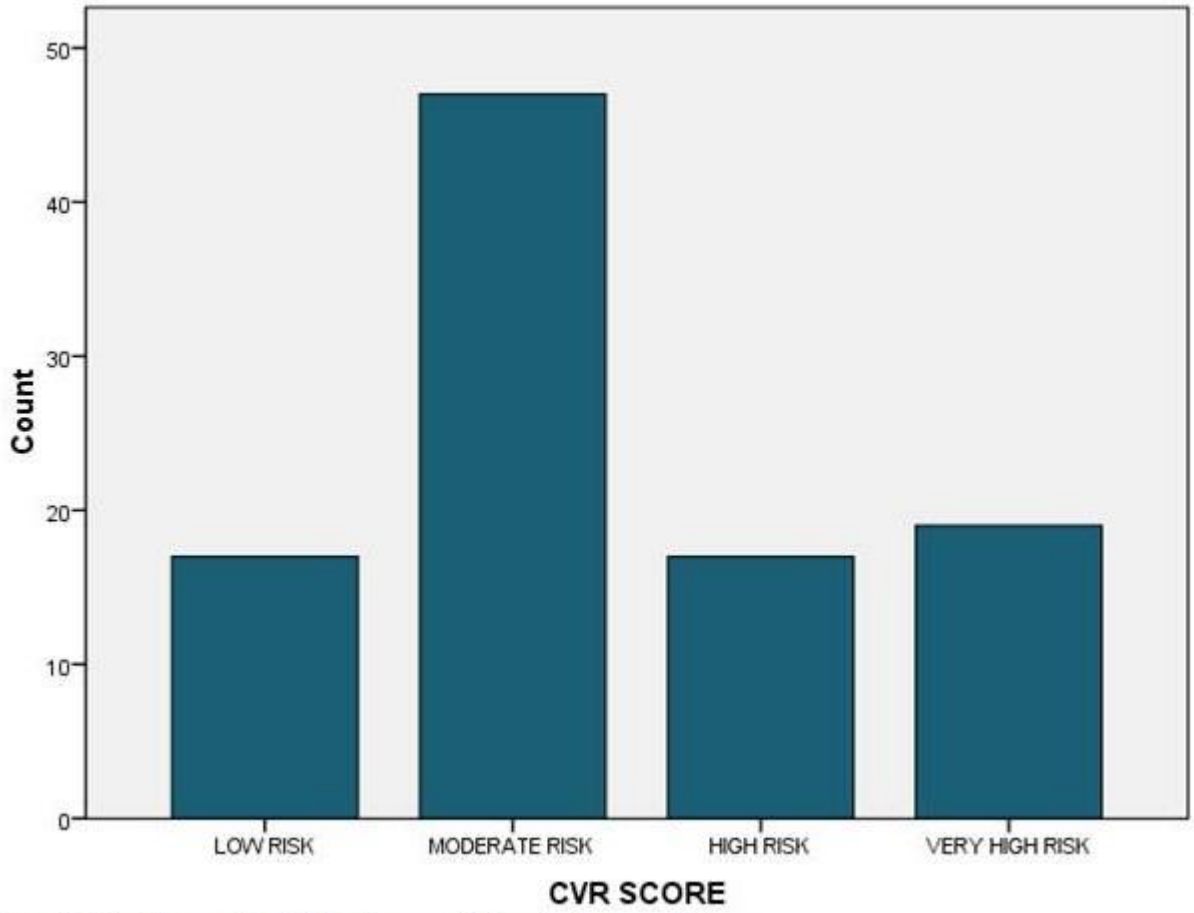

Figure 4. Cardiovascular Risk category of the study population

Low risk 17 (17%); moderate risk (47%); high risk 17 (17%); very high risk 19 (19%)

36 % of patients were found to have high cardiovascular risk while 64% were low to moderate risk.

3.3 Traditional Cardiovascular disease risk factors

3.3.1 History of CVD

The majority of the study population (91%) had no history of CVD, 3 patients (3%) had a previous stroke, 1 (1%) each had heart failure, coronary intervention and stroke, 2 (2%) had heart failure and stroke and 1 (1%) had limb amputation and heart failure. Seventy one (71%) of the study population had no family history of CVD, 13 (13%) reported a family history of stroke, 9 (9%) reported a family history of heart failure, 5 (5%) had family history of acute coronary syndrome and 1 (1%) reported a family history of peripheral vascular disease and arrhythmias.

3.3.2 Hypertension

The systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure and mean arterial pressure (MAP) were significantly higher in the cardiovascular disease high risk groups. The table below shows the mean blood pressures ± SD of the study population.

Table 3.2. Mean ± SD blood pressures of the study population

Mean ± SD	SBP	DBP	PP	MAP
Low risk - N=17	119.18±11.79	76.29±8.98	43.00±7.14	91.59±9.80
Moderate risk - N=47	134.23±15.30	86.34±13.17	49.87±12.18	109.96±13.08
High risk - N=17	137.82±13.40	88.29±10.51	49.53±10.17	105.71±10.60
Very high risk - N=19	136.16±17.11	84.32±13.13	51.37±10.76	101.47±13.93

p=0.021(Anova). SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure; MAP: mean arterial pressure

Eighty seven patients (87%) were hypertensive and on treatment with varying combinations of antihypertensive medications, thirty three of these patients were on at least 3 different medications including calcium channel blockers, alpha blockers, angiotensin converting enzyme inhibitors/angiotensin receptor blockers (ACEi/ARB) and β blockers.

Overall, 46 patients (46%) were on ACEi/ARB and 29 (29%) were on β blockers. Thirty four patients (34%) had SBP of 140-159 mmhg while 7 (7%) had SBP of ≥160 mmhg. Thirty five patients (35%) had DBP of 90-109 mmhg and 3 (3%) had DBP of ≥110 mmhg.

