Relationship of Chronic Inflammatory Markers and Dyslipidaemia to Atherosclerotic Vascular Disease in Different Categories of Chronic Kidney Disease Patients

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Declaration

I, Stephen Olawale Oguntola, declare that the thesis, which I hereby submit for the degree of Doctor of Philosophy in Internal Medicine at the University of the Witwatersrand, Johannesburg, South Africa, is my own work and has not been submitted by me or any other person for degree purposes at any other university.

Stephen Olawale Oguntola

10th day of September 2018

ABSTRACT

Background

Cardiovascular disease (CVD) is the leading cause of mortality among CKD patients, responsible for 40-50 % of all-cause mortality in CKD. Chronic kidney disease patients have been shown to be more likely to succumb to CVD than progress to ESKD. Atherosclerotic vascular disease (AsVD) has been described as an inflammatory disease because of the central role of chronic inflammation and lipid disorders in its aetiopathogenesis. The contributions of these two risk factors have not been well studied in a broad spectrum of CKD patients among black Africans. This study evaluated the relationship of chronic inflammation, dyslipidaemia and *APOL1* risk variants to AsVD among black South Africans with CKD stage 3, peritoneal dialysis (PD) and haemodialysis (HD) patients and kidney transplant recipients (KTRs).

Methods

This was a cross-sectional study of 40 adult (18-65 years) non-diabetic CKD patients, kidney disease outcome quality initiative (KDOQI stage 3), 40 PD patients, 40 HD patients, 41 KTRs and 41 age- and sex-matched healthy controls. An interviewer-administered questionnaire was used to obtain information on participants' sociodemographic and cardiovascular risk factors. Anthropometric parameters were measured. Blood samples were obtained and serum was analysed for baseline tests, lipoprotein and inflammatory biomarkers. Genomic DNA was extracted from whole blood by modified salting out method and *APOL1* genotyping was carried out using restriction fragment length polymorphism. Echocardiography was performed on all patients and carotid intima media thickness (CIMT) was assessed in both right and left carotid arteries at 1cm proximal to the carotid bulb. Atherosclerotic vascular disease was defined by the combination of increased CIMT values (> 0.55 mm) and the presence of carotid plaques.

Results

Prevalence of AsVD was highest among PD patients (70 %), and occurred in 47.5 % of stage 3CKD and HD patients, 46.3 % of KTRs and 17.1 % of controls, ($p < 0.01$). Comparison of the kidney disease groups (CKD stage 3, PD and HD) with controls showed significant difference in waist-hip ratio (WHR), systolic blood pressure (SBP), mean arterial blood pressure (MABP), serum creatinine (Scr), estimated glomerular filtration rate (eGFR), total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C-C), Castelli 1, Castelli 2, atherogenic coefficient (AC), non-HDL-C cholesterol, calcium, phosphate, calcium-phosphate products ($CaXPO_4$), serum albumin, ejection fraction (EF), left atrial diameter (LAD) and left ventricular mass index (LVMI). Comparison between the kidney disease group (CKD stage 3, PD and HD) who had AsVD with those who did not, showed significantly higher age, WHR, LAD, mitral valve deceleration time, LVMI and serum creatinine. Among KTRs, positive correlation was seen between CIMT and LAD, LVMI, Castelli 2 and Lipoprotein combined index (LCI).

Pentraxin-3 levels were significantly higher in all the kidney disease groups (stage 3 CKD, PD, HD and KTRs) compared to controls. high sensitivity C-reactive protein (hsCRP) and tumour necrosis factor-alpha (TNF-α) levels were significantly higher in ESKD patients compared to controls. Pentraxin-3 correlated positively with CIMT among KTRs ($r = 0.336$, $p = 0.032$) and with other inflammatory markers when all kidney disease groups were combined (except for hsCRP). An inverse correlation was seen between pentraxin-3 and eGFR ($r = -0.171$, $p = 0.030$) and serum albumin ($r = -0.168$, $p = 0.033$). The levels of Lp (a) and Lp-PLA2 were increased while levels of APO A1 were reduced in all kidney disease groups compared to controls.

On multivariate analysis, age (> 40 years), male gender, low HDL-C levels and elevated Lp (a) levels independently predicted AsVD after adjusting for BMI, WHR, TC, TG, HDL-C, LDL-

C, inflammatory markers, Lp-PLA2 and APO A1. Lipoprotein (a) predicted AsVD better than other lipid markers evidenced by higher area under the curve (AUC). No significant difference was seen in the utility of the lipid biomarkers in predicting AsVD (except when female kidney disease patients were analysed separately).

The odds of AsVD was more than 11-fold increased in patients who had hypertension-attributable CKD with high risk *APOL1* variants, [OR 11.85, 95 % CI – (1.08 – 129.91), p = 0.043]; this relationship was lost when all kidney disease patients, regardless of aetiology was used in the analysis, (OR 0.84 (95 % CI – 0.22 – 3.28; p – 0.802).

Conclusion

Atherosclerotic vascular disease is common in kidney disease patients and most prevalent among PD patients compared to CKD stage 3, HD and KTRs. Dyslipidaemia and inflammation are common among kidney disease patients. Lipoprotein(a) predicted AsVD better than other lipid biomarkers (Lp-PLA2, APO A1) and lipid profile parameters (LDL-C, TG, TC). Age (> 40 years), male gender, low HDL-C and elevated Lp (a) independently predicted AsVD when all kidney disease patients were combined, while *APOL1* risk variants independently predicted AsVD among patients with hypertension attributable kidney disease.

DEDICATION

This work is dedicated to the overwhelming number of ESKD patients spread all over Africa who neither have access to dialytic therapy nor kidney transplantation. I hope and pray for light at the end of the tunnel someday.

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RESEARCH OUTPUTS

1. S.O. Oguntola[1], M.O. Hassan[4], R. Duarte[3], T. Dix-Peek, C. Dickens, G. Olorunfemi, A. Vachiat[2], G. Paget[1], P. Manga[2], S. Naicker[1]. Atherosclerotic vascular disease and its correlates in stable black South African kidney transplant recipients. **Accepted for publication by the International Journal of Nephrology and Renovascular Disease. Chapter 4**

2. S.O. Oguntola[1]; R. Duarte[3*]; M.O. Hassan[4]; A. Vachiat[2]; P. Manga[2]; S. Naicker.[1]Atherosclerotic vascular disease is more prevalent among black peritoneal dialysis patients in South Africa. **Submitted to BMC Nephrology, under review. Chapter 5**

3. S.O. Oguntola[1], M.O. Hassan[4], R. Duarte[3], T. Dix-Peek, C. Dickens, K. Moodley, A. Vachiat[2], P. Manga[2], S. Naicker.[1] Atherosclerotic vascular disease and inflammation in stage 3 CKD, end-stage kidney disease patients and kidney transplant recipients. **Chapter 6**

4. S.O. Oguntola[1], M.O. Hassan[4], R. Duarte[3], T. Dix-Peek, C. Dickens, T. Snyman, K. Moodley, A. Vachiat[2], P. Manga[2], S. Naicker.[1] The utility and relationship of Lipoprotein markers to Atherosclerotic vascular disease in black CKD and kidney transplant recipients. **Chapter 7**

5. S.O. Oguntola[1], M.O. Hassan[4], R. Duarte[3], T. Dix-Peek, C. Dickens, A. Vachiat[2], P. Manga[2], S. Naicker.[1] *APOL1* two risk variant is associated with atherosclerotic vascular disease among patients with hypertension-attributed nephropathy. **Chapter 8**

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LIST OF ABBREVIATIONS

ABI – ankle brachial index

AC – atherogenic coefficient

AGEP – advanced glycated end-products

APOL1 – apolipoprotein1

APO A1 – apolipoprotein A1

AsVD – atherosclerotic vascular disease

BMI – body mass index

BSA – body surface area

CAC – coronary artery calcification

CIMT – carotid intima media thickness

CKD – chronic kidney disease

CRIC – Chronic Renal Insufficiency Cohorts

CTEP – cholesteryl ester transfer protein

CVD – cardiovascular disease

eGFR – estimated glomerular filtration rate

ESKD – end-stage kidney disease

GFR – glomerular filtration rate

HD – haemodialysis

HDL-C – high density lipoprotein cholesterol

IDEAL – Initiating Dialysis Early And Late trial

IFN-γ – interferon gamma

IHD – ischaemic heart disease

IL-6 – interleukin-6

IL-2R – interleukin-2 receptor

IMA – ischaemia modified albumin

JHS – Jackson Heart Study

KTRs – kidney transplant recipients

KDOQI – kidney disease outcome quality initiative

LCAT – lecithin cholesterol acyltransferase

LCI – lipoprotein combined index

LDL-C – low density lipoprotein cholesterol

Lp (a) – lipoprotein (a)

Lp-PLA2 – lipoprotein phospholipase A2

LVH – left ventricular hypertrophy

LVM – left ventricular mass

LVMI – left ventricular mass index

NCDs – non-communicable diseases

NHANES – National Health And Nutrition Examination Survey

non-HDL-C – non-high density lipoprotein

PCR – polymerase chain reaction

PD – peritoneal dialysis

PVD – peripheral vascular disease

RFLP – restriction fragment length polymorphism

RRT – renal replacement therapy

SCIr – serum catalytic iron

SCr – serum creatinine

sRAGE – soluble receptor of advanced glycation end-products

TC – total cholesterol

TG – triglycerides

VLDL-C – very low density lipoprotein cholesterol

WHI – Women's Health Initiative

WHR – waist-hip ratio

CHAPTER 1

INTRODUCTION

Chronic kidney disease (CKD) is defined as kidney damage for ≥ 3 months as demonstrated by structural and functional abnormalities of the kidney, with or without decreased glomerular filtration rate (GFR), manifesting as either pathological abnormalities or markers of kidney damage, including abnormalities in the composition of blood and urine or abnormalities in imaging tests. It is also defined as GFR < 60 ml/min/1.73m^2 for ≥ 3 months with or without kidney damage.[1]

Available data from the Chronic Disease Directorate of the World Health Organisation (WHO) to support the rising prevalence of premature deaths from non-communicable diseases (NCDs) showed that of the 58 million human deaths in the year 2005, 35 million were attributable to chronic disease with cardiovascular disease (CVD) responsible for 50 %.[2] There was an 82 % rise in the number of deaths caused by CKD from 1990-2010.[3] This rise is the third largest among the 25 common causes of death, behind HIV/AIDS (396 %) and diabetes (93 %).[3]

Currently, CKD is being recognized as a public health problem worldwide. The burden of CKD is estimated to be 19.2 million; this accounts for 11% of the adult population in US,[4] with about 385,000 people with end stage kidney disease (ESKD).[5] The burden of CKD in sub-Saharan African countries has not been well characterized, probably due to the non-availability of registry records in most countries on the continent. Also, only few community based data are in existence while most of the available information is based on hospital acquired data.[6] There is speculation that the actual prevalence of CKD may be more than three times the quoted figures.[7] According to South African Renal Registry 2015 report, the prevalene of ESKD on treatment was 189 per million population.[8] A thirteen year data review of ESKD patients in a tertiary health centre in Nigeria showed that ESKD accounted for about 8% and 22% of all medical admissions and deaths respectively.[9] This huge burden may be the tip of the iceberg,

as the reported renal registry data in South Africa only estimated ESKD patients on renal replacement therapy (RRT). The lack of data therefore justifies the need to improve our knowledge on all categories of CKD, particularly in relation to CVD.

Higher risk of cardiovascular events and CVD mortality has been described in CKD patients compared to healthy controls.[10-12] Several studies have shown that most patients with CKD succumb to CVD before dialysis becomes necessary,[13,14] hence the need to evaluate CKD patients for CVD. The American Heart Association recommended that CKD patients should be classified among those with the maximum risk of developing CVD, because even mild kidney disease contributes significantly to the development of CVD.[15-17]

Although the effect of traditional risk factors for CVD have been widely assessed in healthy populations,[18] credence has also been given to CKD-related factors as risk factors for CVD among CKD patients, notable among which are chronic inflammation and lipid disorders.[19,20]

The contributions of chronic inflammatory markers and dyslipidaemia to CVD and mortality have been well studied in different races and ethnic backgrounds.[21,22] However, data on the relationship of these vital CKD-related risk factors to CVD among black Africans are sparse.

The recent identification of an association between apolipoprotein L1 (*APOL1*) risk alleles and specific forms of non-diabetic CKD such as hypertension-related CKD, HIV-associated nephropathy and focal segmental glomerulosclerosis provided an invaluable basis for further research on the role of *APOL1* in CKD progression and its possible contribution to CVD.[23] Cardiovascular disease burden was assessed among participants of the Jackson Heart Study (JHS) and Women's Health Initiative (WHI) study cohorts with over 2000 participants included in the study. An increased atherosclerotic CVD burden was shown to be associated with the presence of two *APOL1* risk alleles, in addition to confirming the previously known association between these risk alleles and CKD and dialysis dependence;[24] the authors also

proposed the “two hit hypothesis” where the presence of the two risk alleles with an additional comorbidity such as diabetes, hypertension or HIV increases the risk of developing CKD.[24]

In view of the paucity of data from Africa, there is therefore a need to study the *APOL1* risk variants in relation to CKD (including renal allograft recipients) and risk for atherosclerotic vascular disease (AsVD) and also renal allograft function among black Africans since these variants have been described in individuals of African ancestry.[24]

Based on the knowledge gaps identified, we set out to assess the relationship of chronic inflammatory markers and dyslipidaemia to AsVD in different categories of CKD. This study will assess atherosclerotic CVD in CKD with emphasis on chronic inflammatory markers, dyslipidaemia and the association of atherosclerotic CVD in CKD to *APOL1* risk variants among black South Africans.

CHAPTER 2

LITERATURE REVIEW

2.1 Background

Chronic kidney disease (CKD) is a clinical syndrome characterised by a relentless fall in GFR and complicated by heterogenous groups of complications which worsen the clinical outlook of the disease.

Commonest among all the complications of CKD and the highest contributor to morbidity and mortality is CVD.[25,26] Amann et al.[27] reported that CVD was twenty times more common in ESKD patients and was responsible for 40-50 % of mortality.

2.2 Atherosclerotic vascular disease in CKD patients

Atherosclerotic vascular disease (AsVD) is common among CKD patients, frequently manifesting as coronary heart disease including myocardial infarction (MI), carotid vascular disease and peripheral vascular disease (PVD). The burden and morbidity posed by AsVD in CKD patients cannot be over-emphasized. Over the past few decades, evidence has continued to increase concerning the high prevalence of AsVD as a cause of morbidity and mortality in CKD. Lindner et al.[28] studied the contribution of AsVD to death among CKD patients on maintenance HD who were followed-up for an average of 6.5years. It was found that AsVD accounted for 60.9 % of death in this patient group; in addition, it was noted that patients who died from congestive cardiac failure had MI as the cause of their heart failure. Amira et al[29] found a high prevalence of carotid plaque in black ESKD patients on HD, 38.1 % compared to 7.9 % in age and sex-matched healthy controls. Savage et al.[30] reported a prevalence of atherosclerotic plaque of 71 % among non-diabetic ESKD patients compared to 21 % among healthy controls. The high prevalence of atherosclerotic disease reported by Savage et al[30] could have been due to the white population recruited. In addition, the small sample size of the

study was possibly responsible for the lack of differences observed in blood pressures and mean intima media thickness observed between the CKD patients and controls. It has also been reported in several studies that among CKD patients, carotid plaques are likely to be in multiple locations, are calcified and have accentuated media thickness compared to plaques found in healthy populations which are usually in a solitary location and fibroatheromatous.[28,29,31]

In view of the unfavourable sequelae of atherosclerosis, greater attention has been invested in detection of subclinical atherosclerosis using surrogate markers. Ankle brachial index (ABI) and carotid intima media thickness (CIMT) have been used in many studies to assess PVD and carotid vascular disease respectively, with good correlation with atherosclerosis.[28,29,30] The impact of CIMT on long term outcomes in HD patients has shown that patients with CIMT values < 0.97mm had a survival rate of 73.4 % while patients with values > 0.97mm had a survival rate of 16.5 %.[32] The quest for an appropriate risk stratification tool to improve the predictability of CVD in CKD led to the use of coronary artery calcification (CAC) to assess subclinical atherosclerosis in the coronary vessels of CKD patients. In the non-CKD group, coronary calcium deposition can be found in certain clinical conditions such as dyslipidaemia (including inherited lipid disorders), diabetes, ageing and diseases associated with disorders of calcium homeostasis.[33] Coronary artery calcification has also been shown to be more prevalent among CKD patients, with a tendency to occur early and with distinct characteristics differentiating it from CAC in people without CKD.[31,34] Nakamura et al.[34] studied coronary calcification in patients with CKD, who had coronary artery disease; the patients were subdivided into five groups based on eGFR and proteinuria, intimal calcification of plaques was present in all groups, although, it was most frequent and more severe among CKD stage 5 HD patients. Risk factors for intimal calcification were the degree of luminal stenosis, smoking, age, diabetes, high calcium-phosphate product, presence of inflammation and reduced kidney function. On the other hand, medial calcification was seen in a small number of CKD stage 4,

5 and 5D patients with risk factors being calcium containing phosphate binders, use of haemodialysis treatment and duration on HD. The study clearly demonstrated that media calcification occurred only in the late stages of CKD. Freercks et al.[35] studied vascular calcification among a multi-ethnic ESKD population in South Africa on maintenance dialysis; the prevalence of coronary calcification was found to be 38.6% among the study population, with higher calcium score in non-black patients. The study also showed that an abdominal aorta calcium score of ≥ 1 predicted coronary artery calcium score ≥ 10 in 83 % of the patients (AUC of 0.82, sensitivity of 74.1 %, specificity of 88.4%). Lower prevalence of coronary and abdominal aorta calcification was observed among blacks compared to non-blacks in the study despite relatively similar risk exposure. Results from this study could have been more informative if the black population was compared with specific ethnic groups and not multi-ethnic groups. This study also failed to provide the prevalence of coronary artery calcification for specific racial groups. Furthermore, comparison of patients with a minimum duration on maintenance dialysis of one year instead of the 3 months used in the study could have been more informative because the initiation of dialysis may be associated with some level of inflammation due to generation of cytokines.

The presence of carotid plaques, CIMT and CAC are all useful markers of atherosclerosis, as corroborated by the study by Adeseun et al.[36] in which the discriminative ability of non-invasive measures of atherosclerosis including CIMT, carotid plaque, CAC, ascending and descending thoracic aorta calcification and Framingham risk score was examined to predict self-reported CVD. Cardiovascular disease was present in 21 % of the population and there was no difference between CAC, carotid plaque and CIMT as predictors of self-reported prevalent CVD with C-statistics of 0.67, 0.64 and 0.61 respectively ($p > 0.05$). The relatively low prevalence of CVD in this study compared to other studies is probably because the patients

recruited were pre-dialysis CKD patients with over 60 % of the patients in CKD stages 3A and above.

2.3 Left ventricular hypertrophy in CKD patients

Left ventricular hypertrophy (LVH) is a recognized CVD among CKD patients.[29] Amira et al.[29] reported a significantly higher frequency of LVH among black South African ESKD patients on haemodialysis compared to healthy controls (84.14 % versus 17.5 %, $x^2 = 60.72$, $p = 0.001$). Among black South African renal transplant recipients, in whom a much lower prevalence of LVH would have ordinarily been expected, due to anticipated regression of LVH following normalization of renal function after kidney transplantation and improved control of hypertension, Muhammad et al.[37] reported a prevalence of LVH of 76 % among this group of renal transplant recipients; 51 % of the study population had concentric hypertrophy while 25 % had eccentric hypertrophy. Persistence of LVH in the post-renal transplant period underscores the need for further evaluation of CVD risk factors among kidney transplant recipients. The reported high prevalence of LVH could be partly explained by the high prevalence of hypertension among blacks. Hypertension, with the attendant volume and pressure overload in CKD patients, leads to compensatory changes in the cardiac myocytes which result in LVH. Risk factors that predicted LVH were longer duration on dialysis, cigarette smoking, higher cumulative steroid dose, increased CIMT and waist circumference.[37] Negative correlation was also established between LVH and haemoglobin levels similar to reports from other studies.[29,38] Prior to the discovery of erythropoietin, a study of ESKD patients on haemodialysis who had ischaemic heart disease (IHD) revealed that symptoms of IHD may not necessarily denote the presence of large vessel coronary artery disease.[39] It was then hypothesized that features of ischaemia could result from a combined effect of volume overload and LVH, which increase oxygen demand and small vessel coronary disease which reduces oxygen supply.[39] Small vessel coronary disease generally arises from increased

thickening of the intra-myocardial coronary artery wall and myocardial fibrosis which compromises the calibre of the arteries and thereby reduces myocardial blood supply. Although the study by Rostand et al[39] was carried out in the pre-erythropoietin era, with the possible significant contribution of low haemoglobin to the symptoms of angina observed in the patients, more recent studies have been able to demonstrate the impact and presence of arterial stiffening in CKD patients.[40,41] The combination of increased thickening of the intra-myocardial coronary artery and myocardial fibrosis could explain why clinical presentation of angina incorrectly diagnosed acute MI in CKD patients on haemodialysis in 45 % of patients.[42] Left ventricular hypertrophy is common among CKD patients and is associated with a 60 % increase in risk of sudden cardiac death and greater than two-fold increased risk of stroke.[43] Left ventricular hypertrophy is associated with diastolic dysfunction and this may be complicated by intra-dialytic hypotension because small alterations in atrial filling will cause a significant reduction of cardiac output due to the hypertrophied left ventricular wall, reduced relaxation of the wall and activation of the Bezold-Jarisch reflex through pressure sensors on the posterior wall of the hypertrophied highly contractile ventricle.[44] Left ventricular hypertrophy also tends to herald the occurrence of other sinister cardiac complications such as heart failure and cardiomyopathy.[45] The Chronic Renal Insufficiency Cohorts (CRIC) study reported LVH to be present in three-quarters of stage 4 CKD patients, without any clinical evidence of heart failure; only 10% of this group had normal left ventricular geometry.[44]

2.4 Congestive cardiac failure and cardiomyopathy in CKD patients

Congestive cardiac failure, a multifactorial clinical syndrome, commonly presents as diastolic dysfunction and less often as systolic dysfunction or as a combination of both in CKD patients.[29,37,38] Cardiac remodelling in CKD patients can result from pressure overload, with a discrepant increase in wall to lumen dimension resulting in increased wall-lumen ratio. Pressure overload is caused by hypertension and decreased arterial compliance due to increased

stiffness of the arterial wall resulting in concentric LVH.[44] Another potent stimulus for cardiac remodelling is volume overload, caused by anaemia, fluid overload and arteriovenous fistulae and manifests as eccentric hypertrophy with left ventricular dilatation. These structural alterations in the heart may result in diastolic and/or systolic dysfunction. Yamada et al.[45] studied the echocardiographic findings of ESKD patients and found that 85-90 % had left ventricular ejection fraction of ≥ 50% despite a high prevalence of congestive cardiac failure. This is not unexpected, bearing in mind that left ventricular ejection fraction is a measure of systolic function. It is therefore conceivable that previous studies found diastolic dysfunction to be more common than systolic dysfunction in CKD.[29] The prevalence of congestive cardiac failure (CCF) among ESKD patients was reported to be 40 % in their first year with most cases of CCF attributed to diastolic dysfunction and circulatory overload.[46]

2.4.1 Cardiomyopathy in advanced CKD

Cardiomyopathy may present either as IHD or heart failure. The term "cardiomyopathy of advanced CKD" is currently being used to replace the previous terminology "uraemic cardiomyopathy" which has been jettisoned due to its inappropriateness.[44] A report from the CRIC study having studied CKD patients with eGFR < 20ml/min/1.32m^2, found no difference in the left ventricular mass index before dialysis and after initiation of haemodialysis; the study also reported a marginal drop in ejection fraction after initiating dialysis (from 53 % to 50 %).[47] This observation was corroborated by findings of the Initiating Dialysis Early and Late (IDEAL) trial.[48]

Other notable CVDs with significant impact on morbidity and mortality among CKD patients include sudden cardiac death, stroke and arrhythmias (including atrial fibrillation). The 2012 US Renal Data Registry shows that stroke was responsible for 3% of deaths among ESKD patients.[49] The relative risk of stroke in stage 5 CKD patients on dialysis was 5-10 times that

of age and sex matched healthy controls with the overall stroke incidence estimated to be 4% per year.[50] The Choices for Healthy Outcomes In Caring for ESRD (CHOICE) study, which recruited over one thousand ESKD patients on dialysis, found that the stroke rate was about 4.2 % per year, 87 % of which was ischaemic.[51] Atrial fibrillation has a prevalence of 15-20 % and it is the most prevalent cardiac dysrhythmia among CKD patients associated with increased risk of stroke.[52,53]

2.5 Risk factors for cardiovascular disease in CKD patients

Historically, traditional risk factors such as age, hypertension, diabetes, dyslipidaemia, smoking and obesity have been implicated in CVD in the general population. [54-56] However, the high prevalence of CVD among CKD patients necessitated the search for CKD-related risk factors such as, but not limited to, reduced GFR, chronic inflammation, advanced glycation end-products (AGEP), endothelial dysfunction, anaemia, hyperparathyroidism and high calcium-phosphate product.

2.5.1 Hypertension

Hypertension is present in about 75 % of CKD patients and its prevalence increases with worsening GFR.[57] Amira et al.[29] documented a significantly higher mean systolic blood pressure ($p < 0.0001$) among ESKD patients on haemodialysis compared to healthy controls. Endothelial dysfunction appears to be the link between hypertension and atherosclerosis. Disturbed laminar flow, which may be accentuated by hypertension, alters the integrity of the endothelial layer, reducing its production of vasodilatory nitric oxide synthase. Ultimately, there may be increased leakiness in the barrier function of the endothelium, leading to accumulation of lipoproteins in the intima layer. Hypertension may accelerate the change of fatty streaks to raised lesions.[58] Fogo et al[59] evaluated renal biopsy of 39 AASK cohorts and reported the presence of arteriosclerosis and or arteriolosclerosis in 38.

2.5.2 Diabetes mellitus

Diabetes mellitus is one of the causes of CKD. A retrospective longitudinal cohort study which assessed CKD prevalence, progression and associations with all-cause mortality in over 12,500 elderly diabetics showed that 48% had CKD.[60] A three-year follow-up of the cohorts showed progressively rising mortality rates as the GFR declined.[60] Diabetes mellitus is a metabolic disorder that has been intricately linked to other cardiovascular risk factors such as hypertension, obesity and dyslipidaemia. Diabetes mellitus is associated with insulin resistance in all target organs of insulin (skeletal muscle, fat and liver). Insulin resistance in the liver leads to inability to suppress gluconeogenesis and subsequent hyperglycaemia in the fasting and postprandial states. Similarly, insulin resistance in the adipose tissues results in increased generation of free fatty acids, very low density lipoprotein, triglycerides, decreased high density lipoprotein (HDL-C) and elevated low density lipoprotein (LDL-C), particularly the small dense LDL-C fraction, which is very atherogenic.[61,62] Diabetes, glucose intolerance and hyperinsulinaemia have been associated with AsVD in the general population[63,64] and more so in CKD.[29] Diabetes mellitus can be likened to a double edged sword, implicated in the progression of CKD and AsVD; as renal function declines, AsVD worsens.[65]

2.5.3 Dyslipidaemia

Dyslipidaemia can be described as a metabolic abnormality of lipid fractions. Although it is classified historically as a traditional cardiovascular risk factor, many studies have identified several distinct abnormalities of lipid metabolism in CKD, especially in the uraemic milieu.[66-68] Chronic kidney disease patients differ in the size of the lipid particle and composition when compared to those without CKD. Triglyceride (TG) levels are generally elevated in CKD patients and this includes the TG-rich APOB-containing very low density lipoprotein (VLDL-C) and intermediate-density lipoprotein (IDL) particles believed to be due to reduction in the

activity of lipoprotein lipase.[66,67] Similarly, imbalance between the activity of lecithin cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CTEP) contributes to reduction in HDL-C levels.[68] Furthermore, coupled with reduced HDL-C levels, the antioxidant and anti-inflammatory efficacy of HDL-C in CKD is reduced.[68] High serum levels of TG and low HDL-C levels with or without normal levels of total cholesterol and LDL-C levels have been documented in most HD patients.[69] Patients with CKD have also been shown to have abnormalities in the particle size of LDL-C cholesterol and elevated oxidized LDL-C.[69,70] Some studies have suggested that dyslipidaemia, evidenced as an increase in LDL-C, TG, Lipoprotein (a) and reduced HDL-C, is more commonly seen in PD than HD patients.[71,72] In many instances, development and progression of CKD is often accompanied by heavy proteinuria leading to superimposition of nephrotic dyslipidaemia on CKD-induced lipid disorders.[73] Risk of atherosclerosis and cardiovascular morbidity and mortality is enhanced by pro-atherogenic conditions created in CKD.[74]

2.5.4 Chronic inflammation and inflammatory biomarkers

Chronic inflammation is one of the major CKD-related risk factors for CVD.[75] Inflammatory markers that have been studied include serum albumin, white cell count, fibrinogen, C-reactive protein (CRP), interleukins including interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-α), pentraxin-3, the ligand of the receptor for advanced glycation end-products (EN-RAGE), soluble receptor of advanced glycation end products (sRAGE) and procalcitonin. The most studied of all these in relation to chronic inflammation and AsVD in CKD is CRP.[76] C-reactive protein has been shown to be elevated in ESKD patients on HD[29,77] Similarly, both highly sensitive-CRP (hs-CRP) and pentraxin-3 levels among ESKD patients on HD, PD and KTRs showed significantly higher mean value compared to the healthy population ($p < 0.001$).[77] Pentraxin-3, also known as the long pentraxin, an acute phase reactant similar to CRP, is thought to be more specific for inflammation than CRP. This is probably attributable to the fact

that pentraxin-3 is produced at the site of inflammation by inflammatory cells such as macrophages and vascular endothelial cells that are primarily involved in inflammation and atherosclerosis.[78] Although some researchers believe that pentraxin-3 may be atheroprotective,[79] reports from the Multi-Ethnic Study showed a positive association between pentraxin-3 and cardiovascular risk factors including age, obesity, fasting insulin, systolic blood pressure, inflammatory markers (such as CRP, IL-6) and surrogate markers of subclinical atherosclerosis including CIMT, ABI and CAC.[80] The predictive value of IL-6 for cardiovascular and all-cause mortality has been shown to be superior to CRP.[81,82] In a cross-sectional study involving 218 HD patients with CVD, a strong correlation between IL-6 and CRP [$r = 0.68$ ($p < 0.001$)] was demonstrated, together with a stronger predictive value for CVD.[81] In view of the abundant evidence linking chronic inflammation to CVD among Caucasian CKD patients on dialysis and the sparse pockets of evidence among black African CKD patients on dialysis, there is therefore a need to evaluate the relationship between chronic inflammation and CVD in the two dialysis modalities, HD and PD and in pre-dialysis CKD patients.

Available evidence upholds the fact that mild to moderate renal dysfunction is associated with poor cardiovascular outcome.[83] Worsening proteinuria and GFR have been associated with adverse cardiovascular and all-cause mortality with progressive rise in hazard ratio as GFR declines.[84,85] It is therefore not surprising that the American Heart Association recommended that CKD be considered a coronary heart disease risk equivalent.[86] As GFR falls, uraemic toxins such as asymmetric dimethyl arginine (ADMA), indoxyl sulfate, AGEP, p-cresol, guanidine, accumulate and may have a pro-atherogenic effect.[87] Advanced glycation end-products may accumulate in clinical conditions such as diabetes and CKD.[88,89] Binding of the ligand of receptor for advanced glycation end-products (EN-RAGE) to the soluble receptor for advanced glycation end-products (sRAGE), leads to transcription of genes necessary for

cellular activation and stimulation and maintenance of inflammation that may contribute to atherosclerosis.[90] In a prospective study that evaluated 184 haemodialysis patients and 50 healthy controls, the effect of sRAGE and EN-RAGE on mortality was evaluated. The study found that both sRAGE and EN-RAGE were elevated in ESKD patients on haemodialysis and patients with history of CVD had higher levels of EN-RAGE compared to those without a history of CVD ($p = 0.016$). The levels of EN-RAGE predicted both all-cause and cardiovascular mortality.[91] Ischaemia modified albumin (IMA), a biomarker of myocardial ischaemia, was shown to be highly sensitive but poorly specific for myocardial ischaemia.[92] In the course of myocardial ischaemia, several conformational alterations occur in the amino-terminal of the plasma protein, albumin and these changes alter the binding of albumin to cobalt during the IMA test.[93] In a cross-sectional study, the levels of IMA were found to be significantly higher in KTRs, HD and PD patients compared to the healthy population.[77] Ischaemia modified albumin also positively correlated with hs-CRP, pentraxin-3 and neutrophil-to-lymphocyte ratio.[77]

In the setting of intact immunity, following presentation of antigen to T lymphocytes and the subsequent stimulation of CD3 molecules, proliferation and differentiation of T-helper cells is initiated by binding of IL-2 to the interleukin-2 receptor (IL-2R). This results in cytokine-dependent differentiation of naïve T-helper cells (Th0) into any of Th1, Th2 or Th17 cells. Differentiation into T-helper 1 cells is driven by IL-12 and IL-2 and their effector cytokine is IFN-γ.[94] Differentiation along the T-helper 2 pathway is typically driven by IL-4 and their effector cytokines are IL-4, IL-5, IL-6, IL-9, IL-10, IL-13.[95] The T-helper 17 pathway is a newly identified pathway of differentiation of naïve T-helper cell and the fate of Th17 cells can either be generation of pro-inflammatory or anti-inflammatory Th17 cells triggered by IL-23, IL-1β and IL-6, TGFβ respectively; the effector cytokines of Th17 cells are IL-17A, IL-17F, IL-21, IL-22.[96] Chronic inflammation is an established risk factor for CVD in CKD; therefore,

determination of the predominant T-helper pathway involved in CKD inflammation could be promising for therapeutic interventions in the future, particularly in terms of preventing or retarding CVD in CKD patients.

A study of the role of serum catalytic iron (SCIr) in the development of occlusive coronary disease among CKD patients on HD showed that high levels of SCIr were associated with significant coronary artery disease in HD patients.[97] Serum catalytic iron is free iron, unbound to transferrin in plasma,[98] and remains a huge reservoir for the generation of oxygen-derived free radicals which may have harmful vascular effects via myriads of mechanisms such as acceleration of atherosclerosis by production of oxidized low density lipoprotein (ox-LDL-C) and endothelial dysfunction.[99]

2.5.5 Endothelial dysfunction

The endothelium serves important physiological functions[100,101] such as maintenance of vascular tone via the production of vasoactive substances namely, nitric oxide (NO), prostacycline (PGI_2) and endothelin (ET). The endothelium is also involved in the production of cytokines, provision of an anti-thrombotic surface as well as acting as a permeable membrane through which exchange of substances occur between the arterial wall and the blood. Alteration of a single or any combination of these functions can be described as endothelial dysfunction (ED). The integrity of the vascular endothelium can be assessed by its secretion of endothelial-derived relaxation factor called NO.[101] In normal physiologic state, nitric oxide is secreted at the basal level and in response to stimulation. Stimulatory secretion of NO can either be via a flow-mediated (non-receptor pathway) or a receptor-mediated pathway. Flow-mediated release occurs in response to an increase in blood flow and shear stress of the vessel wall[102] while receptor-mediated release of NO occurs in response to acetylcholine, serotonin and substance P.[103] Endothelial dysfunction plays a significant and

central role in the pathogenesis of atherosclerosis, typified by the "response to injury hypothesis".[104] Reduction in NO, which leads to an unopposed vasoconstrictive effect and a paradoxical vasoconstriction following exposure to acetylcholine,[105] is the most studied endothelial alteration. Endothelial dysfunction, defined by paradoxical vasoconstriction following exposure to acetylcholine, was found to be associated with reduction in renal function in patients with essential hypertension.[106] The contributions of uraemic toxins to ED and ultimately to the development of atherosclerosis have been studied.[107-111] Asymmetric dimethylarginine, an endogenously-derived inhibitor of nitric-oxide synthase, which is cleared by the kidneys, accumulates when there is decline in renal function, and has been implicated in the pathogenesis of CVD and endothelial dysfunction.[112,113] The functional integrity of the endothelium was first assessed by an intracoronary acetylcholine test. It was performed by administering increasing doses of acetylcholine during coronary angiography and a paradoxical vasoconstrictive effect is seen in coronary arteries with luminal irregularities.[105] However, in view of the invasiveness of the procedure, it cannot be used in a clinical setting for predictive purpose or in large population studies; therefore less invasive methods have been devised to assess endothelial function in the peripheral vessels based on the concept that atherosclerosis is a systemic disease.[114] The second method is semi-invasive and it involves cannulation of the brachial artery with possible complications of injury to the artery or the median nerve. This method, known as forearm plethysmography, measures changes in blood flow after administration of acetylcholine or after reactive hyperaemia.[115] The third method is flow-mediated vasodilation (FMD) of the brachial artery. It is a non-invasive evaluation of endothelial function.[116] The limitations of FMD include inter-observer variability and presence of factors that influence FMD results such as smoking, caffeine, lipid-rich meals, high glucose meals.[117,118]

2.5.6 Oxidative stress

Oxidative stress has been implicated in CKD.[119] As discussed above, abnormalities in the synthesis of NO are a potent cause of reduced NO bioavailability. It is however very pertinent to highlight that peripheral metabolism of NO and its interaction with other molecules, especially those with thiol group contributes to the reduction in the bioavailability of NO and generation of nitrosative oxidative stress.[120] Nitric oxide has been shown to contribute significantly to the nitrosative components of oxidative stress by binding to molecules containing a functional thiol group such as protein thiol, glutathione, homocysteine and acetylcysteine to form S-nitrosothiols.[120] S-nitrosothiol, a potent vasodilator, functions physiologically as a huge reserve and harbinger of NO, which is released following reaction with transition metal ions, glutathione peroxidase ascorbic acid and thiol compounds.[120-122] Significantly higher levels of S-nitrosothiol were reported in maintenance HD patients compared to healthy controls ($p < 0.0001$).[123] Apart from the NO metabolic pathways, oxidative species can also be generated by myeloperoxidase (MPO), which is secreted by neutrophils and monocytes at site of cellular inflammation, persists in and around the vascular endothelium[124,125] and can oxidize tyrosine to produce 3-chlorotyrosine.[126] Myeloperoxidase oxidative pathways occur in a well-specialised pattern in humans and has also been implicated in the generation of other reactive species such as peroxinitrites and nitrotyrosine.[127,128] A significant positive association was reported between serum malondialdehyde and prevalent CVD among haemodialysis patients.[129] In accordance with the belief that oxidative stress contributes to the cardiovascular burden in CKD patients, the effect of antioxidants in reducing CVD has been studied.[130,131] The effect of acetylcysteine on cardiovascular events was assessed by a prospective, randomised, placebo-controlled trial. The results showed that acetylcysteine reduces composite cardiovascular end-points (cardiovascular death, MI, need for coronary angioplasty or coronary bypass surgery) in maintenance haemodialysis patients.[131]

2.5.7 Anaemia

Anaemia is commonly present in CKD patients and is more severe in ESKD.[132] Anaemia has been shown to occur early in CKD patients. Data from the National Health And Nutrition Examination Survey (NHANES) showed that decline in haemoglobin levels is seen from eGFR < 75 ml/min/1.73m^2 in men and eGFR < 45 ml/min/1.73m^2 in women.[133] Anaemia is a recognised CKD-related risk factor for CVD.[134] Pooled data from four community-based studies (Atherosclerotic Risk in Communities study, Cardiovascular Health Study, Framingham Heart study and Framingham Offspring study) was studied. Anaemia was taken as haematocrit < 36 % in females and < 39 % in males and LVH was defined based on electrocardiographic findings. Anaemia was found to be associated with increased risk of composite outcome (MI, stroke and death).[135] Anaemia, a common phenomenon in CKD patients, probably occurs because erythropoietin (EPO) production from the interstitial fibroblasts near the tubular epithelial cells and peritubular capillaries in the kidneys, significantly exceeds the EPO production capacity of hepatocytes and perisinusoidal cells of Ito (which is more important in the foetal and perinatal periods), and therefore hepatic production of EPO cannot compensate in renal disease. [44,136,137] The exact mechanism responsible for the reduction in EPO production is yet to be fully elucidated, although reduction in EPO production was traditionally thought to be due to local injury to the kidneys, leading to decreased expression of the EPO gene.[138] Current thinking favours the presence of considerable EPO production capacity in the kidneys, even in ESKD.[137] It has therefore been speculated that the problem may be the inability of the kidneys to respond to chronic hypoxia, since an additional hypoxic stimulus causes significant increase in EPO production in CKD patients with anaemia.[137] The role of hypoxia-inducible factors (HIF-1 and HIF-2) and HIF stabilizers in the transcription of genes responsible for increasing the production of EPO in both hypoxic

and normoxic states have been studied; however, there are no data on the association of HIF-2 to atherosclerosis.[139-141]

2.5.8 Calcium-phosphate abnormalities, secondary hyperparathyroidism and vascular calcification

Alteration in calcium-phosphate-vitamin D-parathyroid hormone is commonly encountered in CKD.[142,143] Secondary hyperparathyroidism in CKD patients is typically heralded by one or a combination of hypocalcaemia, hyperphosphataemia due to increased retention as a result of a falling GFR, deficiency of vitamin D due to lack of 1-α hydroxylation, decreased expression of vitamin D and calcium receptors, and resistance to parathyroid hormone.[144-148] The association between serum phosphate levels and LVH has been demonstrated by various studies.[149,150] Block et al[151] using data from the United States Renal Data System (USRDS), showed an association between phosphate, calcium-phosphate product and mortality. Analysis of follow-up data revealed that hyperphosphataemia was also associated with cause-specific cardiovascular mortality.[151,152] Interestingly, apart from altered calcium and phosphate metabolism with an attendant increase in calcium-phosphate product, and possible risk of metastatic deposition,[151] vascular calcification, has been associated with alterations in the serum levels of regulators of metastatic calcification such as fetuin-A (also known as alpha-2 Heremans-Schmidt's glycoprotein),[153] GLA-matrix protein and osteoprotegerin.[154,155] Morena et al.[156] reported that elevated plasma osteoprotegerin levels predicted all-cause (RR - 2.67; 95 % CI - 1.32-5.41; p = 0.006) and cardiovascular mortality (RR 3.15; 95 % CI – 1.14-8.69; p = 0.03) in HD patients followed up for 2 years; the association between osteoprotegerin and all-cause mortality was stronger in patients with higher C-reactive protein levels.