Higher blood pressure had a positive correlation with duration on dialysis (p= 0.017), UPCR (p= 0.001), waist circumference (p= 0.005), cumulative steroid dosage (p= 0.003), serum total cholesterol (p= 0.031), HDL cholesterol (p= 0.011), triglycerides (p= 0.044), LDL cholesterol (p= 0.035), LVMI (p= 0.022) and cIMT (p= 0.002). There was an inverse correlation of higher blood pressure with HLA match (p = 0.027), as with GFR, but tending towards significance (p = 0.055). Thirty patients (30%) were on treatment with statins and 8 patients (8%) were on aspirin. Four patients (4%) were on Anti Retroviral therapy.

3.3.3 Diabetes mellitus

Of the fifteen patients (15%) that were diabetic, 8 had diabetes as the cause of their ESRD, with 7 as new onset diabetes after transplantation. The mean ± SD for HbA1c was 9.31 ± 1.93 g/dl. Twelve of these patients were on insulin and 6 were on oral hypoglycaemic agents, 3 of the diabetics were on combination of insulin and OHAs. The association between diabetes, CNIs and rapamycin was not statistically significant. Diabetes was associated with proteinuria X^2 =5.94. df = 1 and p=0.015.

3.3.4 Obesity

The study population had a mean BMI of 26.40 ± 4.81 kg/m^2 and the waist circumference had a mean of 90.73 ± 14.76 cm. Fourteen (14%) of KTR who were obese had moderate CVD risk profile, 8 (8%) who were obese had high CVD risk while 6 (6%) had very high CVD risk, only 1 (1%) KTR with obesity had low CVD risk. Table 3.3 shows the different CVD risk groups in term of their BMI and waist circumference.

Table 3.3 BMI and waist circumference of the study population

CVD risk group	**BMI (kg/m2)**	**Waist circumference (cm)**
Low risk	23.30 ± 3.51	76.41 ± 10.79
Moderate risk	26.30 ± 4.99	90.59 ± 13.87
High risk	28.71 ± 4.75	96.82 ± 11.38
Very high risk	27.36 ± 4.11	98.42 ± 13.98
Anova	*P=0.007*	*P<0.0001*

There was significantly higher BMI and waist circumference in the higher CVD risk groups.

High BMI showed a positive correlation with LVMI (p= 0.001), serum total cholesterol, LDL cholesterol and triglycerides (p<0.0001). Positive correlation was also seen with cIMT (p<0.0001).

Waist circumference correlated positively with SBP (p=0.005), pulse pressure (p=0.003), MAP (p=0.043) and presence of plaques in the carotid artery (p=0.001). Waist circumference correlated with all the variables that BMI correlated with.

3.3.5 Dyslipidaemia

The mean ± SD total cholesterol of the study population was 4.76 ± 1.31 mmol/L, HDL cholesterol was 1.26 ± 0.46 mmol/L, triglycerides was 1.69 ± 0.87 mmol/L and LDL cholesterol was 2.67 ± 1.04 mmol/L. Fifty patients (50%) had LDL cholesterol levels above 2.59 mmol/l.

Table 3.4 below shows the total cholesterol and HDL cholesterol for the different CVD risk group.

Table 3.4 Serum cholesterol and HDL cholesterol of the study population

Cardiovascular risk group	**Total cholesterol (mmol/l)**	**HDL cholesterol (mmol/l)**	**LDL cholesterol (mmol/l)**
Low risk	3.66 ± 1.03	1.25 ± 0.49	1.80 ± 0.69
Moderate risk	4.91± 1.29	1.27 ± 0.44	2.81 ± 1.11
High risk	5.02 ± 1.35	1.12 ± 0.34	2.88 ± 1.10
Very high risk	5.12 ± 1.11	1.35 ± 0.55	2.94 ± 0.84

p= 0.583(Anova).

There was no significant difference in the lipid profiles of the various risk groups.

3.3.6 Smoking

Ten patients were active smokers, 30 (30%) reported to have stopped smoking while 60 (60%) never smoked cigarettes in the past; table 3.5.

Table 3.5 Shows smoking habit of the study population

SMOKING STATUS	CVR SCORE				
	1	2	3	4	Total
ACTIVE SMOKER	1	6	1	2	10
FORMER SMOKER	1	11	9	9	30
NON SMOKER	15	30	7	8	60
Total	17	47	17	19	100

X^2 = 14.203, df = 6 and p = 0.07

There was statistically significant difference in smoking history in the higher CVD risk group P=0.027, there was a significant association of smoking with left ventricular hypertrophy X^2 = 9.996, df = 2 and p= 0.007, as well as with increased LVMI X^2 = 8.654, df = 2 and p= 0.013.

3.3.7 Exercise

Thirty seven patients (37%) reported no physical activities that meet the definition of exercise, 39 (39%) were engaged in mild physical exercise while 24 (24%) engage in regular exercise; table 3.6. When the level of exercise was compared across the different CVD risk strata, there was no statistically significant difference.

Table 3.6 Levels of physical exercise of the study population.

LEVEL OF EXERCISE	CVR SCORE				
	1	2	3	4	Total
SEDENTARY	6	18	4	9	37
MILD EXERCISE	8	19	6	6	39
REGULAR EXERCISE	3	10	7	4	24
Total	17	47	17	19	100

$X^2 = 4.646$, $df = 6$ and $p = 0.59$

3.3.8 Gender

There was no difference when the gender of the study populations were compared with the different CVD risk groups as table 3.7 below shows.

Table 3.7 Gender and CVD risk group

GENDER	CVR SCORE				
	1	2	3	4	Total
M	6	32	13	12	63
F	11	15	4	7	37
Total	17	47	17	19	100

$X^2 = 7.443$, $df = 3$ and $p = 0.059$

3.3.9 Age

The mean ± SD age of the study population was 42.20 ± 12.42 with a range of 19 to 70 years. Table 3.8 shows the comparison of age with CVD risk group.

Table 3.8 Age and CVD risk group

Cardiovascular risk	**Mean age ± SD**
Low risk	27.53 ± 9.57
Moderate risk	41.74 ± 9.59
High risk	51.59 ± 6.29
Very high risk	48.08 ± 13.15

p<0.0001(Anova)

There was a statistically significant difference in the ages of patients across the CVD risk strata.

Age showed significant positive correlation with pulse pressure (p= 0.009), serum total cholesterol (p= 0.01), triglycerides (p<0.0001) LDL cholesterol (p= 0.003), cIMT (p= 0.0001) and LVMI (p= 0.0001).

3.4 Transplant-associated cardiovascular risk factors

3.4.1 Graft dysfunction

The serum creatinine for the study population had a mean (± SD) 136.33 ± 69.86 µmol/l with a range of 46 to 400 µmol/l and the mean (± SD) eGFR of the study population was 66.28 ± 31.36 mil/min with a range of 13 to 121 ml/min/1.73m^2.

Table 3.9 shows the serum creatinine and eGFR according to the CVD risk profile.

Table 3.9 CVD risk group and eGFR

Cardiovascular risk group		**Mean ± SD**
Low risk	serum creatinine (µmol/l)	121.24 ± 50.82
	eGFR (ml/min/1.73m^2)	72.88 ± 32.87
Moderate risk	serum creatinine (µmol/l)	141.94 ± 68.59
	eGFR (ml/min/1.73m^2)	63.07 ± 31.61
High risk	serum creatinine (µmol/l)	108.82 ± 32.01
	eGFR (ml/min/1.73m^2)	77.00 ± 24.36
Very high risk	serum creatinine (µmol/l)	160.58 ± 99.91
	eGFR (ml/min/1.73m^2)	58.68 ± 32.96

p = 0.294(Anova)

There was no statistically significant difference in the serum creatinine and eGFR of the different CVD risk groups. The eGFR had a significant positive correlation with physical exercise (p= 0.018) and a negative correlation with UPCR (p= 0.002).

3.4.2 Proteinuria

Proteinuria was present in 51 patients (51%) and the mean ± SD 24 hour urinary protein excretion per day (UPCR) was 1.67 ± 2.00 g/day with a range of 0.4 to 9.4 g/day. When proteinuria as a categorical variable was compared across the CVD risk strata, there was a statistically significant difference, $X^2 = 14.687$, df = 3 and p= 0.002.

3.4.3 Anaemia

The mean ± SD haemoglobin in the study population was 13.19 ± 2.11 with a range of 7 to 18 g/dl. Eight patients (8%) had haemoglobin of < 10 g/dl. Comparison of haemoglobin between the different cardiovascular risk group showed no statistical difference p= 0.091.

3.4.4 Rejection

There were 34 biopsy proven rejection episodes, of which 26 were acute cell mediated rejections, 4 were acute humoral rejections and 4 were combined cell mediated and humoral. There was no statistically significant difference when HLA match was compared with number of rejection episodes $X^2 = 9.77$, df = 12 and p= 0.637. There was also no significant difference when the CVD risk groups were compared in terms of rejection episodes $X^2 = 5.71$, df = 6 and p= 0.456.

3.4.5 Immunosuppressive medication

3.4.5.1 Calcineurin inhibitors (CNIs)

Forty four patients (44%) were on tacrolimus as part of their maintenance immunosuppression protocol, 34 (34%) were on cyclosporine, nineteen (19%) were on rapamycin and 3 (3%) were on leflunamide in addition to steroids. There was statistically significant difference when all the CNIs were compared with the different CVD risk groups. Tacrolimus and higher CVD risk X^2 = 12.63, df = 3 and p = 0.006. Cyclosporin and higher CVD risk X^2 = 9.14, df = 3 and p = 0.027.

3.4.5.2 Steroids

All but one patient were on steroids (99%) and the mean ± SD steroid dose for the study population was 11275.94 ± 12288.11 grams. There was no statistically significant difference when cumulative steroid dose was compared with the CVD risk strata.

3.4.5.3 Rapamycin

Nineteen patients (19%) were on rapamycin as part of their immunosuppression protocol. Rapamycin was associated with higher serum total cholesterol (p= 0.029), lower HDL cholesterol (p= 0.007) and higher serum triglycerides (p= 0.04) but had no association with LDL cholesterol (p= 0.266). When rapamycin was compared with CVD risk groups, there was no significant difference noted; X^2 = 2.74, df = 3 and p= 0.433.

3.5 Left ventricular hypertrophy

LVH was found in 76 (76%) of the study population; of these 51 (51%) had concentric LVH and 25 (25%) had eccentric LVH. Systolic dysfunction was found in 12 (12%) of the patients and diastolic dysfunction was seen in 64 (64%). The mean ± SD LVMI of the study population was 129.01 ± 45.28, males had a mean ± SD LVMI of 139.91 ± 45.55 g/m^2 and females had 110.46 ± 38.77 g/m^2 respectively p = 0.001.

Table 3.10 shows the echocardiographic characteristics of the study population.

Table 3.10 Echocardiographic values for the study population

Echo parameter	**Low risk**	**Moderate risk**	**High risk**	**Very high risk**	***P* value**
IVSd	0.98±0.17	1.13±0.17	1.11±0.21	1.23±.26	0.004
PWTd	0.96±0.15	1.09±0.19	1.18±0.20	1.23±0.18	0.015
LVIDd	4.07±0.56	4.74±.79	4.72±0.63	5.05±.80	0.002
LVM	152.89±57.36	237.44±96.45	247.90±72.23	288.57±94.95	0.001
LVMI	94.35±29.53	128.88±47.11	135.44±36.72	154.60±42.02	0.006
LVEF	69.47±7.65	69.87±9.34	73.11±13.19	68.21±8.73	0.874
LVSF	37.47±8.28	39.72±8.07	43.59±12.47	35.74±9.71	0.331
Systolic dysfunction	1	1	3	7	0.026
Diastolic dysfunction	6	28	14	16	0.006

LVMI: left ventricular mass index, LVM: left ventricular mass, LVSF: left ventricular shortening fraction, LVEF: left ventricular ejection fraction, LVIDd: left ventricular internal dimension in diastole, PWTd: left ventricular posterior wall thickness in diastole, IVSd: Interventricular septal thickness in diastole.