2.6 *APOL1* risk variants in CKD and atherosclerotic vascular disease

The recent discovery of the association between *APOL1* and different forms of CKD and atherosclerosis has improved the understanding of the pathogenesis of CKD and AsVD. Circulating *APOL1* was identified as an interacting protein of APO A1 and a minor component of high density lipoprotein subfraction-3 (HDL-C3) in 1997.[157] A vital breakthrough in the investigation of *APOL1* concerning complex diseases of humans was the discovery of two haplotypes of *APOL1*, which accommodates three coding sequence mutations, as risk variants for non-diabetic CKD in African Americans.[158,159] Individuals with one copy of the variants resist being infected by trypanosomes; however, individuals who have two copies of either variant have higher susceptibility of developing non-diabetic CKD such as primary focal segmental glomerulosclerosis, hypertension-related kidney disease, HIV-associated nephropathy, sickle cell disease associated nephropathy, IgA nephropathy and lupus nephritis.[23,159-163] In renal allograft recipients of African American ethnicity, an association has been established between *APOL1* and kidney transplant rejection and graft failure.[164,165] Report from USRDS 2011 showed that hypertension was the aetiology of ESKD in approximately 35 % of African Americans starting dialysis.[25] However, this clinical diagnosis has been shown to be incorrect in most cases[166,167] because *APOL1*-associated glomerulosclerosis is the aetiology of renal dysfunction in the majority of African Americans tagged as hypertension-associated ESKD or hypertension-associated nephropathy.[168,169] This has been corroborated by findings from the African American Study of Kidney disease and hypertension trial (AASK).[170] Apolipoprotein L1 risk alleles showed statistically significant association in CKD cases (OR 2.57); this association was further strengthened in the subsets of patients with more severe renal impairment (serum creatinine of 3 mg/dL or with a baseline urine protein creatinine ratio greater than 0.6 g/g), (OR 4.61 and 6.29, respectively)[170]

Severe kidney disease is more commonly seen in Americans of African descent compared to European populations. However, African Americans have not been shown to have higher rates of early nephropathy.[171] The above, together with the markedly weaker *APOL1* association with milder forms of nephropathy, strengthens the hypothesis that *APOL1* is a risk factor for nephropathy progression.[172] A study by Mukamal et al.[173] demonstrated that the *APOL1* genotypes are associated with increased levels of albumin in urine, clinically-inapparent atherosclerosis, new onset acute MI and death in older African Americans.

The distribution of the variants associated with kidney disease is more prevalent in western parts of Africa, less so in northern and eastern populations of Africa and is almost non-existent in Ethiopia.[174] Among the Yoruba people in the southwestern Nigeria, the prevalence of G1 and G2 risk alleles were found to be 40 % and 8 % [175] while Kasembeli et al[163] found a prevalence of 7.3 % and 11.1 % among black South Africans; and an association between *APOL1* risk variants and HIV-associated nephropathy in seventy-nine percent of patients with HIV-associated nephropathy. Only two percent of population controls carried the *APOL1* risk alleles, and patients with any combination of two *APOL1* risk alleles had 89-fold higher odds of developing HIV-associated nephropathy compared with HIV positive controls without nephropathy.[163]

2.7 Pathogenesis of atherosclerosis

The process of formation of an atherosclerotic lesion has been described as a normal response to diverse injuries to the vascular endothelium and smooth muscle cells of the vessel wall, characterized by formation of fibrofatty and fibrous lesions, heralded and closely linked to inflammation.[176] This underscores the importance of inflammation in the pathogenesis of the lesions of atherosclerosis. Therefore, inflammation may be the missing link responsible for the limited or lack of a positive effect on mortality of lipid-lowering medication (statins) in CKD

patients despite the established effect of statins in preventing mortality in the general population. [177,178]

2.7.1 Histology of the arterial wall

The wall of the artery is, ultrastructurally, made up of three distinct parts, namely, intima, media and adventitia from the lumen to the periphery. The intima is composed of extracellular connective tissue matrix and few smooth muscle cells and it is bordered by a single layer of endothelial cells at the luminal end and the internal elastic lamina at the peripheral end. The components of the intima increase with age in a well-controlled pattern. The media, bordered by the internal and external elastic lamina at the luminal and peripheral ends respectively, as shown in fig. 2.1, is composed of well-patterned, diagonally arranged smooth muscle cells, with varying amounts of collagen and proteoglycans surrounding the cells. Each smooth muscle cell is attached to the other by a tight junctional complex. The adventitia is the outermost layer of the arterial wall that is composed of fibroblasts and smooth muscle cells embedded in collagen and proteoglycans.[179]

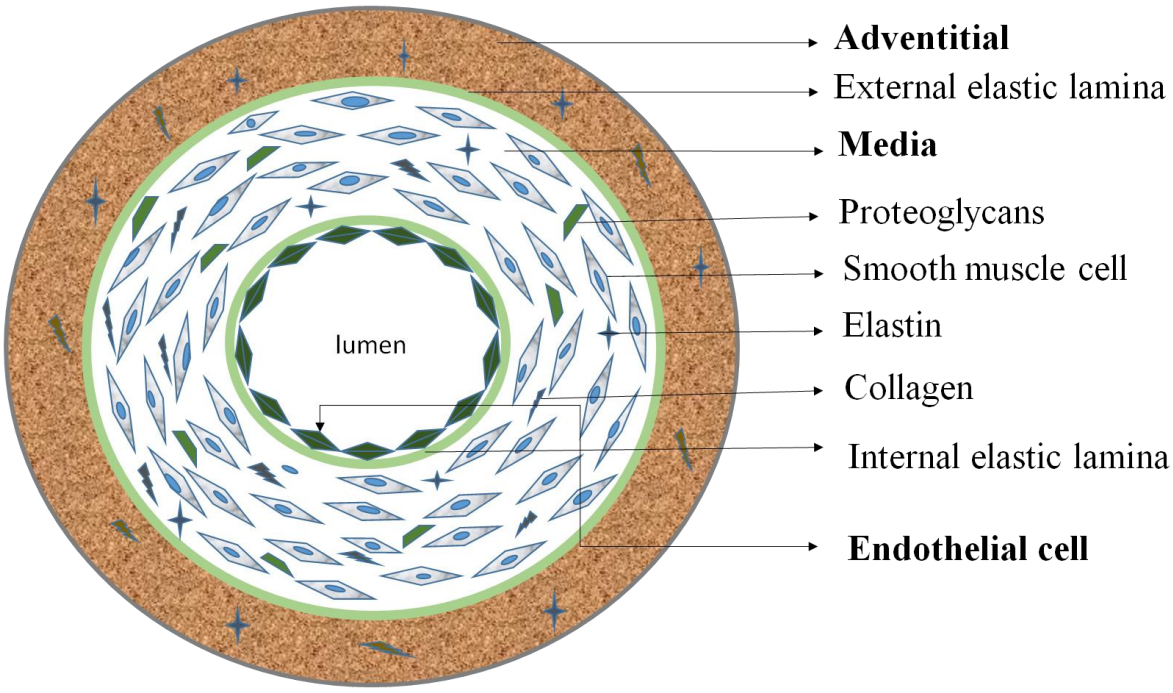

Figure 2.1 Normal cross-sectional structure of an artery. Adapted from the Medical gallery of Blausen Medical 2014[180]

2.7.2 The atherosclerotic lesion

Based on ultrastructural morphology, three distinct types of lesions have been described in atherosclerosis.[181] The first recognizable lesion of atherosclerosis is the fatty streak, yellowish in colour because the smooth muscle cells and macrophages trapped in the lesion are lipid-laden (cholesterol and cholesteryl ester).[182] The fatty streak is also flat in configuration and may be widespread and commences commonly in childhood.[183] However, with increasing age, the morphology of the fatty streak may change to the more advanced atherosclerotic lesion.[183] Fatty streaks have been shown to be the lesions that herald the development of the fibrous plaque,[183,184] which is the second atherosclerotic lesion. The fibrous plaque is whitish, elevated, protrudes into the lumen of the artery and characterizes advanced atherosclerotic lesions.[185] Fibrous plaques are composed of an intimal layer with lipid-laden smooth muscle cells. The third lesion, known as the complex lesion, is a complicated fibrous plaque. Haemorrhagic,

thrombotic, necrotic and calcific complications have been described as a consequence of the fibrous plaque; the calcified plaque remains the typical finding.[186]

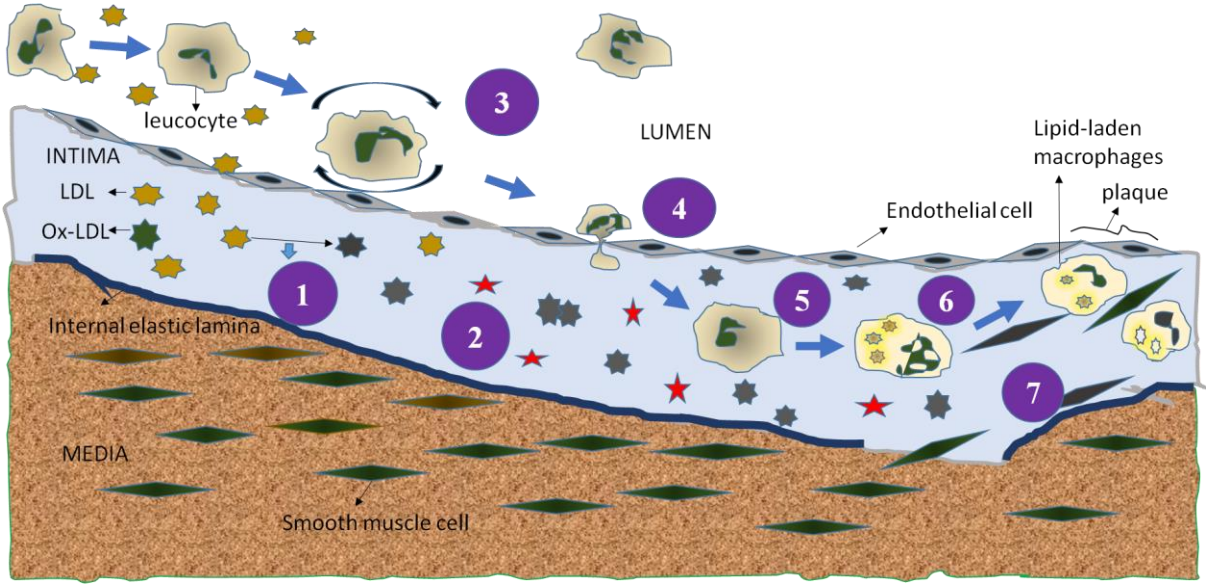

Figure 2.2 sequence of events in the pathogenesis of atherosclerosis

Adapted from Harrison's Principles of Medicine, 19th edition, with permission from McGraw Hill Education Material, (Appendix1).

Key: Ox-LDL-C – oxidized low density lipoprotein; – low density lipoprotein;

1 – LDL-C traversing the arterial endothelium and conversion to Ox-LDL-C

2 – Ox-LDL-C stimulates production of inflammatory markers including leucocyte chemoattractants

3 – Leucocyte margination on the endothelium

4 – leucocyte diapedesis into the arterial media layer

5 – Differentiation of monocytes into macrophages

6 – Ingestion of LDL-C and formation of foam cells.

7 – Migration of smooth muscle cells from the media into the sub-endothelial space following a break in the internal elastic lamina

2.7.3 The sequence of events in the pathogenesis of atherosclerosis

As previously alluded to, one of the physiological functions of the vascular endothelium is to act as a barrier between the intravascular content and the extracellular matrix of the vascular wall.[187] Low density lipoprotein molecules may traverse the endothelial barrier into the intimal layer of the vessel wall, which may be due to increased permeability of the vessel wall.[188] The LDL-C molecule then becomes tightly bound to extracellular material in the intima, particularly proteoglycans.[189] The binding to proteoglycans increases the transit time of the LDL-C molecule in the intima and sequesters the LDL-C from exposure to the normally occurring anti-oxidants in the blood, thereby increasing its risk of oxidation and modification of LDL-C into ox-LDL-C, as shown in fig 2.[190] Oxidized LDL-C generates inflammatory responses by producing leucocyte chemoattractants and by stimulating various cellular components of the vessel wall to produce cytokines, growth factors, adhesion molecules and vasoactive substances[191-193] Vascular endothelial cells secrete macrophage chemoattractant protein-1 (MCP-1), prostacyclin (PGI_2) and platelet-derived growth factor (PDGF).[191,192] Similarly, smooth muscle cells have receptors for PDGF and LDL-C and can secrete several vasoactive substances including prostacyclin (PGI_2) and prostaglandin E_2 (PGE_2).[194,195] Studies have shown that some specific chemoattractants such as IL-1 and leukotriene B4, secreted by endothelial cells and macrophages preferentially favour neutrophilic and monocytic adherence to the vascular endothelium.[196,197] After adhesion to the vascular endothelium, migration of monocytes into the subendothelial space occurs, followed by differentiation of the monocytes into macrophages which then ingest the LDL-C molecules in the intima layer, eventually forming the first recognizable lesion of atherosclerosis, the fatty streak.

JUSTIFICATION FOR THE STUDY

Diseases of the heart and blood vessels are amongst the commonest causes of morbidity and death in CKD. Traditional and non-traditional risk factors have been implicated in the aetiopathogenesis of atherosclerotic vascular disease in CKD.

Dyslipidaemia is an important traditional risk factor for CVD in CKD. Studies on the role of cardiovascular biomarkers have concluded that they potentiate the effect of the traditional risk factors in individuals with near optimal renal function[198] and those with mild to moderate renal impairment. [199,200] More recent studies are investigating the role of lipoprotein (a), Apo A1 and *APOL1* as predictors of CVD in CKD.

A novel biomarker of chronic inflammatory cardiovascular disease, pentraxin-3, needs to be evaluated as a possible surrogate marker for atherosclerosis in CKD patients.

The synergistic effect of dyslipidaemia and chronic inflammation may contribute to CVD in CKD with attendant morbidity and mortality.

AIM

To evaluate the relationship of chronic inflammatory markers and dyslipidaemia to atherosclerotic vascular disease in different categories of chronic kidney disease patients (predialysis CKD stages 3, patients on HD, patients on PD, renal transplant recipients).

OBJECTIVES

1. To establish the prevalence of atherosclerotic vascular disease in CKD stage 3, haemodialysis, peritoneal dialysis patients and kidney transplant recipients.
2. To establish the prevalence of dyslipidaemia and its relationship with atherosclerotic vascular disease in CKD stage 3, haemodialysis, peritoneal dialysis patients and kidney transplant recipients

3. To determine the relationship of inflammation to atherosclerotic vascular disease in CKD stage 3, haemodialysis, peritoneal dialysis patients and kidney transplant recipients
4. To determine relationship of inflammation, dyslipidaemia and atherosclerotic vascular disease in CKD stage 3, haemodialysis, peritoneal dialysis patients and kidney transplant recipients
5. To determine utility of lipoprotein biomarkers and inflammatory markers in predicting atherosclerotic vascular disease
6. To determine relationship of *APOL1* genotypes to AsVD.

References

1. NKF KDOQI Clinical practice guidelines for chronic kidney disease: Evaluation, Classification and Stratification. Am J Kidney Dis 2002; 39: S1-S266
2. Morabia A, Abel T. The WHO report "Preventing chronic diseases: a vital investment" and us. Soz Praventivmed 2006; 51 (2): 74
3. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012; 380: 2095-2128
4. Coresh J, Astor BC, Greene T, Eknoyan G, Levey AS. Prevalence of chronic kidney disease and decreased kidney function in adult US population. Third National Health and Nutrition Evaluation Survey. Am J Kidney Dis. 2003; 41(1): 1-12
5. USRDS Annual Data Report. Atlas of End stage renal disease in the united states. Bethesda MD: National Institute of Health, National institute of Diabetes, Digestive and Kidney Diseases: 2003
6. Arogundade FA, Barsoum RS. CKD prevention in Sub-Saharan Africa; a call for governmental. Non-governmental and community support. Am J Kidney Dis 2008; 51:515-523
7. Naicker S. End-stage renal disease in Sub-Saharan Africa. Ethn Dis 2009; 19:1-15
8. Davids MR, Marrais N, Jacobs JC. South African renal registry Annual report 2015. South African renal society, Cape Town, 2017
9. Ulasi II, Ijoma CK. The enormity of chronic kidney disease in Nigeria: the situation in teaching hospital in South-East Nigeria. J Trop Med. 2010; 2010: 501957. Doi: 10.1155/2010/501957

10. Foley RN, Murray AM, Li S, Herzog CA, McBean AM, Eggers PW, et al. Chronic kidney disease and the risk for cardiovascular disease, renal replacement, and death in the United States Medicare population, 1998 to 1999. J Am Soc Nephrol 2005; 16: 489–495
11. Yamamoto S, Kon V. Mechanisms for increased cardiovascular disease in chronic kidney dysfunction. Curr Opin Nephrol Hypertens 2009; 18: 181-188
12. Baigent C, Burbury K, Wheeler D. Premature cardiovascular disease in chronic renal failure. Lancet 2000; 356: 147-152
13. Van Biesen W, De Bacquer D, Verbeke F, Delanghe J, Lameire N, Vanholder R, et al. The glomerular filtration rate in an apparently healthy population and its relation with cardiovascular mortality during 10 years. Eur Heart J 2007 ; 28: 478-483
14. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events and hospitalization. N Engl J Med 2004; 351: 1296-1305
15. Shulman NB, Ford CE, Hall WD, Blaufox MD, Simon D, Langford HG, et al. Prognostic value of serum creatinine and effect of treatment of hypertension on renal function. Results from the hypertension detection and follow-up program. The hypertension detection and follow-up program cooperative group. Hypertension 1989; 13:180-193
16. Herzog CA, Asinger RW, Berger AK, Charytan DM, Diez J, Hart RG, et al. Cardiovascular disease in chronic kidney disease. A clinical update from kidney disease improving global outcomes (KDIGO). Kidney Int 2011; 80(6): 572-586
17. Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, et al. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart association councils on kidney in cardiovascular disease, High Blood

pressure research, clinical cardiology, and Epidemiology and prevention. Circulation 2003; 108: 2154-2169

18. Urbano L, Portilla E, Munoz W, Hofman A, Sierra-Torres CH. Prevalence and risk factors associated with peripheral arterial disease in an adult population from Columbia. Arch Cardiol Mex. 2017; doi: 10.1016/j.acmx.2017.02.002
19. Peyster E, Chen J, Feldman HI, Go AS, Gupta J, Mitra N, et al. Inflammation and arterial stiffness in chronic kidney disease. Findings from the CRIC study. Am J Hypertens. 2017; 30(4): 400-408
20. Reiss AB, Voloshyna I, DeLeon J, Miyawaki N, Mattana J. Cholesterol metabolism in CKD. Am J Kidney Dis. 2015; 66(6): 1071-1082
21. Norata GD, Marchesi P, Pulakazhi Venu VK, Pasqualini F, Anselmo A, Moalli F, et al. Deficiency of long Pentraxin-3 promotes vascular inflammation and atherosclerosis. Circulation 2009; 120(8): 699-708
22. Lewington S, Whitlock G, Clarke R, Sherlicker P, Embertson J, Halsey J, et al. Blood cholesterol and vascular mortality by age, sex and blood pressure: A meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. Lancet 2007; 370: 1829-1839
23. Kopp JB, Nelson GW, Sampath K, Johnson RC, Genovese G, An P, et al. ApoL1 genetic variants in focal segmental glomerulosclerosis and HIV-associated nephropathy. J Am Soc Nephrol. 2011; 22: 2129-2137
24. Ito K, Bick AG, Flannick J, Friedman DJ, Genovese G, Parfenov MG, et al. Increased burden of cardiovascular disease in carriers of Apo L1 genetic variants. Circ. Res. 2014; 114(5): 845-850

25. US Renal Data Systems USRDS 2011 Annual data report: Atlas of end stage renal disease in United States. National Institute of Health, National Institute of Diabetes and Digestive and Kidney diseases; Bethesda, MD, 2011
26. Muntner P, He J, Hamm L, Loria C, Whelton PK. Renal insufficiency and subsequent death resulting from cardiovascular disease in the United States. J Am Soc Nephrol. 2002; 13(3): 745-753
27. Amann K, Tyralla K, Gross ML, Eifert T, Adamczak M, Ritz E. Special characteristics of atherosclerosis in chronic renal failure. Clin Nephrol. 2003; 60(1): 13-20
28. Lindner A, Charra B, Sherrard DJ Scribner BH. Accelerated atherosclerosis in prolonged maintenance hemodialysis. New Engl. J Med. 1974; 290: 697-701
29. Amira OC, Naicker S, Manga P, Sliwa K, Mia A, Raal F, et al. Adiponectin and atherosclerosis risk factors in African haemodialysis patients: A population at low risk for atherosclerotic cardiovascular disease. Hemodialysis Int. 2012; 16(1): 59-68
30. Savage T, Clarke AL, Giles M, Tomson CR, Raine AE. Calcified plaques are common in carotid and femoral arteries of dialysis patients without clinical vascular disease. Nephrol Dial Transplant 1998; 13: 2004-2012
31. Schwarz U, Buzello M, Ritz E, Stein G, Raabe G, Wiest G, et al. Morphology of coronary atherosclerotic lesions in patients with end stage renal failure. Nephrol Dial Transplant 2000; 15:218-223
32. Patel ML, Radheyshyam, Verma A, Sachan R, Kamal R. Impact of carotid intima-media thickness on long-term outcome in haemodialysis patients. N Am J Med Sci. 2015; 7(6): 281-287
33. Abedin M, Tintut Y, Demer LL. Vascular calcifications: mechanisms and clinical ramifications. Atherosclerosis, Thrombosis and Vascular Biology 2004; 24: 1161 - 1170

34. Nakamura S, Ishibashi-Ueda H, Niizuma S, Yoshihara F, Horio T, Kawano Y. Coronary calcification in patients with chronic kidney disease and coronary artery disease. Clin J Am Soc Nephrol. 2009; 4: 1892-1900
35. Freercks R, Swanepoel C, Carrara H, Moosa S, Lachman A, Rayner B. Vascular calcification in South African dialysis patients: Ethnic variation, prevalence, detection and haemodynamic correlates. Nephrology 2012; 17: 607-617
36. Adeseun GA, Xie D, Wang X, Joffe MM, Mohler ER 3rd, Townsend RR, et al. Carotid plaque, carotid intima media thickness and coronary calcification equally discriminates prevalent cardiovascular disease in kidney disease. Am J Nephrol. 2012; 36(4): 342 - 347
37. Muhammad AS, Naidoo S, Manga P, Nazir MS, Naicker S. Left ventricular hypertrophy in kidney transplant recipients in Sub-Saharan Africa. Sub-Saharan Afr J Med. 2015; 2: 70-73
38. Dzemidzic J, Rasic S, Saracevic A, Rebic D, Uncanin S, Srna A, et al. Predictors of left ventricular remodelling in kidney transplant recipients in the first post-transplant year. Bosn J Basic Med Sci 2010; 10 Suppl 1: S51-55
39. Rostand SG, Kirk KA, Rutsky EA. Dialysis-associated ischaemic heart disease: Insights from coronary angiography. Kidney Int. 1984; 25: 653-659
40. Blacher J, Guerin AP, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness on survival in end stage renal disease. Circulation. 1999; 99: 2434-2439
41. Guerin AP, Blacher J, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness attenuation on survival of patients in end-stage renal failure. Circulation. 2001; 103: 987-992
42. Herzog CA, Littrel K, Arko C, Frederick PD, Blaney M. Clinical characteristics of dialysis patients with acute myocardial infarction in United States: A collaborative

project of the United States Renal Data System and the National Registry of Acute Myocardial Infarction. Circulation 2007; 116(13): 1465-1472

43. Krane V, Heinrich F, Meesmann M, Olschewski M, Lilienthal J, Angermann C, et al. Electrocardiography and outcome in patients with diabetes mellitus on haemodialysis. Clin J Am Soc Nephrol. 2009; 4: 394-400
44. Stenvinkel P, Herzog CA. Cardiovascular disease in chronic kidney disease. Comprehensive clinical Nephrology. 5th Edition, 2014; Chapter 82, page 957- 967.
45. Yamada S, Ishii H, Takahashi H, Aoyama T, Morita Y, Kasuga H, et al. Prognostic value of reduced left ventricular ejection fraction at start of haemodialysis therapy on cardiovascular and all-cause mortality in end stage renal disease. Clin J Am Soc Nephrol. 2010; 5: 1793-1798
46. US Renal Data Systems USRDS 2007 Annual data report: Atlas of chronic kidney disease and end stage renal disease in United States. Bethesda MD, National institute of health, National institute of Diabetes and digestive and Kidney diseases; 2007
47. Park M, Hsu CY, Li Y, Mishra RK, Keane M, Rosas SE, et al. Associations between kidney function and subclinical cardiac abnormalities in CKD. J Am Soc Nephrol. 2012; 23: 1725-1734
48. Whalley GA, Marwick TH, Doughty RN, Cooper BA, Johnson DW, Pilmore A, et al. Effect of early initiation of dialysis on cardiac structure and function: Result from the echo sub-study of the IDEAL trial. Am J Kid Dis 2013; 61: 262-270
49. US Renal Data System USRDS 2012 Annual data report: Atlas of chronic kidney disease and end stage renal disease in United States. Bethesda MD, National Institute of Health, National Institute of Diabetes and Digestive and Kidney diseases; 2012

50. Seliger SL, Gillen DL, Longstreth Jr WT, Kestenbaum B, Stehman-Breen CO. Elevated risk of stroke among patients with end-stage renal disease. Kidney Int. 2003; 64: 603-609
51. Sozio SM, Armstrong PA, Coresh J, Jaar BG, Fink NE, Plantinga LC, et al. Cerebrovascular disease incidence, characteristics, and outcomes in patients initiating dialysis: the choices for healthy outcomes in caring for ESRD (CHOICE) study. Am J kidney Dis 2009; 54(3): 468-477
52. Kanel WB, Abbott RD, Savage DD, McNamara PM. Epidemiologic features of chronic atrial fibrillation: The Framingham study. N Engl J Med 1982; 306(17): 1018-1022
53. Go AS, Fang MC, Udaltsova N, Chang Y, Pomernacki NK, Borowsky L, et al. Impact of proteinuria and glomerular filtration rate in risk of thromboembolism in atrial fibrillation: The anticoagulation and risk factors in atrial fibrillation (ATRIA) study. Circulation 2009; 119(10): 1363-1369
54. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004; 364: 937-952
55. Vasan RS, Sullivan LM, Wilson PW, Sempos CT, Sundstrom J, Kannel WB, et al. Relative importance of borderline and elevated levels of coronary heart disease risk factors. Ann Intern Med 2005; 142: 393-402
56. Patel SA, Winkel M, Ali MK, Narayan KM, Mehta NK. Cardiovascular mortality associated with 5 leading risk factors: National and state preventable fractions estimated from survey data. Ann Intern Med 2015; 163(4): 245-255
57. Coresh J, Wei GL, McQuillan G, Brancati FL, Levey AS, Jones C, et al. Prevalence of high blood pressure and elevated serum creatinine level in the United States: Findings

from the third National Health and Nutrition Examination Survey (1988-1994). Arch Intern Med. 2001; 161(9): 1207-1216

58. McGill HC Jr, McMahan CA, Tracy RE, Oalmann MC, Cornhill JF, Herderick EE, et al. Relation of a post-mortem renal index of hypertension to atherosclerosis and coronary artery size in young men and women. Pathobiological determinants of atherosclerosis in youth (PDAY) research group. Arterioscler Thromb Vasc Biol. 1998; 18(7): 1108-1118
59. Fogo A, Breyer JA, Smith MC, Cleveland WH, Agodoa L, Kirk KA, et al. Accuracy of the diagnosis of hypertensive nephrosclerosis in African Americans: A report from the African American Study of Kidney Disease (AASK) trial. Kidney Int. 1997; 5(1): 244-252
60. Patel UD, Young EW, Ojo AO, Hayward RA. CKD Progression and mortality among older patients with diabetes. Am J Kidney Dis 2005; 46(3): 406-414
61. Miccoli R, Bianchi C, Penno G, Del Prato S. Insulin resistance and lipid disorders. Future Lipidology 2008; 3(6): 651-664
62. Gerber PA, Thalhammer C, Schmied C, Spring S, AmaNn-vesti B, Spinas GA, et al. Small dense LDL-C particles predict changes in intima media thickness and insulin resistance in men with type 2 diabetes and prediabetes- A prospective study. 2013 doi.org/10.1371/journal.pone.0072763
63. Kannel WB, McGee DL. Diabetes and glucose tolerance as risk factors for cardiovascular disease: The Framingham Study. Diabetes Care 1979; 2: 120-126
64. Reaven GM. Banting lecture. Role of insulin resistance in human disease. Diabetes 1988; 37: 1595-1607

65. Ishimura E, Shoji T, Emoto M, Motoyama K, Shinohara K, Matsumoto N, et al. Renal insufficiency accelerates atherosclerosis in patients with type 2 diabetes mellitus. Am J Kidney Dis. 2001; 38: S186-S190
66. Attman PO, Knight-Gibson C, Tavella M, Samuelsson O, Alaupovic P. The compositional abnormalities of lipoproteins in diabetic renal failure. Nephrol Dial Transplant 1998;13: 2833-2841.
67. Lee DM, Knight-Gibson C, Samuelsson O, Attman PO, Wang CS, Alaupovic P. Lipoprotein particle abnormalities and the impaired lipolysis in renal insufficiency. Kidney Int 2002; 61:209-218
68. Schuchardt M, Tolle M, Van der Giet M. High density lipoprotein- Structural and functional changes under uraemic conditions and the therapeutic consequences. Handbook of Experimental Pharmacology 2015; 224: 423-453
69. Avram MM, Goldwasser P, Burrell DE, Antiqnani A, Fein PA, Mittman N. The uremic dyslipidaemia: A cross-sectional and longitudinal study. Am J Kidney Disease 1992; 20: 324-335.
70. Deighan CJ, Caslake MJ, McConnell M, Boulton-Jones JM, Packard CJ. Atherogenic lipoprotein phenotype in end-stage renal failure: Origin and extent of small dense low-density lipoprotein formation. Am J Kidney Dis 2000; 35: 852-862
71. Shurraw S, Tonelli M. Statins for treatment of dyslipidaemia in chronic kidney disease. Perit Dial Int 2006; 26:523-539.
72. Moberly JB, Attman PO, Samuelsson O, Johansson AC, Knight-Gibson, Alaupovic P. Alteration in lipoprotein composition in peritoneal dialysis patients. Perit Dial Int 2002; 22: L220-228
73. Vasri ND. Molecular mechanisms of lipid dysregulation in nephrotic syndrome. Kidney Int. 2003; 63:1964-1976

74. Abrass CK, Cellular lipid metabolism and the role of lipids in progressive renal disease. Am J Nephrol. 2004; 24: 46-53
75. Carrero JJ, Stenvinkel P. Inflammation in end-stage renal disease- what have we learned in 10 years? Semin Dial. 2010; 23:498-509
76. Stuveling EM, Hilege HL, Bakker SJ, Gans RO, De Jong PE, De Zeeuw D. C-reactive protein is associated with renal function abnormalities in a non-diabetic population. Kidney Int 2003; 63:654-661
77. Turkmen K, Tonbul HZ, Toker A, Gaipov A, Erdur FM, Cicekler H, et al. The relationship between oxidative stress, inflammation, and atherosclerosis in renal transplant and end-stage renal disease. Renal Failure 2012; 34 (10)1229-1237
78. Mantovani A, Garlanda C, Bottazzi B, Peri G, Doni A, Martinez de la Torre Y, et al. The long pentraxin (PTX3) in vascular pathology. Vascul Pharmacol 2006; 45: 326-330
79. Salio M, Chimenti S, De Angelis N, Molla F, Maina V, Nebuloni M, et al. Cardioprotective function of the long pentraxin-3 in acute myocardial infarction. Circulation 2008; 117: 1055-1064
80. Jenny NS, Blumenthal RS, Kronmal RA, Rotter JI, Siscivick DS, Psaty BM, et al. Associations of Pentraxin-3 with cardiovascular disease: the multi-ethnic study of Atherosclerosis. J Thromb Haemost. 2014;12(6):999-1005.
81. Tripepi G, Mallamaci F, Zoccali C. Inflammation markers, Adhesion molecules and all-cause and cardiovascular mortality in patients with ESRD: Searching for the best risk marker by multivariate modelling. J Am Soc Nephrol 2005; 16: S83-S88
82. Panichi V, Maggiore U, Taccola D, Migliori M, Rizza GM, Consani C, et al. Interleukin-6 is a stronger predictor of total and cardiovascular mortality than C-

reactive protein in haemodialysis patients. Nephrol Dial Transplant 2004; 19: 1154-1160

83. Van der Velde M, Matsushita K, Coresh J, Astor BC, Woodward M, Levey A, et al. Lower estimated glomerular filtration rate and higher albuminuria are associated with all-cause and cardiovascular mortality. A collaborative meta-analysis of high-risk population cohorts. Kidney Int 2011; 79: 1341
84. Matsushita K, Vander Velde M, Astor BC, Woodward M, Levey AS, de Jong PE, et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. Chronic kidney disease prognosis consortium. Lancet 2010; 375(9731): 2073-81
85. Leoncini G, Viazzi F, Pontremoli R. Overall health assessment: a renal perspective. Lancet 2010; 375: 2053
86. Antman EM, Anbe DT, Armstrong PW, Bates ER, Green LA, Hand M, et al. ACC/AHA guideline for the management of patients with ST-elevation myocardial infarction- Executive summary: a report of the American College of Cardiology/ American Heart Association task force on practice guidelines (writing committee to revise the 1999 guidelines for the management of patients with acute myocardial infarction). Circulation 2004; 110(5): 588-636
87. Vanholder R, Massy Z, Argiles A, Spasovski G, Verbeke F, Lameire N, et al. Chronic kidney disease as cause of cardiovascular morbidity and mortality. Nephrol Dial Transplant 2005; 20(6): 1048-1056
88. Brownlee M, Cerami A, Vlassara H. Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. N Engl J Med 1988; 318: 1315-1321
89. Miyata T, Hori O, Zhang J, Yan S, Ferran L, Iida Y, et al. The receptor for advanced glycation end products (RAGE) is a central mediator of the interaction of AGE-beta-2

micrglobulin with human mononuclear phagocytes via an oxidant-sensitive pathway. Implications for the pathogenesis of dialysis-related amyloidosis. J Clin Invest 1996; 98: 1088-1094

90. Rodriguez-Ayala E, Anderstam B, Suliman M, Seeberger A, Heimburger O, Lindholm B, et al. Enhanced RAGE-madiated NFkappa B stimulation in inflamed haemodialysis patients. Atherosclerosis 2005; 180: 333-340
91. Nakashima, Carrero JJ, Qureshi AR, Miyamoto T, Anderstam B, Barany P, et al. Effect of circulating soluble receptor for advanced glycation end products (sRAGE) and the proinflammatory RAGE ligand (EN-RAGE, S100A12) on mortality in haemodialysis patients. Clin J Am Soc Nephrol 2010; 5: 2213-2219
92. Anwaruddin S, Januzzi JL, Baggish AL, Lewandrowski EL, Lewandrowski KB. Ischaemia-modified albumin improves the usefulness of standard cardiac biomarkers for the diagnosis of myocardial ischaemia in the emergency department setting. Am J Clin Pathol. 2005; 123(1): 140-145
93. Bar-Or D, Curtis G, Rao N, Bampos N, Lau E. Characterization of Co2+ and Ni2+ binding amino-acid residues of the N-terminus of human albumin: an insight into the mechanism of a new assay for myocardial ischaemia. Eur J Biochem 2001; 268: 42-47
94. Coffman RL, Carty J. A T-cell activity that enhances polyclonal IgE production and its inhibition by interferon-γ. J Immunol 1986; 136: 949-954
95. Mosmann TR, Moore KW. The role of IL-10 in cross-regulation of Th1 and Th2 responses. Immunol Today 1991: 12: A49-A45
96. Locksley RM. The roaring Twenties. Immunity 2008; 28(4): 437-439
97. Rajapurkar MM, Lele SS, Malavade TS, KansarabMR, Hegede UN, Gohel KD, et al. Serum catalytic iron: A novel biomarker for coronary artery disease in patients on maintenance haemodialysis. Indian J Nephrol. 2013; 23(5): 332-337

98. Kooistra MP, Kersting S, Gosriwatana I, Lu S, Nijhoff-Schutte J, Hider RC, et al. Non-transferrin bound iron in the plasma of haemodialysis patients after intravenous iron saccharate infusion. Eur J Clin Invest 2002; 32 Suppl 1: 36-41
99. Becker BN, Himmelfarb J, Henrich WL, Hakim RM. Reassessing the cardiac risk profile in chronic haemodialysis patients: A hypothesis on the role of oxidant stress and other non-traditional cardiac risk factors. J Am Soc Nephrol. 1997; 8: 475-486.
100. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med 1993; 27: 2002-2012
101. Goligorsky MS. Endothelial cell dysfunction: can't live with it, how to live without it. Am J Physiol Renal Pysiol 2005; 288: F871-880
102. Rubanyi GM, Romero JC, Vanhoutte PM. Flow-induced release of endothelium-derived relaxing factor. Am J Physiol 1986; 250: H1145-1149
103. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: Physiology, pathophysiology and pharmacology. Pharmacol Rev 1991; 43: 109-142
104. Ross R. Response to injury hypothesis. N Engl J. Med. 1986; 314: 488-500
105. Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW, et al. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med 1986; 315: 1046-1051
106. Perticone F, Maio R, Tripepi G, Zoccali C. Endothelial dysfunction and mild renal insufficiency in essential hypertension. Circulation. 2004; 110: 821-825
107. Dou L, Bertrand E, Cerini C, Faure V, Sampol J, Vanholder R, et al. The uraemic solutes p-cresol and indoxyl sulfate inhibit endothelial proliferation and wound repair. Kidney Int. 2004; 65: 442-451