There were significant differences in all the left ventricular echocardiographic parameters across the CVD risk groups except in their systolic and diastolic functions as shown in table 3.10.

Table 3.11 LVMI and its correlation with some CV risk variables.

LVMI	Spearman's correlation	P value
Age	0.479	<0.0001
Duration on dialysis	0.231	0.021
HLA match	-0.317	0.001
Type of dialysis	-0.12	0.522 (NS)
BMI	0.326	0.001
Waist circumference	0.442	<0.0001
SBP	0.229	0.022
DBP	0.227	0.023
Pulse pressure	0.207	0.038
MAP	0.204	0.030
UPCR	0.206	0.04
CVD risk score	0.437	0.001
Carotid IMT	0.511	0.001
Rapamycin	0.039	0.689 (NS)

UPCR: urine protein creatinine ratio, MAP: mean arterial pressure, SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index, NS: not significant, HLA: human leucocyte antigen, CVD: cardiovascular disease, LVMI: left ventricular mass index.

LVMI and level of physical exercise showed a tendency towards significance $X^2 = 5.84$, df = 2 and p= 0.054. Kidney Transplant Recipients of cadaver kidney had a higher LVMI $X^2 = 10.127$, df = 2 and p= 0.006; table 3.11.

Table 3.12 Risk factors for increased LVMI

Duration on dialysis	*P=0.017*
Cigarette smoking	*P=0.032*
Exercise	*P=0.016*

Though many factors were associated with increased LVMI on univariate analysis, on multivariate analysis using regression model only duration on dialysis, cigarette smoking and lack of physical exercise were found to be risk factors for increased LVMI; table 3.12.

3.6 Carotid intima-media thickness (cIMT)

The mean ± SD cIMT of the study population was 0.62 ± 0.21 mm with a range of 0.42 to 1.45 mm; 14 patients (14%) had a carotid artery plaque. Twenty five patients (25%) had cIMT of >0.7 mm

Table 3.13 Mean ± SD cIMT of the study population

CVD risk group	Low risk	Moderate risk	High risk	Very high risk	P value
RIMT	0.48±0.09	0.55±0.12	0.67±0.21	0.73±0.26	<0.0001
LIMT	0.52±0.09	0.59±0.11	0.75±0.26	0.81±0.36	<0.0001
CIMT	0.50±0.09	0.58±0.11	0.71±0.23	0.78±0.31	<0.0001

RIMT; right carotid intima-media thickness, LIMT; left carotid intima-media thickness, CIMT; averaged carotid intima-media thickness for right and left.

There was a statistically significant difference in both measurements of the common carotid artery across the different CVD risk groups; table 3.13.

Correlation of cIMT with some CV risk variables is shown in table 3.14

Table 3.14 Correlation of cIMT with some CV risk variables

cIMT	Spearman's correlation	P value
Age	0.60	0.001
HLA match	0.14	0.165 (NS)
Duration on dialysis	0.142	0.158 (NS)
BMI	0.416	<0.0001
Waist circumference	0.567	<0.0001
SBP	0.309	0.002
DBP	0.201	0.045
Pulse pressure	0.291	0.009
MAP	0.228	0.022
UPCR	0.181	0.071 (NS)
Total cholesterol	0.179	0.075 (NS)
Haemoglobin	-0.058	0.564 (NS)
LVMI	0.511	<0.0001
GFR	-0.152	0.13 (NS)
Male/Female	-0.184	0.067 (NS)
Plaque	0.523	<0.0001

UPCR: urine protein creatinine ratio, MAP: mean arterial pressure, SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index, NS: not significant, HLA: human leucocyte antigen, CVD: cardiovascular disease, LVMI: left ventricular mass index, GFR: glomerular filtration rate.

On univariate analysis, age, blood pressure, LVMI, presence of a plaque, BMI and waist circumference showed positive correlation with cIMT; table 3.14. When these significant associations with increased cIMT were subjected to a regression model analysis, all the significance was lost. No single factor was found to be an independent predictor of increased cIMT.

Predictors of higher cardiovascular risk were found to be UPCR ($p= 0.022$), high cumulative steroid dosage ($p= 0.028$), serum triglycerides ($p= 0.04$) and the presence of plaques ($p= 0.012$).

CHAPTER FOUR

4.0 DISCUSSION

Cardiovascular diseases (CVD) are more common in kidney transplant recipients (KTRs) than in the general population [1]. The majority of deaths following kidney transplantation are due to CVD, accounting for more than 50% of mortality, and it is also the major cause of graft loss [1]. This study was carried out to determine the cardiovascular risk profile of KTRs and to evaluate the relationship of CV risk with carotid artery intima-media thickness.

4.1 Demographic data

The majority of the study populations were black South African, who constitute over 80% of patients recruited into the renal replacement therapy program every year. Though CVD has traditionally been more common in white patients, the relatively small proportion of white patients in this study made comparison based on race to be inappropriate. The gender distribution of these patients was similar to that reported by other studies [5, 8]. Our patients were younger than those reported in other studies [4-6] but similar to the study of Foley et al [8]. In South Africa, patients who are not transplantable (those older than 60 years, patients with advanced CVD and diabetic patients with CVD) are not accepted onto the chronic dialysis program because of limited resources [57]. This would explain the relatively younger patients in this study. Hypertension was a major cause of end stage renal disease (ESRD) in our patients as opposed to diabetes, which was the predominant cause of ESRD in several studies from the United States and Europe [2, 4-6], this may be explained by the fact that the majority of the patients were black, a racial group with a higher prevalence of hypertension and hypertensive nephrosclerosis [137]. However, diabetics are often excluded from RRT in our situation.

4.2 Cardiovascular risk profile of the study population

High CVD risk was prevalent in our study population, similar to other studies [4, 6 and 7].