108. Cerini C, Dou L, Anfosso F, Sabatier F, Moal V, Glorieux G, et al. P-cresol, a uraemic retention solute, alters the endothelial barrier function in vitro. Thromb Haemost. 2004; 92: 140-150

109. Bammens B, Evenepoel P, Keuleers H, Verbeke K, Vanrenterghem Y. Free serum concentrations of the protein-bound retention solute p-cresol predicts mortality in haemodialysis patients. Kidney Int. 2006; 69: 1081-1087

110. Vallance P, Leiper J. Cardiovascular biology of the asymmetric dimethyl arginine: dimethylaminohydrolase pathway. Arterioscler Thromb Vasc Biol 2004; 24: 1023-1030

111. Vallance P, Leone A, Calver A, Collier J, Moncada S. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. Lancet 1992; 339: 572-575

112. Kielstein JT, Zoccali C. Asymmetric dimethyl arginine: A cardiovascular risk factor and a uraemic toxin coming of age. Am J Kidney Dis 2006; 46: 186-202

113. Calgar Y, Yilmax MI, Sonmez A, Cakir E, Kaya A, Acikel C, et al. ADMA, proteinuria and insulin resistance in non-diabetic stage 1 chronic kidney disease. Kidney Int. 2006; 70: 781-787

114. Sorensen KE, Kristensen IB, Celermajer DS. Atherosclerosis in the human brachial artery. J Am Coll Cardiol. 1997; 29: 318-322

115. Playford DA, Watts GF. Non-invasive measurement of endothelial function. Clin Exp Pharmacol Physiol 1998; 25: 640-643

116. Calermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 1992; 340: 1111-1115

117. Bots ML, Westerink J, Rabelink TJ, de Koning EJ. Assessment of flow-mediated vasodilation (FMD) of the brachial artery: effects of technical aspects of FMD measurement on the FMD response. Eur Heart J 2005; 26: 363-368
118. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the international brachial artery reactivity task force. J Am Coll Cardiol 2002; 39: 257-265.
119. Mathur S, Devaraj S, Jialal I. Accelerated atherosclerosis, dyslipidaemia, and oxidative stress in end-stage renal disease. Curr Opin Nephrol Hypertens. 2002; 11: 141-147.
120. Loscalzo J. Nitric oxide insufficiency, platelet activation and arterial thrombosis. Circ Res 2001; 88: 756-762
121. Hogg N. Biological chemistry and clinical potential of S-nitrosothiols. Free Radic Biol Med 2000; 28: 1478-1486
122. De Man JG, De Winter BY, Moreels TG, Herman AG, Pelckmans PA. S-nitrosothiols and the nitrergic neurotransmitter in the rat gastric fundus: effects of antioxidants and metal chelation. Br J Pharmacol 1998; 123:1039-1046
123. Massy ZA, Borederie D, Nguyen-Khao T, Drueke TB, Ekindjian OG, Lacour B. Increased plasma S-nitrosothiol levels in chronic haemodialysis patients. Nephrol Dial Transplant 2003; 18: 153-157
124. Hampton MB, Kettle AJ, Winterbourn CC. Inside the neutrophil phagosome: oxidants, myeloperoxidase and bacterial killing. Blood 1998; 92: 3007-3017
125. Kettle AJ, Winterbourn CC. Myeloperoxidase: a key regulator of neutrophil oxidant production. Redox Rep 1997; 3(1): 3-15

126. Hazen SL, Crowley JR, Mueller DM, Heinecke JW. Mass spectrometric quantification of 3-chlorotyrosine in human tissues with attomole sensitivity: a sensitive and specific marker for myeloperoxidase-catalysed chlorination at sites of inflammation. Free Radic Biol Med. 1997; 23(6): 909-916

127. Pennathur S, Bergt C, Shao B, Byun J, Kassim SY, Singh P, et al. Human atherosclerotic intima and blood of patients with established coronary artery disease contain high density lipoprotein damaged by reactive nitrogen species. J Biol Chem 2004; 279(41): 42977-42983

128. Eiserich JP, Hristova M, Cross CE, Jones AD, Freeman BA, Halliwell B, et al. Formation of nitric oxide-derived inflammatory oxidants by myeloperoxidase in neutrophils. Nature 1998; 391(6665): 393-397

129. Boaz M, Matas Z, Biro A, Katzir Z, Green M, Fainaru M, Smetana S. Serum malondialdehyde and prevalent cardiovascular disease in haemodialysis. Kidney Int. 1999; 56: 1078-1083

130. Scholze A, Rinder C, Beige J, Riezler R, Zidek W, Tepel M. Acetylcysteine reduces plasma homocysteine concentration and improves pulse pressure and endothelial function in patients with end-stage renal disease. Circulation 2004; 109: 369-374

131. Tepel M, Van der Giet M, Statz M, Jankowski J, Zidek W. The antioxidant acetylcysteine reduces cardiovascular events in patients with end-stage renal failure. Circulation 2003; 107: 992-995

132. Babitt JL, Lin HY. Mechanisms of anaemia in CKD. J Am Soc Nephrol 2012; 23: 1631-1634

133. Astor BC, Muntner P, Levin A, Eustace JA, Coresh J. Association of kidney function with anaemia: The third National Health and Nutrition Examination Survey (1988-1994). Arch Intern Med. 2002; 24162(12): 1401-1408

134. Vlagopoulos PT, Tighiouart H, Weiner DE, Griffith J, Pettitt D, Salem D, et al. Anaemia as a risk factor for cardiovascular diasease and all-cause mortality in diabetes: The impact of chronic kidney disease. J Am Soc Nephrol 2005; 16: 3403-3410

135. Weiner DE, Tighiouart H, Vlagopoulos PT, Griffith JL, Salem DN, Levey AS, et al. Effects of Anaemia and left ventricular hypertrophy on cardiovascular disease in patients with chronic kidney disease. J Am Soc Nephrol 2005; 16: 1803-1810

136. Jelkmann W. Molecular biology of Erythropoietin. Intern Med. 2004; 43: 649-659

137. Eckardt KU. Erythropoietin: Oxygen-dependent control of erythropoiesis and its failure in renal disease. Nephron 1994; 67: 7-23

138. Maxwell PH, Ferguson DJ, Nicholls LG, Johnson MH, Ratcliffe PJ. The interstitial response to renal injury: fibroblast-like cells show phenotypic changes and have reduced potential for erythropoietin gene expression. Kidney Int 1997; 52: 715-724

139. Tanaka T, Nangaku M. Recent advances and clinical application of erythropoietin and erythropoiesis-stimulating agents. Exp Cell Res. 2012; 318(9): 1068-1073

140. Rosenberger C, Mandriota S, Jurgensen JS, Wiesener MS, Horstrup JH, Frei U et al. Expression of hypoxia inducible factor-1 alpha and -2alpha in hypoxic and ischaemic rat kidneys. J Am Soc Nephrol. 2002; 13(7): 1721-1732

141. Masson N, William C, Maxwell PH, Pugh CW, Ratcliffe PJ. Independent functions of two destruction domains in hypoxia-inducible factor-alpha chains activated by prolyl hydroxylation. EMBO J. 2001; 20(18): 5197-5206

142. Gonzalez EA, Martin KJ. Renal Osteodystrophy: Pathogenesis and management. Nephrol Dial Transplant 1995; 10(Suppl 3): 13-21

143. Takamoto S, Onishi T, Morimoto S, Imanaka S, Tsuchiya H, Seino Y, et al. Serum Phosphate, parathyroid hormone and vitamin D metabolites in patients with chronic

renal failure: Effect of aluminium hydroxide administration. Nephron 1985; 40: 286-291

144. Yamamoto M, Igaroshi T, Miramatsu M, Fukagawa M, Motokura T, Ogata E. Hypocalcaemia increases and hypercalcaemia decreases the steady state level of parathyroid messenger ribonucleic acid in the rat. J Clin Invest 1989; 83: 1053-1056
145. Goldman R, Bassett SH. Phosphorus excretion in renal failure. J Clin Invest 1954; 33: 1623-1630
146. Portale AA, Booth BE, Tsai HC, Morris RC Jr. Reduced plasma concentration of 1,25-dihydroxyvitamin D in children with moderate renal insufficiency. Kidney Int. 1982; 21: 627-732
147. Korkor AB. Reduced binding of [^{3}H] 1,25-dihydroxyvitamin D_3 in the parathyroid glands of patients with renal failure. N Engl J Med 1987; 316: 860-864
148. Llach F, Massry SG, Singer FR, Kurokara K, Kaye J, Coburn JW. Skeletal resistance to endogenous parathyroid hormone in patients with early renal failure. A possible cause for secondary hyperparathyroidism. J Clin Endocrinol Metab 1975; 41: 339-345
149. Strozecki P, Adamowicz A, Nartowicz E, Odrowaz-Sypniewska G, Wlodarczyk Z, Manitius J. Parathormone, calcium, phosphorus and left ventricular structure and function in normotensive haemodialysis patients. Ren Fail. 2001; 23(1): 115-126
150. Galetta F, Cupisti A, Franzoni F, Femia FR, Rossi M, Barsotti G, et al. Left ventricular function and calcium phosphate plasma levels in uraemic patients. J Intern Med. 2005; 258(4): 378-384
151. Block GA, Hulbert-Shearon TE, Levin NW, Port FK. Association of serum phosphorus and calcium x phosphate product with mortality risk in chronic haemodialysis patients. Am J Kidney Dis 1998; 31: 607-617

152. Ganesh SK, Stack AG, Levin NW, Hulbert-Shearon T, Port FK. Association of elevated serum PO_4, Ca x PO_4 product, and parathyroid hormone with cardiac mortality risk in chronic haemodialysis patients. J Am Soc Nephrol 2001; 12: 2131-2138

153. Schafer C, Heiss A, Schwarz A, Westenfeld R, Ketteler M, Floege J, et al. The serum protein alpha 2-Heremans-Schmidt glycoprotein/fetuin-A is a systemically acting inhibitor of ectopic calcification. J Clin Invest 2003; 112: 357-366

154. Hofbauer LC, Brueck CC, Shanahan CM, Schoppet M, Dobnig H. Vascular calcification and osteoporosis. From clinical observation towards molecular understanding. Osteoporosis Int 2007; 18: 251-259

155. Kazama JJ, Shigematsu T, Yano K, Tsuda E, Miura M, Iwasaki Y, et al. Increased circulating levels of osteoclastogenesis inhibitory factor (osteoprotegerin) in patients with chronic renal failure. Am J Kidney Dis 2002; 39: 525-532

156. Morena M, Terrier N, Jaussent I, Leray-Moragues H, Chalabi L, Rivory J, et al. Plasma osteoprotegerin is associated with mortality in haemodialysis patients. J Am Soc Nephrol 2006; 17: 262-270

157. Duchateau PN, Pullinger CR, Orellana RE, Kunitake ST, Naya-Vigne J, O'Connor PM, et al. Apolipoprotein L, a new human high density lipoprotein apolipoprotein expressed by the pancreas. Identification, cloning, characterization and plasma distribution of apolipoprotein L. J Biol Chem 1997; 272: 25576-25582

158. Vanhamme L, Paturiaux-Hanocq F, Poelvoorde P, Nolan DP, Lins L, Van Den Abbeele J, et al. Apolipoprotein L1 is the trypanosome lytic factor of human serum. Nature 2003; 422: 83-87

159. Genovese G, Friedman DJ, Ross MD, Lecordier L, Uzureau P, Freedman BI. et al. Association of Trypanolytic *APOL1* variants with Kidney disease in African-American. Science 2010; 329 (5993): 841–845

160. Hu CA, Klopfer EI, Ray PE. Human Apolipoprotein L1 in cancer and chronic kidney disease. FEBBS LETT. 2012; 586(7): 947-955

161. Parsa A, Kao WH, Xie D, Astor BC, Li M, Hsu CY, et al. *APOL1* risk variants, race and progression of chronic kidney disease. N Engl J Med 2013; 369: 2183-2196

162. Kopp JB, Smith MW, Nelson GW, Johnson RC, Freedman BI, Bowden DW, et al. *MYH9* is a major-effect risk gene for focal segmental glomerulosclerosis. Nat Genet. 2008; 40: 1175-1184

163. Kasembeli AN, Duarte R, Ramsay M, Mosiane P, Dickens C, Dix-Peek, et al. *APOL1* Risk variants are strongly associated with HIV-Associated Nephropathy in Black South Africans. J Am Soc Nephrol 2015;26(11):2882-2890

164. Reeves-Daniel AM, Depalmer JA, Bleyer AJ, Rocco MV, Murea M, Adams PL, et al. The *APOL1* gene and allograft survival after kidney transplantation. Am J Transplant 2011; 11: 1025-1030

165. Cohen DM, Mittalhenkle A, Scott DL, Young CJ, Norman DJ. African American living-kidney donor should be screened for *APOL1* risk alleles. Transplantation 2011; 92:722-725

166. Schelling JR, Zarf L, Sehgal A, Lyenger S, Sedor JR. Genetic susceptibility to end stage renal disease. Curr Opin Nephrol Hypertens. 1999; 8: 465-472

167. Freedman BI, Bowden DW, Rich SS. Genetic basis of kidney disease. Brenner and Rector's The kidney. 9th edition. Taal MW, Chertow GM, Marsden PA, Skorecki K, Yu ASI, eds. Elsevier, Inc. Philadelphia: 2012. P. 1554-1569

168. Freedman BI, Hicks PJ, Bostrom MA, Cunningham ME, Liu Y, Divers J, et al. Polymorphism in non-muscle myosin heavy chain 9 gene (MYH9) are strongly associated with end stage renal disease historically attributed to hypertension in African Americans. Kidney Int. 2009; 75: 736-745

169. Freedman BI, Langefeld CD. The new era of *APOL1*-associated glomerulosclerosis. Nephrol Dial Transplant. 2012; 27: 1288-1291

170. Lipkowitz MS, Freedman BI, Langefeld CD, Comeau ME, Bowden DW, Kao WH, et al. Apolipoprotein L1 gene variant associate with hypertension-attributed nephropathy and the rate of kidney function decline in African Americans. Kidney Int. 2013; 83: 114-120

171. McClellan W, Warnock DG, McClure L, Campbell RC, Newsome BB, Howard V, et al. Racial difference in the prevalence of chronic kidney disease among participants in the reasons for geographical and racial differences in stroke (REGARDS) Cohort study. J Am Soc Nephrol 2006; 17:1710-1715

172. Friedman DJ, Kozlitina J, Genovese G, Jog P, Pollak MR. Population-based risk assessment of *APOL1* on renal disease. J Am Soc Nephrol. 2011; 22: 2098-2105

173. Mukamal KJ, Tremaglio J, Friedman DJ, Ix JH, Kuller LH, Tracy RP, et al. *APOL1* Genotype, Kidney and Cardiovascular Disease and death in older adults. Arteriosclerosis, Thrombosis and Vascular Biology.2016; 36: 398-403

174. Tzur S, Rosset S, Shemer R, Yudkovsky G, Selig S, Tarekeqn A, et al. Missense mutations in the *APOL1* gene are highly associated with end stage kidney disease risk previously attributed to the MYH9 gene. Human Genetics 2010; 128 (3): 345–350

175. Rosset S, Tzur S, Behar DM, Wasser WG, Skorecki K. The population genetics of chronic kidney disease: insights from the *MYH9-APOL1* locus. Nat Rev Nephrol. 2011; **7** (6): 313–326

176. Ross R, Glomset JA. The pathogenesis of atherosclerosis. N Engl J Med. 1976; 295; 369-77, 420-425

177. Wanner C, Krane V, Winfried M, Atorvastatin in patients with type 2 diabetes mellitus undergoing haemodialysis. N Engl J Med. 2005; 353: 238-248

178. Fellstrom BC, Jardine AG, Schmieder RE, Rosuvastatin and cardiovascular events in patients undergoing haemodialysis. N Engl J Med. 2009; 360: 1395-1407

179. SEER Training modules, classification and structures of blood vessels. U.S National Institutes of Health, National Cancer Institute.2018; training.seer.cancer.gov, 25th March, 2018

180. Bruce Blaus. Medical gallery of Blausen Medical. Wikijournal of Medicine1. 2014; doi:10.15347/wjm/2014.010, 25th March, 2018

181. A report by the National Heart and Lung Institute Task Force on Arteriosclerosis. (DHEW Publication). Arteriosclerosis 1971; 2: 72-219

182. McGill HC Jr., Strong JP. The geographic pathology of atherosclerosis: Baltimore. Ann N Y Acad Sci 1968;149(2): 923-927

183. Stary HC. Evolution of atherosclerotic plaques in the coronary arteries of young adults. Arterosclerosis 1983; 3: 471a. Abstract

184. Robertson WB, Geer JC, Strong JP, McGill HC Jr. The fate of the fatty streak. Exp Mol Pathol. 1963; Suppl 1: 28-39

185. Geer JC, McGill HC Jr, Strong JP. The fine structure of human atherosclerotic lesions. Am J Pathol 1968; 33: 263-287

186. Wexler L, Brundage B, Crouse J, Detrano R, Fuster V, Maddahi J, et al. Coronary artery calcification: Pathophysiology, epidemiology, imaging methods and clinical implications. Circulation 1996; 94:1175-1192

187. Huttner I, Boutet M, More RH. Studies on protein passage through arterial endothelium. Structural correlate of permeability in rat arterial endothelium. Lab Invest 1973; 288: 672-677

188. Steinberg D. Lipoprotein and atherosclerosis. A look back and a look ahead. Arteriosclerosis 1983; 3: 283-301

189. Nivelstein-Post P, Mottino G, Fogelman A, Frank J. An ultrastructural study of lipoprotein accumulation in cardiac valves of the rabbit. Arterioscler Thromb. 1994; 14: 1151-1161

190. Hazen SL, Heinecke JW. Chlorothyrosine, a specific marker of myeloperoxidase-catalysed oxidation, is markedly elevated in low density lipoprotein isolated from human atherosclerotic intima. J Clin Invest. 1997; 99: 2075-2081

191. Moncada S, Herman AG, Higgs EA, Vane JR. Differential formation of prostacyclin (PGX or PGI_2) by layers of the arterial wall. An explanation for the anti-thrombotic properties of vascular endothelium. Thromb Res 1977; 11: 323-344

192. Gajdusek CM, Dicorleto P, Ross R, Schwartz SM. An endothelial cell-derived growth factor. J Cell Biol 1980; 85: 467-472

193. Castelot JJ Jr, Addonizio ML, Rosenberg R, Karnovsky MJ. Cultured endothelial cells produced a heparin-like inhibitor of smooth muscle cell growth. J Cell Biol 1981; 90: 372-379

194. Bowen-Pope DF, Seifert RA, Ross R. The platelet-derived growth factor receptor. New York Academic Press 1985; 1: 281-312

195. Witte LD, Cornicelli JA. Platelet derived growth factor stimulates low density lipoprotein receptor activity in cultured human fibroblasys. Proc Natl Acad Sci USA 1980; 77: 5962-5966.

196. Bevilacqua MP, Pober JS, Cotran RS, Gimbrone MA Jr. Interleukin-1 acts upon vascular endothelium to stimulate procoagulant activity and leucocyte adhesion. J Cell Biochem 1985; Suppl 9A: 148. Abstract

197. Martin TR, Altman LC, Albert RK, Henderson WR. Leucotriene B_4 production by human alveolar macrophage: a potential mechanism for amplifying inflammation in the lung. Am Rev Respir Dis 1984; 129: 106-111

198. Wang TJ, Gona P, Larson MG, Tofler GH, Levy D, Newton Cheh C, et al. Multiple biomarkers for the prediction of first major cardiovascular events and death. N Engl J Med 2006; 355: 2631-2639

199. Addabbo F, Mallamaci F, Leonardis D, Tripepi R, Tripepi G, Goligorsky MS, et al. Searching for biomarker patterns characterizing carotid atherosclerotic burden in patients with reduced renal function. Nephrol Dial Transplant 2007; 22:3521-3526

200. Honda H, Qureshi AR, Heimburger O, Barany P, Wang K, Pecoits-Filho R, et al. Serum albumin, C-reactive protein, interleukin 6, and fetuin A as predictors of malnutrition, cardiovascular disease, and mortality in patients with ESRD. Am J Kidney Dis 2006; 47: 139-148

CHAPTER 3

METHODOLOGY

Study location

The study was carried out at the Division of Nephrology, Charlotte Maxeke Johannesburg Hospital (CMJAH), Johannesburg, South Africa. The Charlotte Maxeke Johannesburg Academic Hospital is a major academic public hospital in the Gauteng province, fed by other health centers and hospitals within the province and beyond. The Nephrology Division offers a wide range of nephrology services such as peritoneal dialysis, haemodialysis and renal transplantation. The Division of Cardiology is also a fully equipped unit with facilities for echocardiography, electrocardiography, non-invasive assessment of AsVD such as CIMT, doppler ultrasound of blood vessels as well as coronary angiography.

Study population

This was a comparative cross-sectional study of 41 adult (age 18-65 years) non-diabetic stable KTRs, 41 ESKD patients on PD, 41 ESKD patients on HD, 41 stage 3 CKD patients and 41 age- and sex-matched healthy controls, recruited from hospital staff and students, at a large urban public hospital in South Africa from 2 January 2017 to 31 August 2017. Consecutive patients fulfilling the inclusion and exclusion criteria were recruited into the study.

Inclusion criteria

Age 18-65 years

Patients need to be stable on dialysis (HD/PD) for at least 3 months after initiation of RRT

Renal transplant patients had to be at least a year post transplant

Exclusion criteria

Age < 18 years or > 65 years

Patients on Sirolimus

Presence of diabetes mellitus or new onset diabetes after transplant (NODAT)

Presence of acute kidney injury

Clinical signs of active and chronic infection

Patients on anti-inflammatory medications

Seropositivity for Hep B, C and HIV

Clinically hypervolaemic patients

Presence of primary cardiovascular disease, i.e evidence to suggest that cardiovascular disease predated kidney disease

Presence of inherited lipid abnormalities

Presence of inflammatory bowel disease

Presence of connective tissue disease or vasculitides

Sample size calculation:

Using STATA version 13, the sample size was calculated based on the difference in atherosclerosis and biomarkers of inflammation.

The power to detect differences in levels of atherosclerosis was calculated. With a sample size of 40 in each group, a 5 % significance level and atherosclerosis proportion of 38.1 % in haemodialysis patients compared to 7.93 % in controls,[1] this study had a power of 90.89 to detect differences in these groups.

For the CRP and biomarker evaluation

With the sample size of 40 in each group, a 5 % significance level and CRP mean of 8.06 ± 1.31 in haemodialysis patients compared to 2.27 ± 0.34 in healthy controls,[1] this study had a power close to 100 to detect differences in these groups. Similarly, with a sample size of 40 in each group, a 5 % significance level and a pentraxin-3 mean of 6.9 ± 2.8 in haemodialysis patients compared to 2.4 ± 0.6 in healthy controls,[2] the study had a power close to 100 to detect differences in these groups.

Ethical considerations

Ethical approval for the study was obtained from the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg, South Africa; clearance certificate number M160614, (Appendix 2). All information obtained from the patients was handled with strictest confidentiality with all procedures duly explained to the patients before being carried out.

The subject and control information sheet was administered to participants, and a written informed consent was obtained from all participants before they were recruited into the study, (Appendix 3, 4).

Study protocol

All patients participated in a 4-step protocol.

Protocol 1: Biodata and History

A questionnaire was completed for all participants, (Appendix 5). Basic demographic statistics including age, sex, occupation, marital status, religion, educational status, hospital number and phone number were documented. The number of years on haemodialysis, peritoneal dialysis, number of years post-renal transplantation, and current medications were documented. The cause of CKD, history suggestive of vascular disease such as intermittent claudication, precordial chest pain radiating to the jaw and left shoulder tip, fatigue, breathlessness, orthopnea, paroxysmal nocturnal dyspnoea, fainting spells, dizziness, previous history of amaurosis fugax, previous transient ischaemic attack or stroke, history of risk factors for AsVD

such as history of hypertension, diabetes, previous percutaneous coronary intervention, previous carotid endarterectomy, previous surgery for peripheral arterial disease.

Protocol 2: Physical Examination

A brief general physical examination focusing on eliciting the signs of vascular compromise on the extremities such as dystrophic nail changes, loss of hair, darkening of the skin/bluish discoloration of the limbs was carried out on all study participants. Body mass index (BMI) was calculated from weight and height measurements while the body surface area (BSA) was calculated using the Mosteller formula,[3] waist-hip ratios (WHR) was also calculated from waist and hip circumference measurements. Cardiovascular examination was carried out with emphasis on peripheral pulses, blood pressure (taken on left arm with appropriate size cuff, in sitting position after patient had rested for 5 minutes) and features of heart failure such as displaced apex beat, elevated jugular venous pressure and presence of third heart sound were documented.

Protocol 3: Blood and Urine investigations

Blood samples were taken to assess the stage of kidney disease; serum electrolyte, urea and creatinine, serum total protein and albumin, serum magnesium, calcium, phosphate and uric acid. The e-GFR of the patient was calculated using 2009 CKD-EPI creatinine equation.[4]

GFR (ml/min/1.73m^2) = 141 min (S_{cr}/κ, 1)$^{\alpha}$ x max (S_{cr}/κ, 1)$^{1.209}$ x 0.993^{Age} x 1.018 (if female) x 1.157 (if black)

Where κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of S_{cr}/κ or 1and max indicates the maximum of S_{cr}/κ or 1.

Blood samples were taken in a non-fasting state for lipid profile [total cholesterol (TC), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), triglycerides (TG)] and serum levels were analysed by an enzymatic colorimetric method using the Roche Cobas 8000 modular analyser series, module c701 Analyzer (Roche, Japan).

For biomarker studies whole blood was collected in serum separating tubes. Blood was allowed to clot for ten minutes and then centrifuged at 5000 rpm at 4^0C for 10 minutes in Sorvall RT6000B refrigerated centrifuge, (Dupont, USA). The serum was aliquoted into eppendorf tubes for long term storage at -80^0C. Highly sensitive C-reactive protein, IL-6, TNF-α, pentraxin-3, EN-RAGE, Lipoprotein (a), Apo A1 and Lp-PLA2, measurements were carried out by solid phase enzyme linked immunosorbent and luminex assays from commercial suppliers. Highly sensitive CRP (hsCRP) levels were determined using HycultBiotech human HK369 Elisa kit, (HycultBiotech, Netherlands), (Appendix 6).Serum levels of pentraxin-3, IL-6, TNF-α and EN-RAGE were determined by a Magnetic Luminex Assay, Human Premixed Multi-Analyte Kit by R&D Systems using BioPlex 200 Systems (Bio-Rad Laboratories Inc., USA), (Appendix 7). Serum levels of Lipoprotein (a) were determined by using Beckman Immage 800 Immunochemistry System (Beckman Coulter, USA), while the serum levels of apolipoprotein A1 (APO A1) and lipoprotein phospholipase A2 (Lp-PLA2) were determined by Magnetic Luminex Assay, Human Premixed Multi-Analyte Kit by R&D Systems using the BioPlex 200 Systems (Bio-Rad Laboratories Inc., USA). Interleukin-6 concentrations were determined using a DuoSet Ancillary Reagent Kit 2 (R&D Systems, USA) but because many of the readings obtained were below the levels of detection, the data was excluded from the final analysis.

Additional whole blood was obtained in EDTA tubes for genomic DNA extraction using modified salting-out method,[5] (Appendix 8). The concentration of DNA was determined using Nanodrop™ 2000 Spectrophotometer (Thermo Scientific, USA). For *APOL1* genotyping 50 ng of DNA was amplified using sequence specific primers (Appendix 9) on MJ Mini personal Thermal cycler (BioRAD, Mexico), and the polymerase chain reaction (PCR) products subjected to restriction fragment length polymorphisms (RFLP), (Appendix 10 - 12), using restriction enzymes HindI III, NspI and mLuCI for *APOL1* single nucleotide polymorphism

(SNPs) G1 (rs73885319, rs60910145) and G2 insertion/deletion (rs71785313) respectively, (Appendix 9). Agarose gel electrophoresis was done using Owl^{TM} EC-105 compact power supply (Thermofisher Scientific, USA) and the gel was viewed using a Gel Doc EZ Imager (BioRaD, U.S.A). For *APOL1* genotyping 50 ng of DNA was amplified using sequence specific primers and the PCR product subjected to restriction fragment length polymorphisms, (Appendix 10).

Urine sample was taken from KTRs and control for Albumin-Creatinine ratio (ACR) estimation as part of routine standard of care.

Protocol 4: Procedures

All participants had echocardiography and CIMT measurements in accordance with the guidelines of the American Society of Echocardiography,[6] using a Philips iE33 echocardiography machine (Philips Corporation, USA). Carotid intima media thickness (CIMT) was assessed using the vascular probe of the echocardiography machine, Philips iE33 (S5-1 probe) by focussing on the far wall of the carotid artery, 1cm proximal to the dilatation of the carotid bulb along the long axis of the artery. Automatic echo-generated measurements with percentage quality of 95% were recorded. The procedure was done for left and right carotid arteries and the average was used in the analysis. Atherosclerotic vascular disease was defined by the combination of CIMT > 0.55 mm and presence of carotid plaques.

Echocardiography was done to determine left ventricular hypertrophy, systolic and diastolic function. Echocardiography and CIMT measurements were undertaken by a single cardiology technologist and reviewed by the same cardiologist.

Study Design

This was a descriptive observational study.

A total of 202 consecutive patients, age 18-65 years, who met the inclusion criteria and who gave their consent to be part of the study were recruited, 40 CKD stage 3 patients, 40 HD

patients, 40 PD patients, 41 KTRs and 41 healthy volunteers who served as controls. The healthy volunteers were recruited among students and hospital staff who gave their consent to be part of the study while the PD, HD, KTRs and CKD stage 3 patients were recruited from the respective clinics. Patients' demographic characteristics and pertinent history focused on eliciting risk factors for AsVD such as history of hypertension, paroxysmal nocturnal dyspnoea (PND), orthopnea, and dyspnoea on exertion were documented. The patients who completed the 4 study protocols were included in the analysis.

Data collection method

Patients were recruited from the general nephrology clinic, HD unit, PD and KTRs clinic.

A questionnaire was administered to all patients who gave written consent to be part of the study after reading through the information sheets, to obtain relevant information concerning their socio-demographic characteristics and pertinent clinical details to elicit risk factors to developing AsVD. Waist and hip circumference and WHR were measured with patients in an erect position and after PD fluid had been drained out in PD patients. Weight was measured by using a weighing scale; in HD patients, the weight was obtained before initiation of dialysis while weight was obtained after the PD fluid was drained in PD patients. Height was assessed by a stadiometer. Body mass index was calculated using the formula weight/height^2, while the body surface area was calculated using the Mosteller formula.[15] Blood pressure was taken after the patient had rested for 5 minutes in a sitting position, on the left arm (fistula arm was avoided in the HD and transplant patients) with an appropriate sized cuff. Blood samples were taken before dialysis in HD patients. Echocardiography and CIMT measurements were done just before the mid-week HD session in HD patients and after draining PD fluid in PD patients.

Data Analysis

Data obtained from the patients was analyzed using Stata 13 (Statacorp, USA). Descriptive statistics were used. Categorical data were expressed as frequencies and percentages. The

percentage of patients who had AsVD was determined in the different study groups. The frequency of risk factors such as age (> 40 years), gender, BMI, WHR, hypertension and lipid disorders was noted. The mean of normally distributed data were compared using student t-test while non-normally distributed data were compared using Wilcoxon rank-sum test. Comparison of means was done between the five groups using the Kruskal-Wallis test, followed by a post-hoc test using a Wilcoxon pairwise test to determine differences in the groups for the mean levels of all continuous variables including inflammatory markers (hsCRP, IL-6, TNF-a, pentraxin-3, EN-RAGE, albumin), lipid profile (TC, TG, LDH, HDL-C) and lipid biomarkers including Lp (a), Lp-PLA2 and APO A1. Wilcoxon rank-sum test was used to compare the means of inflammatory markers and lipid profiles and lipid biomarkers among those with AsVD and those without AsVD.

Correlation between BMI, WHR, urea, creatinine, calcium, phosphate, calcium-phophate product, inflammatory markers (hs-CRP, IL-6, pentraxin-3, EN-RAGE, albumin), lipid profile (TC, TG, LDL-C, HDL-C), lipid biomarkers (Lp (a), Lp-PLA2, APO A1) and CIMT was determined using the Spearman's rank correlation.

A multivariate binary logistic regression analysis was done to determine the predictors of AsVD among kidney disease patients. The logistic regression analysis was carried out at a 0.05 level of significance to find out if there is a significant relationship between AsVD and various risk factors. The utility of the inflammatory markers for predicting AsVD was assessed using receiver operating characteristics curve and it was also used to compare lipoprotein biomarkers and traditional lipid profiles with Lp (a) to determine whether there was significant difference in the utility of these markers in predicting AsVD.

References

1. Amira OC, Naicker S, Manga P, Sliwa K, Mia A, Raal F, et al. Adiponectin and atherosclerosis risk factors in African haemodialysis patients: A population at low risk for atherosclerotic cardiovascular disease. Hemodialysis Int. 2012; 16(1): 59-68
2. Turkmen K, Tonbul HZ, Toker A, Gaipov A, Erdur FM, Cicekler H, et al. The relationship between oxidative stress, inflammation and atherosclerosis in renal transplant and end-stage renal disease patients. Renal Failure. 2012; 34(10): 1229-1237
3. Mosteller RD. Simplified calculation of body surface area. N Engl J Med. 1987; 317(17):1098
4. Early A, Miskulin D, Lamb EJ, Levey AS, Uhliq K. Estimating equations for glomerular filtration rate in the era of creatinine standardization. A systematic review. Ann Intern Med. 2012; 156: 785-795
5. Nasiri H, Forouzandeh M, Rasaee MJ, Rahbarizadeh F. Modified salting-out method: High yield, high-quality genomic DNA extraction from whole blood using laundry detergent. J Clin Lab Anal 2005; 19: 229-232
6. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al. Recommendations for cardiac quantification. A report from the American Society of Echocardiography's guidelines and standard committee and the chamber quantification writing group, developed in conjunction with the European association of Echocardiography, a branch of the European association of Cardiology. J Am Soc Echocardiogr 2005; 18: 1440-1463

CHAPTER 4

Atherosclerotic vascular disease and its correlates in stable black South African kidney transplant recipients

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Abstract

Background

Despite remarkable improvements in renal function attributable to kidney transplantation, the burden of cardiovascular disease (CVD) among kidney transplant recipients (KTRs) remains high in the post-transplant period. Aggressive use of statins in kidney transplant recipients (KTRs) may make lipoprotein ratios correlate better with atherosclerotic vascular disease (AsVD) when compared to traditional lipid profile parameters. We therefore evaluated the clinical and echocardiographic correlates of AsVD among non-diabetic, stable black KTRs in South Africa.

Methods

This was a cross-sectional study of 41 adult (18-65 years) non-diabetic stable KTRs and 41 age- and sex-matched healthy controls. An interviewer-administered questionnaire was used to obtain information on participants' socio-demographic and cardiovascular risk factors. Anthropometric parameters were measured. Urine and blood samples were obtained and analysed. Echocardiography was performed and carotid intima media thickness (CIMT) was assessed in both right and left carotid arteries. Spearman's rank correlation and binary logistic regression were performed to determine the relationship between CVD risk factors and AsVD.

Results

Atherosclerotic vascular disease was present in 46.3 % of KTRs compared to 17.1 % of healthy controls ($p = 0.004$). Left ventricular hypertrophy was present in 92.7 % of the KTRs. There were statistically significant differences in waist-hip ratio, systolic blood pressure, mean arterial pressure, urine albumin creatinine ratio (urine-ACR), serum fibrinogen, serum creatinine, estimated glomerular filtration rate (eGFR), left atrial diameter (LAD), left ventricular mass (LVM) and left ventricular mass index (LVMI) between KTRs and controls. A positive relationship was present between CIMT and certain risk factors for CVD including LVM, LVMI and mitral valve deceleration time, ($p < 0.001$). Castelli 2 index and lipoprotein combine index (LCI) showed positive correlation with CIMT. On multivariate analysis, increasing age and kidney transplantation were independent predictors of AsVD after controlling for other risk factors.

Conclusion

Atherosclerotic vascular disease was common among KTRs. Older age and kidney transplant status independently predicted AsVD. Castelli index 2 and LCI correlated with AsVD more strongly than serum lipid parameters.

Keywords: atherosclerotic vascular disease, carotid intima media thickness, kidney transplant recipients, lipoprotein ratios.

Introduction

Kidney transplantation offers a greater survival benefit compared to dialytic therapy in end-stage kidney disease (ESKD) patients;[1] however, a high prevalence of cardiovascular disease (CVD) among kidney transplant recipients (KTRs) predisposes them to increased mortality in comparison to healthy controls.[2,3] Risk factors for vascular disease identified among KTRs in an American study included age, gender, cigarette smoking, pre-transplant splenectomy and

serum albumin.[4] Traditionally, a favourable lipid profile among black Africans was identified as the reason for a lower prevalence of atherosclerotic vascular disease (AsVD) and ischaemic heart disease, despite the presence of other cardiovascular risk factors.[5] However, an increase in the prevalence of dyslipidaemia and ischaemic heart disease has been reported in the urban black population of South Africa.[6,7] This trend has been attributed to increasing urbanization, changes in diet and a reduction in physical activity.[8] Additionally, the high prevalence of both traditional and CKD-related cardiovascular risk factors among ESKD patients has contributed to the high risk of AsVD among this group of patients. A case control study among black South African ESKD patients reported a higher prevalence of carotid plaques among ESKD patients on maintenance haemodialysis (38.1%) when compared to controls (7.9%).[9] Furthermore, Muhammad et al[10] found left ventricular hypertrophy (LVH) to be prevalent among KTRs and also reported that a longer duration on dialysis, cigarette smoking, higher cumulative steroid dose, increased carotid intima media thickness (CIMT) and increased waist circumference predicted the presence of LVH. In view of the aggressive use of lipid lowering medications in CKD and post-kidney transplantation, use of conventional lipid profiles for cardiovascular risk assessment in this group of patients may not provide the whole picture. Lipoprotein ratios may correlate better with AsVD than lipid profile parameters. The discriminatory and predictive power of total cholesterol/HDL-C was found to be superior to either total cholesterol or HDL-C.[11] Lipoprotein ratios have been shown to be superior to conventional lipid profiles in predicting coronary heart disease.[12] Furthermore, socio-demographic characteristics and possibly genetic variations may impact the cardiovascular risk among KTRs in our environment. Although some studies have evaluated CVD among black ESKD, maintenance dialysis and renal transplant patients,[9,10] a significant knowledge gap of the risk factors for AsVD among stable black KTRs in the absence of diabetes and inflammatory conditions still exists.

In view of the paucity of knowledge about AsVD among black South African KTRs, we evaluated the relationship of dyslipidaemia and lipoprotein ratios to AsVD among non-diabetic stable black KTRs.

Methods

This was a comparative cross-sectional study of 41 adult (age 18-65 years) non-diabetic stable KTRs and 41 age- and sex-matched healthy controls at a large urban public hospital in South Africa from 2 January 2017 to 31 August 2017. The study was approved by the Human Research Ethics Committee, University of the Witwatersrand, Johannesburg, South Africa (Study number 160614). All participants provided signed informed consent before enrolment in the study. An interviewer-administered questionnaire was used to obtain information on the participants' socio-demographic and cardiovascular risk factors.

Waist and hip circumferences were measured with patients in an erect position and waist-hip ratios (WHR) calculated. Body mass index (BMI) was calculated using the formula mass/height^2, while body surface area was calculated using the Mosteller formula.[13] Serum fibrinogen was determined using STA-R Max (Stago, France). Serum lipogram (total cholesterol-TC, low density lipoprotein-LDL-C, triglyceride-TG, high density lipoprotein-HDL-C) was determined by an enzymatic colorimetric method using the Cobas 8000 modular analyser series, module c701 Analyzer (Roche, Japan). Lipoprotein ratios were calculated as follows: Atherogenic index of plasma (AIP) = Log (Tg/HDL-C); Castelli index 1 = TC/HDL-C; Castelli index 2 = LDL-C/HDL-C; non-HDL-C = TC-HDL-C; Atherogenic index (AI) = non-HDL-C/HDL-C; Lipoprotein combine index (LCI) = (TC × TG × LDL-C)/HDL-C.[14] The urine albumin-creatinine ratio was determined by an immunoturbidimetric assay, using a Tina-quint Albumin Gen.2 serum/plasma Application kit, on the Cobas 8000 modular analyser, module c502 (Roche, Japan). Estimated GFR was calculated using the 2009 CKD-EPI formula.[15] All participants had echocardiocardiography and CIMT measurements in

accordance with the guidelines of the American Society of Echocardiography,[16] using a Philips iE33 echocardiography machine (Philips Corporation, USA). Carotid intima media thickness was assessed using the vascular probe of the echocardiography machine, Philips IE33 (S5-1 probe) by focussing on the far wall of the common carotid artery, 1cm proximal to the dilatation of the carotid bulb along the long axis of the artery. Automatic echo-generated measurements with percentage quality of 95% were recorded. The procedure was performed on both the left and right carotid arteries and the average used in analysis.

Data analysis

Stata version13.1, (StataCorp, USA) was used for statistical analysis. Categorical variables were expressed as frequencies and percentages and compared using the chi-square test. A Shapiro-Wilk test of normality was performed on all continuous variables and normally distributed data were presented as mean ± SD while non-normally distributed data were presented as median and interquartile range (IQR). Comparisons were performed between KTRs and controls, and between participants who had AsVD and those who did not, using the student t-test for normally distributed data and the Wilcoxon rank-sum test for non-normally distributed data.

Spearman correlation was used to determine the relationship between CIMT and cardiovascular risk factors among KTRs and controls. Multivariate regression analysis was performed to determine the relationship and contribution of cardiovascular risk factors to AsVD. Significance was taken as $p < 0.05$. Post regression analysis was performed to determine the goodness of fit of the final model.

Results

The median age was 39 years (IQR: 30-52) among KTRs while the median age among the control group was 41 years (IQR: 29-48) as shown in Table 1. The most common causes of

ESKD in the KTR population were hypertension-attributed CKD and glomerulonephritis (both n = 19, 46.3%).

Table 1. Sociodemographic and clinical characteristics of the study population

Parameter	**KTR (n=41) (%)**	**Control (n=41) (%)**
Age (years)	39 (30 - 52) *	41 (29 – 48) *
Gender		
Female	20 (49.0)	23 (56.0)
Male	21 (51.0)	18 (44.0)
Marital status		
Married	23 (56.0)	30 (73.0)
Single	18 (44.0)	11 (27.0)
Cause of ESKD		
Hypertension	19 (46.3)	
Glomerulonephritis	19 (46.3)	
Primary oxalosis	1 (2.4)	
Dysplastic kidneys	2 (4.9)	
Donor source		
Deceased	38 (92.7)	
RLD	3 (7.3)	
CNI		
Prograf	37 (90.2)	
Cyclosporin	4 (9.8)	
Pre-transplant dialysis duration (years)	5.0 (4.0 – 6.0)*	
Post-transplant duration (years)	4.0 (1.0 – 7.0)*	
Proteinuria		
≤3mg/mmol	19 (46.3)	39 (95.1)
>3mg/mmol	22 (53.7)	2 (4.9)
Hypertension		
Absent	16 (39.0)	34 (82.9)
Present	25 (61.0)	7 (17.1)

*median (interquartile range); ESKD - end stage kidney disease, RLD - related living donor; CNI – calcineurin inhibitor

All the KTRs were on calcineurin inhibitor (CNI) based immunosuppressive therapy (with more than 90 % on Tacrolimus while less than 10 % were on cyclosporine), antimetabolites [30 (73.2 %) were on mycophenolate mofetil, 5 (12.2 %) were on mycophenolic acid, 4 (9.8 %) were on azathioprine and 2 (4.9 %) were on Leflunomide] and 5mg maintenance prednisonedaily. The majority (n=38/41, 92.7 %) of the KTRs had received deceased donor organs while 3 (7.3 %) had related living donor transplantation. The median post-transplant

follow-up duration was 4 years (IQR: 1-7) while median pre-transplant dialysis duration was 5 years (IQR: 4–6). Left ventricular hypertrophy was present in 34 (82.9 %) of the KTRs and in 15/17 (88.2 %) of KTRs with eGFR < 60ml/min/173m^2 and in 13/18 (72.2 %) KTRs with elevated blood pressure. Among the KTRs, 24 (58.5%) had concentric hypertrophy (relative wall thickness (RWT) > 0.42 and LVMI > 95g/m^2 for females or > 115g/m^2 for males), 10 (24.4 %) had eccentric hypertrophy (RWT ≤ 0.42 and LVMI > 95g/m^2 for females or > 115g/m^2 for males), 5 (12.2 %) had concentric remodelling (RWT > 0.42 and LVMI ≤ 95g/m^2 for females or ≤ 115g/m^2 for males) and 2 (4.9 %) had normal geometry. Using the combination of increased CIMT values (> 0.55 mm) and the presence of plaques, AsVD was present in 19 (46.3 %) KTRs compared to 7 (17.1 %) healthy controls, (p = 0.004). Carotid intima media thickness was significantly increased among the KTRs compared to the controls (p = 0.021). As shown in Table 2, significant differences were seen between other echocardiographic measurements when KTRs were compared to controls. There was no association between CNI agents and AsVD, (x^2 – 1.46, p = 0.321).

Table 2. Comparison of cardiovascular risk factors between kidney transplant recipients and controls

Parameters	KTR (n = 41)	Control (n = 41)	p-value
Age (years)	39 (30 – 52)	41 (29 – 48)	0.824*
BMI (kg/m^2)	25.7 (23.1 – 28.5)	28.7 (22.9 – 31.2)	0.081*
WHR	0.89 ± 0.07	0.85 ± 0.06	0.002$^{\#}$
SBP (mmHg)	139.7 ± 15.8	125.4 ± 11.3	< 0.001$^{\#}$
MAP (mmHg)	106.9 ± 13.8	94.6 ± 11.4	< 0.001$^{\#}$
TC (mmol/l)	4.46 (3.93 – 4.93)	4.27 (3.71 - 4.70)	0.330*
LDL-C (mmol/l)	2.63 (2.15 – 3.08)	2.59 (2.08 – 3.17)	0.777*
TG (mmol/l)	1.31 (1.06 – 1.92)	1.25 (0.80 – 1.53)	0.399*
HDL-C (mmol/l)	1.29 (1.05 – 1.47)	1.15 (0.98 – 1.54)	0.467*
Urine-ACR	4.60 (1.20 – 16.2)	0.40 (0.20 – 0.80)	< 0.001*
Fibrinogen (µmol/l)	3.00 (2.60 – 3.5)	2.50 (2.20 – 3.20)	0.027*
SCr (µmol/l)	123 (91 -152)	80 (63 -89)	< 0.001*
eGFR (ml/min/1.73m^2)	71 (49 – 85)	113 (99 – 124)	< 0.001*
LAD (mm)	35.9 (32.3-34.6)	31.02 (29.0-32.2)	< 0.001$^{\#}$
CIMT (mm)	0.60 (0.51 - 0.66)	0.53 (0.47 - 0.60)	0.021*
LVM (g)	219 (187 - 284)	174 (142 - 226)	0.001*
LVMI (g/m^2)	130.0 (108.4 -165.2)	101.1 (73.8 – 115.7)	< 0.001*

Key: $^{\#}$mean ± SD, student t-test; *median (IQR), Wilcoxon rank sum test

KTR- kidney transplant recipient; BMI - body mass index; WHR - waist hip ratio; SBP - systolic blood pressure; MAP – mean arterial pressure; TC - total cholesterol; LDL-C - low density lipoprotein; TG - triglyceride; HDL-C - high density lipoprotein; urine-ACR- urine albumin-creatinine ratio; SCr – serum creatinine; eGFR - glomerular filtration rate; LAD- left atrial diameter; CIMT- carotid intima media thickness; LVM- left ventricular mass; LVMI- left ventricular mass index.

There was a statistically significant difference in Castelli 1 and 2 indices, AI, non-HDL-C and LCI in those with AsVD compared to those without AsVD (Table 3). There were no significant differences in the serum levels of TC, TG, LDL-C and HDL-C.

Table 3. Comparison between participants with and without atherosclerotic vascular disease

Parameter	**KTRs and Controls (n = 82)**		**p-value**	**KTRs (n = 41)**		**p-value**
	ASVD Present (n = 26)	**ASVD Absent (n = 56)**		**AsVD Present (n = 19)**	**AsVD Absent (n = 22)**	
Age (years)	48.0 (41.0 – 55.0)	32.5 (27.0 – 44.0)	< 0.001*	48.0 (39.0 – 62.0)	30.5 (26.0 -40.0)	< 0.001
WHR	0.91 (0.86 – 0.93)	0.86 (0.83 – 0.90)		0.91 (0.88 – 0.96)	0.87 (0.84 – 0.90)	0.069
BMI (kKg.m^2)	26.5 (24.0 – 31.2)	27.1 (21.5 – 30.0)	0.028$^{\#}$	26.2 (23.7 – 28.2)	25.3 (20.7 -28.7)	0.513
SBP (mmHg)	139.2 (132.6 – 145.9)	140.1 (132.3 – 147.0)	0.273*	138.0 (128.0 – 147.0)	141.1 (126.0 -151)	0.794
AIP	0.03 (-0.03 – 0.11)	0.02 (-0.08 – 0.12)	0.855$^{\#}$	0.02 (-0.13 – 0.20)	0.01 (-0.15 – 0.22)	0.917
Castelli 1	4.1 (3.1 – 4.9)	3.1 (2.5 – 4.1)	0.729*	3.53 (3.00 – 4.80)	3.36 (2.57 – 4.16)	0.403
Castelli 2	2.5 (1.6 – 3.2)	1.8 (1.3 – 2.6)	0.033*	2.09 (1.53 – 2.73)	1.67 (1.12 – 2.55)	0.158
LCI	15.4 (10.1 – 18.9)	8.7 (4.4 – 19.6)	0.048*	13.38 (9.13 – 16.54)	8.65 (4.61 -24.32)	0.308
non-HDL-C	3.4 (2.8 – 4.0)	2.9 (2.3 – 3.5)	0.013*	3.31 (2.76 – 3.77)	3.03 (2.36 – 3.76)	0.381
AI	3.0 (2.1 – 3.9)	2.1 (1.5 – 3.2)	0.019*	2.53 (2.00 – 3.80)	2.36 (1.57 3.36)	0.403
TC (mmol/l)	4.5 (4.1 – 5.1)	4.3 (3.5 – 4.8)	0.034*	4.46 (4.14 – 4.93)	4.47 (3.46 – 5.09)	0.666
TG (mmol/l)	1.3 (1.1 – 2.1)	1.3 (0.8 – 1.7)	0.071*	1.29 (0.99 – 1.89)	1.32 (1.12 – 1.95)	0.855

LDL-C (mmol/l)	2.7 (2.2 – 3.3)	2.6 (2.0 – 2.9)	0.139*	2.65 (2.19 – 3.24)	2.49 (1.45 – 2.73)	0.205
HDL-C (mmol/l)	1.1 (0.9 – 1.4)	1.3 (1.1 – 1.6)	0.087*	1.16 (1.01 – 1.60)	1.32 (1.09 – 1.47)	0.456
Fibrinogen (µmol/l)	3.0 (2.6 – 3.5)	2.5 (2.2 – 3.2)	0.163*	3.00 (2.70 – 3.60)	2.95 (2.50 – 3.30)	0.582
Urine-ACR	2.3 (0.8 – 8.1)	0.8 (0.3 – 2.6)	0.041*	2.60 (1.40 – 10.7)	9.65 (1.20 – 30.8)	0.296
eGFR	86.5 (54.0 – 117)	97.5 (70.5 – 116.0)	0.056*	71.0 (44.0 – 115.0)	70.0 (49.0 – 84.0)	0.497
(ml/min/1.73m^2)	37.5 (32.0 40.0)	32.0 (29.0 – 35.0)	0.386*	38.0 (35.0 – 41.0)	34.5 (30.0 – 36.0)	0.011
LAD (mm)	135.1 (118.2 – 168.2)	103.9 (81.6 – 123.5)	< 0.001$^{\#}$	141.2 (128.2 – 178.0)	116.2 (100.7 – 131.8)	< 0.001
LVMI (g/m^2)	172.5 (155.0 – 201.0)	148.0 (129.5 –171.0)	<	176.0 (158.0 – 204.0)	135.5 (120.0 – 175.0)	0.003
Dec. time (ms)	73.0 (64.0 – 79.0)	68.0 (62.5 – 74.0)	0.001*	76.0 (64.0 – 80.0)	69.0 (64.0 79.0)	0.574
EF (%)			<			
			0.001*			
			0.229*			

Key: $^{\#}$ mean ±SD, student t-test; *median (IQR), Wilcoxon rank sum; ASVD atherosclerotic vascular disease; WHR - waist-hip ratio; BMI - body mass index; SBP - systolic blood pressure; AIP - atherogenic index of plasma; LCI - lipoprotein combine index; non-HDL-C - non-high density lipoprotein; AI – atherogenic index; TC-total cholesterol; HDL-C- high density lipoprotein; LDL-C - low density lipoprotein; TG- triglyceride; urine-ACR- urine albumin-creatinine ratio; LAD- left atrial diameter; LVM- left ventricular mass; LVMI- left ventricular mass index, Dec. time- mitral valve deceleration time; EF- ejection fraction.

Spearman's correlation between CIMT and risk factors for CVD among KTRs revealed a positive relationship with LVM ($r = 0.52$, $p < 0.001$), LVH ($r = 0.53$, $p < 0.001$), LAD ($r = 0.43$, $p = 0.007$), age ($r = 0.43$, $p = 0.005$) and WHR ($r = 0.39$, $p = 0.012$) as shown in Table 4. Of the lipid profile parameters and lipoprotein ratios in KTRs, only Castelli 2 index and LCI showed correlation with CIMT.

Table 4. Correlation of carotid intima media thickness to risk factors for cardiovascular disease among kidney transplant recipients

Parameter	KTR (n = 41)		Controls (n = 41)	
	rho	p-value	rho	p-value
Age (years)	0.42	0.006*	0.24	0.138
WHR	0.39	0.013*	-0.13	0.431
BMI (Kg/m^2)	0.17	0.296	0.11	0.512
eGFR ($ml/min/1.73m^2$)	0.29	0.071	-0.08	0.617
TC (mmol/l)	0.20	0.203	0.19	0.230
LDL-C (mmol/l)	0.30	0.050	0.08	0.619
TG (mmol/l)	0.09	0.566	-0.02	0.919
Castelli I	0.27	0.082	0.06	0.710
Castelli II	0.33	0.035*	0.03	0.845
AIP	0.13	0.408	-0.03	0.834
non-HDL-C	0.29	0.071	0.12	0.465
Atherogenic index	0.28	0.082	0.06	0.710
LCI	0.31	0.047*	0.04	0.813
EF (%)	0.32	0.044*	0.06	0.710
FS (%)	0.27	0.090	0.02	0.884
e/e'	0.21	0.180	0.28	0.079
Dec. time (ms)	0.59	<0.001*	0.15	0.341
LVM (g)	0.50	<0.001*	0.24	0.132
LVMI (g/m^2)	0.50	<0.001*	0.25	0.111
LAD (mm)	0.42	0.007*	0.14	0.373

Key: *statistically significant, $p < 0.05$; KTR – kidney transplant recipient; WHR - waist-hip ratio; BMI - body mass index; SBP - systolic blood pressure; eGFR - estimated glomerular filtration rate; TC-total cholesterol; HDL-C- high density lipoprotein; LDL-C - low density lipoprotein; TG- triglyceride; AIP - atherogenic index of plasma; non-HDL-C - non-high density lipoprotein; LCI - lipoprotein combined index; EF- ejection fraction; FS- fractional shortening; Dec. time- mitral valve deceleration time; LVM- left ventricular mass; LVMI- left ventricular mass index; LAD- left atrial diameter;

The multivariable model of the predictors of AsVD showed that KTR status confers an 11-fold risk of developing AsVD (OR = 11.22, 95 % CI= 1.82-68.93, $p = 0.009$); Table 5. The odds of developing AsVD was 17 times higher in subjects ≥ 40 years ($p = 0.001$). Age and KTR status were independent predictors of AsVD after correcting for WHR, proteinuria, eGFR, Castelli

index 2 and LVH. The goodness of fit of the model was assessed by the Hosmer Lemmeshow test (p = 0.319). Binary logistic regression analysis among KTRs showed that age > 40 years independently predicted AsVD after adjusting for post-transplant follow up duration, duration of dialysis and LVH, Table 6.

Table 5. Logistic regression showing relationship of atherosclerotic vascular disease to risk factors of cardiovascular disease

Risk factors	OR	95 % CI	p-value
Age (years)	17.12	3.36 – 87.12	0.001*
WHR	0.56	0.14 – 2.17	0.401
GFR (ml/min/1.73m^2)	0.34	0.67 – 1.73	0.193
Proteinuria	1.29	0.32 – 5.20	0.720
Kidney transplant status	11.22	1.82 – 68.93	0.009*
Castelli index 2	1.95	0.57 – 6.73	0.288
LVH (g/m^2)	2.49	0.42 – 14.60	0.312

Key: *statistically significant, p < 0.05; 95 % CI- 95 % confidence interval; OR- odds ratio; eGFR - estimated glomerular filtration rate; WHR - waist-hip ratio; KTR - kidney transplant recipient, LVH - left ventricular hypertrophy.

Table 6. Logistic regression showing relationship of atherosclerotic vascular disease to risk factors among kidney transplant recipients.

Risk factors	OR	95% CI	p-value
Age at transplant (> 40 years)	8.54	1.77 – 41.23	0.008*
Post-transplant duration (> 3 years)	1.09	0.25 – 4.81	0.914
Duration of dialysis (> 3 years)	1.58	0.37 – 6.80	0.542
LVH (g/m^2)	2.12	0.11 – 40.53	0.618

Key: *statistically significant, p < 0.05; 95 % CI- 95% confidence interval; OR - odds ratio; LVH - left ventricular hypertrophy.

Discussion

Atherosclerotic vascular disease was significantly more prevalent among KTRs compared to controls in our study, with nearly half of the KTRs having AsVD. This is comparable with findings from previous studies.[17,18,19] Basiratnia et al[17] demonstrated a higher mean CIMT in KTRs compared to healthy controls. Similarly, Cader et al[17] found a significantly higher

prevalence of increased CIMT among their cohort of KTRs. Although the control group in our study and the study by Basiratnia et al[17] were similar, in the study by Cader et al[19] the controls were CKD-stage and cardiovascular risk matched. Prevalence of AsVD in our study (46.3 %) is very similar to that reported in a previous study by Japichino et al (46.5 %).[18] The higher prevalence of AsVD (66.7 %) reported by Cader et al[19] could be due to visualization of carotid plaques and measurement of CIMT at the carotid bulb and also because of differences in participants' profiles with diabetes mellitus accounting for one-third of subjects recruited in their study; we had excluded patients with diabetes mellitus and current smokers. The high prevalence of AsVD in our study can be explained by the significant differences in the levels of some established cardiovascular risk factors such as blood pressure, WHR, proteinuria, decreased eGFR, LVH and LAD in KTRs compared to controls.

We found a strong association between AsVD and cardiovascular risk factors. Age, WHR, Castelli indices 1 and 2, non-HDL-C, AI, LCI, serum fibrinogen levels, LAD, LVH and mitral valve deceleration time were also significantly associated with the presence of AsVD in our study, comparable to results from previous studies.[18-24] Kolonko et al [20] found age, pre-transplant diabetes, LVH and CVD to be related to CIMT similar to our findings, despite the exclusion of diabetic KTRs from our study based on the known association between diabetes, dyslipidaemia and atherosclerosis.[25] Serum fibrinogen has been shown to increase inflammatory and atherosclerotic conditions, possibly through the elaboration of inflammatory cytokines by macrophages involved in atherosclerosis and subsequent stimulation of fibrinogen production by these cytokines.[21] In our study population, serum fibrinogen was significantly higher among KTRs compared to controls and also significantly higher among participants who had AsVD compared to those without AsVD. Atherosclerotic vascular disease is currently viewed as an inflammatory disease and serum levels of fibrinogen have been influenced by several risk factors of CVD such as hypertension, diabetes and inflammation.[22,23] Among a

cohort of ESKD patients on chronic haemodialysis, serum fibrinogen was found to be higher among patients with fatal and non-fatal cardiovascular events compared to event-free patients.[24] Our study, which demonstrates an association between AsVD and LVH is supported by reports from an earlier study which described LVH as both a CVD and a risk factor for CVD.[26]

Age, WHR, Castelli 2, LAD, LVM and LVMI had positive correlations with CIMT in our study; the association between increased CIMT and age had been described in a previous study.[27] Advancing age has been associated with diminished nitric oxide-mediated vasodilatation and reduction in total nitric production,[28] resulting in endothelial dysfunction.[28] Increasing age has also been associated with other risk factors for AsVD such as diabetes, hypertension and vascular calcification.[27] Left ventricular hypertrophy is an important predictor of all-cause mortality among KTRs.[29] Our study showed a negative correlation between LVH and renal function ($r = -0.25$, $p = 0.02$), consistent with earlier studies in pre-dialysis CKD patients[23] and KTRs.[11] An increased waist-hip ratio has been associated with cardiovascular events among CKD patients;[30] the finding of a positive correlation between WHR and CIMT in our study is probably due to the use of steroids by the KTRs but may also be explained by the fact that restoration of renal function after kidney transplantation reduces CKD-related inflammation and malnutrition, enhancing appetite and weight gain.

In our study population, among all the lipid profile and lipoprotein indices analysed, only Castelli 2 index and LCI correlated positively with CIMT. This finding could be due to the aggressive treatment of dyslipidaemia with statins in our patients. Sub-analysis within the KTR group showed that Castelli 2 index retained its positive correlation with CIMT ($r = 0.33$ $p = 0.035$) while LDL-C showed a marginally significant correlation. There was no correlation between blood pressure and CIMT, possibly due to the aggressive blood pressure treatment in our KTRs who had a median blood pressure of 139.7/89.5mmHg. Age and KTR status were

independent predictors of AsVD even after correcting for WHR, proteinuria, GFR, Castelli index 2 and LVH (Table 4). Among KTRs, only age >40 years predicted AsVD even when adjusted for LVH, duration of dialysis and post-transplant duration. Increasing age has been demonstrated to be an important predictor of vascular injury[28] and atherosclerosis has been shown to be associated with changes in CIMT early in the post-kidney transplant period.[31]

This study excluded patients with diabetes mellitus, connective tissue diseases and inflammatory disorders, acute and chronic infections and smokers. Furthermore, only stable black KTRs were recruited. This could have contributed to the lower prevalence of AsVD seen in this study compared to the other studies alluded to in the discussion.

In conclusion, AsVD is common among KTRs. Strong correlations exist between CIMT and age, WHR, LVH, LAD, EF and mitral valve deceleration time. Among the KTRs lipoprotein indices, namely Castelli index 2 and LCI, showed a better correlation with CIMT than conventional lipid profile parameters. Age and KTR status were independent predictors of AsVD. The findings of this study suggest that serum fibrinogen, Castelli index 2 and LCI may be important surrogate markers of atherosclerosis in KTRs. It is recommended that the levels of these markers be determined before renal transplantation and monitored in the post-transplant period. In addition, we recommend more aggressive surveillance for AsVD among KTRs older than 40 years of age.

Conflict of Interest

No competing interests in relation to this study.

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Authors' Contribution

S.O. Oguntola, R. Duarte and S. Naicker were involved in study design. S.O. Oguntola, M.O. Hassan, A. Vachiat, P. Manga, G. Paget and S. Naicker participated in data collection. T. Dix-Peek, C. Dickens, S.O Oguntola and R. Duarte were involved in laboratory analysis. Data analysis was done by S.O. Oguntola and G. Olorunfemi. First draft of the manuscript was prepared by S.O. Oguntola and all authors reviewed the manuscript.

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References

1. Schnuelle P, Lorenzo D, Trede M, Van Der Woude FJ. Impact of cadaveric transplantation on survival in end-stage renal failure: Evidence for reduced mortality risk compared with haemodialysis during long term follow-up. J Am Soc Nephrol 1998; 9: 2135 – 2141.
2. Ribic CM, Holland D, Howell J, Jevnikar A, Kim SJ, Knoll G, et al. Study of cardiovascular outcomes in renal transplantation: A prospective, multicentre study to determine the incidence of cardiovascular events in renal transplant recipients in Ontario, Canada. Canadian Journal of Kidney Health and Disease 2017; 4: 1-9
3. Ojo AO, Hanson JA, Wolfe RA, Leichtman AB, Agodoa LY, Port FK. Long-term survival in renal transplant recipients with graft function. Kidney Int. 2000; 57: 307-313.

4. Kasiske BL, Guijarro C, Massy ZA, Wiederkehr MR, Ma JZ. Cardiovascular disease after renal transplantation. J Am Soc Nephrol. 1996; 7: 158-165.
5. Vorster HH, The emergence of cardiovascular disease during urbanisation of Africans. Public Health Nutr 2002; 5: 239-243.
6. Peer N, Steyn K, Lombard C, Gaziano T, Levitt N. Alarming rise in prevalence of atherogenic dyslipidaemia in the black population of Cape Town: The Cardiovascular Risk in Black South Africans (CRIBSA) study. Eur. J. Prev. Cardiol 2014; 21(12): 1549-1556.
7. Ramjeeth A, Butkow N, Raal FJ, Maholwana-Mokgatihe M. The evaluation of low-density lipoprotein cholesterol goals achieved in patients with established cardiovascular disease and/or hyperlipidaemia receiving lipid-lowering otherapy: The South African Not at Goal Study (SA-NAG). Cardiovasc J Afr 2008; 19: 88-94.
8. Sumner AE, Zhou J, Doumatey A, Imoisili OE, Amoah A, Acheampong J, et al. Low HDL-C-cholesterol with normal triglyceride levels is the most common lipid pattern in West Africans and African Americans with metabolic syndrome: Implication for Cardiovascular disease prevention. CVD Prev Control 2010; 5: 75-80.
9. Amira OC, Naicker S, Manga P, Sliwa K, Mia A, Raal F, et al. Adiponectin and atherosclerosis risk factors in African haemodialysis patients: A population at low risk for atherosclerotic cardiovascular disease. Hemodialysis Int. 2012; 16(1): 59-68.
10. Muhammad AS, Naidoo S, Manga P, Nazir MS, Naicker S. Left ventricular hypertrophy in kidney transplant recipients in Sub-Saharan Africa. Sub-Saharan Afr J of Med. 2015; 2: 70-73.
11. Kinosian B, Glick H, Garland G. Cholesterol and coronary heart disease: predicting risks by levels and ratios. Ann Intern Med. 1994 121(9); 641-647.

12. Zhu L, Lu Z, Zhu L, Ouyang X, Yang Y, He W. etal. Lipoprotein ratios are better than conventional lipid parameters in predicting coronary heart disease in Chinese Han people. Kardiol Pol 2015; 73: 931-938.
13. Mosteller RD. Simplified calculation of body surface area. N Engl J Med. 1987; 317(17):1098.
14. Cai G, Shi Ganwei, Xue Sheliang Lu Wei. The atherogenic index of plasma is a strong and independent predictor for coronary artery disease in the Chinese Han population. Medicine 2017; 96: 37(e8058).
15. Earley A, Miskulin D, Lamb EJ, Levey AS, Uhliq K. Estimating equations for glomerular filtration rate in the era of creatinine standardization. A systematic review. Ann Intern Med. 2012; 156: 785-795.
16. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al. Recommendations for cardiac quantification. A report from the American Society of Echocardiography's guidelines and standard committee and the chamber quantification writing group, developed in conjunction with the European association of Echocardiography, a branch of the European association of Cardiology. J Am Soc Echocardiogr 2005; 18: 1440-1463.
17. Basiratnia M, Fazel M, Lotfi M, Al-Hashemi GH, Fallahzadeh MH, Derakhshan A. Subclinical atherosclerosis and related risk factors in renal transplant recipients. Pediatr Nephrol 2010; 25:343-348.
18. Japichino GG, Bonati L, Rubini P, Capocasale E. Prevalence of atherosclerosis in renal transplant recipients. Minerva Cardioangiol. 2001; 49(4) 229-238.
19. Cader RA, Zakaria NI, Yaacob Y, Shah SA. Carotid intima-media thickness in kidney transplant recipients. Hong Kong Journal of Nephrology 2016; 19: 36-41.

20. Kolonko A, Chudek J, Szotowska M, Kuczera P, Wiecek A. Cardiovascular risk factors and markers of atherosclerosis in stable kidney transplant recipients. Transplant Proc. 2016; 48(5) 1543-1550.
21. Koenig W. Fibrinogen in cardiovascular disease: An update. Thromb Haemost. 2003; 89: 601-609.
22. Lee AJ, Lowe GD, Woodward M, Tunstall-Pedoe H. Fibrinogen in relation to personal history of prevalent hypertension, diabetes, stroke, intermittent claudication, coronary heart disease and family history: The Scottish Heart Health Study. Br Heart J 1993; 69: 338-342.
23. Ridker PM. Inflammation, atherosclerosis and cardiovascular risk: an epidemiologic view. Blood Coagul Fibrinolysis 1999; 10 Suppl 1: S9-S12.
24. Zoccali C, Mallamaci F, Tripepi G, Cutrupi S, Parlongo S, Malatino LS, et al. Fibrinogen, mortality and incident cardiovascular complications in end-stage renal failure. Journal of Internal Medicine 2003; 254: 132-139.
25. Hong EG, Ohn JH, Lee SJ, Kwon HS, Kim SG, Kim DJ, et al. Clinical implications of carotid artery intima media thickness assessment on cardiovascular risk stratification in hyperlipidaemic Korean adults with diabetes: The Alto study. BMC Cardiovasc Disord. 2015; 15: 114
26. Leigh JA, O'Neal WT, Soliman EZ. Echocardiographic left ventricular hypertrophy as a predictor of cardiovascular disease independent of left ventricular anatomy in persons ≥ 65 years of age. Am J Cardiol. 2016; 117(11): 1831-1835
27. Khutan H, Aggarwal S, Kajal KS, GargR, Kaur R, Kaur A. Study of carotid intima media thickness in essential hypertension with or without left ventricular hypertrophy. Ann Afr Med. 2017; 16(4): 192-195.

28. Lyons D, Roy S, Patel M, Benjamin N, Swift CG. Impaired nitric oxide-mediated vasodilatation and total body nitric oxide production in healthy old age. Clinical Science. 1997; 93: 519-525.
29. Paolletti E, Cannella G. Reducing the risk of left ventricular hypertrophy in kidney transplant recipients: the potential role of mammalian target of rapamycin. Transplant Proc 2009; 41: S3-S5.
30. Elsayed EF, Tighiouart H, Weiner DE, Griffith J, Salem D, Levey AS, et al. Waist-hip ratio and body mass index as risk factors for cardiovascular events in chronic kidney disease. Am J Kidney Dis. 2008; 52(1): 49-57.
31. Nafar M, Khatami F, Kardavani B, Farjad R, Pour-Reza-Gholi F, Firoozan A. et al. Atherosclerosis after kidney transplantation: changes of intima media thickness of carotids during early post-transplant period. Urol J. 2007; 4(2): 105-110.

CHAPTER 5

Atherosclerotic vascular disease is more prevalent among black peritoneal dialysis patients in South Africa

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Abstract

Background

Occurrence of cardiovascular disease (CVD) in the setting of chronic kidney disease (CKD) can be described as a "cruel alliance", with CVD responsible for about half of all deaths among CKD patients. Chronic kidney disease patients are more likely to die from CVD than progress to end stage kidney disease (ESKD). Dyslipidaemia, a known traditional risk factor for CVD, is highly prevalent among CKD patients and with an even higher frequency among ESKD patients on dialytic therapies. In view of widespread statin use among CKD patients, we hypothesized that lipoprotein ratios may correlate better than lipid profile parameters with AsVD. In this study, we investigated the relationship of atherosclerotic vascular disease (AsVD) to clinical and echocardiographic parameters among black South Africans with CKD (stage 3) and ESKD on peritoneal dialysis (PD) and haemodialysis (HD).

Methods

This was a cross-sectional study of 40 adult (18-65 years) non-diabetic CKD patients (kidney disease outcome quality initiative, KDOQI stage 3), 40 ESKD patients on PD, 40 ESKD patients on HD and 41 age and sex-matched healthy controls. An interviewer-administered questionnaire was used to obtain information on participants' sociodemographic and cardiovascular risk factors. Anthropometric parameters were measured. Serum blood samples were analysed for creatinine, albumin and lipid profile; lipoprotein ratios were calculated. Echocardiography was performed on all patients and carotid intima media thickness (CIMT)

was measured in both right and left carotid arteries at 1cm proximal to the carotid bulb. Spearman's rank correlation and binary logistic regression were conducted to determine the relationship of AsVD to clinical and echocardiographic parameters.

Results

Atherosclerotic vascular disease was most prevalent among ESKD patients on PD (70 %, n = 28/40). Chronic kidney disease and HD patients exhibited a similar prevalence (47.5 %, n = 19/40), while the prevalence in controls was 17.1 % (n = 7/41). Presence of AsVD was associated with significantly higher values for age, WHR, urea, creatinine and left ventricular mass index (LVMI). Significant differences in clinical and echocardiographic parameters were observed when the study groups were compared. Age and LVH were independent predictors of AsVD.

Conclusion

Atherosclerotic vascular disease was more prevalent among PD patients compared to pre-dialysis CKD and HD patients. There was an association between AsVD and age, WHR, serum urea, creatinine and LVMI. Age (> 40 years) and presence of LVH were independent predictors of AsVD.

Keywords Atherosclerotic vascular disease, peritoneal dialysis, haemodialysis, chronic kidney disease, end-stage kidney disease, left ventricular hypertrophy.

Introduction

Robust association exists between chronic kidney disease (CKD) and cardiovascular disease (CVD),[1] of which, atherosclerotic vascular disease (AsVD) contributes significantly to morbidity and mortality in CKD.[2] Atherosclerotic vascular disease was found to be related to 60.9 % of deaths among non-diabetic end stage kidney disease (ESKD) patients on

maintenance haemodialysis (HD).[3] As the estimated glomerular filtration rate (eGFR) deteriorates, cardiovascular complications emerge and increase in frequency as CKD progresses.[4] Report of the baseline characteristics of participants from the Chronic Renal Insufficiency Cohort (CRIC) study showed that lower levels of eGFR were associated with a higher prevalence of CVD.[5] Similarly, the Atherosclerotic Risk In Community (ARIC) study found a consistent association between increased left ventricular mass and low eGFR.[6] In the sub-Saharan African setting, a study found cardiac lesions to be highly prevalent among ESKD patients on maintenance HD with left ventricular hypertrophy (LVH) being the most common cardiac lesion.[7] A case control study among black South African ESKD patients found a high prevalence of carotid plaques (38.1 %) among ESKD patients on maintenance haemodialysis compared to controls (7.9 %).[8] Similarly, Freercks et al[9] found that the prevalence of coronary calcification was 38.6 % among ESKD patients and was associated with older age and previous CVD. Although the study by Freercks et al[9] recruited both HD and PD patients, nearly 90 % of the participants were on HD.[9] Studies designed to assess CVD in black South African ESKD patients on PD are sparse, despite increased CVD-related morbidity and mortality seen in this group of patients. A study on the predictors of mortality among rural dwelling ESKD patients on chronic dialysis found continuous ambulatory peritoneal dialysis (CAPD) to be a predictor of all-cause mortality,[10] hence the need to investigate CVD in both HD and PD patients.

Dyslipidaemia is recognized as a traditional risk factor for CVD; furthermore, abnormalities in lipid metabolism related to uraemia have been reported.[11] A rise in the prevalence of atherogenic dyslipidaemia in the black population in an urban settlement in Cape Town, South Africa, has been noted; 59% of ischaemic heart disease and 29% of ischaemic stroke burden in males and females ≥30 years were attributable to increased cholesterol levels in this population.[12] Some studies have reported that dyslipidaemia, such as an increase in low density

lipoprotein (LDL-C), triglycerides (TG), lipoprotein (a) (LPa) and reduced high density lipoprotein (HDL-C) are more commonly seen in PD than HD.[13,14]

The high prevalence of dyslipidaemia in the urban population of South Africa, the association between dyslipidaemia and CKD and the evidence of higher mortality in PD when compared to HD, impelled us to evaluate the determinants of atherosclerotic vascular disease in CKD (stage 3) and ESKD (PD and HD) patients.

Methods

This was a comparative cross-sectional study of 40 adult (age 18-65 years) non-diabetic ESKD patients on HD, 40 ESKD patients on PD, 40 stage 3 CKD patients and 41 age- and sex-matched healthy controls at a large urban public hospital in South Africa from 2 January 2017 to 31 August 2017. The study was approved by the human research ethics committee (HREC) of the University of the Witwatersrand, study number M160614. An interviewer-administered questionnaire was used to obtain information on participants' sociodemographic and cardiovascular risk factors including age, gender, waist-hip ratio (WHR), body mass index (BMI).

Waist and hip circumference and WHR were measured with patients in an erect position and after PD fluid had been drained out in PD patients. Weight was measured by using a weighing scale; in haemodialysis patients, the weight was obtained before initiation of dialysis while weight was obtained after the PD fluid was drained in PD patients. Height was assessed by a stadiometer. Body mass index was calculated using the formula weight/height^2, while the body surface area was calculated using the Mosteller formula.[15] Blood pressure was taken after the patient had rested for 5 minutes in a sitting position, on the left arm with an appropriate sized cuff. Blood samples were taken before dialysis in HD patients. Serum lipogram (total cholesterol-TC, low density lipoprotein-LDL-C, triglyceride-TG, high density lipoprotein-

HDL-C) was determined by an enzymatic colorimetric method using Roche cobas 8000 modular analyser series, module c701 analyzer (Roche, Japan). Lipoprotein ratios were calculated as follows: Atherogenic index of plasma (AIP)= Log (Tg/HDL-C), Castelli index 1 = TC/HDL-C, Castelli index 2 = LDL-C/HDL-C, non-HDL-C=TC-HDL-C, Atherogenic index= non-HDL-C/HDL-C, Lipoprotein combined index (LCI) = (TC × TG × LDL-C)/HDL-C.[16] Estimated GFR was calculated in stage 3 CKD patients and PD patients using the 2009 CKD-EPI formula.[17] All participants had echocardiography and CIMT measurements in accordance with the guidelines of the American Society of Echocardiography,[18] using a Philips iE33 echocardiography machine (Philips Corporation, USA). Carotid intima media thickness was assessed using the vascular probe of the echocardiography machine, Philips iE33 (S5-1 probe) by focussing on the far wall of the carotid artery, 1cm proximal to the dilatation of the carotid bulb along the long axis of the artery. Automatic echo-generated measurements with percentage quality of 95% were recorded. The procedure was done for both left and right carotid arteries and the average was used in the analysis. Atherosclerotic vascular disease was defined by using a combination of increased CIMT (> 0.55mm) and presence of plaques.