4.3 Traditional risk factors

4.3.1 Hypertension

Hypertension was highly prevalent in our study patients. This was also found in other studies in which prevalence ranged from 60 to 95% [2, 5 and 137]. The high prevalence of hypertension could be explained by the predominantly black racial composition of our study patients and the use of CNIs and steroids as part of maintenance immunosuppression [2, 5, 7 and 137]. Systolic blood pressure was higher in the high CVD risk group, which is not surprising considering that the Framingham's risk score (FRS) utilized SBP in patient's stratification. However, DBP, pulse pressure and MAP were higher in the high CVD risk groups and; this was similar to the study of Kasiske et al [20, 33].

4.3.2 Serum lipids

Serum total cholesterol and LDL cholesterol were higher in the high CVD risk group. The Framingham's risk scoring system utilizes serum total cholesterol and HDL cholesterol to risk stratify patients. Low density lipoprotein cholesterol and triglycerides, though not utilized in the risk stratification of patients by the FRS, were higher in the high CVD risk group and similar observations were reported by Massy [4] and Roodnat et al [37]. The use of rapamycin as part of the maintenance immunosuppression in this study was associated with higher serum total

cholesterol, triglycerides and lower HDL cholesterol. Dyslipidaemia is a recognized adverse effect of rapamycin, steroids and CNIs in this patient population [40]. Dyslipidaemia has been associated with graft dysfunction, cardiovascular and all cause mortality [37]. In this study, we found serum total cholesterol to be associated with high BMI and waist circumference, all of which are recognized cardiovascular risk factors [48-51]. Graft dysfunction was not found to be associated with dyslipidaemia in this study, as contrary to the study of Massy et al [36]. This difference can be explained by the small sample size in this study.

4.3.3 Diabetes Mellitus

Diabetic KTRs, by the FRS have a high CV risk, ie both NODAT and those KTRs who had diabetes mellitus prior to kidney transplant. The low prevalence of NODAT we found in this study could be explained by small sample size and the sampling technique used. NODAT was reported to be present in 15.6% of patients in our unit [45]. The prevalence of NODAT reported in other studies was considerably higher than ours [44, 45]. Diabetes was associated with proteinuria in our KTRs, but not with graft dysfunction as reported by Miles et al [45]. The use of steroids and CNIs as part of maintenance immunosuppression was not associated with NODAT in this study, in contrast to report of several other studies [43, 44 and 45]. This disparity could be explained by small sample size in this study. However, diabetics are often excluded from renal replacement therapy in our situation.

4.3.5 Obesity

Weight gain after kidney transplant has been associated with the development of the metabolic syndrome and poor graft outcome [48]. There was a high prevalence of obesity in our patients; high CVD risk KTRs had a higher BMI and waist circumference. Similar to other reports, obesity was found to be associated with higher blood pressure, higher LVMI, dyslipidaemia, graft dysfunction, cumulative steroid dose and is a marker of atherosclerosis; with an increased cIMT [51, 52-54]. The duration of time post transplant was also associated with increased obesity, confirming the role of cumulative steroid exposure in its development, this has been confirmed by many other studies [49, 52].

4.3.6 Smoking

Cigarette smoking after kidney transplant has been associated with adverse CV outcome and graft dysfunction [55]. The prevalence of smoking was high when both active and former smokers were considered; and this was comparable to what was reported in other studies [55, 56]. Smoking was utilized in stratifying patients according to the FRS. It was also found to be a risk factor for LVH and was associated with graft dysfunction in our study.

4.3.7 Age

Considering that the FRS utilizes age in addition to other variables to categorize patients into different CVD risk group, it is not surprising that the higher CVD risk KTRs were older in this

study. The correlation of age with blood pressure, dyslipidaemia and increased cIMT was similar to that observed in other studies [71, 77].

4.3.8 Physical exercise

Lack of physical exercise is both a CV risk factor and also a risk factor for graft dysfunction with proteinuria [58]. It is inversely associated with the metabolic syndrome, a history of cardiovascular disease, fasting insulin, and triglyceride concentration, and positively associated with kidney function and 24-hour urinary creatinine excretion [58]. Twenty four percent of our KTRs were involved in regular physical exercise, with the remainder being either sedentary or involved in only mild exercise. As reported by Darien et al [58], lack of physical exercise was found to be associated with an increased LVMI; our study did not show an association with graft dysfunction and proteinuria, probably due to the relatively small sample size.

4.3.9 Gender

The risk of CVD in males seen in the general population was also seen in KTRs [6, 17]. In this study however, there was no difference observed in the CVD risk profile of KTRs based on their gender. A male preponderance of high CVD risk may have been observed with a larger sample size.

4.4 NON-TRADITIONAL CARDIOVASCULAR RISK FACTORS

4.4.1 Graft function

An increase in cardiac death, non-cardiovascular death, all cause mortality and major adverse cardiac events were reported in KTRs with decreased graft function [29, 59]. Graft dysfunction was highly prevalent (52%) in our KTRs and similar to other reports, graft dysfunction was associated with rejection episodes and LVMI [59]. Graft function was not different amongst the different CVD risk groups in this study; this finding is in contrast with reports from other studies, where it was found to be associated with higher CVD risk [29, 40 and 59]. In those studies, sample sizes were larger as opposed to our study which had a smaller sample size.

4.4.2 Proteinuria

Proteinuria is an excellent marker of poor long term graft survival and an independent risk factor for total and cardiovascular mortality in the transplant population [61]. The prevalence of proteinuria in our study was high (51%) in contrast to that reported by Fernandez et al in which a prevalence of 15.3% was reported at 1 year post transplant [61]. This difference could be explained by the fact that, our study patients were recruited at least 3 years after transplant, with some having their graft for 7 years. Proteinuria was associated with high CVD risk and graft dysfunction in our KTRs. Similar observations were made in other studies [61, 62]. Anaemia, LVMI, high BP and higher cumulative steroid dose were found to be associated with proteinuria. These associations were also observed in the Spanish Chronic Allograft Nephropathy study [61]. This finding is not surprising, as graft function deteriorates, blood pressure control becomes even more difficult and anaemia becomes more pronounced, all impacting negatively on left ventricular function. The use of rapamycin in KTRs has been associated with proteinuria [64],

but this association was not seen in our study, probably because only 19 of our study patients were on rapamycin and tend to be converted to other immunosuppressive agents if proteinuric.