Power calculation and Data analysis

Stata version13.1 (StataCorp, USA), was used for statistical analysis. The power to detect differences in levels of atherosclerosis was calculated. With sample size of 40 in each group, a 5% significance level and atherosclerosis proportion of 38.1% in haemodialysis patients[8] compared to 7.93% in controls,[8] there will be a power of 90.89 to detect differences in these groups.

Categorical variables were expressed as frequencies and percentages. Test of normality (Shapiro-Wilk) was performed on all continuous variables and data was presented as median and interquartile ranges (IQR). Comparison was performed between HD, PD, CKD and

controls using the Kruskal-Wallis test and a post-hoc analysis was performed using the pairwise Wilcoxon rank-sum. Comparison was also performed between those who had AsVD and those who did not, using the Wilcoxon rank-sum test.

Spearman correlation was used to determine the relationship between CIMT and cardiovascular risk factors among stage 3 CKD, PD, HD patients and the combination of the three groups. Multivariate regression analysis was performed to determine the relationship and contribution of cardiovascular risk factors to AsVD. Test of significance was taken as p-value < 0.05. Post regression analysis was done to determine the goodness of fit of the final model.

Results

The median age was 41 years (IQR = 36.0 – 51.5) among CKD and 39.5 years (IQR = 35.0 – 46.5) among PD patients, Table 1. Increased WHR was most prevalent among PD patients. Hypertension was most prevalent among HD patients 82.5 % (n = 33) compared to 17.1 % (n = 7) among controls, (p < 0.001). Increased total cholesterol was present in 52.5 % (n = 21/40) of PD patients compared to 22.5 % (n = 9/40) CKD and 12.2 % (n = 5/41) controls, (p = 0.006 and < 0.001 respectively). However, total cholesterol levels were not elevated in HD patients. Atherosclerotic vascular disease was most prevalent among PD patients in 70 % (n = 28/40) and showed significant difference compared to 47.5 % (n = 19/40) among HD and 17.1 % (n = 7/41) among controls; p = 0.041 and < 0.001 respectively; Table 1

.

Table 1. Sociodemographic and clinical characteristics of study population

Parameter	**Total (n=161)**	**CKD (n=40)**	**PD (n=40)**	**HD (n=40)**	**Controls (n=41)**
Age (years)	41.0 (35.0 – 48.0)	41.0 (36.0 - 51.5)	39.5 (35.0 - 46.5)	40.5 (36.0 - 49.0)	41.0(29.0 – 48.0)
Gender					
Female	80 (49.7)	20 (50.0)	19 (47.5)	18 (45.0)	23 (56.1)
Male	81 (50.3)	20 (50.0)	21 (52.5)	22 (55.0)	18 (43.9)
BMI					
< 30kg/m^2	107 (66.6)	19 (47.5)	31 (77.5)	32 (80.0)	25 (61.0)
> 30kg/m^2	54 (33.4)	21 (52.5)	9 (22.5)	8 (20.0)	16 (39.0)
WHR					
Normal	64 (40.0)	12 (30.0)	9 (22.5)	15 (37.5)	28 (68.3)
Increased	97 (60.0)	28 (70.0)	31 (72.5)	25 (62.5)	13 (31.7)
Hypertension[a]					
No	68 (42.2)	15 (37.5)	12 (30)	17 (17.5)	34 (82.9)
Yes	93 (57.8)	25 (62.5)	28 (70)	33 (82.5)	7 (17.1)
TC (mmol/l)					
< 5.17	126 (78.3)	31 (77.5)	19 (47.5)	40 (100)	36 (87.8)
> 5.17	35 (21.7)	9 (22.5)	21 (52.5)		5 (12.2)
LDL-C |(mmol/l)					

< 2.59	90 (55.9)	19 (47.5)	14 (35.0)	36 (90.0)	21 (51.2)
> 2.59	71 (44.1)	21 (52.5)	26 (65.0)	4 (10.0)	20 (48.8)
HDL-C[b](mmol/l)					
Low	89 (55.3)	30 (75.0)	21 (52.5)	15 (37.5)	23 (56.1)
Normal	72 (44.7)	10 (25.0)	19 (47.5)	25 (62.5)	18 (43.9)
AsVD					
Absent	88 (54.7)	21 (52.5)	12 (30.0)	21 (52.5)	34 (82.9)
Present	73 (45.3)	19 (47.5)	28 (70.0)	19 (47.5)	7 (17.1)

CKD – chronic kidney disease; PD – peritoneal dialysis; HD – haemodialysis; BMI - Body mass index; WHR - Waist-hip ratio; TC – Total cholesterol; LDL-C – Low density cholesterol; HDL-C – High density cholesterol; AsVD – Atherosclerotic vascular disease; [a]Systolic blood pressure>140mmHg ± diastolic blood pressure > 90mmHg, Low HDL-C = < 1.03 in male and < 1.29mmol/l in female

All tested cardiovascular risk factors showed significant differences between the four groups using the Kruskal-Wallis test, except for age and atherogenic index of plasma (AIP). Pairwise comparison with p-value is shown in Table 2. Significantly higher serum phosphate levels and calcium phosphate product ($CaXPO_4$) were seen when PD patients were compared with controls [1.6 (1.2 – 1.9) vs 1.0 (0.9 – 1.1), $p < 0.001$] and [40.1 (32.1 – 53.7) vs 29.5 (26.7 – 32.5), $p < 0.001$] respectively. Comparison between PD and stage 3 CKD also showed significantly higher levels of serum phosphate and $CaXPO_4$ in PD patients; Table 2. Although serum levels of phosphate and $CaXPO_4$ were higher among HD patients compared to controls, they were not statistically significant.

Table 2. Comparison of clinical characteristics and echocardiographic parameters among chronic kidney disease, haemodialysis, peritoneal dialysis patients and controls

Parameter	K-W test (n=161)	Wilcoxon pairwise ranksum (p-value)				
	x^2 (p-value)	A	B	C	D	E
Age (years)	1.38 (0.71)					
BMI Kg/m^2	16.3 (0.001)	0.067	0.199	0.075	0.001	<0.001
WHR	20.6 (<0.001)	0.002	<0.001	0.002	0.758	0.310
SBP (mmHg)	39.1 (<0.001)	<0.001	<0.001	<0.001	0.204	0.016
MABP (mmHg)	89.1 (<0.001)	<0.001	<0.001	<0.001	0.077	0.015
TC (mmol/l)	50.6 (<0.001)	0.189	0.001	<0.001	0.029	<0.001
TG (mmol/l)	14.0 (0.003)	0.511	0.364	0.018	0.780	0.001
LDL-C (mmol/l)	35.9 (<0.001)	0.660	0.062	<0.001	0.019	<0.001
HDL-C (mmol/l)	10.6 (0.014)	0.296	0.872	0.051	0.148	0.001
Castelli1	12.4 (0.006)	0.966	0.030	0.436	0.008	0.256
Castelli2	10.9 (0.012)	0.261	0.249	0.085	0.011	0.373
AC	12.4 (0.006)	0.966	0.030	0.436	0.008	0.256
AIP	3.3 (0.354)					
non-HDL-C	39.8 (<0.001)	0.308	<0.001	0.001	0.008	<0.001
eGFR (ml/min/1.73m^2)	144.0 (<0.001)	<0.001				
Calcium(mmol/l)	8.3 (0.041)	0.928	0.025	0.088	0.024	0.092
Phosphate(mmol/l)	35.7 (<0.001)	0.052	<0.001	0.073	<0.001	0.514
$CaXPO_4$(mg^2/dl^2)	22.2 (<0.001)	0.132	<0.001	0.228	<0.001	0.755
Albumin(g/l)	47.4 (<0.001)	0.014	<0.001	<0.001	<0.001	0.006
LAD	96.4 (<0.001)	<0.001	<0.001	<0.001	0.287	0.003
EF (%)	32.3 (<0.001)	<0.001	<0.001	<0.001	0.419	0.172
LVMI (g/m^2)	24.2 (<0.001)	0.136	<0.001	<0.001	0.010	0.023
CIMT (mm)	36.3 (<0.001)	<0.001	<0.001	<0.001	0.006	0.758

A - comparison between CKD patients and controls; B - Comparison between ESKD on PD and controls; C - Comparison between ESKD on HD and controls; D - Comparison between CKD and ESKD on PD; E - Comparison between CKD and ESKD on HD; BMI - body mass index; WHR - waist-hip ratio; SBP - systolic blood pressure; MABP – mean arterial blood pressure; TC-total cholesterol; TG - triglyceride; LDL-C - low density lipoprotein; HDL-C - high density lipoprotein; AC – atherogenic coefficient; AIP - atherogenic index of plasma; non-HDL-C- non- high density lipoprotein; eGFR - estimated glomerular filtration rate; LAD - left atrial diameter, EF - ejection fraction; LVMI- left ventricular mass index.

Comparison between those who had AsVD and those who did not, showed statistically significant differences in age, WHR and LVMI. High density lipoprotein and left atrial diameter (LAD) showed a tendency towards significance; Table 3. Significantly higher values were observed with age, WHR, urea, LVMI; Table 3. No association was seen between serum lipids, lipoprotein ratios and AsVD.

Table 3. Comparison between those who had atherosclerotic vascular disease and those without atherosclerotic vascular disease

Risk factors	AsVD present (n = 66)	AsVD Absent (n = 54)	p-value
Age (years)	43.5 (36.0 – 50.0)	37.0 (30.0 – 44.0)	0.002*
BMI (Kg/m^2)	27.0 (22.5 – 31.9)	26.4 (22.0 – 30.5)	0.394
WHR	0.92 (0.88 – 0.96)	0.89 (0.85 – 0.94)	0.028*
SBP (mmHg)	148.5 (131.0 – 164.0)	147.0 (127 – 167)	0.411
MABP (mmHg)	139.8 (116.3 – 161.0)	141.8 (130.3 – 155.3)	0.792
TC (mmol/l)	4.4 (3.4 – 5.3)	4.1 (3.5 – 4.7)	0.342
TG (mmol/l)	1.2 (0.8 – 1.6)	1.3 (0.7 – 1.8)	0.444
LDL-C (mmol/l)	2.5 (1.7 – 3.3)	2.3 (1.8 – 2.7)	0.150
HDL-C (mmol/l)	1.2 (1.0 – 1.4)	1.1 (0.9 – 1.4)	0.065
Urea (mmol/l)	16.7 (11.3 – 23.5)	13.2 (8.5 – 18.9)	0.042*
Calcium (mmol/l)	2.29 (2.18 – 2.41)	2.34 (2.17 – 2.42)	0.594
Phosphate (mmol/l)	1.20 (1.00 – 1.60)	1.15 (0.9 – 1.4)	0.253
$CaXPO_4$ (mg^2dl^2)	36.7 (29.5 – 44.7)	33.5 (26.8 – 41.1)	0.310
Castelli1	3.5 (2.8 – 4.4)	3.6 (3.1 – 4.6)	0.560
Castelli2	1.9 (1.5 – 2.9)	2.0 (1.6 – 2.6)	0.914
AC	2.5 (1.8 – 3.4)	2.6 (2.1 – 3.6)	0.560
AIP	0.0 (-0.5 – 0.4)	0.1 (-0.4 – 0.4)	0.202
non-HDL-C	3.2 (2.3 – 4.1)	3.0 (2.5 – 3.3)	0.518
LCI	9.5 (4.8 – 21.7)	10.1 (4.8 – 18.3)	0.868
EF (%)	58.5 (53.0 – 64.0)	61.0 (56.0 – 64.0)	0.257
LAD	3.9 (3.5 – 4.5)	3.8 (3.2 – 4.2)	0.050
LVMI (g/m^2)	122.9 (103.5 – 146.0)	98.6 (83.0 – 117.1)	< 0.001*

* - statistically significant, p<0.05; AsVD – atherosclerotic vascular disease; BMI - body mass index; WHR - waist-hip ratio; SBP - systolic blood pressure; MABP – mean arterial blood pressure; TC-total cholesterol; TG - triglyceride; LDL-C - low density lipoprotein; HDL-C - high density lipoprotein; SCr – serum creatinine; eGFR – estimated glomerular filtration rate; $CaXPO_4$ – calcium-phosphate product; AC – atherogenic coefficient; AIP - atherogenic index of plasma; non-HDL-C- non- high density lipoprotein; LCI – lipoprotein combine index; EF - ejection fraction; LAD - left atrial diameter; LVM - left ventricular mass; LVMI- left ventricular mass index.

Age showed positive correlation with CIMT in all the kidney disease groups with the strongest seen in CKD (r = 0.48, p = 0.002; Spearman correlation). Moderate positive correlation was

seen between CIMT and LVMI across all kidney disease groups. There was a negative correlation between CIMT and Castelli indices 1 & 2 and AC among HD patients, ($r = -0.43$, 0.006; $r = -0.44$, $p = 0.004$; $r = -0.43$, $p = 0.006$; respectively); no correlation was seen between CIMT and lipoprotein ratios in stage 3 CKD and PD patients, and when all the kidney disease groups were combined; Table 4.

Table 4. Correlation between carotid intima media thickness and risk factors for cardiovascular disease among chronic kidney disease, peritoneal dialysis and haemodialysis patients

Parameter	CKD (n=40) rho (p-value)	PD (n=40) rho (p-value)	HD (n=40) rho (p-value)	TOTAL (n=120) rho (p-value)
Age (years)	0.48 (0.002)	0.28 (0.077)	0.27 (0.099)	0.33 (< 0.001)
BMI (Kg/m^2)	0.21 (0.205)	0.10 (0.538)	0.03 (0.845)	0.04 (0.651)
WHR	0.36 (0.023)	0.31 (0.051)	0.13 (0.429)	0.27 (0.003)
SBP (mmHg)	0.16 (0.318)	0.21 (0.187)	- 0.09 (0.579)	0.12 (0.187)
TC (mmol/l)	-0.05 (0.760)	- 0.04 (0.834)	- 0.13 (0.428)	0.08 (0.411)
TG (mmol/l)	- 0.19 (0.240)	-0.07 (0.681)	-0.03 (0.845)	-0.05 (0.606)
LDL-C (mmol/l)	0.19 (0.244)	0.11 (0.501)	- 0.33 (0.040)	0.13 (0.173)
HDL-C (mmol/l)	-0.29 (0.069)	0.17 (0.291)	0.48 (0.002)	0.14 (0.130)
Castelli1	0.16 (0.322)	- 0.19 (0.238)	- 0.43 (0.006)	- 0.06 (0.500)
Castelli2	0.19 (0.245)	- 0.07 (0.662)	- 0.44 (0.004)	- 0.03 (0.772)
AC	0.16 (0.322)	- 0.19 (0.238)	- 0.43 (0.006)	- 0.06 (0.500)
AIP	- 0.01 (0.955)	- 0.14 (0.383)	- 0.20 (0.219)	-0.09 (0.349)
non-HDL-C	0.09 (0.574)	- 0.11 (0.500)	- 0.29 (0.070)	0.04 (0.662)
LCI	0.04 (0.824)	- 0.10 (0.557)	- 0.20 (0.219)	0.01 (0.893)
Calcium (mmol/l)	-0.12 (0.456)	-0.02 (0.889)	-0.09 (0.566)	-0.12 (0.184)
Phosphate (mmol/l)	0.00 (0.982)	0.09 (0.602)	-0.13 (0.425)	0.10 (0.277)
$CaXPo_4$ (mg^2/dl^2)	-0.02 (0.898)	0.05 (0.749)	-0.13 (0.425)	0.06 (0.508)
EF (%)	- 0.19 (0.230)	- 0.01 (0.930)	0.01 (0.893)	- 0.11 (0.244)
LAD	0.20 (0.217)	0.55 (<0.001)	- 0.17 (0.300)	0.21 (0.025)
LVMI (g/m^2)	0.40 (0.012)	0.42 (0.006)	0.44 (0.005)	0.43 (<0.001)

CKD- chronic kidney disease; PD- peritoneal dialysis; HD- haemodialysis; BMI - Body mass index; WHR - Waist-hip ratio; SBP - systolic blood pressure; MABP – mean arterial blood pressure; TC-total cholesterol; TG - Triglyceride; LDL-C - low density lipoprotein; HDL-C - High density lipoprotein; eGFR – estimated glomerular filtration rate; AC – Atherogenic coefficient; AIP - Atherogenic index of plasma; non-HDL-C- non- high density lipoprotein; LCI – Lipoprotein combine index; EF - Ejection fraction; LAD - Left atrial diameter; LVMI- Left ventricular mass index.

Binary logistic regression analysis showed that age and LVH were independent predictors of AsVD after adjusting for gender, hypertension, TC and LDL-C and TG, Table 5. The regression model showed that the presence of LVH confers > 49-fold risk of AsVD among ESKD patients. Similarly, age > 40 years confers a 3-fold risk of developing AsVD.

Table 5. Relationship between atherosclerotic vascular disease and risk factors for cardiovascular disease among peritoneal dialysis and haemodialysis patients

Risk factors	OR	95 % CI (n = 80)	p-value
Age (> 40 years)	3.11	1.00 – 9.65	0.049*
Gender	1.58	0.48 – 5.20	0.453
Hypertension[a]	1.49	0.37 – 5.97	0.575
LVH	49.79	2.58 – 959.68	0.010*
TC (> 5.17 mmol/l)	12.15	0.98 – 150.60	0.052
LDL-C (> 2.59 mmol/l)	1.64	0.34 – 7.99	0.541
TG (> 1.69 mmol/l)	0.69	0.17 – 2.80	0.599

* - Statistically significant, $p < 0.05$; LVH - left ventricular hypertrophy; TC – total cholesterol; LDL-C - low density lipoprotein; BMI - body mass index; [a]systolic hypertension (systolic blood pressure >140mmHg)

Discussion

Carotid intima media thickness is a reliable surrogate marker of subclinical atherosclerosis in the general population and increased CIMT has been associated with risk of cardiovascular events.[19] Similar findings have been reported among CKD and ESKD patients.[20] Our study showed a significantly higher prevalence of AsVD among PD, CKD and HD patients compared to controls. Almost three-quarters of the PD patients and almost half of the CKD and HD patients had AsVD compared to less than one-fifth in the control group. This is consistent with results from previous studies.[8,9] The finding of prevalence of 17.1 % in the controls in our study is comparable to prevalence of 29.3 % for peripheral arterial disease found among black rural dwellers in South Africa;[21] lower prevalence observed in our study may be due to exclusion of elderly people from our study. Although the finding of high prevalence of AsVD in our stage

3 CKD and HD groups can be explained by the high prevalence of cardiovascular risk factors such as hypertension and reduced renal function among the kidney disease groups, we suggest that the very high prevalence of AsVD seen in PD may be associated with additional PD-related factors. Firstly, we suggest a synergistic interplay between high glucose exposure and increased levels of inflammation contributing to the high prevalence of AsVD among PD patients. Exposure of PD patients to high glucose concentrations via the use of glucose-containing PD fluids, in the presence of high levels of inflammation, could result in increased glucose absorption and consequent hyperinsulinaemia, which has been associated with hypertension, obesity, dyslipidaemia and glucose intolerance.[22] In the setting of chronic hyperinsulinaemia, experimental evidence supports marked increase in lipogenesis in white adipose tissue and liver.[23] From our study, the finding of higher prevalence of increased TC and LDL-C among PD patients compared to stage 3 CKD and HD patients further buttressed a possibility of hyperinsulinaemia among PD patients. Secondly, we found significantly higher levels of uraemic retention solutes such as serum creatinine and urea among PD patients compared to HD and stage 3 CKD. In comparison to PD patients, the lower levels of uraemic retention solutes in HD patients, seen in our study may be explained by the effectiveness of current HD procedure in the clearance of uraemic toxins using biocompatible membranes, high flux dialyzers, dialysate flow rate of 500ml/min and moderate blood flow rate of 350ml/min, with dialysis ensured thrice weekly and four hours per session. However, PD patients were dialysing at home and a higher dialysis dose of PD may have been required. Thirdly, higher uraemic retention solutes among PD patients may likely connote the presence of high levels of uraemic toxins such as middle molecules and protein-bound solutes among the PD patients which have been associated with CVD.[24-26] Bammens et al[24] found that increasing PD dose may compensate for declining residual renal function by increasing elimination of water soluble uraemic solutes but not middle molecules like p-cresol. Several studies have demonstrated an

association between uraemic toxins such as indoxyl sulphate, p-cresyl sulphate and CVD.[25,26] Both total and free indoxyl sulphate and p-cresyl sulphate were found to be independently associated with structural and functional markers of CVD.[26] Further studies on the relationship of chronic inflammation and uraemic toxins to AsVD in ESKD among black Africans will be required to establish this association. Serum levels of phosphate and $CaXPO_4$ were higher among kidney disease patients with AsVD compared to patients without AsVD, but were not statistically significant possibly because all ESKD patients were on phosphate binders and vitamin D.

Statistically significant differences were found in clinical and echocardiographic cardiovascular risk factors when each of the kidney disease groups was compared with controls; Table 2. Of particular importance are systolic blood pressure (SBP), mean arterial blood pressure (MABP), WHR, non-HDL-C cholesterol, LAD, LVMI and CIMT which showed a near consistent value of $p < 0.001$ when the kidney disease groups were compared with controls. These findings are consistent with those documented in previous studies designed to evaluate the CVD risk factors in CKD.[27,28]

When those who had AsVD were compared with those who did not, significantly increased differences were observed with age, WHR, LVMI, while LAD showed tendency towards significance. The association between age and AsVD in our study is consistent with previous observations[29] and could be due to the fact that advancing age is associated with known cardiovascular risk factors such as hypertension, diabetes and vascular disease;[30] in addition, advancing age predisposes to endothelial dysfunction.[31] The finding of a significantly higher LVMI among patients who had AsVD and an association between AsVD and LVH among kidney disease patients is consistent with a previous study.[32] In addition to being a CVD, LVH has been shown as a cardiovascular risk factor in the general population[33] and in ESKD patients.[34] Significantly higher frequency of LVH was reported among black South African

ESKD patients on haemodialysis compared to controls.[8] In the presence of hypertension and renal dysfunction, pressure and volume overload causes the cardiac myocytes to undergo conformational changes in order to compensate for haemodynamic alterations, resulting in LVH.[35]

Age (> 40 years) and LVH independently predicted AsVD among ESKD patients after adjusting for gender, WHR, systolic hypertension, LDL-C and TG. We found that LVH confers a 49-fold risk of AsVD among our ESKD patients. This highlights the robust relationship between LVH and AsVD among our ESKD group and also underscores the importance of LVH as a CVD risk factor and the need to direct treatment strategies towards LVH reduction among CKD patients.

We recommend that longitudinal follow-up studies designed to evaluate the relationship of AsVD to inflammation, hyperinsulinaemia and uraemic toxins, especially middle molecules, be conducted among black ESKD on PD and HD to ascertain the contribution of these risk factors to AsVD.

This study has some limitations. We did not assess serum concentration of insulin and insulin resistance in these patient; these could have helped to confirm hyperinsulinaemia as a risk factor for AsVD among PD patients. The exclusion of the elderly from this study could have reduced the overall prevalence of AsVD in the different patient groups investigated. The cross-sectional nature of this study allowed for measurements of the various parameters at a single point; a longitudinal study will provide data on the evolution of atherosclerosis over the period of CKD and dialysis.

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Ethics Approval and consent to participate in the study

This study was approved by the Human Research and Ethics Committee (HREC) of the University of the Witwatersrand, Johannesburg, South Africa; clearance certificate number M160614. All participants' gave written informed consent before enrolment

Availability of data or material

The dataset used in the analysis is available with the corresponding author and will be released on request.

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Consent for publication

All the authors gave their consent for the publication of this article

Authors' Contributions

S.O. Oguntola, M.O. Hassan, R. Duarte and S. Naicker were involved in study design. S.O. Oguntola, M.O. Hassan, A. Vachiat, P. Manga and S. Naicker participated in data collection. S.O. Oguntola and R. Duarte did the laboratory analysis of samples. Data analysis was done by S.O. Oguntola. First draft of manuscript was prepared by S.O. Oguntola and all authors reviewed the manuscript.

References

1. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification and stratification. Am J Kid. Dis. 2002; 39(suppl 1): S1 – S266

2. Collins AJ, Li S, Gilbertson DT, Liu J, Chen SC, Herzog CA. Chronic kidney disease and cardiovascular disease in the medicare population. Kidney Int Suppl. 2003; 87: S24 – S31

3. Lindner A, Charra B, Sherrard DJ Scribner BH. Accelerated atherosclerosis in prolonged maintenance hemodialysis. New Eng. J Med. 1974; 290: 697-701

4. Rahman M, Brown CD, Coresh J, Davis BR, Eckfeldt J, Kopyt N, et al. The prevalence of reduced glomerular filtration rate in older hypertensive patients and its association with cardiovascular disease: a report from the anti-hypertensive and lipid-lowering treatment to prevent heart attack trial. Arch Intern Med. 2004; 164(9): 969-976

5. Lash JP, Go AS, Appel LJ, He J, Ojo A, Rahman M, et al. Chronic renal insufficiency cohort (CRIC) Study: Baseline characteristics and associations with kidney function. Clin J Am Soc Nephrol 2009; 4: 1302-1311

6. Matsushita K, Kwak L, Sang Y, Ballew SH, Skali H, Shah AM, et al. Kidney disease measures and left ventricular structure and function: The atherosclerosis risk in communities' study. J Am Heart Assoc. 2017; 6: e006259. DOI: 10.1161/JAHA.117.006259

7. Kaze FF, Kengne A, Djalloh AA, Ashuntantang G, Halle MP, Menanga AP, et al. Pattern and correlates of cardiac lesions in a group of sub-Saharan African patients on maintenance haemodialysis. Pan African Medical Journal. 2014; 17: 03 doi: 10.11604/pamj.2014.17.3.3422

8. Amira OC, Naicker S, Manga P, Sliwa K, Mia A, Raal F, et al. Adiponectin and atherosclerosis risk factors in African haemodialysis patients: A population at low risk for atherosclerotic cardiovascular disease. Hemodialysis Int. 2012; 16(1): 59-68

9. Freercks R, Swanepoel C, Carrara H, Moosa S, Lachman A, Rayner B. Vascular calcification in South African dialysis patients: Ethnic variation, prevalence, detection and haemodynamic correlates. Nephrology 2012; 17: 607-615

10. Tamayo-Isla RA, Ameh O, Mapiye D, Swanepoel CR, Bello AK, Ratsela AR, et al. Baseline Predictors of mortality among predominantly rural dwelling end-stage renal disease patients on chronic dialysis therapies in Limpopo, South Africa. PLoS ONE 2016; 11(6): e0156642. doi:10.1371/journal.pone.0156642

11. Schuchardt M, Tolle M, Van der Giet M. High density lipoprotein structural and functional changes under uraemic conditions and the therapeutic consequences. Handbook of experimental pharmacology 2015; 224: 423-453

12. Norman R, Bradshaw D, Steyn K, Gaziano T, the South African Comparative Risk Assessment Collaborating Group. Estimating the burden of disease attributable to high cholesterol in South Africa in 2000. S Afr Med J. 2007; 97(8): 708-715

13. Shurraw S, Tonelli M. Statin for treatment of dyslipidaemia in chronic kidney disease. Perit Dial Int 2006; 26: 523-539

14. Moberly JB, Attman PO, Samuelsson O, Johansson AC, Knight-Gibson, Alaupovic P. Alteration in lipoprotein composition in peritoneal dialysis patients. Perit Dial Int 2002; 22: L220-228

15. Mosteller RD. Simplified calculation of body surface area. N Engl J Med. 1987; 317(17):1098

16. Cai G, Ganwei S, Sheliang X, Wei L. The atherogenic index is a strong and independent predictor for coronary artery disease in the Chinese Han population. Medicine 2017; 96: 37(e8058)

17. Early A, Miskulin D, Lamb EJ, Levey AS, Uhliq K. Estimating equations for glomerular filtration rate in the era of creatinine standardization. A systematic review. Ann Intern Med. 2012; 156: 785-795

18. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al. Recommendations for cardiac quantification. A report from the American Society of Echocardiography's guidelines and standard committee and the chamber quantification writing group, developed in conjunction with the European association of Echocardiography, a branch of the European association of Cardiology. J Am Soc Echocardiogr 2005; 18: 1440-1463

19. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction. Circulation. 1997; 96: 1432-1437

20. Adeseun GA, Xie D, Wang X, Joffe MM, Mohler ER, Townsend RR, et al. Carotid plaque, carotid intima-media thickness and coronary calcification equally discriminate prevalent cardiovascular disease in kidney disease. Am J Nephrol 2012; 36: 342-347

21. Paul AK, Mash B, Rupesinghe G. Peripheral arterial disease – High prevalence in rural black South Africans. S Afri Med J. 2007; 97: 285-288

22. Modan M, Halkin H, Almog S, Lusky A, Eshkol A, Shefi M, et al. Hyperinsulinaemia: A link between hypertension obesity and glucose intolerance. J. Clin. Invest. 1985; 75: 809-817

23. Assimacopoulos-Jeannet F, Brichard S, Rencurel F, Cusin I, Jeanrenaud B. In-vivo effects of hyperinsulinaemia on lipogenic enzymes and glucose transporter expression in rat liver and adipose tissue. Metabolism 1995; 44(2): 228-233.

24. Bammens B, Evenepoel P, Verbeke K, Vanrenterghem Y. Time profiles of peritoneal and renal clearances of different uraemic solutes in incident peritoneal dialysis patients. Am J Kid. Dis. 2005; 46(3): 512-519

25. Schmidt S, Westhoff TH, Krauser P, Zidek W, Van der Giet M. The uraemic toxin phenylacetic acid increases the formation of reactive oxygen species in vascular smooth muscle cells. Nephrology Dialysis Transplantation. 2008; 23(1): 65-71

26. Rossi M, Campbell K, Johnson D, Stanton T, Pascoe E, Hawley C, et al. Uraemic toxins and cardiovascular disease across the chronic kidney spectrum: An observational. study. Nutrition, Metabolism and cardiovascular diseases 2014; 24: 1035-1042

27. Parikh NI, Hwang S, Larson MG. Cardiovascular disease risk factors in chronic kidney disease. Overall burden and rates of treatment and control. Arch Intern Med. 2006; 166(17): 1884-1891

28. Burmeister JE, Mosmann CB, Costa VB, Saraiva RT, Grandi RR, Bastos JP. Prevalence of cardiovascular risk factors in haemodialysis patients- The CORDIAL Study. Arq. Bras. Cardiol. 2014; 102(5): 473-480

29. Stein JH, Doug;as PS, Srinivasan SR, Bond MG, Tang R, Li S, et al. Distribution and cross-sectional age-related increases of carotid artery intima-media thickness in young adults. Stroke 2004; 35: 2782-2787

30. Khutan H, Aggarwal S, Kajal KS, Garg R, Kaur R, Kaur A. Study of carotid intima media thickness in essential hypertension with or without left ventricular hypertrophy. Ann Afr Med. 2017; 16(4): 192-195

31. Lyons D, Roy S, Patel M, Benjamin N, Swift CG. Impaired nitric oxide-mediated vasodilatation and total body nitric oxide production in healthy old age. Clinical Science. 1997; 93: 519-525

32.Tirmenstajn-Jankovic B, Dimkovic N, Radojicic Z, Bastac D, Zivanovic M, Zikic S. Association between age and cardiovascular status by Echosonography in asymptomatic predialysis patients with chronic kidney disease. Saudi J Kidney Dis Transplant 2017; 28(4): 818-829

33. Messerli FH, Schmieder R. Left ventricular hypertrophy. A cardiovascular risk factor in essential hypertension. Drugs. 1986; 31 Suppl 4: 192-201

34. Charytan D. Is left ventricular hypertrophy a modifiable risk factor in end-stage renal disease. Curr Opin Nephrol Hypertens. 2014; 23(6): 578-585

35. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. N Engl J. Med. 1990; 322: 1561-1566

CHAPTER 6

Atherosclerotic vascular disease and inflammation in CKD and dialysis patients and kidney transplant recipients

Submitted to BMC Nephrology

Abstract

Background

Chronic inflammation is a known risk factor for cardiovascular disease (CVD) among chronic kidney disease (CKD) patients. Current evidence supports the pivotal role of inflammation in atherosclerosis, bolstered by the association between commonly used inflammatory markers, such as high sensitivity C-reactive protein (hsCRP), and CVD. We evaluated the association between inflammatory markers and atherosclerotic vascular disease (AsVD) in stage 3 CKD, end-stage kidney disease (ESKD) patients on dialysis and kidney transplant recipients (KTRs).

Methods

This was a cross-sectional study of 40 adult (18-65years) non-diabetic stage 3 CKD patients, 40 peritoneal dialysis (PD) patients, 40 haemodialysis (HD) patients, 41 KTRs and 41 age- and sex-matched healthy controls. An interviewer-administered questionnaire was used to obtain information on participants' sociodemographic and cardiovascular risk factors. Anthropometric parameters were measured. Blood samples were obtained and serum was analysed for creatinine, albumin, lipid profile and inflammatory markers including highly sensitive CRP (hsCRP), pentraxin-3, tumour necrosis factor alpha (TNF-α), the ligand of the receptor for advanced glycation end-products (EN-RAGE). Echocardiography was performed on all patients and carotid intima media thickness (CIMT) was assessed in both right and left carotid arteries at 1cm proximal to the carotid bulb. Associations between AsVD and cardiovascular risk factors were determined using Wilcoxon rank sum. Correlates of AsVD and pentraxin-3 and the predictors of AsVD and elevated pentraxin-3 were determined using

Spearman's rank correlation and binary logistic regression. The utility of the inflammatory markers studied in predicting AsVD was assessed using receivers operating characteristics (ROC) and the area under curve (AUC), sensitivity and specificity were documented.

Results

The levels of total cholesterol were significantly higher in stage 3 CKD, PD, HD and KTRs compared to controls while the levels of serum albumin were significantly lower in PD, HD and stage 3 CKD compared to controls. Serum phosphate and $CaXPO_4$ levels were significantly higher in PD compared to controls. Among the inflammatory markers, only pentraxin-3 levels were significantly higher in all the kidney disease groups (stage 3 CKD, PD, HD and KTRs) compared to controls while hsCRP levels were significantly increased in stage 3 CKD, PD and HD patients. Tumour necrosis factor alpha was significantly higher in PD patients only compared to controls. EN-RAGE was significantly higher among KTRs compared to controls. Age, increased WHR and low HDL-C levels were associated with AsVD. Pentraxin-3 correlated positively with other inflammatory markers (except for hsCRP) and negatively with eGFR ($r = -0.171$, $p = 0.030$) and serum albumin levels ($r = -0.168$, $p = 0.033$) when the kidney disease groups (stage 3 CKD and ESKD and KTRs) were combined. Pentraxin-3 correlated positively with CIMT among KTRs ($r = 0.336$, $p = 0.032$), but there was no correlation between hsCRP and CIMT in any of the kidney disease groups. Predictors of high pentraxin-3 levels were hypertension, elevated total cholesterol, TNF-α and EN-RAGE levels while age (> 40 years), male gender and low HDL-C independently predicted AsVD.

Conclusion

Inflammation is present in all the kidney disease groups and persists in the post transplant period. Pentraxin-3 correlated positively with inflammatory markers including TNF-α and ENRAGE, but negatively with eGFR and serm albumin. Age (> 40 years), male gender and

low HDL-C independently predicted AsVD. Aggressive treatment of lipid disorders will be beneficial in CKD to reduce the burden of CVD.

Keywords Atherosclerotic vascular disease, peritoneal dialysis, haemodialysis, chronic kidney disease, end-stage kidney disease, inflammatory markers, pentraxin-3.

Introduction

Chronic inflammation is an established CKD-related risk factor for CVD. Surrogate markers of inflammation that are commonly used in the general population and chronic kidney disease (CKD) patients are interleukin-6 (IL-6) and C-reactive protein (CRP).[1,2] Interleukin-6, an immunomodulatory cytokine, plays a central role in inflammation and atherosclerosis by manifesting both pro-inflammatory and anti-inflammatory properties.[3,4] In response to diverse stimuli such as persistent infection, oxidative stress, volume overload, declining renal function and dialysis-related factors, IL-6 is secreted by several inflammatory cells such as monocytes, lymphocytes, macrophages, vascular smooth muscle and endothelial cells.[5-7] Although, the predictive value of IL-6 for cardiovascular and all-cause mortality was reported to be better than CRP among haemodialysis patients,[8,9] highly sensitive CRP has been widely used as an inflammatory marker in HD and PD patients and KTRs.[1,2]

Pentraxin-3, the long pentraxin, shares some similarities with IL-6, including secretion by multiple inflammatory cells at the site of inflammation or vascular injury in response to TNF-α and IL-1.[10,11] Similarly, pentraxin-3 expression in atherosclerotic lesions increases in response to inflammatory stimuli, in the same manner as IL-6, suggesting that pentraxin-3 may have a role in the pathogenesis of atherosclerosis.[12] An association has been established between pentraxin-3 and CVD in the general population and in ESKD patients.[13,14] Pentraxin-3 was shown to predict CVD better than hsCRP in maintenance HD patients.[14] The detection of pentraxin-3 in renal epithelial and mesangial cells[15,16] and the association between pentraxin-

3 and reduced glomerular filtration rate (GFR) signifies a link between renal disease and pentraxin-3.[17]

The ligand of the receptor for advanced glycation end-products (EN-RAGE), secreted by neutrophils, monocytes and macrophages, functions as a pro-inflammatory ligand of the receptor for advanced glycation end-products (RAGE).[18] EN-RAGE stimulates the expression of other pro-inflammatory cytokines such as interleukin-1 and TNF-α.[19] It also stimulates chemotaxis of monocytes and macrophages to the site of damaged vessels, [20] hence enhances the inflammatory process of atherosclerosis at the site of vascular injury.[21] Association between EN-RAGE and atherosclerosis has been established in both PD[22] and HD patients.[18] This study investigated the clinical and radiographic correlates of atherosclerosis in association with inflammatory biomarkers in stage 3 CKD and dialysis patients and KTRs.

Methods

This was a comparative cross-sectional study of 40 adult (18-65 years) non-diabetic stage 3 CKD patients, 40 patients on HD, 40 patients on PD, 41 KTRs and 41 age- and sex-matched healthy controls at a large urban public hospital in South Africa from 2 January 2017 to 31 August 2017. The study was approved by the University of the Witwatersrand Human Research Ethics Committee (HREC), study number M160614. An interviewer-administered questionnaire was used to obtain information on participants' sociodemographic and cardiovascular risk factors including age waist-hip ratio (WHR), body mass index (BMI). Measurements and calculation for WHR, BMI, body surface area, blood pressure and serum albumin and lipogram levels were performed as described in the Methods section, Chapter 3. Inflammatory markers (hsCRP, pentraxin-3, TNF-α and EN-RAGE) were determined by Magnetic Luminex Assay, Human Premixed Multi-Analyte Kit by R&D Systems using BioPlex 200 Systems (Bio-Rad Laboratories Inc., USA). High sensitivity C-reactive protein

levels were determined using HycultBiotech human HK369 Elisa kit. (HycultBiotech, Netherlands). Estimated GFR calculation, echocardiographic procedure and CIMT measurements were performed as decribed in Methods section, Chapter 3.

Data analysis

Stata version13.1 (Stata Corp, USA), was used for statistical analysis. Categorical variables were expressed as frequencies and percentages and tested using chi-squared test. Test of normality (Shapiro-wilk) was performed on all continuous variables and data was presented as median and interquartile ranges (IQR). Comparison of means was performed between the kidney disease groups (HD, PD, stage 3 CKD, KTRs) and controls using Wilcoxon rank-sum test.

Spearman's rank correlation was used to determine the relationship between pentraxin-3 and cardiovascular risk factors. Receiver operating characteristics (ROC) was performed to determine the utility of the inflammatory markers in predicting AsVD.