4.4.3 Anaemia

Anaemia is common in KTRs and some studies have reported incidences approaching 40% at 1 year after transplant and it was associated with impaired graft function [66, 67]. In this study, we found a low incidence of anaemia (only 8% had haemoglobin less than 10 g/dl), despite a high prevalence of graft dysfunction. The reason for the low incidence of anaemia could be diligent care of these patients, including optimal iron therapy and the use of erythropoietin.

4.4.4 Rejection

Graft rejection appears to be a cardiovascular risk factor in KTRs [14, 29]. The use of high dose corticosteroids to manage acute graft rejection possibly accounts for this relationship [20, 36]. We found multiple rejection episodes in our KTRs, and rejection was strongly associated with graft dysfunction but no such association was found with HLA match. Treatment of rejection requires high dose steroids and augmentation of immunosuppression which are all associated with increased CVD risk. In contrast to other studies [20, 36] where rejection episodes predicted a higher CVD risk, this study found rejection episodes to be similar among the different CVD risk groups. However, graft dysfunction consequent upon rejection episodes will ultimately aggravate the CVD risk of the patients. A long term prospective study will be better suited to look at the effect of rejection on CVD risk.

4.4.5 Immunosuppressive medication

4.4.5.1 Calcineurin inhibitors (CNIs**)**

Immunosuppression protocol using CNIs have been implicated in the development and worsening of hypertension post transplant [88, 89]. Calcineurin inhibitor based immunosuppression protocol was widely used in our study population with 44% on tacrolimus and 34% on cyclosporin, a combined 78%. Both cyclosporin and tacrolimus were associated with high CVD risk in this study, but the risk appears to be higher with tacrolimus which is contrary to reports from the literature, reason for this may be explained by high number of our patients on tacrolimus and the small sample size of this study, a longitudinal study with larger sample may explain this observation. Several studies reported higher hypertension and dyslipidaemia rates with cyclosporin than tacrolimus [80, 81 and 88]. The risk of NODAT appears to be increased with tacrolimus compared to cyclosporin [82], but this was not demonstrated in our study.

Steroids increase the risk of NODAT in a dose dependent manner [83]. In this study, 99% of our KTRs were on steroids. We found a strong association between cumulative steroid dose and increased waist circumference, but there was no difference in cumulative steroid dose across the CVD risk strata. Steroids, especially in combination with cyclosporine are associated with dyslipidaemia in KTRs [90]. These associations were not observed in our study cohorts. Dyslipidaemia is a recognized side effect of rapamycin in KTRs [92]. Our patients who had rapamycin as part of their immunosuppression protocol had a significantly higher total serum cholesterol and triglycerides and lower HDL cholesterol. Comparison of different CVD risk

groups in terms of rapamycin showed no difference. Despite dyslipidaemia, the development of atherosclerosis is not increased with the use of rapamycin, at least in animal models [138].

4.4.6 Carotid intima-media thickness (cIMT)

Increased IMT in the common carotid artery is related to generalized atherosclerosis and increased risk of future MI and cerebrovascular disease. Clinical trials have provided outcomes that support the role of cIMT measurements for predicting cardiovascular events (ie, the thicker the c-IMT, the higher the rate of MI or stroke) in KTRs [6, 71]. Holland et al found increased cIMT to be correlated with angiographically proven coronary artery disease in a South African black population [72]. In our study, KTRs with higher CVD risk had a thicker mean cIMT and this observation was similar to that reported in other studies [6, 71 and 72]. This increase in cIMT of our KTRs was seen across the different CVD risk groups.

Increased cIMT has been shown to be associated with most of the risk factors for atherosclerosis [6, 71]. In our study population, increased cIMT correlated with age, BMI and waist circumference, blood pressure, LVMI and presence of plaque in the carotid artery. The correlation of different CVD risk factors to increased cIMT was significant only on univariate analysis. When subjected to multiple regression analysis, no single risk factor was found to predict increased cIMT, suggesting a role for multiple factors. In this study, the presence of plaques in the carotid artery mirrored thickened cIMT; and this observation was also made by Barbagallo et al [6].

4.4.7 Left Ventricular Hypertrophy

Left ventricular hypertrophy is present in 40–60% of KTRs, and its persistence in the first year after renal transplantation is associated with reduced patient survival. It has also been shown to be the strongest predictor of all-cause mortality, together with diabetes in KTRs [25-27, 75]. In this study, we found the prevalence of LVH in our KTRs to be 76%; which was similar to the prevalence found by Middleton et al [76], but lower than what was reported by Parfrey et al [26]. The high prevalence of LVH in our study could be due to the fact that the majority of our patients had hypertension as the cause of their ESRD and also majority of our KTRs were black, a racial group with preponderance of hypertensive heart disease. The commonest type of LVH in this study was concentric LVH and it was found to be associated with diastolic dysfunction. The same observation has been reported in several studies [25, 27 and 75]. Eccentric LVH was seen in 25% of our study patients and it was associated with systolic dysfunction.

Comparison of the echocardiographic characteristics of the study population revealed a significant difference in all left ventricular dimensions, left ventricular systolic and diastolic dysfunction across the different CVD risk groups. Increased LVMI correlated with graft dysfunction, age, and longer duration on dialysis, HLA mismatch, obesity, smoking, blood pressure, proteinuria, high CVD risk and increased cIMT. These are strong CVD risk factors and these associations have been reported in several studies [25, 26, 27 and 75].

Risk factors for LVH in our study were lack of physical exercise, duration on dialysis and cigarette smoking. Similar observations were reported in another study [58]. Longer dialysis duration has been found to increase the risk of CVD and death in KTRs [2, 3, 59 and 65].

4.5 LIMITATIONS OF THIS STUDY

The following were limitations of this study

1. The small sample size of this study made some conclusions inappropriate. While high cardiovascular risk is traditionally more common in the white and Asian races, the relatively small number of whites (7%) and Asians (5%) against blacks (77%) in this study made such comparison inappropriate.

2. The cross sectional nature of the study which only allowed for patients to be seen once.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The findings from this study demonstrate that:

1. Kidney transplant recipients have several factors which carry a risk for CVD. The CVD score is high.
2. Carotid intima-media thickness correlates with high CVD score.
3. No single factor was found to be an independent predictor of increased carotid IMT.
4. Predictors of higher cardiovascular risk were proteinuria, high cumulative steroid dosage, serum triglycerides and the presence of plaques.

5.2 Recommendation for clinical practice

Ultrasound assessment of the carotid artery should be performed as part of the routine follow up of kidney transplant recipients; this will help identify patients who will benefit from more aggressive management. It's a simple, cheap and reproducible screening tool for high risk KTRS. Calculating the CVD score of KTRs is also recommended since it correlates with increased carotid intima-media thickness, a proven surrogate of atherosclerosis.

5.3 Recommendations for future work

1. A study with a large sample size from multiple centers.

2. A longitudinal study that will follow patients from predialysis time through dialysis to the post transplant period. This will adequately provide information on changes in the cardiovascular status of patients as they transit through CKD to the various modalities of renal replacement therapy.

APPENDIX A

PARTICIPANT INFORMATION SHEET

Introduction:

Good day, my name is Dr Aminu Muhammad Sakajiki. I am a Doctor at the Charlotte Maxeke Johannesburg Academic Hospital, in the department of Internal Medicine.

I wish to ask you to consider participating in a research study for my masters degree, entitled; **"CARDIOVASCULAR RISK PROFILE OF KIDNEY TRANSPLANT RECIPIENTS AT THE CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL."** It is important that you read and understand the purpose of this study, procedures, benefits, risks and discomforts. This information leaflet is to help you to decide if you would like to participate. If you have any questions, do not hesitate to ask me.

Purpose of the study:

Cardiovascular diseases cause significant morbidity and mortality after kidney transplant and are important causes of graft failure. This study is designed to identify individuals at high risk of cardiovascular disease so that early appropriate intervention can be instituted.

Procedures:

I will ask you regarding smoking, alcohol intake, level of exercise, family history of heart disease, and previous heart disease. I will also take some information about your transplant from your file. You will also be examined; your weight, height, waist circumference and blood pressure will be taken. This will take about ten minutes.

You will require an ultrasound of your neck: for this, you will be asked to lie flat and the probe of the machine which is about half the size of your fist will be applied on your neck. It is completely painless and it will take about five minutes to take pictures of the artery. Pictures of your heart will also be taken using the same probe and it is also a painless procedure which will take about five minutes.

Risks:

Ultrasound of your neck and heart are painless procedure and carry no risk.

Benefits:

You may receive additional treatment based on the findings or special counseling on lifestyle changes. There is no monetary benefit for taking part in this study.

Confidentiality:

All the data will be grouped, analyzed and the results will be presented for my thesis and possibly published in scientific journals. All information obtained in this study, including your hospital records, personal data and research data will be kept strictly confidential. Data that may be reported in scientific journals will not include any information that identifies you as a participant in this study and individual results will not be made available to anyone without your express and written permission.

I have obtained approval for my study from the Human Research Ethics Committee of the University of the Witwatersrand. If you have any questions regarding your rights as a participant in this study or any complaints please contact Ms Anisa Keshav from the Ethics Committee on 011 717 1234.

Participation in this study is voluntary and you are allowed to withdraw at any time. If after reading this information sheet you decide against participating in the study please be assured that any further treatment will not be affected. If you have further questions please ask me.

I would like to ask you please to participate in the study and confirm your willingness to do so by signing the consent form overleaf.

Sincerely

Dr Aminu Muhammad Sakajiki 0789391962

APPENDIX B

CONSENT TO ACT AS A PARTICIPANT IN RESEARCH

I, ……being 18 years or older, consent to participating in a research project entitled:

CARDIOVASCULAR RISK PROFILE OF KIDNEY TRANSPLANT RECIPIENTS AT THE CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

The procedures/questionnaires have been explained to me and I understand and appreciate their purpose, any risks involved, and the extent of my involvement. I have read and understand the attached information leaflet.

I understand that the procedures form part of a research project, and may not provide any direct benefit to me.

I understand that all experimental procedures have been reviewed and approved by the Human Research Ethics Committee, University of the Witwatersrand, Johannesburg. If you have any questions regarding your rights as a participant in this study or any complaints please contact Ms Anisa Keshav from the Ethics Committee on 0117171234.

I understand that my participation is voluntary, and that I am free to withdraw from the project at any time without prejudice.

…… ……..

Participant Signature Date

…… ……..

Investigator Signature Date

APPENDIX C

DATA COLLECTION SHEET; FROM FILES

TITLE: CARDIOVASCULAR RISK PROFILE OF KIDNEY TRANSPLANT RECIPIENTS AT CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

STUDY NUMBER:

INVESTIGATOR: DR AMINU MUHAMMAD SAKAJIKI

Participant number…….. Enrollment date……..

1. Personal Data:

Age……. Sex……. Race……. Age at transplant……

2. Cause of ESRD…….

3. Previous cardiovascular event (YES/NO):

None…..

Angina……..

ACS…..……

Coronary intervention……..

Stroke……..

HF……..

PVD……..

Arrhythmias……..