Multivariate binary regression analysis was performed to assess the determinants of high pentraxin-3 levels and also to determine the predictors of AsVD among kidney disease patients. Post-hoc test was done after each logistic regression to ensure good model fit. Test of significance was taken as p-value < 0.05.

Results

The median age was 41 years (IQR = 36.0 – 50.5) among stage 3 CKD patients, 39.5 years (IQR = 35.0 – 46.5) among PD patients, 40.5 years (IQR = 36.0 – 49.0) among HD patients, 39 years (IQR = 30.0 – 52.0) among KTRs and 41.0 years (29.0 – 48.0) among controls; no significant difference in age was observed between the groups. There was an increased prevalence of hypertension in CKD, PD, HD and KTRs patients when compared with controls, $p < 0.001$, Table 1. Serum levels of hsCRP were significantly increased in stage 3 CKD, PD

and HD patients compared to controls, Table 1. Significantly higher levels of pentraxin-3 were seen among stage 3 CKD, PD and HD patients and KTRs compared to controls. Significantly higher levels of TNF-α were seen among PD patients compared to controls. Serum levels of TNF-α were increased in other kidney disease groups (CKD stage 3, HD and KTRs) but were not statistically significant. In addition significantly higher levels of EN-RAGE were seen in KTRs compared to controls, however, levels of EN-RAGE were higher among stage 3 CKD and PD patients but it was not statistically significant. Of all the inflammatory biomarkers assessed, only pentraxin-3 levels were significantly elevated in all the four kidney disease groups compared to controls.

Table 1. Sociodemographic and clinical characteristics of study the population

Parameter	Control (n = 41)	PD (n = 40)	p-value	HD (n = 40)	p-value	CKD STAGE 3 (n = 40)	p-value	KTRs (n = 41)	p-value
Age									
< 40 years	20 (48.8)	20 (50.0)	0.913#	19 (47.5)	0.908#	16 (40.0)	0.427#	29 (70.7)	0.618#
≥ 40 years	21 (52.2)	20 (50.0)		21 (52.5)		24 (60.0)		12 (29.3)	
Gender									
Female	23 (56.1)	19 (47.5)	0.439#	18 (45.0)	0.318#	20 (50.0)	0.582#	20 (48.8)	0.507#
male	18 (43.9)	21 (52.5)		22 (55.0)		20 (50.0)		21 (51.2)	
BMI									
< 25 Kg/m^2	14 (34.2)	19 (47.5)	0.221#	19 (47.5)	0.221#	6 (15.0)	0.046#	18 (43.9)	0.365#
≥ 25 Kg/m^2	27 (65.8)	21 (52.5)		21 (52.5)		34 (85.0)		23 (56.1)	
Hypertension									
Absent	34 (82.9)	12 (30)	<0.001#	17 (17.5)	<0.001#	15 (37.5)	<0.001#	23 (56.1)	0.001#
Present	7 (17.1)	28 (70)		33 (82.5)		25 (62.5)		18 (43.9)	
Creatinine (µmol/l)	80 (63 – 89)					124 (106 – 166)	<0.001*	123 (91 – 152)	<0.001*
Albumin (mmol/l)	44.0 (42 – 45)	35.5 (33.0 – 40.0)	<0.001*	38.5 (35.0 – 40.0)	<0.001*	41.5 (38.5 – 44.0)	0.014*	43.0 (42.0 – 48.0)	0.505*
Calcium (mmol/l)	2.33 (2.28 – 2.45)	2.29 (2.12 – 2.40)	0.025*	2.29 (2.15 – 2.40	0.088*	2.34 (2.27 – 2.44)	0.928*	2.44 (2.34 – 2.59)	0.004*
Phosphate (mmol/l)	1.0 (0.9 – 1.1)	1.6 (1.2 – 1.9)	<0.001*	1.2 (0.8 – 1.4)	0.073*	1.1 (0.9 – 1.2)	0.052*	0.97 (0.85 – 1.07)	0.516*
$CaXPO_4$ (mg^2/dl^2)	29.5 (26.7 – 32.5)	40.1 (32.1 – 53.7)	<0.001*	35.4 (22.4 – 41.0)	0.228*	31.4 (26.5 – 37.5)	0.132*	29.8 (22.9 – 35.9)	0.756*
TC (mmol/l)	4.3 (3.7 – 4.7)	5.2 (4.2 – 6.1)	0.001*	3.4 (3.0 – 3.9)	<0.001*	4.4 (4.1 – 5.1)	0.189*	4.46 (3.93 – 4.93)	0.330*

TG (mmol/l)	1.3 (0.8 – 1.5)	1.3 (1.0 – 2.0)	0.364*	0.9 (0.6 – 1.2)	0.018*	1.3 (1.0 – 1.9)	0.511*	1.3 (1.1 – 1.9)	0.399*
LDL-C (mmol/l)	2.6 (2.1 – 3.2)	3.0 (2.4 – 4.1)	0.062*	1.8 (1.4 – 2.3)	<0.001*	2.7 (1.9 – 3.1)	0.660*	2.6 (2.2 – 3.1)	0.777*
HDL-C (mmol/l)	1.2 (1.0 – 1.5)	1.2 (0.9 – 1.4)	0.872*	1.0 (0.9 – 1.3)	0.051*	1.3 (1.1 – 1.5)	0.296*	1.3 (1.1 – 1.5)	0.467*
hsCRP (mg/l)	1.5 (0.6 – 4.5)	7.4(2.7 – 13.0)	<0.001*	6.7 (3.0 – 12.3)	<0.001*	4.4 (1.5 – 11.7)	0.013*	2.5 (1.0 – 6.4)	0.239*
Pentraxin-3 (ng/ml)	1.1 (0.4 – 1.9)	3.4 (1.6 – 5.7)	<0.001*	1.9 (1.1 – 3.4)	0.009*	2.8 (0.7 – 4.1)	0.009*	1.6 (0.9 – 2.7)	0.023*
TNF-alpha (pg/ml)	2.5 (0.5 – 26.9)	29.1 (7.0 – 78.3)	<0.001*	4.0 (0.8 – 20.7)	0.576*	4.0 (2.1 – 26.7)	0.309*	14.1 (3.3 – 26.3)	0.083*
EN-RAGE (ng/ml)	36.5 (16.7 – 88.9)	61 (28.8 – 110.6)	0.139*	34.6 (17.0 – 66.2)	0.491*	42.5 (30.0 – 70.9)	0.590*	91.1 (33.2 – 260.8)	0.004*
CIMT (mm)	0.49 (0.46 – 0.52)	0.63 (0.55 – 0.71)	<0.001*	0.55 (0.50 – 0.62)	<0.001*	0.55 (0.48 – 0.61)	<0.001*	0.55 (0.48 – 0.63)	0.001

[#] frequency (percentage), Chi-squared test; * median (IQR), Wilcoxon rank-sum test; ESKD- end-stage kidney disease; BMI – body mass index; eGFR – estimated glomerular filtration rate; TC – total cholesterol; LDL-C – low density lipoprotein; TG – triglyceride; HDL-C – high density lipoprotein; TNF-α – tumour necrosis factor-alpha; EN-RAGE – ligand for receptor of advance glycation end product; CIMT – carotid intima media thickness

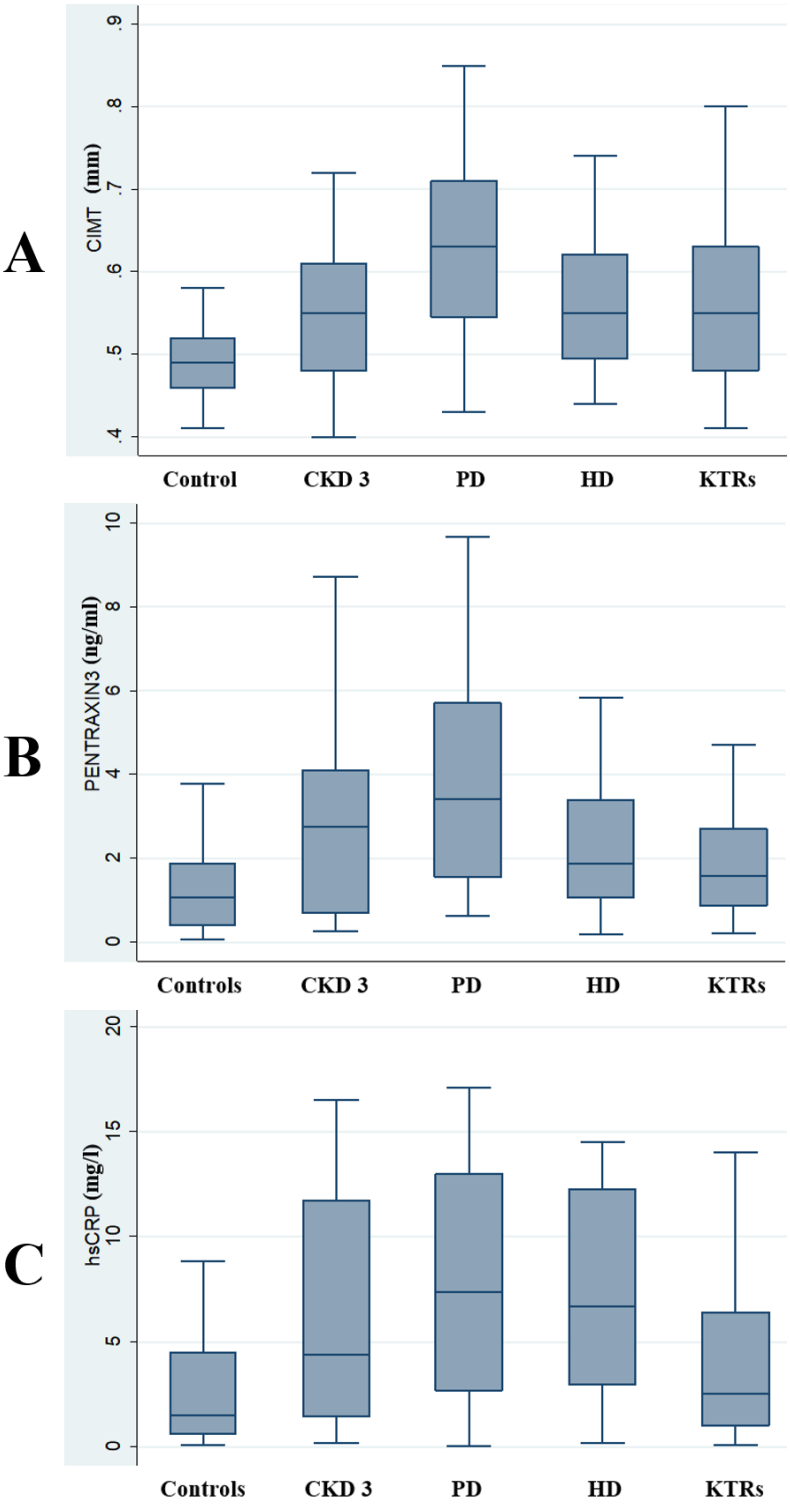

Figure 6.1 Carotid intima media thickness, pentraxin-3 and highly sensitive CRP levels in the study population

CIMT – Carotid intima media thickness, **(A)**; pentraxin-3 – **(B)**; hsCRP - Highly sensitive CRP, **(C)**; CKD – chronic kidney disease, KDOQI stage 3; PD – peritoneal dialysis; HD – haemodialysis; KTRs – kidney transplant recipients

Table 2. Correlates atherosclerotic vascular disease in kidney disease patients

Risk factors	AsVD present (n = 85)	AsVD Absent (n = 76)	p-value
Age (years)	44.0 (38.0 – 52.0)	37.0 (27.0 – 43.0)	0.002*
BMI (Kg/m^2)	26.7 (23.4 – 31.2)	26.2 (21.9 – 29.7)	0.196
WHR	0.92 (0.88 – 0.96)	0.89 (0.85 – 0.93)	0.003*
SBP (mmHg)	145.0 (130.0 – 160.0)	145.5 (126.5 – 158)	0.428
TC (mmol/l)	4.3 (3.5 – 5.1)	4.2 (3.5 – 5.1)	0.806
TG (mmol/l)	1.3 (0.8 – 1.7)	1.2 (0.9 – 1.8)	0.792
LDL-C (mmol/l)	2.4 (1.9 – 3.1)	2.4 (1.8 – 3.0)	0.466
HDL-C (mmol/l)	1.1 (0.9 – 1.4)	1.3 (1.1 – 1.4)	0.010*
Urea (mmol/l)	14.0 (7.2 – 22.0)	10.9 (7.4 – 18.0)	0.192
SCr (µmol/l)	456 (124 – 1018.0)	210.5 (126 – 635.0)	0.116
Calcium (mmol/l)	2.3 (2.2 – 2.4)	2.4 (2.2 – 2.5)	0.309
Phosphate (mmol/l)	1.2 (0.9 – 1.4)	1.1 (0.9 – 1.3)	0.218
$CaXPO_4$ (mg^2dl^2)	35.5 (26.5 – 41.4)	31.8 (26.9 – 38.4)	0.356
hsCRP (mg/l)	5.7 (1.5 – 11.8)	4.2 (1.5 12.4)	0.473
Pentraxin-3 (ng/ml)	2.3 (0.9 – 3.6)	2.4 (1.1 – 4.2)	0.767
TNF-α (pg/ml)	9.1 (3.3 – 33.1)	7.6 (2.0 – 33.6)	0.457
EN-RAGE (ng/ml)	46.2 (25.9 – 99.4)	54.7 (28.6 – 100.7)	0.480

*statistically significant, $p < 0.05$; WHR – waist-to-hip ratio; SBP – systolic blood pressure; TC – total cholesterol; LDL-C – low density lipoprotein; TG – triglyceride; HDL-C – high density lipoprotein; SCr – serum creatinine; hsCRP – high sensitivity C-reactive protein; TNF-α – tumour necrosis factor-alpha; EN-RAGE – ligand of receptor for advanced glycation end products; $CaXPO_4$ – calcium-phosphate product

High density lipoprotein levels were significantly lower among the kidney disease patients who had AsVD compared to those without it, [1.1mmol/l (0.9 – 1.4) vs 1.3mmol/l (1.1 – 1.4), $p = 0.010$]. None of the other traditional lipid profile parameters (TC, TG or LDL-C) was associated with AsVD; nor was there an association with inflammatory markers.

Table 3. Correlation between pentraxin-3 and cardiovascular risk factors among stage 3 CKD and dialysis patients and Kidney transplant recipients

Parameter	Dialysis patients (n = 80)		Stage 3 CKD (n=40)		KTRs (n = 41)	
	Rho	p-value	Rho	p-value	rho	p-value
Age (years)	-0.113	0.317	-0.368	0.020*	0.128	0.427
BMI (Kg/m^2)	-0.037	0.746	0.193	0.234	-0.073	0.651
WHR	0.142	0.208	0.014	0.931	0.099	0.537
Creatinine (µmol/l)			-0.218	0.176	-0.150	0.351
Albumin	-0.316	0.004*	-0.016	0.920	0.208	0.193
TC (mmol/l)	0.226	0.044*	0.093	0.570	0.271	0.087
LDL-C (mmol/l)	0.255	0.023*	0.199	0.217	0.283	0.073
TG (mmol/l)	0.143	0.207	0.108	0.509	0.146	0.363
HDL-C (mmol/l)	0.010	0.127	-0.045	0.782	-0.001	0.997
TNF-α (pg/ml)	0.404	<0.001*	0.340	0.032*	0.289	0.067
EN-RAGE (ng/ml)	0.260	0.020*	0.117	0.471	0.456	0.003*
hsCRP(mg/l)	0.173	0.125	-0.052	0.750	0.070	0.673
EF (%)	-0.152	0.178	0.204	0.206	0.313	0.046*
LAD	-0.141	0.212	0.127	0.435	0.021	0.899
LVMI (g/m^2)	-0.151	0.182	0.028	0.862	-0.020	0.900
CIMT (mm)	-0.074	0.515	-0.273	0.088	0.336	0.032*

*statistically significant, $p < 0.05$; ESKD- End-stage kidney disease; BMI – body mass index; WHR – waist-to-hip ratio; eGFR – estimated glomerular filtration rate; TC – total cholesterol; LDL-C – low density lipoprotein; TG – triglyceride; HDL-C – high density lipoprotein; TNF-α – tumour necrosis factor-alpha; EN-RAGE – ligand of receptor for advanced glycation end product; EF – ejection fraction; LAD – left atrial diameter; LVMI – left ventricular mass index; CIMT – carotid intima media thickness

Table 4. Correlation between carotid intima media thickness and inflammatory markers in kidney disease patients.

Parameters	Stage 3 CKD (n = 40)		PD (n = 40)		HD (n = 40)		KTRs (n = 41)	
	r	p-value	r	p-value	r	p-value	r	p-value
HsCRP	-0.038	0.818	0.124	0.445	-0.079	0.628	0.115	0.475
Pentraxin-3	-0.273	0.088	-0.245	0.127	-0.128	0.430	0.336	0.032*
TNF-α	-0.163	0.316	-0.090	0.581	0.088	0.588	0.099	0.535
EN-RAGE	-0.076	0.640	0.086	0.596	-0.070	0.669	0.144	0.370
Albumin	-0.033	0.841	-0.095	0.560	0.060	0.714	-0.021	0.895

* - Statistically significant, $p < 0.05$; CKD – chronic kidney disease; PD – peritoneal dialysis; HD – Haemodialysis; KTRs – Kidney transplant recipients; TNF-α – Tumour necrosis factor alpha; EN-RAGE – ligand for receptor of advance glycation end product.

Pentraxin-3 correlated positively with TNF-α, EN-RAGE and negatively with serum albumin in kidney disease patients while pentraxin-3 correlated positively only with TNF-α among stage 3 CKD patients. Among the KTRs, pentraxin-3 correlated positively with EN-RAGE while correlation with TNF-α showed tendency towards significance, Table 2. Pentraxin-3 correlated positively with total cholesterol and low density lipoprotein among dialysis patients but no correlation with serum lipids was seen among stage 3 CKD and KTRs. Among KTRs, pentraxin-3 correlated with ejection fraction and CIMT, Table 3. No correlation was seen between pentraxin-3 and echocardiographic parameters among stage 3 CKD and ESKD patients. No correlation was seen between hsCRP and CIMT.

The inflammatory markers (hsCRP, pentraxin-3, TNF-α and EN-RAGE) poorly predicted AsVD with very low AUC, skewed sensitivity and specificity, Table 5.

Table 5. Receiver operating characteristics of inflammatory markers for predicting atherosclerotic vascular disease

Inflammatory markers	AUC	95 % CI	Sensitivity	Specificity
HsCRP	0.50	0.37 – 0.63	12,77	100.00
Pentraxin-3 (ng/ml)	0.44	0.31 – 0.56	14.89	93.94
TNF-α (pg/ml)	0.56	0.42 – 0.69	97.87	18.18
EN-RAGE (ng/ml)	0.49	0.36 – 0.62	10.64	96.97

AUC – Area under curve; TNF-α – tumour necrosis factor-alpha; EN-RAGE – ligand for receptor of advanced glycation end-products

Table 6. Predictors of elevated pentraxin-3 and atherosclerotic vascular disease among kidney disease patients

Parameters	Predictors of high pentraxin-3			Predictors of AsVD		
	OR	95 % CI	p-value	OR	95 % CI	p-value
Age (> 40years)	0.80	0.37 – 1.72	0.568	5.18	2.35 – 11.44	< 0.001*
Gender	1.22	0.54 – 2.76	0.631	2.45	1.08 – 5.54	0.031*
WHR	1.00	0.45 – 2.25	0.992	1.05	0.45 – 2.46	0.910
Hypertension (mmHg)	0.38	0.17 – 0.88	0.024*	1.01	0.44 – 2.32	0.982
TC (≥ 5.17 mmol/l)	3.57	1.17 – 10.97	0.026*	0.93	0.31 – 2.77	0.890
LDL-C (≥ 2.59 mmol/l)	0.65	0.27 – 1.55	0.329	1.74	0.70 – 4.35	0.235
Low HDL-C (mmol/l)	1.05	0.50 – 2.231	0.883	2.93	1.31 – 6.57	0.009*
LVH (g/m^2)	0.35	0.56 – 9.97	0.085	1.60	0.49 – 5.18	0.433
hsCRP (≥ 4.5mg/l)	1.53	0.73 – 3.20	0.261	1.27	0.60 – 2.71	0.529
TNF-α (≥ 26.87pg/ml)	3.56	1.50 – 8.44	0.004*	0.98	0.42 – 2.28	0.960
EN-RAGE (≥ 88.9ng/ml)	3.12	1.34 – 7.26	0.008*	0.92	0.39 – 2.16	0.839
Pentraxin-3 (ng/ml)				0.98	0.44 – 2.18	0.954

* - statistically significant, $p < 0.05$; WHR – waist-hip ratio - > 0.85 in female and > 0.90 in male; AsVD – atherosclerotic vascular disease; TC – total cholesterol; LDL-C – low density lipoprotein; Low HDL-C = < 1.03 in male and < 1.29mmol/l in female; hsCRP – highly sensitive C-reactive protein; TNF-α – tumour necrosis factor-alpha, EN-RAGE – ligand for receptor of advance glycation end-product; LVH – Left ventricular hypertrophy.

Multivariate binary logistic regression showed that presence of hypertension, high total cholesterol (> 5.17mmol/l), TNF-α (≥ 26.87pg/ml) and EN-RAGE (≥ 88.9ng/ml) were independent predictors of high pentraxin-3 levels after adjusting for age, gender, WHR, LDL-C, HDL-C, hsCRP and LVH. High total cholesterol, TNF-α and EN-RAGE conferred a 3-fold risk for high pentraxin-3 levels, Table 6. Similarly, age > 40 years, male gender and low HDL-C independently predicted AsVD after adjusting for WHR, TC, LDL-C, inflammatory markers and LVH. Low HDL-C conferred a 2.9-fold risk of AsVD among patients with kidney disease.

Discussion

Inflammatory markers and renal disease

Inflammation is common in ESKD and has been shown to be worsened by the initiation of dialysis.[23] Plasma levels of pentraxin-3, hs-CRP and ischaemia-modified albumin were found to be significantly increased among HD and PD patients compared to healthy controls.[2] In our study, we found significantly higher levels of pentraxin-3 among stage 3 CKD, PD, HD patients, and KTRs compared to controls. The levels of tumour necrosis factor alpha (TNF-α) and the ligand of the receptor for advanced glycation end-products (EN-RAGE) were higher in all the kidney disease groups compared to controls but were significantly higher in PD. We found a significantly elevated hsCRP levels in stage 3 CKD patients, and PD and HD patients compared to controls. Increased levels of hsCRP were also seen in KTRs compared to controls but this was not significant. These findings suggest that chronic inflammation in kidney disease starts long before CKD patients became dialysis-requiring, and persists with ESKD and in the post-transplant period. Similar results were reported in a cross-sectional study in HD and PD patients.[2,14, 24] Plasma levels of pentraxin-3 were shown to be significantly higher among HD patients when compared with healthy controls; pentraxin-3 levels increased rapidly after a single session of HD.[14]

In our study, when levels of pentraxin-3 were compared between PD and HD patients, we found significantly higher levels of pentraxin-3 among PD patients. This contradicts findings from a previous study which reported higher levels of pentraxin-3 among HD compared to PD patients.[2] In comparison to PD, the lower levels of inflammatory markers found among HD patients in our study could be as a result of recurrent exposure of the HD patients to the HD procedure which is probably more effective in removing toxic molecules using high-flux biocompatible membranes and strict adherence to the dialysis dose delivered thrice per week.

Our findings of very high levels of inflammatory markers in PD which signifies a higher level of inflammation among our PD patients can be explained by some PD-related factors; firstly, insertion of peritoneal catheter and filling of peritoneum with PD fluid triggers inflammatory response in the peritoneum.[25] A study demonstrated an increase in the levels of inflammatory markers after initiation of PD; persistent rise in levels of inflammatory markers were seen with increasing duration of exposure to PD.[25] Secondly, persistent exposure of peritoneal mesothelial cells to glucose-based peritoneal dialysis fluid triggers an inflammatory response which can self-persist by continuous activation of inflammatory markers.[26,27] Similarly, formation of glucose degradation product (GDP) which occurs during heat sterilization of glucose-based PD fluids has been implicated in the morphological changes seen in the peritoneum of patients treated with glucose-based PD fluids.[28] Therefore, the combination of low P^H (5.2), high lactate levels (40 mmol/l), glucose-induced high osmolarity (395 mOsm) which can precipitate GDP in our glucose-based PD fluid can explain the high levels of inflammation among our PD patients. In addition, a previous history of peritonitis among the PD patients can worsen an underlying inflammatory response in PD patients. Peritonitis triggers an inflammatory response which leads to release of inflammatory cytokines from inflammatory cells to the site of infection, where pentraxin-3 activates the complement system via the classical pathway[29] and enhances phagocytosis of apoptotic cells by dendritic cells and

macrophages.[30] A previous study found the levels of pentraxin-3 in peritoneal effluent to be higher among patients with a history of peritonitis (> 6 months to the time of patient recruitment) and prolonged PD duration (> 8 years) compared to patients without such history.[31]

We found that pentraxin-3 correlated positively with inflammatory markers (TNF-α and EN-RAGE) while negative correlation was seen between pentraxin-3 and serum albumin. This is consistent with findings from a previous study[2] whereby Pentraxin-3 correlated positively with neutrophil-to-lymphocyte ratio and hsCRP and negatively with serum albumin levels.[2] In our study, significantly higher levels of TNF-α were seen among PD patients compared to controls, and in all kidney disease patients when compared to controls. Our finding is consistent with those of a previous study that assessed inflammatory markers that are monocyte activators in pre-dialysis CKD, PD and HD patients. These monocyte-activating inflammatory biomarkers, including TNF-α, were significantly increased among dialysis patients.[32] In our study, we found higher levels of EN-RAGE among PD patients when compared with controls, although this was not statistically significant; this is comparable to the findings in a previous study among long-term HD patient.[33] We found significantly higher levels of inflammatory biomarkers among PD patients compared to haemodialysis patients, reflecting higher level of inflammation among PD patients compared to HD patients.

Pentraxin-3, renal function and AsVD

When stage 3 CKD, ESKD and KTRs were combined, we found a negative correlation between pentraxin-3 levels and eGFR, ($r = -0.171$, $p = 0.030$); this is similar to findings of previous studies which demonstrated that increase in pentraxin-3 levels was related to a decrease in eGFR in stage 3 and 4 CKD and ESKD patients.[2,33] Increased expression of pentraxin-3 in mesangial cells, proximal tubular epithelial cells and in renal fibroblasts has been previously

demonstrated; this was also observed to be augmented in the presence of pro-inflammatory cytokines and was thought to have a role in renal inflammation.[15] Pentraxin-3 correlated with CIMT among KTRs, similar to findings reported in previous studies.[13,33] Earlier studies on the role of pentraxin-3 in CVD were largely without consensus; some authors believed pentraxin-3 was mainly pro-atherogenic,[13] while the anti-atherogenic and cardioprotective roles of pentraxin-3 have been championed by other reseachers.[34,35] However, the cardioprotective role seems to have gained wider acceptance. A study compared cardiac lesions in pentraxin-3-deficient mice and the wild type mice in a model of acute myocardial infarction induced by ligation of the coronary artery.[36] The study found an increase in the circulating levels and mRNA expression of pentraxin-3, with more severe heart lesions among pentraxin-3- deficient mice. The cardiac damage was associated with increased polymorphonuclear cell infiltration, increased number of apoptotic cardiomyocytes, reduced number of capillaries and no-reflow area.[35] Similar study assessed the role of pentraxin-3 in promoting vascular inflammation and atherosclerosis using apolipoprotein E-knockout mice and double knockout mice lacking both apolipoprotein E and pentraxin-3;[37] vascular lesions were significantly increased in the double knockout mice and in those who were heterozygous for pentraxin-3 compared with apolipoprotein E-knockout mice. An increased inflammatory profile in the vessel wall among pentraxin-3-deficient mice was reported;[37] these results favour an atheroprotective role for pentraxin-3.

We found that advancing age (> 40 years), male gender and low HDL-C independently predicted AsVD in the combined group of kidney disease patients after adjusting for WHR, hypertension, TC, LDL-C, LVH and inflammation. Increasing age has been identified as a predictor of vascular injury due to alteration in the levels of vasoactive nitric oxide.[38] A study found increased large HDL-C sub-fractions among ESKD patients compared to the finding of small HDL-C sub-fractions among healthy individuals; the study concluded that this difference

seen in the HDL-C sub-fractions may be responsible for the higher rates of atherosclerosis in CKD patients.[39] Current concepts on the alteration in HDL-C in CKD patients, with consequent CVD, goes beyond merely a reduction in quantity. An emphasis has now been placed on the reduction in the quantity of HDL-C in CKD.[40]

Determinants of elevated pentraxin-3

We found that hypertension, elevated total cholesterol, TNF-α and EN-RAGE were independent predictors of high pentraxin-3 levels when all kidney disease patients were combined. The presence of elevated total cholesterol, TNF-α and EN-RAGE conferred a > 3-fold risk of higher pentraxin-3 levels; this result aligns with the literature.[11] Tumour necrosis factor-α is one of the main pro-inflammatory activators of pentraxin-3 production.[11] This positive relationship, seen in our study, has also been documented in previous studies, as discussed above. Similarly, EN-RAGE has been demonstrated as an amplifier of immune and inflammatory responses by stimulating the release of pro-inflammatory cytokines (IL-1 and TNF-α), which are the activators of pentraxin-3.[20]

In conclusion, levels of inflammatory markers were significantly increased in CKD stage 3 and ESKD dialysis patients and KTRs. Serum levels of pentraxin-3 correlated positively with TNF-α and EN-RAGE and negatively with renal function and serum albumin among kidney disease patients. There was a weak correlation between pentraxin-3 and CIMT among KTRs. Determinants of higher levels of pentraxin-3 among the kidney disease patients were hypertension, elevated total cholesterol, TNF-α and EN-RAGE levels while the predictors of AsVD were age (> 40 years), male gender and low HDL-C levels.

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Ethics Approval and consent to participate in the study

This study was approved by the Human Research and Ethics Committee (HREC) of the

University of the Witwatersrand, Johannesburg, South Africa; clearance certificate number M160614. All participants' gave written informed consent before enrolment

Availability of data or material

The dataset used in the analysis is available with the corresponding author and will be released on request.

Conflict of Interest

No competing interests in relation to this study.

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Authors' Contributions

S.O. Oguntola, R. Duarte and S. Naicker were involved in study design. S.O. Oguntola, M.O. Hassan, A. Vachiat, P. Manga, G. Paget and S. Naicker participated in data collection. K Moodley, T. Dix-Peek, C. Dickens, S.O. Oguntola and R. Duarte were involved in laboratory analysis of samples. Data analysis was done by S.O. Oguntola. First draft of the manuscript was prepared by S.O. Oguntola and all authors reviewed the manuscript.

References

1. Amira OC, Naicker S, Manga P, Sliwa K, Mia A, Raal F, et al. Adiponectin and atherosclerosis risk factors in African haemodialysis patients: A population at low risk for atherosclerotic cardiovascular disease. Hemodialysis Int. 2012; 16(1): 59-68
2. Turkmen K, Tonbul HZ, Toker A, Gaipov A, Erdur FM, Cicekler H, et al. The relationship between oxidative stress, inflammation and atherosclerosis in renal transplant and end-stage renal disease patients. Renal Failure. 2012; 34(10): 1229-1237
3. Okada M, Kitahara M, Kishimoto S, Matsuda T, Hirano T, Kishimoto T. IL-6/BSF-2 functions as a killer helper factor in the in vitro induction of cytotoxic T cells. J Immunol 1988; 141(5): 1543-1549
4. Stakie R, Ostrowski SR, Jauffred S, Febbraio M, Pedersen BK. Exercise and IL-6 infusion inhibit endotoxin-induced TNF-alpha production in humans. The FASEB journal 2003; 17: 884-886
5. Niessner A, Kaun C, Zorn G, et al. Polymorphic membrane protein (PMP) 20 and PMP 21 of Chlamydia pneumoniae induce proinflammatory mediators in human endothelial cells in vitro by activation of the nuclear factor-kappaB pathway. J Infect Dis 2003; 188: 108-113
6. Sharma R, Bolger AP, Li W, Davlouros PA, Volk HD, Poole-wilson PA, et al. Elevated circulating levels of inflammatory cytokines and bacterial endotoxin in adults with congenital heart disease. Am J Cardiol. 2003; 92(2): 188-193
7. Descamps-Latscha B, Herbelin A, Nguyen AT, Roux-Lombard P, Zingraff J, Moynot A, et al. Balance between IL-1 beta, TNF-alpha and their specific inhibitors in chronic renal failure and maintenance dialysis. Relationships with activation markers of T-cells, B cells and monocytes. J Immunol. 1995; 154(2): 882-892

8. Tripepi G, Mallamaci F, Zoccali C. Inflammation markers, Adhesion molecules and all-cause and cardiovascular mortality in patients with ESRD: Searching for the best risk marker by multivariate modelling. J Am Soc Nephrol 2005; 16: S83-S88
9. Panichi V, Maggiore U, Taccola D, Migliori M, Rizza GM, Consani C, et al. Interleukin-6 is a stronger predictor of total and cardiovascular mortality than C-reactive protein in haemodialysis patients. Nephrol Dial Transplant 2004; 19: 1154-1160
10. Alle VV, Bottazzi B, Peri G, Golay J, Introna M, Mantovani A. Inducible expression of PTX3, a new member of the pentraxin family, in human mononuclear phagocytes. Blood 1994; 84: 3483-3493
11. Mantovani A, Garlanda C, Bottazzi B, Peri G, Doni A, Martinez de la Torre Y, et al. The long Pentraxin in vascular pathology. Vascular Pharmacol 2006; 45: 326-330
12. Savchenko A, Imamura M, Ohashi R, Jiang S, Kawasaki T, Hasegawa G, et al. Expression of pentraxin-3 (PTX3) in human atherosclerotic lesion. J Pathol. 2008; 215(1): 48-55
13. Jenny NS, Blumenthal RS, Kronmal RA, Rotter JI, Siscovick DS, Psaty BM. Associations of pentraxin-3 with cardiuovascular disease: The Multi-Ethnic study of Atherosclerosis. J Thromb Haemost 2014; 12: 999-1005
14. Xu Y, Ding X, Zou J, Liu Z, Jiang Z, Xu S, et al. Plasma Pentraxin-3 is associated with cardiovascular disease in haemodialysis patients. Ren Fail, 2011; 33(10): 998-1004
15. Nauta AJ, de Haij S, Bottazzi B, Mantovani A, Borrias MC, Aten J, et al. Human renal epithelial cells produce the long pentraxin PTX3. Kidney Int 2005; 67; 543-553
16. Bussolati B, Peri G, Salvidio G, Verzola D, Mantovani A, Camussi G. The long pentraxin PTX3 is synthesized in IgA glomerulonephritis and activates mesangial cells. J Immunol 2003; 170: 1466-1472.

17. Sjoberg B, Qureshi AR, Heimburger O, Stenvinkel P, Lind L, Larsson A, et al. Associations between levels of pentraxin-3 and incidence of chronic kidney disease in the elderly. J Intern Med 2016; 279: 173-179
18. Mori Y, Kosaki A, Kishimoto N, Kimura T, Lida K, Fukui M, et al. Increased plasma S100A12 (EN-RAGE) levels in haemodialysis patients with atherosclerosis. Am J Nephrol 2009; 9: 18-24
19. Mahajan N, Malik N, Bahl A, Dhawan V. Receptor for advanced glycation end products (RAGE) and its inflammatory ligand EN-RAGE in non-diabetic subjects with premature coronary artery disease. Atherosclerosis 2009; 207: 597-602
20. Schmidt AM, Yan SD, Yan SF, Stern DM. The multi-ligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. J Clin Invest 2001; 108: 949-955
21. Nakashima A, Carrero JJ, Qureshi AR, Miyamoto T, Anderstam B, Barany P, et al. Effect of circulating soluble receptor for advanced glycation end products (sRAGE) and the proinflammatory rage ligand (EN-RAGE, S100A12) on mortality in haemodialysis patients. Clin J Am Soc Nephrol 2010; 5: 213-219
22. Yayar O, Eser B, Buyukbakkal M, Erdogan B, Ercan Z, Merhametsiz O, et al. Do elevated plasma S100A12 levels predict atherosclerosis in peritoneal dialysis? Ren Fail, 2015; 37(5): 845-850
23. Kenneth Y, Dogra G, Boudville NC, Lim WH. Increased inflammatory response in association with the initiation of haemodialysis compared with peritoneal dialysis in a prospective study of end-stage kidney disease patients. Perit Dial Int 2018; 38(1): 18-23

24. Palomar-Fontanet R, Lavin-Gomez BA, Quintanar-Lartundo JA, Garcia-Unzueta MT, Gago-Fraile M, Torrealba-Rodriguez MI, et al. Markers of inflammation before and during peritoneal dialysis.
25. Farhat K, Van Ittersum FJ, Ter Wee PM, Paauw NJ, Beelen RHJ, Douma CE. Initiation of peritoneal dialysis in the first weeks after catheter insertion: A comparison of a neutral P^H, low GDP PD fluid and a conventional PD fluid. Clin Nephrol 2018; 89(2): 75-82
26. Li XY, Wu J, Luo D, Chen WX, Zhu GL, Zhang YX, et al. Effect of high glucose-based peritoneal dialysis fluid on NLRP3-IL-1β in peritoneal mesothelial cells. Beijing Da Xue Xue Bao Yi Xue Ban 2017; 49(6): 954-960
27. Chu Y, Wang Y, Zheng Z, Lin Y, He R, Liu J, et al. Pro-inflammatory effect of high glucose concentrations on HMrSV5 cells via the autocrine effects of HMGB1. Front. Physiol. 2017; 8:762. Doi: 10.3389/fphys.2017.00762
28. Johnson DW, Clarke M, Wilson V, Woods F, Brown FG. Rationale and design of the balANZ trial. A randomised controlled trial of low GDP, neutral P^H versus standard peritoneal dialysis solution for the preservation of residual renal function. BMC Nephrol. 2010; 11:25 doi: 10.1186/1471.2369-11-25
29. Nauta AJ, Bottazzi B, Mantovani A, et al. Biochemical and functional characterization of the interaction between pentraxin-3 and C1q. Eur J Immunol 2003; 33: 465-473
30. Jaillon S, Jeannin P, Hamon Y, Fremaux I, Doni A, Bottazzi B, et al. Endogenous pentraxin-3 translocates at the membrane of late apoptotic human neutrophils and is involved in their engulfment by macrophages. Cell Death Differ 2009; 16: 465-474
31. Kanda R, Hamada C, Kaneko K, Nakano T, Wakabayashi K, Lo H, et al. Pentraxin-3 as a new biomarker of peritoneal injury in peritoneal dialysis patients. J Artificial Organs 2013; 16(1): 66-73

32. Liang M, Wang L, Hou FF, Liu J, Liu ZQ, Tian JW. Inflammatory status in patients with end-stage renal disease: Role of monocyte activation. Di Yi Jun Yi Da Xue Xue Bao. 2003; 23(8): 781-784

33. Tong M, Carrero JJ, Qureshi AR, Anderstam B, Heimburger O, Barany P, et al. Plasma pentraxin-3 in patients with chronic kidney disease: Associations with renal function, protein-energy wasting, cardiovascular disease and mortality. Clin J Am Soc Nephrol 2007; 2:889-897

34. Camozzi M, Zacchigna S, Rusnati M, Coltrini D, Ramirez-Corea G, Botazzi B. Pentraxin-3 inhibits fibroblast growth factor 2-dependent activation of smooth muscle cells in-vitro and neointima formation in-vivo. Arterioscler Thromb Vasc Biol. 2005; 25: 1837-1842.

35. Deban LRusso RC, Sironi M, Moalli F, Scanziani M, Zambelli V, Cuccovillo I. Regulation of leukocyte recruitment by the long pentraxin PTX3. Nat Immunol 2010; 11: 328-334

36. Salio M, Chimenti S, De Angelis N, Molia F, Maina V, Nebuloni M, et al. Cardioprotective function of the long pentraxin PTX3 in Acute myocardial infarction. Circulation. 2008; 117: 1055-1064

37. Norata GD, Marchesi P, Pulakazhi venu VK, Pasqualini F, Anselmo A, Moalli F, et al. Deficiency of the long pentraxin PTX3 promotes vascular inflammation and atherosclerosis. Circulation. 2009; 120: 699-708

38. Lyons D, Roy S, Patel M, Benjamin N, Swift CG. Impaired nitric oxide-mediated vasodilatation and total body nitric oxide production in healthy old age. Clinical Science. 1997; 93: 519-525.

39. Gluba-Brzozka A, Franczyk B, Banach M, Rysz-Gorzynska M. Do HDL-C and LDL-C subfractions play a role in atherosclerosis in end-stage renal disease (ESRD) patients? Int Urol Nephrol. 2017; 49(1): 155-164
40. Kronenberg F. High density lipoprotein in chronic kidney diseases- the Devil is in the Detail. J Am Soc Nephrol. 2018; doi:10.1681/ASN.2017070798

CHAPTER 7

The utility and relationship of lipoprotein markers to atherosclerotic vascular disease in black CKD and kidney transplant recipients

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Abstract

Background

Dyslipidaemia is a well-recognized risk factor for cardiovascular disease (CVD) in chronic kidney disease (CKD), to a greater degree in end-stage kidney disease (ESKD) and kidney transplant recipients (KTRs). The widespread use of statins in kidney disease patients may alter the relationship between the traditional lipid profile and atherosclerotic vascular disease (AsVD). We evaluated the relationship of lipoprotein biomarkers to AsVD among stage 3 CKD, end stage kidney disease (ESKD) patients on peritoneal dialysis (PD) and haemodialysis (HD) and KTRs.

Methods

This was a cross-sectional study of 40 adult (18-65 years) non-diabetic stage 3 CKD patients, 40 PD and 40 HD patients, 41 KTRs and 41 age- and sex-matched healthy controls. An interviewer-administered questionnaire was used to obtain information on participants' sociodemographic and cardiovascular risk factors. Anthropometric parameters were measured. Blood samples were obtained and serum was analysed for creatinine, albumin, lipid profile, lipoprotein markers including lipoprotein(a) [Lp (a)], Lipoprotein-associated phospholipase A2 (Lp-PLA2) and apolipoprotein A1 (APO A1) and inflammatory markers [hsCRP, pentraxin-3, tumour necrosis factor-alpha (TNF-α), ligand for advanced glycation end-products

(EN-RAGE)]. Echocardiography was performed on all patients and carotid intima media thickness (CIMT) was assessed in both right and left carotid arteries at 1cm proximal to the carotid bulb.

Results

The levels of Lp (a) were increased in all kidney disease groups and significantly higher levels of Lp (a) were seen in PD, HD and the combined kidney disease group compared with controls. Lp (a) levels in CKD stage 3 showed tendency towards significance when compared with controls. The levels of Lp-PLA2 were increased in all kidney disease groups and significantly higher levels of Lp-PLA2 were seen in PD patients compared to controls, [158.4ng/ml (77.3 – 204.5) vs 89.8ng/ml (50.9 – 139.2), p = 0.007], a tendency towards significance was seen in HD and the combined kidney disease group when compared to controls. Comparison of ESKD dialysis patients (PD and HD) with KTRs showed significantly lower Lp-PLA2 levels in KTRs [158.4ng/ml (77.3 – 204.5) vs 92.4ng/ml (55.7 – 149.0); p=0.004] and [129.3ng/ml (71.5 – 187.6) vs 92.4ng/ml (55.7 – 149.0), p=0.048] when compared to PD and HD, respectively. Significantly lower levels of Lp (a) were seen in KTRs when compared to PD patients. Levels of APO A1 were lower in all kidney disease groups compared to controls but this was not statistically significant. On multivariate analysis, age (> 40 years), male gender, low HDL-C levels and elevated Lp (a) levels independently predicted AsVD after adjusting for BMI, WHR, TC, TG, HDL-C, LDL-C, inflammatory markers, Lp-PLA2 and APO A1. Lipoprotein (a) predicted AsVD better than other lipid markers evidenced by higher AUC. No significant difference was seen in the utility of the lipid biomarkers in predicting AsVD.

Conclusion

The levels of Lp (a) and Lp-PLA2 were increased in all the kidney disease groups with the strongest association seen among PD patients. The levels of APO A1 were lower in all kidney

disease patients. The burden of abnormal lipid biomarkers is greater among ESKD patients with the highest burden in PD patients. Kidney transplant recipients have a more favourable lipid biomarkers profile compared to dialysis patients. Lipoprotein (a) was a better surrogate marker of AsVD compared with otherlipoprotein biomarkers and lipid profile parameters among female kidney disease patients. Therapeutic effort directed at lowering Lp (a) levels may be beneficial in CKD patients.

Introduction

Despite increasing knowledge, sophistication in clinical diagnostic modalities and improvements in therapeutic options, CVD remains a prominent and feared complication among CKD patients. Dyslipidaemia has been demonstrated as an incontrovertible risk factor for CVD among the general population.[1] However, in the setting of CKD, dyslipidaemia is more profound due to characteristic metabolic alterations in lipoprotein moieties.[2] In view of the current concept of "reverse epidemiology in CKD" and the widespread use of statins, traditional lipid profile parameters including total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL-C) and high density lipoprotein (HDL-C) may have weaker associations with AsVD compared to lipoprotein biomarkers such as Lp (a), Lp-PLA2 and APO A1.

Lipoprotein (a) is a cholesteryl-ester-rich macromolecular complex secreted by the liver.[3] Lipoprotein (a) is made up of two components, apo (a) and apo B-100 in LDL-C bound together by a disulphide bond.[4] The levels of Lp (a) are not affected by age, gender or anthropometric parameters,[5,6] but levels may be lowered by moderate alcohol and saturated fat.[7,8] The atherogenic risk of LDL-C and Lp (a) was studied among patients with myocardial infarction; Lp (a) was reported to be ten times more atherogenic than LDL-C.[9] Association between elevated plasma levels of Lp (a) and premature coronary atherosclerosis was demonstrated in

a cross-sectional study.[10] Similarly, serum levels of Lp (a) were shown to correlate well with the presence and severity of atherosclerotic vascular lesions on coronary angiography.[11] These clinical studies all showed the strong relationship between AsVD and levels of Lp (a). Apart from its association with the presence of AsVD, the propagation of atherosclerotic plaque has also been shown to be influenced by Lp (a) levels.[12] Experimental studies have also demonstrated the involvement of Lp (a) in the initiation and formation of atherosclerotic plaques.[13-15]

Another lipoprotein biomarker of importance is Lp-PLA2, also known as platelet activating factor acetylhydrolase (PAF-AH), found to be expressed by cells critical for the formation of atherosclerotic plaques such as macrophages, T-lymphocytes and mast cells.[16] Lipoprotein phospholipase A2 circulates in blood mainly bound to LDL-C, with about 66 % bound to LDL-C and the remaining bound to HDL-C and very low density lipoprotein (VLDL-C).[17] Oxidation of LDL-C particles in the arterial wall provides the appropriate substrate for the hydrolytic action of Lp-PLA2, thus cleaving the oxidized phosphatidylcholine component of the LDL-C particle. This releases mediators such as oxidized non-esterified fatty acids, arachidonic acid and lysophosphatidylcholine (Lyso-PC) which are pro-inflammatory and pro-atherogenic.[18,19] The Atherosclerotic Risk in Community (ARIC) Study reported that Lp-PLA2 was an independent predictor of coronary heart disease among patients with low LDL-C.[20]

Among the traditional lipid profile parameters, HDL-C is unique because it is involved in reverse cholesterol transport (RCT) and also has some anti-atherogenic properties.[21,22] Apolipoprotein A1, the main protein component of HDL-C, accounting for approximately 70% of HDL-C protein, is critical in the process of RCT via the ATP-binding cassette transporter ABCA1 in macrophages.[23,24]

Because of the crucial role of lipoprotein biomarkers and inflammation in AsVD, we evaluated the relationship of lipoprotein biomarkers (Lp (a), Lp-PLA2, APO A1) and inflammation to AsVD and determined the utility of lipoprotein biomarkers in predicting AsVD

Results

There was a statistically significant difference when the levels of Lp (a) were compared between kidney disease groups (PD, HD, combined kidney disease) and controls, Table 1. Levels of Lp (a) in CKD stage 3 patients showed tendency towards significance when compared to controls. Similarly, significant difference was seen when levels of Lp-PLA2 were compared between PD and controls

Table 1. Comparison between chronic kidney disease stage 3, peritoneal dialysis, haemodialysis patients, kidney transplant recipients and controls

Parameters	**Controls (n = 41) Median (IQR)**	**CKD (n = 40) Median (IQR) p-value**	**PD (n = 40) Median (1QR) p-value**	**HD (n = 40) Median (IQR) p-value**	**KTR (n = 41) Median (IQR) p-value**	**KD (n = 161) Median (IQR) p-value**
Age (years)	41.0 (29.0 – 48.0)	41.0 (36.0 – 50.5) **0.323**	39.5 (35.0 – 46.5) **0.561**	40.5 (36.0 – 49.0) **0.564**	39.0 (30.0 – 52.0) **0.824**	40.0 (35.0 – 50.0) **0.452**
Gender						
male	18 (43.9)	20 (50.0)	21 (52.5)	22 (55.0)	20 (48.8)	83 (51.5)
female	23 (56.1)	20 (50.0)	19 (47.5)	18 (45.0)	21 (51.2)	78 (48.5)
WHR	0.85 (0.82 – 0.89)	0.92 (0.84 – 0.99) **0.002**	0.92 (0.87 – 0.96) **< 0.001**	0.90 (0.87 – 0.94) **0.002**	0.89 (0.86 – 0.94) **0.003**	0.91 (0.86 – 0.96) **< 0.001**
SBP (mmHg)	125 (119 – 132)	138 (125 – 156) **< 0.001**	147 (127 – 162) **< 0.001**	155 (138 – 171) **< 0.001**	139 (128 – 151) **< 0.001**	145 (128 – 159) **< 0.001**
TC (mmol/l)	4,27 (3.71 – 4.70)	4.40 (3.95 – 5.05) **0.326**	5.20 (4.15 – 6.05) **0.001**	3.40 (3.00 – 3.80) **< 0.001**	4.46 (3.93 – 4.93) **0.330**	4.20 (3.50 – 5.10) **0.944**
TG (mmol/l)	1.25 (0.80 – 1.53)	1.30 (1.00 – 1.85) **0.398**	1.30 (1.00 – 2.00) **0.364**	0.80 (0.65 – 1.20) **0.005**	1.31 (1.06 – 1.92) **0.399**	1.21 (0.80 – 1.70) **0.950**
LDL-C(mmol/l)	2.59 (2.08 – 3.17)	2.30 (1.85 – 2.90)	3.00 (2.35 – 4.10)	1.80 (1.40 – 2.30)	2.63 (2.15 – 3.08)	2.40 (1.80 – 3.03)

		0.416	**0.062**	**< 0.001**	**0.777**	**0.218**
HDL-C(mmol/l)	1.15 (0.98 – 1.54)	1.30 (1.10 – 1.45)	1.20 (0.90 – 1.40)	1.05 (0.90 – 1.25)	1.29 (1.05 – 1.47)	1.20 (1.00 – 1.40)
		0.400	**0.872**	**0.072**	**0.467**	**0.905**
APO A1	14.5 (12.3 – 17.3)	13.9 (11.7 – 18.1)	13.5 (10.1 – 15.4)	12.1 (10.3 – 15.6)	13.1 (11.1 – 16.5)	13.4 (10.5 – 16.3)
		0.951	**0.078**	**0.063**	**0.282**	**0.141**
Lp-PLA2	89.8 (50.9 – 139.2)	110.2 (73.9 – 153.3)	158.4 (77.3 – 204.5)	129.3 (71.5 – 187.6)	92.4 (55.7 – 149.0)	114.9 (66.7 – 171.6)
		0.225	**0.007**	**0.060**	**0.978**	**0.068**
Lp (a)(mg/dl)	40.2 (29.3 – 65.4)	65.3 (29.1 – 99.9)	123.0 (80.8 – 173.0)	55.1 (41.8 – 105.5)	55.2 (25.9 – 97.6)	69.3 (36.4 – 125.0)
		0.056	**< 0.001**	**0.019**	**0.113**	**< 0.001**

*statistically significant, ($p < 0.05$); CIMT carotid intima media thickness; CKD – chronic kidney disease; PD – peritoneal dialysis; HD – haemodialysis; KTRs – kidney transplant recipients; KD – combined kidney disease; TC – total cholesterol; TG – triglyceride; LDL-C – low density lipoprotein; HDL-C – high density lipoprotein; APO A1 – apolipoprotein A1; L p-PLA2 – lipoprotein phospholipase A2; Lp (a) – lipoprotein(a)

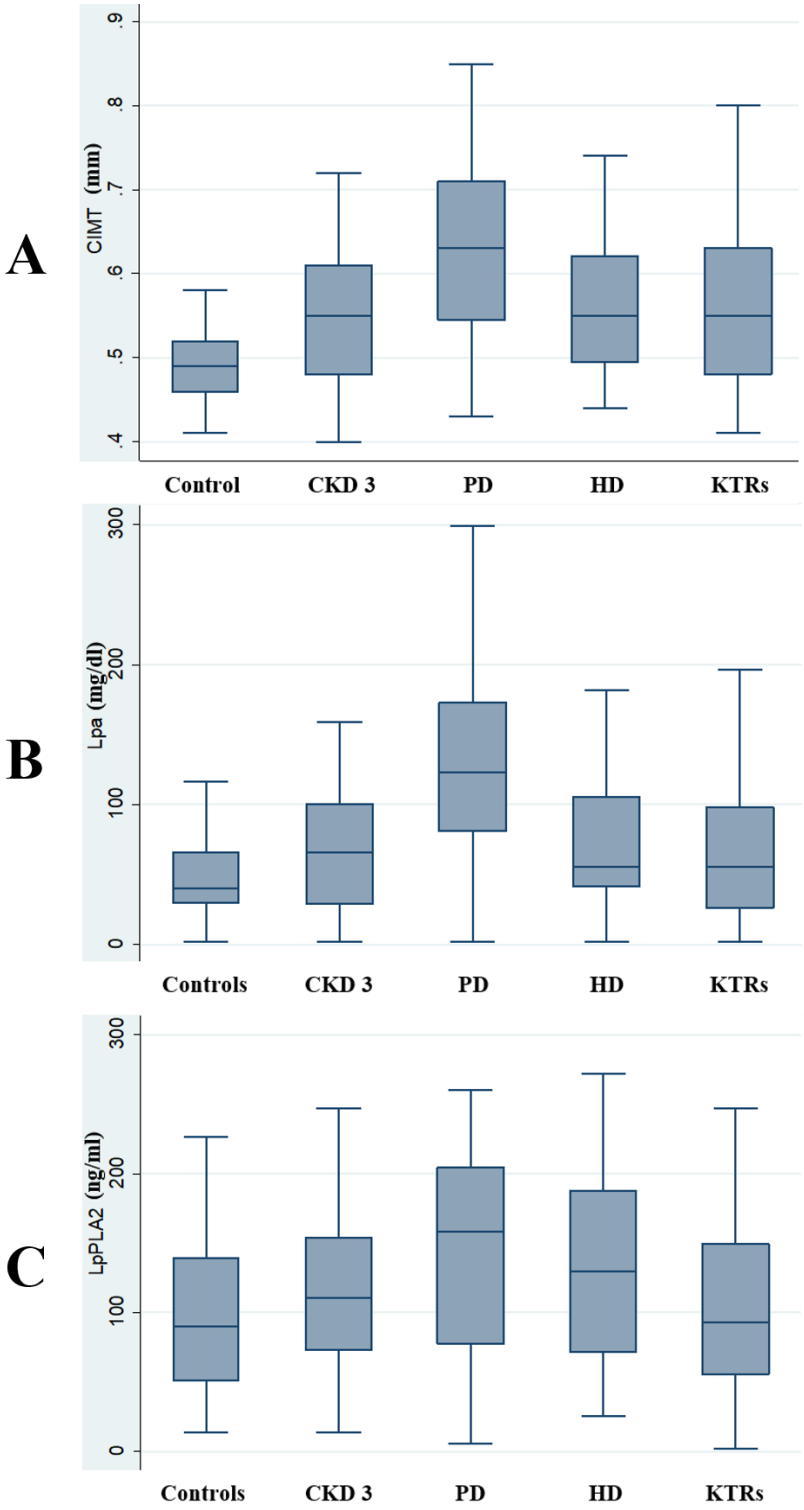

Figure 7.1 Carotid intima media thickness, lipoprotein (a) and lipoprotein phospholipase A2 levels in the study population

CIMT – carotid intima media thickness, (A); Lpa – lipoprotein (a), (B); Lp-PLA2 – lipoprotein phospholipase A2, (C); CKD 3 – chronic kidney disease, KDOQI stage 3; PD – peritoneal dialysis; HD – haemodialysis; KTRs – kidney transplant recipients

Table 2. Comparison of lipoprotein markers by presence of atherosclerotic vascular disease and by kidney disease

Parameters	**CKD (n = 40)** **AsVD present** **AsVD absent** **(p-value)**	**PD (n = 40)** **AsVD present** **AsVD absent** **(p-value)**	**HD (n = 40)** **AsVD present** **AsVD absent** **(p-value)**	**KTR (n = 41)** **AsVD present** **AsVD absent** **(p-value)**	**KD (n = 161)** **AsVD present** **AsVD absent** **(p-value)**
APO A1 (g/l)	13.9 (10.5 – 20.2) 13.8 (11.7 – 17.4) **0.745**	11.6 (10.1 – 14.7) 14.6 (12.5 – 17.4) **0.108**	12.8 (10.8 – 16.1) 11.0 (10.1 – 15.1) **0.424**	13.6 (10.6 – 17.1) 12.8 (11.2 – 16.2) **0.744**	13.1 (10.5 – 15.8) 14.5 (12.4 – 17.4) **0.642**
Lp-PLA2 (ng/ml)	88.9 (50.0 – 145.6) 122.1 (93.7 – 160.9) **0.091**	162.0 (77.3 – 195.3) 155.6 (76.6 – 214.6) **0.859**	153.0 (61.6 – 205.1) 110.2 (73.6 – 164.1) **0.228**	125.4 (59.9 – 154.2) 83.1 (39.1 – 13.5) **0.239**	129.8 (61.6 – 179.8) 90.7 (54.9 – 136.7) **0.385**
Lp (a) (mg/dl)	73.2 (27.4 – 111.0) 62.1 (31.5 – 81.5) **0.695**	119.0 (68.7 – 164.0) 145.5 (87.6 – 232.5) **0.288**	60.4 (44.0 – 125.0) 54.5 (39.8 – 84.8) **0.310**	61.7 (36.4 – 161.0) 43.9 (22.9 – 74.1) **0.166**	84.5 (44.0 – 140.0) 59.5 (32.6 – 111.0) **0.091**

*statistically significant, ($p < 0.05$); AsVD – atherosclerotic vascular disease; CKD – chronic kidney disease; PD – peritoneal dialysis; HD – haemodialysis; KTRs – kidney transplant recipients; KD – combined kidney disease; APO A1 – apolipoprotein A1; Lp-PLA2 – lipoprotein phospholipase A2; Lp (a) – lipoprotein(a)

Table 3. Correlation of lipoprotein markers with CIMT and inflammatory markers by kidney disease groups

Parameter	Stage 3 CKD		PD		HD		KTRs		KD	
	rho	p	rho	p	rho	p	rho	p	rho	p
CIMT										
APO A1	0.170	0.293	-0.225	0.163	0.053	0.747	0.020	0.902	-0.012	0.884
Lp-PLA2	-0.194	0.230	0.046	0.780	0.242	0.133	0.107	0.507	0.126	0.110
Lp (a)	0.159	0.327	-0.254	0.113	0.077	0.638	0.121	0.450	0.111	0.161
Pentraxin-3										
APO A1	-0.181	0.263	0.067	0.682	-0.265	0.099	0.098	0.541	-0.075	0.342
Lp-PLA2	0.221	0.171	0.439	0.005*	0.444	0.004*	0.045	0.781	0.306	<0.001*
Lp (a)	-0.278	0.082	0.059	0.716	0.250	0.119	0.109	0.498	0.097	0.220
TNF-α										
APO A1	-0.132	0.417	0.091	0.576	-0061	0.710	0.391	0.012	0.037	0.637
Lp-PLA2	0.310	0.052	0.522	<0.001*	0.279	0.081	0.112	0.486	0.341	<0.001*
Lp (a)	-0.314	0.048	0.125	0.443	0.081	0.620	-0.117	0.467	0.053	0.505
EN-RAGE										
APO A1	0.033	0.841	0.136	0.403	0.017	0.919	0.414	0.007	0.178	0.024
Lp-PLA2	-0.192	0.235	-0.085	0.600	-0.043	0.792	0.153	0.341	-0.072	0.363
Lp (a)	0.111	0.497	-0.202	0.211	0.156	0.336	-0.023	0.888	0.033	0.675

*statistically significant, ($p < 0.05$); CIMT carotid intima media thickness; CKD – chronic kidney disease; PD – peritoneal dialysis; HD – haemodialysis; KTRs – kidney transplant recipients; KD – combined kidney disease; TNF-α – tumour necrosis factor-alpha; EN-RAGE – ligand of receptor for advanced glycation end-products; APO A1 – apolipoprotein A1; L p-PLA2 – lipoprotein phospholipase A2; Lp (a) – lipoprotein(a)

There was a positive correlation between Lp-PLA2 and pentraxin-3 among KTRs, HD and PD patients, Table 3. There was a positive correlation between Lp-PLA2 and TNF-α among KTRs and PD patients. No correlation was seen between lipoprotein biomarkers and CIMT. No significant difference was seen when the AUC of lipid biomarkers were compared among all kidney disease patients

Table 4. AUC among men and women in the study population.

Parameter	MEN		WOMEN	
	AUC	95% CI	AUC	95% CI
Lp(a)	0.476	0.346 – 0.607	0.697	0.579 – 0.814
Lp-PLA2	0.497	0.371 – 0.623	0.581	0.450 – 0.711
APO A1	0.595	0.464 – 0.725	0.367	0.223 – 0.502

Table 4 showed the gender difference in the contribution to the overall AUC among kidney disease patients (stage 3 CKD, PD, HD and KTRs). Based on the strength of the AUC, Lp(a) and Lp-PLA2 predicted AsVD better among women than men.

Table 5. Comparison of utility of lipid biomarkers in predicting atherosclerotic vascular disease in kidney disease patients

Parameter	ROC (AUC)	95% CI	p-value
Kidney disease (n = 161)			
Lp (a)	0.577	0.488 – 0.666	0.382
APO A1	0.521	0.432 – 0.611	
Lp (a)	0.577	0.488 – 0.666	0.544
Lp-PLA2	0.540	0.450 – 0.630	
CKD (stage 3) (n = 40)			
Lp (a)	0.536	0.348 – 0.724	0.965
APO A1	0.530	0.339 – 0.721	
Lp (a)	0.536	0.348 – 0.724	0.290
Lp-PLA2	0.657	0.477 – 0.836	
PD (n = 40)			
Lp (a)	0.607	0.402 – 0.812	0.721
APO A1	0.662	0.455 – 0.869	
Lp (a)	0.607	0.402 – 0.812	0.552
Lp-PLA2	0.518	0.299 – 0.737	
HD (n = 40)			
Lp (a)	0.594	0.414 – 0.774	0.871
APO A1	0.574	0.389 – 0.759	
Lp (a)	0.594	0.414 – 0.774	0.879
Lp-PLA2	0.612	0.426 – 0.797	
KTRs (n = 40)			
Lp (a)	0.627	0.449 – 0.805	0.457
APO A1	0.530	0.346 – 0.714	
Lp (a)	0.627	0.449 – 0.805	0.874
Lp-PLA2	0.608	0.429 – 0.786	

ROC – receiver operating characteristics; AUC – area under curve; APO A1 – apolipoprotein A1; Lp-PLA2 – lipoprotein phospholipase A2; Lp (a) – lipoprotein(a); TC – total cholesterol; TG – triglyceride; LDL-C – low density lipoprotein; HDL-C – high density lipoprotein

Table 6. Comparison of the utility of lipid biomarkers and lipid profile parameters in predicting atherosclerotic vascular disease in female kidney disease patients

Parameters	ROC (AUC)	95 % CI	p-value
Kidney disease (n = 161)			
Lp (a)	0.697	0.579 – 0.814	0.472
APO A1	0.633	0.498 – 0.768	
Lp (a)	0.697	0.579 – 0.814	0.190
Lp-PLA2	0.581	0.450 – 0.711	
Lp (a)	0.697	0.579 – 0.814	0.062*
TC	0.517	0.385 – 0.649	
Lp (a)	0.697	0.579 – 0.814	0.034**
TG	0.504	0.368 – 0.640	
Lp (a)	0.697	0.579 – 0.814	0.709
HDL-C	0.665	0.540 – 0.791	
Lp (a)	0.697	0.579 – 0.814	0.066*
LDL-C	0.538	0.405 – 0.671	

**statistically significant, $p < 0.05$; *tending towards significance, $p < 0.09$; ROC – receivers operating characteristics; AUC – area under curve; APO A1 – apolipoprotein A1; Lp-PLA2 – lipoprotein phospholipase A2; Lp (a) – lipoprotein(a); TC – total cholesterol; TG – triglyceride; LDL-C – low density lipoprotein; HDL-C – high density lipoprotein

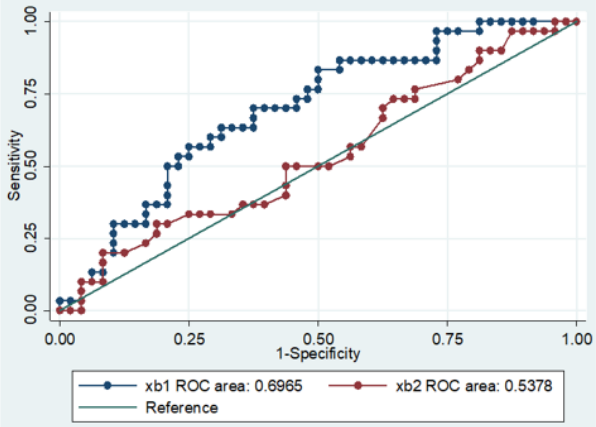

Figure 7.2a Comparison between Lp (a) and LDL-C for predicting atherosclerotic vascular disease in female kidney disease patients

xb1 – Lp (a); xb2 – LDL-C; [0.697 (0.579 – 0.814) vs 0.538 (0.405 – 0.671); p = 0.066]

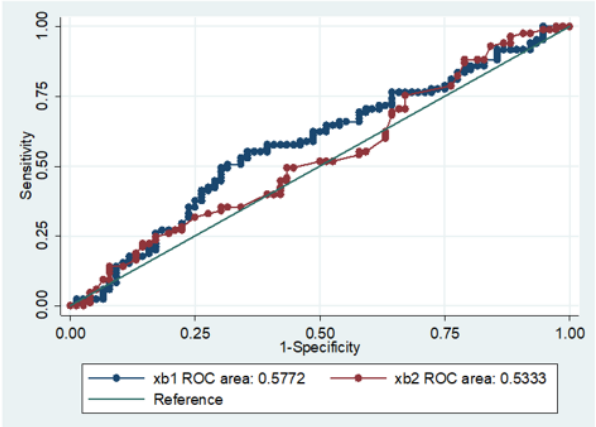

Figure 7.2b Comparison between Lp (a) and LDL-C for predicting atherosclerotic vascular disease in kidney disease patients

xb1 – Lp (a); xb2 – LDL-C; [0.577 (0.488 – 0.666) vs 0.533 (0.443 – 0.623); p = 0.433]

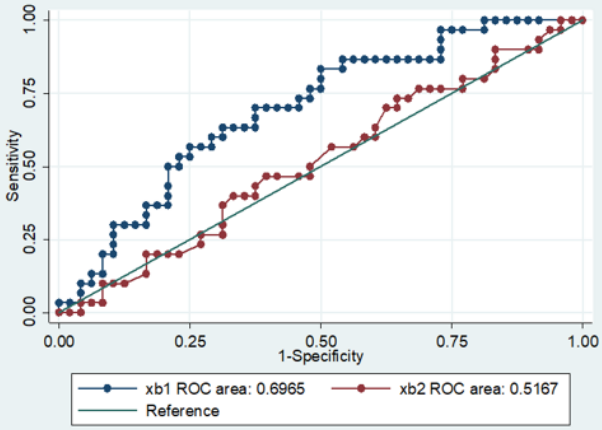

Figure 7.3a Comparison between Lp (a) and TC for predicting atherosclerotic vascular disease in female kidney disease patients

xb1 – Lp (a); xb2 – total cholesterol (TC); [0.697 (0.579 – 0.814) vs 0.517 (0.385 – 0.649); p = 0.062]

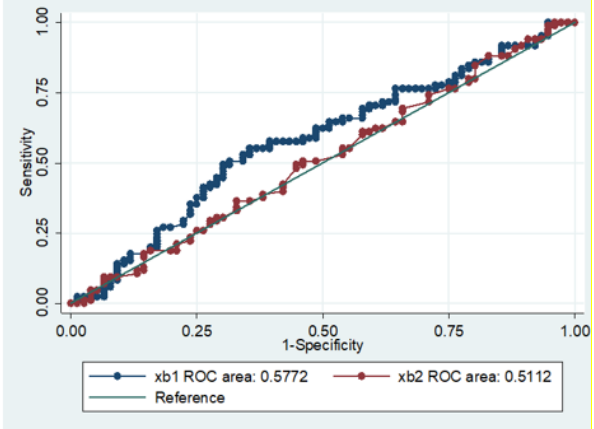

Figure 7.3b Comparison between Lp (a) and TC for predicting atherosclerotic vascular disease in kidney disease patients

xb1 – Lp (a); xb2 – total cholesterol (TC); [0.577 (0.488 – 0.666) vs 0.511 (0.421 – 0.601); p = 0.248]

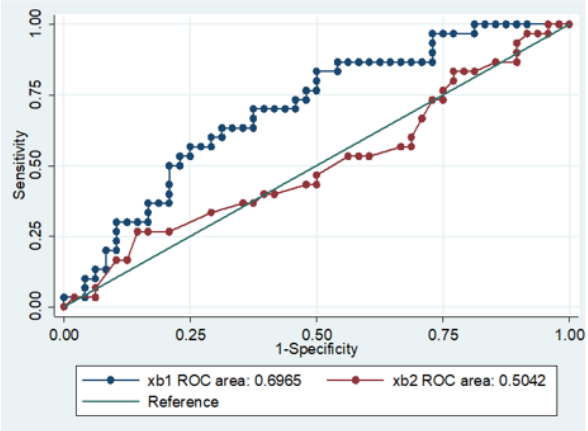

Figure 7.4a Comparison between Lp (a) and TG for predicting atherosclerotic vascular disease in female kidney disease patients

xb1 – Lp (a); xb2 – total cholesterol (TC); [0.697 (0.579 – 0.814) vs 0.504 (0.368 – 0.640); p = 0.034]

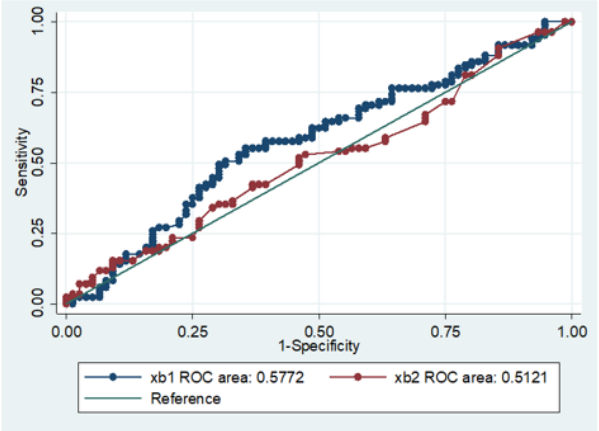

Figure 7.4b Comparison between Lp (a) and TG for predicting atherosclerotic vascular disease in kidney disease patients

xb1 – Lp (a); xb2 – total cholesterol (TC); [0.577 (0.488 – 0.666) vs 0.512 (0.422 – 0.602); p = 0.332]

When female patients with kidney disease were analysed separately, there was a statistically significant difference when the AUC was compared between Lp (a) and TG, Table 5. Comparison between Lp (a) and TC and between Lp (a) and LDL-C showed tendency towards significance.

Table 6 Binary logistic regression showing predictors of atherosclerotic vascular disease

Parameter	**OR**	**95% CI**	**p-value**
Age	5.00	2.14 – 11.68	<0.001*****
Gender	3.24	1.42 – 7.39	0.005*
BMI (kg/m^2)	1.05	0.98 – 1.13	0.175
WHR	0.15	0.01 – 60.24	0.534
TC (> 5.17mmol/l)	1.01	0.33 – 3.10	0.982
TG (> 1.69mmol/l)	1.00	0.38 – 2.38	0.924
LDL-C (> 2.59mmol/l)	1.51	0.59 – 3.87	0.387
Low HDL-C[a] (mmol/l)	3.14	1.35 – 7.31	0.008*
hsCRP (mg/l)	0.92	0.40 – 2.11	0.848
Pentraxin-3 (ng/ml)	0.80	0.36 – 1.75	0.571
APO A1 (g/l)	0.94	0.42 – 2.10	0.888
Lp-PLA2 (ng/ml)	1.34	0.61 – 2.96	0.471
Lp (a) (mg/dl)	2.23	1.01 – 4.94	0.048*

*statistically significant, ($p < 0.05$); [a] < 1.03 in male and < 1.29mmol/l in female; CIMT - carotid intima media thickness; CKD – chronic kidney disease; PD – peritoneal dialysis; HD – haemodialysis; KTRs – kidney transplant recipients; KD – combined kidney disease; TNF-α – tumour necrosis factor-alpha; EN-RAGE – ligand of receptor for advanced glycation end-products; APO A1 – apolipoprotein A1; Lp-PLA2 – lipoprotein phospholipase A2; Lp (a) – lipoprotein(a)

Multivariate analysis showed that age (> 40 years), male gender, low HDL-C levels and elevated Lp (a) levels independently predicted AsVD when all kidney disease patients were combined. Elevated Lp (a) and low HDL-C confer a 2- and 3-fold risk respectively for AsVD in kidney disease patients, Table 6.

Discussion

Lipoprotein (a) and kidney diseases

Increased levels of Lp (a) were present in all kidney disease groups in this study compared to controls and significantly increased levels were seen in PD, HD, and combined kidney disease group compared to controls, with the largest increase seen in PD patients. This is similar to report from previous studies.[25, 26] Plasma and Lp (a)-associated Lp-PLA2 activity was evaluated in mild-moderate chronic renal failure patients, PD and HD patients; all patient groups showed significantly increased Lp (a) levels.[25] Factors associated with elevated Lp (a) levels in CKD patients were evaluated; CKD-related factors including higher levels of proteinuria, c-reactive protein (CRP) and TG levels were associated with elevated Lp (a) levels.[27]

Inflammation provides part of the explanation for our finding of high levels of Lp (a) among the ESKD (PD and HD) patients. Higher levels of inflammation among ESKD patients, especially PD patients, evidenced by significantly increased inflammatory markers, possibly contributed to the increased levels of Lp (a) in this group of patients. Our study also found higher levels of Lp (a) among the study participants; median levels in our control group was 40.2 (29.3 – 65.4) mg/dl, which was well above the atherogenic cut-off of 30mg/dl. Significantly higher median levels of Lp (a) have been demonstrated among blacks compared to whites.[27]

Some studies have demonstrated that Lp (a) levels in the general population are determined by the molecular size of apo(a), individuals with small apo(a) molecular size have higher levels of Lp (a) and vice versa.[26, 27] Could it be that the apo(a) small isoform is more prevalent among blacks compared to whites? Therefore, there is need for further studies to compare the relationships of the apo(a) molecular size isoforms and Lp (a) to renal disease and

cardiovascular disease among black CKD patients. Studies have demonstrated differences in the apo(a) sizes associated with elevated Lp (a) in PD and HD patients. An increase in Lp (a) levels of up to 4-fold was seen only among HD patients with the large apo(a) isoform;[28] contrary to this, a study among PD patients demonstrated an increase in Lp (a) levels that is not apo(a) size dependent.[29,30]

In our study, we found a negative correlation between Lp (a) levels and eGFR when CKD stage 3, PD and KTRs patients were combined, ($r = -0.305$, $p < 0.001$). Although, Uhlig et al[27] found no association between eGFR and plasma levels of Lp (a), a study on Lp (a), apo (a) isoforms and kidney disease found an inverse relationship between Lp (a) levels and GFR.[31] In our study, the findings of an inverse correlation between eGFR and Lp (a) levels among kidney disease patients, the presence of very high levels of Lp (a) among PD and HD patients and the increasing strength of the association between eGFR and Lp (a) with declining eGFR in our study [GFR < 60ml/min/1.73m^2 (χ^2 - 6.36, $p = 0.012$); GFR < 30ml/min/1.73m^2 (χ^2 - 8.55, $p = 0.003$)] bolsters the previous suggestion that Lp (a) levels rises as GFR falls due to reduced clearance by the diseased kidney.[32]

We found that serum levels of Lp-PLA2 were increased in all kidney disease groups; PD patients had the highest increase which was statistically significant when compared to controls. Levels of Lp-PLA2 in HD and the combined kidney disease group also showed a tendency toward significance. This is similar to findings in a previous study where plasma levels of Lp-PLA2 activity were found to be significantly higher in both HD and PD patients, with a more profound activity seen in PD patients.[25] In our study, we found that levels of APO A1 were lower in all kidney disease groups compared to controls; this is similar to the report from Atherosclerotic Risk in Community (ARIC) Study where lipoprotein ratios and APO A1 and APOB/APO A1 ratios were evaluated for associations with coronary heart disease.[33] Lower levels of APO A1 were found among patients with CKD compared to individuals without

CKD; the study found no evidence that APO A1 and APO A1/APOB ratio were more strongly associated with coronary heart disease incidence in CKD compared to non-HDL-C/HDL-C.[33]

Lipoprotein biomarkers and AsVD

In our study, Lp (a) independently predicted AsVD, after adjusting for confounders, when all kidney disease patients were combined. This is consistent with findings in previous studies.[34,35] The association between elevated Lp (a) levels and incident myocardial infarction and mortality was assessed among the participants of the Chronic Renal Insufficiency Cohort (CRIC) study followed up for 7.5 years; the study found that the highest quartile of Lp (a), with baseline levels > 61.3mg/dl was associated with increased risk of myocardial infarction (HR 1.49; 95 % CI - 1.05 – 2.11) and death (HR 1.28; 95 % CI - 1.05 – 1.57).[34] In another similar study in haemodialysis patients, a significant positive association was seen between Lp (a) and CIMT.[35]

Reports in existing literature shows that apart from the association between Lp (a) and AsVD, which was also demonstrated in our study, gender-based association between Lp (a) and AsVD have also been evaluated in some studies.[36,37] We found an association between Lp (a) and AsVD among female kidney disease patients when all the kidney disease groups were combined, (χ^2 – 4.36, p = 0.037). In the general population, similar findings of an association between Lp (a) and CVD have been reported among women and Lp (a) has been reported as a predictor of coronary heart disease in younger women (65 years of age or younger).[36] Lipoprotein (a) was found to be a predictor of significant coronary artery disease among patients who had coronary angiography; interestingly, the study also reported that Lp (a) predicted coronary artery disease better in women compared to men.[37, 38] However, some studies have demonstrated association between CVD and Lp (a) levels in young and middle aged men in the general population,[39,40] In contrast, other studies have either found a stronger

association in women compared to men[37,38] or demonstrated no association between Lp (a) levels and CVD in men.[41,42]

We also found higher mean levels of Lp (a) among females compared to males, although this was not statistically significant. This is comparable to a previous report by Simon et al,[43] which evaluated more than 2,500 men and women and found higher mean levels of Lp (a) among women than men.