4. Duration on dialysis……

5. Type of dialysis at transplant…...

6. Years post transplant……

7. Number of transplants……

8. HLA match……

9. Donor type……..

10. Number of rejection…….

11. Type of rejection…….

12. Blood pressure…….

13. Pulse pressure……

14. Mean arterial pressure……

15. Diabetes……

16. HbA1c……

17. Haemoglobin……..

18. Total Cholesterol……

19. HDL Chol……

20. Triglycerides……..

21. LDL Chol……

22. Serum Creatinine……

23. GFR…….

24. Proteinuria……

25. UPCR……

26. HIV……

Immunosuppressive drugs (YES/NO):

27. Cumulative steroid dose…….

28. Cyclosporine….

29. Tacrolimus….

30. MMF……

31. Azathioprine…….

32. Rapamycin……

Other medications:

33. ACEi/ARB…..…..

34. Statins…..…..

35. Insulin…..…..

36. ARVs……..

37. OHAs…..…..

38. Beta blockers……

39. Aspirin……

DATA COLLECTION SHEET: FROM INTERVIEW AND PHYSICAL EXAMINATION

40. Smoking:

Active smoker……..…

Former smoker…..…

Non smoker……

41. Alcohol:

Non drinker….. ……

Moderate drinker…..……

Heavy drinker……..

42. Weight at transplant…….

43. Weight at 3 months post transplant……

44. Weight at 6 months post transplant…….

45. Weight at 1 year post transplant……

46. Height….. 47. BMI at transplant…….

48. BMI at 3 months post transplant……..

49. BMI at 6 months post transplant…….

50. BMI at 1 year post transplant……

51. Waist circumference …….

52. Exercise:

Sedentary (less than 30 minutes of aerobic exercise per week)……

Mild exercise (2 to 3 exercises per week not less than 30 minutes each, aerobic)…….

Regular exercise (4 or more aerobic exercises per week each lasting atleast 30 minutes)……

53. Family history of cardiovascular disease:

None……..

Angina……..

ACS…..……

Coronary intervention……..

Stroke……..

HF……..

PVD……..

Arrhythmias……..

54. Echocardiography:

Left ventricular diastolic dysfunction (LVDD) E:A….

Left ventricular ejection fraction (LVEF %)……..

Left ventricular shortening fraction (LVSF %)……

Left ventricular end diastolic dimension (LVEDD cm)……

LV posterior wall thickness in diastole (PWTd cm)……

Interventricular septal thickness in diastole (IVSTd cm)…….

Left ventricular mass index (g/m^2)……..

LVM……

BSA…….

RWT…….

Left ventricular geometry:

Normal geometry…….

Concentric LVH…….

Eccentric LVH……

Concentric remodeling……..

55. Carotid Doppler ultrasound:

Intima media thickness:

Right ……..

Left……

cIMT……..

Plaques……..

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Aminu S Muhammed

CLEARANCE CERTIFICATE	**M120596**
PROJECT	Cardiovascular Risk Profile of Kidney Transplant Recipients at the CMJAH
INVESTIGATORS	Dr Aminu S Muhammed.
DEPARTMENT	Department of Internal Medicine/Nephrology
DATE CONSIDERED	04/05/2012
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 11/06/2012 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Prof S Naicker

DECLARATION OF INVESTIGATOR(S)
To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

APPENDIX E

FRAMINGHAM 10-YEAR RISK ASSESSMENT CHART
(RISK OF CVD: CORONARY ARTERY DISEASE, STROKE, PERIPHERAL ARTERY DISEASE OR HEART FAILURE)

FOR MEN

AGE (YEARS)	POINTS
30 – 34	0
35 – 39	2
40 – 44	5
45 – 49	6
50 – 54	8
55 – 59	10
60 – 64	11
65 – 69	12
70 – 74	14
75 +	15

FOR WOMEN

AGE (YEARS)	POINTS
30 – 34	0
35 – 39	2
40 – 44	4
45 – 49	5
50 – 54	7
55 – 59	8
60 – 64	9
65 – 69	10
70 – 74	11
75 +	

FOR MEN

TOTAL CHOLESTOREL mmol/L	POINT
< 4.10	**0**
4.10 - 5.19	**1**
5.2 - 6.19	**2**
6.20 - 7.20	**3**
>7.20	**4**

HDL- CHOLESTOREL mmol/L	POINT
>150	**0**
1.30 – 1.49	**1**
1.20 – 1.29	**2**
0.90 – 1.19	**3**
≤0.90	**4**

SYSTOLIC BP - UNTREATED (mmHg)	POINT
< 120	**-2**
120 – 129	**0**
130 – 139	**1**
140 – 159	**2**
≥ 160	**3**

FOR WOMEN

TOTAL CHOLESTOREL mmol/L	POINTS
< 4.10	**0**
4.10 - 5.19	**1**
5.2 - 6.19	**3**
6.20 - 7.20	**4**
>7.20	**5**

HDL- CHOLESTOREL mmol/L	POINT
≥ 1.50	**-2**
1.30 – 1.49	**-1**
1.20 – 129	**0**
0.90 – 1.19	**1**
<0.90	**2**

SYSTOLIC BP - UNTREATED (mmHg)	POINT
< 120	**-3**
120 – 129	**0**
130- 139	**1**
140 – 149	**2**
150 – 159	**4**

≥160	**5**

SYSTOLIC BP- ON ANTIHYPERTENSIVE TREATMENT (mmHg)	**POINT**
<120	**0**
120 – 129	**2**
130 – 139	**3**
140 – 159	**4**
≥160	**5**

SYSTOLIC BP- (mmHg) NOT ON TREATMENT	**POINT**
<120	**-1**
120 – 129	**2**
130 – 139	**3**
140 – 149	**5**
150 – 159	**6**
≥160	**7**

FOR MEN

POINTS TOTAL	**10 YEAR RISK (%)**
-3 or<	<1
-2	1.1
-1	1.4
0	1.6
1	1.9
2	2.3
3	2.8
4	3.3
5	3.9
6	4.7
7	5.6
8	6.7
9	7.9
10	9.4
11	11.2
12	13.2
23	18.6
14	18.4
15	21.6
16	25.3
17	39.4
18 or >	30>

FOR WOMEN

POINTS TOTAL	**10 YEAR RISK (%)**
-2 or <	<1
-1	1
0	1.1
1	1.5
2	1.8
3	2.1
4	2.5
5	2.9
6	3.4
7	3.9
8	4.6
9	5.4
10	6.3
11	7.4
12	8.6
13	10
14	11.6
15	13.5
16	15.6
17	18.1
18	20.9
19	24
20	27.5
20 >	>30

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