When all the kidney disease groups were combined, we demonstrated an association between APO A1 levels and AsVD in female kidney disease patients, (χ^2 – 4.51, p = 0.034). No association was found between APO A1 and AsVD in male kidney disease patients in this study. In a previous study, lower levels of APO A1 were associated with presence of carotid plaques.[44]

We also found an association between Lp-PLA2 and AsVD among women with kidney disease, with a tendency towards significance, (χ^2 – 3.13, p = 0.077). A previous study evaluated the risk of ischaemic stroke among post-menopausal women and found that Lp-PLA2 was associated with incident ischaemic stroke among non-users of hormone therapy, independent of traditional CVD risk factors and CRP levels.[45]

The utility of the biomarkers for predicting atherosclerotic vascular disease was tested using receivers' operating characteristics (ROC) and the area under curve (AUC) generated was used in comparing Lp (a) to other lipid markers. We found that Lp (a) predicted atherosclerotic vascular disease better than the other lipid markers tested, evidenced by higher AUC but significant difference was seen when Lp (a) was compared with TG. A tendency towards significance was seen when Lp (a) was compared with TC and LDL-C among female kidney disease patients. A sub-analysis of the white participants of the Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) trial

found elevated Lp (a) to be a predictor of CVD risk in statin-treated patients with low LDL-C.[46] The aggressive use of statins in CKD patients possibly makes Lp (a) more predictive of cardiovascular disease in this group of patients.

In conclusion, lipoprotein biomarkers are associated with kidney disease. Age > 40 years, male gender, low HDL-C and elevated Lp (a) were independent predictors of AsVD. Among female kidney disease patients, there was an association between all the lipoprotein biomarkers and AsVD. In addition, Lp (a) predicted AsVD better than other lipoprotein biomarkers and the traditional lipid profile parameters among female kidney disease patients.

This study is not without limitations. The exclusion of the elderly (age > 65 years) from this study could have affected the levels of some of the biomarkers which are influenced by advancing age. The cross-sectional nature of this study allowed for measurements of the various parameters at a single point; a longitudinal study will provide data on the evolution of atherosclerosis over the period of CKD and dialysis.

Methods

This was a comparative cross-sectional study of 40 adult (18-65years) non-diabetic ESKD patients on HD, 40 patients on PD, 40 stage 3 CKD patients, 41 KTRs and 41 age- and sex-matched healthy controls at a large urban public hospital in South Africa from 2 January 2017 to 31 August 2017. The study was approved by the University of the Witwatersrand human research ethics committee (HREC), study number M160614. Measurements and calculations for WHR, BMI, body surface area, blood pressure and serum albumin and lipogram levels were performed as described in the Methods section, Chapter 3. lipid biomarkers (APO A1 and Lp-PLA2) were determined by Magnetic Luminex Assay, Human Premixed Multi-Analyte Kit by R&D Systems using BioPlex 200 Systems (Bio-Rad Laboratories Inc., USA). Lipoprotein (a) assay was determined using Beckman Immage 800 Immunochemistry system (Beckman

Coulter, USA). Estimated GFR calculation, echocardiographic procedure and CIMT measurements were performed as decribed in the Methods section, Chapter 3. Atherosclerotic vascular disease was defined by the combination of CIMT > 0.55mm and presence of carotid plaques.

Data analysis

Stata version13.1 (Stata Corp, USA), was used for statistical analysis. Categorical variables were expressed as frequencies and percentages. Test of normality (Shapiro-wilk) was performed on all continuous variables and data was presented as median and interquartile ranges (IQR). Comparison was performed between kidney disease groups (HD, PD, stage 3 CKD, KTRs) and controls using Wilcoxon rank-sum test.

Spearman's rank correlation was used to determine the relationship between lipid biomarkers and inflammatory markers and cardiovascular risk factors among PD and HD patients. Receiver operating characteristics (ROC) was performed to compare the utility of the lipid biomarkers in predicting AsVD. A comparative AUC from the ROC curve and the p-value quoted was derived from comparing the AUCs obtained from the ROC curve plotted for the biomarkers.

Multivariate binary logistic regression analysis was performed to determine the predictors of AsVD.

Disclosure

No competing interests in relation to this study.

References

1. Lewington S, Whitlock G, Clarke R, Sheliker P, Emberson J, Halsey J, et al. Blood cholesterol and vascular mortality by age, sex and blood pressure: a meta-analysis of

individual data from 61 prospective studies with 55,000 vascular deaths. Lancet 2007; 370(9602): 1829-1839

2. Kaysen GA. Lipid and lipoprotein metabolism in chronic kidney disease. J Ren Nutr 2009; 19: 73-77
3. Lobentanz EM, Dieplinger H. Biogenesis of lipoprotein (a) in human and animal hepatocytes. Electrophoresis 1997; 18(14): 2677-2681
4. Chiesa G, Hobbs HH, Koschinsky ML, Lawn RM, Maika SD, Hammer RE. Reconstitution of lipoprotein (a) by infusion of human low density lipoprotein into transgenic mice expressing human apolipoprotein (a). J Biol Chem. 1992; 267(34): 24369-24374
5. Enas EA, Dhawan J, Petkar S. Coronary artery disease in Asian Indians: lessons from the role of lipoprotein (a). Indian Heart J. 1997; 49(1): 25-34
6. Dahlen CG. Lipoprotein (a) in cardiovascular disease: Review article and view point. Atherosclerosis 1994; 108: 111-126
7. Clevidence BA, Judd JT, Schaefer EJ, Jenner JL, Lichtenstein AH, Muesing RA, et al. Plasma lipoprotein (a) in men and women consuming diets enriched in saturated, cis-, or trans-monounsaturated fatty acids. Arterioscler Thromb Vasc Biol. 1997; 17(9): 1657-1661
8. Passilta M, Kervinen K, Rantala AO, Savolainen MJ, Lilja M, Reunanen A. Social alcohol consumption and low Lp (a) lipoprotein concentrations in middle aged Finnish men: population based study. BMJ 1998; 316(7131): 594-595
9. Lawn RM. Lipoprotein (a) in heart disease. Sci Am. 1992; 266(6): 54-60
10. Scanu AM, Fless GM. Lipoprotein (a); heterogeneity and biological relevance. J Clin Invest. 1990; 85(6): 1709-1715

11. Rosengren A, Wilhelmsen L, Eriksson E, Risberg B, Wedel H. Lipoprotein (a) and coronary heart disease: a propective case-control study in a general population sample of middle aged men. BMJ. 1990; 301(6763): 1248-1251
12. Dangas G, Mehran R, Harpel PC, Sharma SK, Marcovina SM, Dube G, et al. Lipoprotein(a) and inflammation in human coronary atheroma: association with the severity of clinical presentation. J Am Coll Cardiol. 1998; 32(7): 2035-2042
13. Bottalico LA, Keesler GA, Fless GM, Tabas I. Cholesterol loading of macrophages leads to marked enhancement of native lipoprotein (a) and apolipoprotein (a) internalization and degradation. J Biol Chem 1993; 268(12) 8569-8573
14. Smith EB, Cochran S. Factors influencing the accumulation in fibrous plaques of lipids derived from low density lipoprotein II. Preferential immobilization of lipoprotein (a). Atherosclerosis 1990; 84(2-3): 173-181
15. Allen S, Khan S, Tam Sp, Koschinsky M, Taylor P, Yacoub M. Expression of adhesion molecules by Lp (a): a potential novel mechanism for its atherogenicity. FASEB J. 1998; 12(15): 1765-1776
16. Asano K, Okamoto S, Fukunaga K, Shiomi T, Mori T, Iwata M. Cellular sources of platelet-activating factor acetylhydrolase activity in plasma. Biochem. Biophys. Res. Commun. 1999; 261: 511-514
17. Stafforini DM, Tjoelker LW, McCormick SP, Vaitkus D, McIntyre TM, Gray PW, et al. Molecular basis of the interaction between plasma platelet-activating factor acetylhydrolase and low density lipoprotein. J Biol. Chem. 1999; 274: 7018-7024
18. Macphee CH, Moores KE, Boyd HF, Dhanak D, Ife RJ, Leach CA, et al. Lipoprotein-associated phospholipase A2, platelet-activating factor acetylhydrolase, generates two bioactive products during the oxidation of low-density lipoprotein: use of a novel inhibitor. Biochem. J. 1999; 338: 479-487

19. Hannson GK. Mechanisms of disease- Inflammation, atherosclerosis and coronary artery disease. N. Engl J. Med. 2005; 352: 1685-1695
20. Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Heiss G, et al. Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein and risk for incident coronary heart disease in middle-aged men and women in the Atherosclerotic Risk in Communities (ARIC) study. Circulation 2004; 109: 837-842
21. Lund-Katz S, Phillips MC. High density lipoprotein structure-function and role in reverse cholesterol transport. Subcell Biochem 2010; 51: 183-227
22. Besler C, Luscher TF, Landmesser U. Molecular mechanisms of vascular effects of high density lipoprotein: alterations in cardiovascular disease. EMBO Mol Med 2012; 4: 251-268
23. Phillips MC. New insights into the determination of HDL-C structure by apolipoproteins: thematic review series: high density lipoprotein structure, function and metabolism. J Lipid Res 2013; 54: 2034-2048
24. Mukhamedova N, Escher G, D'Souza W, Tchoua U, Grant A, Krozowski Z, et al. Enhancing apolipoprotein A1-dependent cholesterol efflux elevates cholesterol export from macrophages in vivo. J Lipid Res. 2008; 49(11):2312-2322
25. Milionis HJ, Elisaf MS, Karabina SA, Bairaktari E, Tselepis AD, Siamopoulos KC. Plasma and Lp (a)-associated acetyhydrolase activity in uraemic patients undergoing different dialysis procedures. Kidney Int. 1999; 56(6): 2276-2285
26. Kronenberg F, Utermann G, Dieplinger H. Lipoprotein (a) in renal disease. Am J Kidney Dis. 1996; 27(1): 1-25
27. Uhlig K, Wang S, Beck GJ, Kusek JW, Marcovina SM, Greene T, et al. Factors associated with Lipoprotein (a) in chronic kidney disease. Am J Kidney Dis. 2005; 45(1): 28-38

28. Dieplinger H, Lackner C, Kronenberg F, Sandholzer C, Lhotta K, Hoppichler F, et al. Elevated plasma concentrations of lipoprotein (a) in patients with end-stage renal disease are not related to the size polymorphism of apolipoprotein(a). J Clin Invest 1993; 91: 397-401
29. Milionis HJ, Elisaf MS, Tselepis A, Bairaktari E, Karabina SA, Siamopoulos KC. Apolipoprotein(a) phenotypes and lipoprotein(a) concentrations in patients with renal failure. Am J Kidney Dis 1999; 33: 1100-1106
30. Kronenberg F, Konig P, Neyer U, Auinger M, Pribasnig A, Lang U, et al. Multicenter study of lipoprotein(a) and apolipoprotein(a) phenotypes in patients with end-stage renal disease treated by haemodialysis or continuous ambulatory peritoneal dialysis. J Am Soc Nephrol 1995; 6: 110-120
31. Kronenberg F, Kuen E, Ritz E, Junker R, Konig P, Kraatz K, et al. Lipoprotein (a) serum concentrations and apolipoprotein (a) phenotypes in mild and moderate renal failure. J Am Soc Nephrol 2000; 11: 105-115
32. Frischmann ME, Kronenberg F, Trenkwalder E, Schaefer JR, Schweer H, Dieplinger B. In vivo turnover study demonstrates diminished clearance of lipoprotein(a) in haemodialysis patients. Kidney Int. 2007; 71:1036-1043
33. Lamprea-Montealegre JA, Sharrett AR, Matsushita K, Selvin E, Szklo M, Astor BC. Chronic kidney disease, lipids and apolipoproteins and coronary heart disease: the ARIC study. Atherosclerosis 2014; 234(1): 42-46
34. Bajaj A, Damrauer SM, Anderson AH, Xie D, Budoff MJ, Go AS, et al. Lipoprotein (a) and risk of myocardial infarction and death in chronic kidney disease. Findings from the Chronic Renal Insufficiency Cohort (CRIC) Study. Atherosclerosis, Thrombosis and Vascular Biology. 2017 doi:10.1161/ATVBAHA.117.309920

35. Rofieian-Kopaei M, Nasri H. Serum lipoprotein (a) and atherosclerotic changes in haemodialysis patients. J Renal Inj Prev. 2013; 2(2): 47-50
36. Orth-Gome'r K, Mittleman MA, Schenck-Gustafsson K, Wamala SP, Eriksson M, Belkic K, et al. Lipoprotein(a) as a determinant of coronary heart disease in young women. Circulation. 1997; 95: 329-334
37. Solymon BC, Marcil M, Wesolowska E, Gilfix BM, Lesperance J, Campeau L. Relation of coronary artery disease in women < 60 years of age to the combined elevation of serum lipoprotein(a) and total cholesterol to high-density cholesterol ratio. Am J Cardiol. 1993; 72: 1215-1219
38. Labeur C, De Backer G, Vincke J, Muyidermans L, Vanderkerhove Y, Van der Stichele E, et al. Plasma Lipoprotein(a) values and severity of coronary artery disease in a large population of patients undergoing coronary angiography. Clin Chem. 1992; 38: 2261-2266
39. Bostom AG, Cupples LA, Jenner JL, Ordivas JM, Serman LJ, Wilson PW, et al. Elevated plasma lipoprotein(a) and coronary heart disease in men aged 55 years and younger. A prospective study. JAMA 1996; 276(7): 544-548
40. Rosengren A, Wilhelmsen L, Eriksson E, Risberg B, Wedel H. Lipoprotein(a) and coronary heart disease: a prospective case-control study in a general population sample of middle aged men. BMJ 1990; 301(6763): 1248-1251
41. Jauhiainen M, Koskinen P, Ehnholm C, Frick MH, Manttari M, Manninen V, et al. Lipoprotein(a) and coronary heart disease risk: a nested case-control study of the Helsinki Heart Study participants. Atherosclerosis. 1991: 89(1): 59-67
42. Ridker KJ, Hennekens CH, Stampfer MJ. A prospective study of lipoprotein(a) and the risk of myocardial infarction. JAMA. 1993; 270: 2195-2199

43. Simons L, Friedlander Y, Simons J, McCallum J. Lipoprotein(a) is not associated with coronary heart disease in the elderly: Cross-sectional data from the Dubbo Study. Atherosclerosis. 1993; 99: 87-95
44. Abi-Ayad M, Abbou A, Abi-Ayad FZ, Behadada O, Benyoucef M. HDL-C-C, APO A1 and VLDL-C-TG as biomarkers for the carotid plaque presence in patients with metabolic syndrome. Diabetes Metab Syndr. 2017; doi: 10.1016/j.dsx.2017.12.017
45. Wasserthell-Smoller S, Kooperberg C, McGinn AP, Kaplan RC, Hsia J, Hendrix SL, et al. Lipoprotein-associated phospholipase A2, hormone use and the risk of ischaemic stroke in post-menopausal women. Hypertension 2008; 51(4): 1115-1122
46. Khera AV, Everett BM, Caulfield MP, Hantash FM, Wohlgemuth J, Ridker PM, et al. Lipoprotein (a) concentrations, Rosuvastatin therapy and residual vascular risk: An analysis from the Jupiter trial. Circulation 2014; 129(6): 635-642

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CHAPTER 8

APOL1 risk variants are associated with atherosclerotic vascular disease among patients with hypertension-attributed nephropathy

Formatted for BMC Nephrology

Abstract

Background

The discovery of risk variants in the *APOL1* gene has been pivotal in the understanding of the genetic basis of chronic kidney disease (CKD) specifically in the aetiology of HIV-associated nephropathy, focal segmental glomerulosclerosis (FSGS) and hypertension-attributed nephropathy (HAN). Most of the available studies have been among African Americans, however, the high prevalence of the *APOL1* risk alleles among people of African ancestry and the high prevalence of hypertension among blacks justify a need to evaluate black South African CKD patients for a causal relationship between *APOL1* risk alleles and hypertension-attributed CKD.

Methods

This was a cross-sectional study of 40 adult (18-65 years) non-diabetic CKD patients, kidney disease outcome quality initiative (KDOQI stage 3), 40 peritoneal dialysis (PD) patients, 40 haemodialysis (HD) patients, 41 kidney transplant recipients (KTRs) and 41 age- and sex-matched healthy controls. Interviewer-administered questionnaires were used to obtain information on participants' sociodemographic and cardiovascular risk factors and anthropometric parameters recorded. Echocardiography was performed on all patients and carotid intima media thickness (CIMT) assessed in both right and left carotid arteries.Serum

was analysed for creatinine, lipid profile and inflammatory markers. Genomic DNA was extracted from whole blood and *APOL1* genotpyting carried out using restriction fragment length polymorphisms (RFLPs). Binary logistic regression was done to determine the relationship of *APOL1* risk variants to AsVD among patients with hypertension-attributed CKD.

Results

The frequency of 0, 1 and 2 risk alleles among controls was 25/41 (60.98 %), 12/41 (29.27 %) and 4/41 (9.76 %) respectively while the frequency among patients with HAN was 36 (46.2%), 33 (42.3 %) and 9 (11.5 %), respectively. A logistic regression model shows a relationship between 2 *APOL1* risk alleles and AsVD among patients with hypertension-attributed CKD, (OR 11.85; 95 % CI [1.08 – 129.91]; p = 0.043), This relationship was lost when all kidney disease patients, regardless of aetiology, were used in the analysis, (OR 0.84; 95% CI [0.22 – 3.28]; p – 0.802).

Conclusion

The presence of 2 *APOL1* risk variants predicted AsVD among patients with HAN. Incorporation of *APOL1* genotyping into routine CKD and pre-transplant screening of patients with HAN for targeted follow-up of the high risk group may be beneficial.

Introduction

Chronic kidney disease (CKD) has been used as an umbrella diagnosis for all causes of prolonged (> 3 months) kidney dysfunction. Although, this categorization has clinical usefulness, the nomenclature seems to downplay the diverse pathophysiological events and genetic factors involved in the pathogenesis of specific aetiologies of CKD.

The quest to improve our understanding of the pathogenesis of CKD led to the discovery of an association between *APOL1* risk alleles and development and progression of non-diabetic CKD.[1] Apolipoprotein L1 gene encodes APOL1 protein which serves the function of lysing trypanosomes, hence conferring protection against African sleeping sickness.[2] The location of the *APOL1* risk variants is within the serum resistance-associated protein (SRA)-interacting domain, in the terminal exon of *APOL1* gene.[2] A study demonstrated the highest prevalence of the G1 allele in West Africa, among the Yoruba people (39 %) while the G2 allele (3-8 %) was similar across populations evaluated.[3] Kasembeli et al[4] reported an allele frequency of 7.3 % and 11.1 % for the G1 and G2 alleles, respectively, in a South African Black population, and an 89-fold greater odds for HIVAN in the presence of 2 risk alleles. The presence of two copies of *APOL1* risk alleles confers 5-29 fold higher odds of developing severe kidney disease such as HIV-related nephropathy, focal segmental glomerulosclerosis (FSGS) and hypertension-attributed nephropathy (HAN).[2,5,6] Among the participants' of the African American Study of Kidney Disease and Hypertension (AASK), a primary outcome of doubling of serum creatinine or ESKD occurred in 58.1 % of patients who had *APOL1* high-risk variants and in 36.6 % of patients who had *APOL1* low-risk variants, (HR 1.88; $p < 0.001$).[7] A significant association was found between *APOL1* risk variants and hypertension-attributed kidney disease, (OR 2.57); *APOL1* risk variants were also associated with the progression of kidney disease.[7] Similarly, the Chronic Renal Insufficiency Cohort (CRIC) study showed that black patients with *APOL1*-high risk variants had a more rapid decline in estimated glomerular filtration rate (eGFR) and a higher risk of primary outcome of 50 % reduction in eGFR from baseline levels compared to white patients in spite of diabetes status.[8] Some studies have also found associations between *APOL1* risk variants and cardiovascular disease (CVD) and mortality among African Americans.[9,10] Among the Jackson Heart Study (JHS) participants, increased

risk of CVD was observed among patients with *APOL1* risk variants compared to participants without the risk variants, (OR 2.17; $p = 9.4 \times 10^{-4}$).[10]

Among Afican Americans, a high prevalence of *APOL1* risk variants as well as its association with CKD progression has been demonstrated.[8,11] It is plausible to infer that there could be an association between *APOL1* risk variants and atherosclerotic vascular disease (AsVD) among CKD patients since CVD has been shown to worsen with progression of CKD.[12] We evaluated the association between AsVD and *APOL1* risk variants among black South African patients with hypertension-attributed CKD.

Methods

This was a cross-sectional study of 40 adult (18-65 years) non-diabetic ESKD patients on HD, 40 patients on PD, 40 stage 3 CKD patients, 40 KTRs and 41 age- and sex-matched healthy controls at a large urban public hospital in South Africa from 2 January 2017 to 31 August 2017. The study was approved by the University of the Witwatersrand Human Research Ethics Committee (HREC), study number M160614. An interviewer-administered questionnaire was used to obtain information on participants' sociodemographic and cardiovascular risk factors including age, waist-hip ratio (WHR) andbody mass index (BMI). The diagnosis of hypertension-attributed CKD was a clinical diagnosis based on typical features as assessed by the treating physician (presence of hypertension or use of antihypertensive agents, mild or no proteinuria [proteinuria $\leq$ 2.2 g/24h])[13] after excluding of alternate causes of CKD such as diabetes mellitus, connective tissue diseases, autosomal dominant polycystic kidney disease, obtstructive uropathy and secondary causes of hypertension. Measurements and calculation for WHR, BMI, body surface area, blood pressure and serum albumin and lipogram levels were performed as described in the Methods section, Chapter 3.

Inflammatory markers (pentraxin-3, TNF-α and EN-RAGE) were determined by Magnetic Luminex Assay, Human Premixed Multi-Analyte Kit by R&D Systems using BioPlex 200 Systems (Bio-Rad Laboratories Inc., Hercules, CA, USA). Whole blood was used for genomic DNA extraction using a modified salting-out method,[14] and the concentration of DNA determined using Nanodrop 2000 Spectrophotometer. Polymerase chain reaction (40 cycles) was done using MJ Mini personal Thermal cycler (BioRAD, Mexico). Amplified PCR were subjected to RFLPs using the restriction enzymes HindI III, NspI and mLuCI specific for *APOL1* single nucleotide polymorphism (SNPs) rs73885319, rs60910145 (G1) and and (rs71785313) G2 insertion/deletion, respectively. The restricted products were run on 1% agarose gel electrophoresis using Owl^{TM} EC-105 compact power supply (Thermofisher Scientific, USA) and the gel viewed using Gel Doc EZ Imager (BioRaD, U.S.A). Estimated GFR calculation, echocardiographic procedure and CIMT measurements were performed as decribed in the Methods section, Chapter 3.

Data analysis

Stata version13.1 (Stata Corp, USA), was used for statistical analysis. Categorical variables were expressed as frequencies and percentages. The Shapiro–Wilk test of normality was performed on all continuous variables and data was presented as median and interquartile ranges (IQR). Comparisons were made between kidney disease groups (stage 3 CKD, PD, HD and KTRs) and controls using the Wilcoxon rank-sum test.

Multivariate logistic regression analysis was performed to determine the relationship between *APOL1* risk alleles and AsVD. Test of significance was taken as p-value < 0.05.

Results

Table 1 Sociodemographic and clinical characteristics of study population

Parameter	Total (n=161)	CKD (n=40)	PD (n=40)	HD (n=40)	KTR (n=41)	Controls (n=41)
Age (years)						
Absent	76 (47.2)	16 (40.0)	20 (50.0)	19 (47.5)	21 (51.2)	20 (48.8)
Present	85 (52.8)	24 (60.0)	20 (50.0)	21 (52.5)	20 (48.8)	21 (51.2)
Gender						
Female	78 (48.5)	20 (50.0)	19 (47.5)	18 (45.0)	21 (51.2)	23 (56.1)
Male	83 (51.5)	20 (50.0)	21 (52.5)	22 (55.0)	20 (48.8)	18 (43.9)
BMI						
<30kg/m^2	117 (72.7)	19 (47.5)	31 (77.5)	32 (80.0)	35 (85.4)	25 (61.0)
>30kg/m^2	44 (27.3)	21 (52.5)	9 (22.5)	8 (20.0)	6 (14.6)	16 (39.0)
WHR						
Normal	52 (32.7)	12 (30.0)	9 (22.5)	15 (37.5)	16 (41.0)	28 (68.3)
Increased	107 (67.3)	28 (70.0)	31 (72.5)	25 (62.5)	23 (59.0)	13 (31.7)
Hypertension[a]						
No	50 (31.1)	15 (37.5)	12 (30)	7 (17.5)	16 (39.0)	34 (82.9)
Yes	111 (68.9)	25 (62.5)	28 (70)	33 (82.5)	25 (61.0)	7 (17.1)

TC (mmol/l)						
<5.17	124 (78.3)	31 (77.5)	19 (47.5)	40 (100)	33 (80.5	36 (87.8)
>5.17	37 (21.7)	9 (22.5)	21 (52.5)		8 (33.5)	5 (12.2)
LDL-C (mmol/l)						
<2.59	91 (56.5)	19 (47.5)	14 (35.0)	36 (90.0)	20 (48.8)	21 (51.2)
>2.59	70 (43.5)	21 (52.5)	26 (65.0)	4 (10.0)	21 (52.2)	20 (48.8)
HDL-C[b](mmol/l)						
Low	80 (49.7)	30 (75.0)	21 (52.5)	15 (37.5)	14 (34.1)	23 (56.1)
Normal	81 (50.3)	10 (25.0)	19 (47.5)	25 (62.5)	27 (65.9)	18 (43.9)
Pentraxin-3						
<1.87ng/ml	71 (44.1)	17 (42.5)	11 (27.5)	20 (50.0)	23 (56.1)	30 (73.2)
>1.87ng/ml	90 (55.9)	23 (57.5)	29 (72.5)	20 (50.0)	18 (43.9)	11 (26.8)
AsVD						
Absent	76 (54.7)	21 (52.5)	12 (30.0)	21 (52.5)	22 (53.7)	34 (82.9)
Present	85 (45.3)	19 (47.5)	28 (70.0)	19 (47.5)	19 (46.3)	7 (17.1)

CKD – chronic kidney disease stage 3; PD – peritoneal dialysis; HD – haemodialysis; KTR – kidney transplant recipients; Total – CKD + PD + HD + KTR; BMI – body mass index; WHR – waist-hip ratio; TC – total cholesterol; LDL-C – low density lipoprotein; HDL-C – high density lipoprotein; AsVD – atherosclerotic vascular disease

Table 2. Frequency of *APOL1* alleles among patients with hypertension-attributed nephropathy

Parameter	Total (n=78)	CKD (n=21)	PD (n=21)	HD (n=18)	KTR (n=18)	Controls (n=41)
APOL1						
0 risk allele	36 (46.2)	13 (61.9)	9 (42.9)	7 (38.9)	7 (38.9)	25 (60.98)
1 risk allele	33 (42.3)	6 (28.6)	7 (33.3)	11 (61.1)	9 (50.0)	12 (29.27)
2 risk alleles	9 (11.5)	2 (9.5)	5 (23.8)		2 (11.1)	4 (9.76)
G1 mutation						
Absent	55 (70.5)	17 (80.9)	15 (71.4)	12 (66.7)	11 (61.1)	29 (70.7)
Present	23 (29.5)	4 (19.1)	6 (28.6)	6 (33.6)	7 (38.9)	12 (29.3)
G2 mutation						
Absent	53 (67.9)	13 (61.9)	13 (61.9)	13 (72.2)	14 (77.8)	36 (87.8)
Present	25 (32.1)	8 (38.1)	8 (38.1)	5 (27.8)	4 (22.8)	5 (12.2)

CKD – chronic kidney disease stage 3; PD – peritoneal dialysis; HD – haemodialysis; KTR – kidney transplant recipients; *APOL1* – apolipoprotein L1; 1 risk allele – presence of any one of the risk alleles; 2 risk alleles – presence of 2 risk alleles in any combination, G1 G1, G1 G2 or G2 G2; Presence of G1 mutation – presence of any G1 alleles, $G1^{GM}$, $G1^{+M}$ or $G1^{G+}$; G2 mutation – deletion

Table 3 Logistic regression showing relationship of *APOL1* alleles to Atherosclerotic vascular disease among patients with hypertension-attributed kidney disease

Parameter[#]	HAN (n = 78)			Combined kidney disease (n = 199)		
	OR	95 % CI	p-value	OR	95 % CI	p-value
APOL1 2 risk variants	11.85	1.08 – 129.91	0.043*	0.84	0.22 – 3.28	0.802
Presence of G1	3.43	0.16 – 74.03	0.431	2.06	0.22 – 19.42	0.528
Presence of G2	0.35	0.08 – 1.44	0.144	1.07	0.48 – 2.37	0.869
*APOL1*_rs73885319	0.16	0.01 – 2.83	0.213	0.52	0.07 – 3.89	0.525
*APOL1*_rs60910145	0.58	0.05 – 7.02	0.668	1.04	0.22 – 4.86	0.956

[#]-This model was adjusted for age, gender, HDL-C and inflammation; * - statistically significant with $p<0.05$; HAN – Hypertension-attributed nephropathy; *APOL1* 2 risk variants – presence of 2 risk alleles in any combination, G1/ G1, G1/ G2 or G2/ G2; Presence of G1 mutation – presence of any G1 mutation, $G1^{GM}$, $G1^{+M}$ or $G1^{G+}$; Presence of G2 mutation.

The frequency of the no risk allele, 1 risk allele and two risk alleles among controls was 25/41 (60.98 %), 12/41 (29.27 %) and 4/41 (9.76 %) respectively while the frequency among patients with HAN was 36 (46.2 %), 33 (42.3 %) and 9 (11.5 %) respectively. The frequencies of GI and G2 alleles were 12/41 (29.3 %) and 5/41 (12.2 %) respectively in controls while among patients with HAN, the frequency was 23/78 (29.5 %) and 25/78 (32.1 %) respectively.

Discussion

Clear insight into the genetic basis of a disease improves the understanding of its aetiopathogenetic mechanisms and possible therapeutic approaches. This is of great importance in CKD, a disease associated with heterogenous aetiologies. The recent discovery of an association between *APOL1* risk alleles and specific forms of non-diabetic CKD ushered in an era of understanding of the genetic basis of CKD. In our study *APOL1* high risk variants (presence of 2 risk alleles in any combination (G1/G1, G1/G2 or G2/G2) predicted AsVD among patients with hypertension-attributed nephropathy when all the kidney disease groups were combined. This result is similar to findings in a previous study.[10] Ito et al[10] randomly selected 1959 African American participants of the JHS, sequenced *APOL1* and evaluated associations between *APOL1* genotypes and kidney disease and CVD. An increased risk for atherosclerotic CVD was observed among participants with two *APOL1* risk alleles, (OR 2.17, $p=9.4 \times 10^{-4}$). A similar association between *APOL1* risk variants and CVD was demonstrated in a cohort of postmenopausal women who participated in the Women's Health Initiative (WHI) study, (OR 1.98, $p=8.37 \times 10^{-3}$).[10] Furthermore, a study by Mukamal et al[9] found an association between *APOL1* risk variants and albuminuria, subclinical atherosclerosis and incident myocardial infarction. Despite this evidence in favour of an association between *APOL1* risk variants and CVD, a few studies have reported contrary views. Gutierrez et al[14] failed to find an association between *APOL1* risk variants and subclinical atherosclerosis or left ventricular function. The lack of association reported by Gutierrez and colleagues may be

related to the study participants' profile; the mean eGFR of the study population was 102.5 ± 15.9 ml/min. In view of the fact that *APOL1* is not associated with all forms of CKD but HIV-associated nephropathy, FSGS and HAN;[2,5,6] the association of *APOL1* and CVD is probably more robust in the setting of moderate to severe renal disease of specific aetiologies such as HIV infection, FSGS or hypertension. This was demonstrated in our study, when all the kidney disease groups, regardless of the aetiology of CKD, were used in the logistic regression model. We found no association between AsVD and *APOL1* risk variants, which bolsters the previous report of an association with a specific aetiology of CKD, which in ours was HAN.

Although the pathogenetic mechanisms involved in *APOL1*-mediated kidney damage in HIV-associated nephropathy, FSGS and HAN are still unclear,[16] it may be reasonable to suggest that the link between *APOL1* and AsVD may be related to the functions of HDL-C-subfractions since *APOL1* is one of the lipoprotein components of HDL-C. High density lipoprotein has been broadly classified based on density into large HDL-C-2 and small dense HDL-C-3; the HDL-C-2 has been further sub-fractionated into HDL-C-2b and HDL-C-2a, while HDL-C-3 has been sub-fractionated into HDL-C-3a, HDL-C-3b and HDL-C-3c.[17] The dense HDL-C-3 particles have been shown to be rich in *APOL1* and this strongly correlated with the capacity of HDL-C to attenuate LDL-C oxidation, a vital step in atherogenesis.[18] Similarly, HDL-C-3 has been shown to exert myriads of anti-atherogenic properties such as anti-inflammatory, anti-oxidant, anti-thrombotic, immune-modulatory and the control of glucose homestasis,[17,19,20] all of which have been implicated in the aetiopathogenesis of atherosclerosis.[21,22,23] Could it be that *APOL1* two risk alleles switch off the ability of HDL-C-3 particles to function optimally, subsequently leading to increased inflammation, oxidative stress, increased thrombotic tendency and poor glucose homeostasis, resulting in a potentially pro-atherogenic milleu? Paradoxically, increased levels of HDL-C-3 fractions and low levels of HDL-C-2 fractions have been reported in myocardial infarction and acute ischaemic stroke.[24,25] It has been shown

that in some pathological conditions, in addition to changes in the levels of HDL-C subfractions, there could also be structural and functional changes in HDL-C particles, resulting in "dysfunctional HDL-C" molecules which are more pro-atherogenic.[26] It is therefore intuitive to hypothesize that the presence of two *APOL1* risk alleles causes a conformational change in the HDL-C-3 particle that prevents its conversion to HDL-C-2 by cholesteryl ester transfer protein, thereby halting the process of reverse cholesterol transfer which is the primary anti-atherogenic role of the HDL-C particle.

The frequency of the no risk allele, 1 risk allele and two risk alleles in our study among controls was 25/41 (60.98 %), 12/41 (29.2 7%) and 4/41 (9.76 %), respectively, while the frequency among patients with HAN was 36 (46.2 %), 33 (42.3 %) and 9 (11.5 %), respectively. A lower frequency of G2 variants was seen when all the other causes of CKD were combined 7/121 (5.79 %). The observed allele frequencies in the control patients in our study is similar to that reported by Kasembeli et al.[4]

Limitations

The small number of patients who had hypertension-attributed CKD in our study may have reduced the power of the analysis.

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Ethics Approval and consent to participate in the study

This study was approved by the Human Research and Ethics Committee (HREC) of the

University of the Witwatersrand, Johannesburg, South Africa; clearance certificate number M160614. All participants' gave written informed consent before enrolment

Availability of data or material

The dataset used in the analysis is available with the corresponding author and will be released on request.

Conflict of Interest

No competing interests in relation to this study.

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Authors' Contribution

S.O. Oguntola, R. Duarte and S. Naicker were involved in study design. S.O. Oguntola, M.O. Hassan, A. Vachiat, P. Manga, G. Paget and S. Naicker participated in data collection. K Moodley, T. Dix-Peek, C. Dickens, S.O. Oguntola and R. Duarte were involved in laboratory analysis of samples. Data analysis was done by S.O. Oguntola. The first draft of the manuscript was prepared by S.O. Oguntola and all authors reviewed the manuscript.

References

1. Foster MC, Coresh J, Fornage M, Astor BC, Grams M, Franceschini N, et al. *APOL1* variants associate with increased risk of CKD among African Americans. J Am Soc Nephrol 2013; 24: 1484-1491

2. Genovese G, Friedman DJ, Ross MD, Lecordia L, Uzureau P, Freedman BI, et al. Association of trypanolytic *APOL1* variants with kidney disease in African americans. Science. 2010; 329: 841-845
3. Ko WY, Rajan P, Gomez F, Scheinfeldt L, An P, Winkler CA, et al. Identifying Darwinian selection acting on different human *APOL1* variants among diverse African populations. Am J Hum Genet 2013; 93(1): 54-66
4. Kasembeli AN, Duarte R, Ramsay M, Mosiane P, Dickens C, Dix-Peek T, et al. *APOL1* risk variants are strongly associated with HIV-associated nephropathy in Black South Africans. J Am Soc Nephrol. 2015; 26(11): 2882-2890.
5. Kopp JB, Nelson GW, Sampath K, Johnson RC, Geniovese G, An P, et al. *APOL1* genetic variants in focal segmental glomerulosclerosis and HIV-associated nephropathy. J Am Soc Nephrol 2011; 22: 2129-2137
6. Papeta N, Kiryluk K, Patel A, Sterken R, Kacak N, Snyder HJ, et al. *APOL1* variants increase risk for FSGS and HIVAN but not IgA nephropathy. J Am Soc Nephrol 2011; 22: 1991-1996
7. Lipkowitz MS, Freedman BI, Langefeld CD, Comeau ME, Bowden DW, Kao WHL, et al. Apolipoprotein L1 gene variants associate with hypertension-attributed nephropathy and the rate of kidney function decline in African Americans. Kidney Int. 2013; 83(1): 114-120
8. Parsa A, Kao WH, Xie D, Astor BC, Li M, Hsu CY, et al. *APOL1* risk variants, race and progression of chronic kidney disease. N Engl J Med 2013; 369(23): 2183 -2196
9. Mukamal KJ, Tremaglio J, Friedman J, Ix JH, Kuller LH, Russell T, et al. *APOL1* genotype, kidney and cardiovascular disease and death in older adults. Arterioscler Thromb Vasc Biol. 2016; 36(2): 398-403

10. Ito K, Bick AG, Flannick J, Friedman DJ, Genovese G, Parfenov MG, et al. Increased burden of cardiovascular disease in carriers of *APOL1* genetic variants. Circ Res. 2014; 114(5): 845-850
11. McClellan W, Warnock DG, McClure L, Campbell RC, Newsome BB, Howard V, et al. Racial difference in the prevalence of chronic kidney disease among participants in the reasons for geographical and racial differences in stroke (REGARDS) Cohort study. J Am Soc Nephrol 2006; 17: 1710-1715
12. Go AS, Chertow GM, Fan D, McCullouch CE, Hsu CY. Chroic kidney disease and the risks of death, cardiovascular events and hospitalization. N Engl J Med. 2004; 351(13): 1296-1305
13. Wright JT Jr, Kusek JW, Toto RD, Lee JY, Agodoa LY, Kirk KA, et al. Design and baseline characteristics of participants in the African American Study of Kidney Disease and Hypertension (AASK) Pilot Study. Control Clin Trials, 1996. **17**(4 Suppl): 3S-16S
14. Nasiri H, Forouzandeh M, RasaeeMJ, Rahbarizadeh F. Modified salting-out method: High yield, high-quality genomic DNA extraction from whole blood using laundry detergent. J Clin Lab Anal 2005; 19: 229-232
15. Gutierrez OM, Limou S, Lin F, Peralta CA, Kramer HJ, Carr JJ, et al. *APOL1* nephropathy risk variants do not associate with subclinical atherosclerosis or left ventricular mass in middle-aged black adults. Kidney Int. 2017; doi: 10.1016/j.kint.2017.08.019
16. Pollak MR, Genovese G, Friedman DJ. *APOL1* and kidney disease. Curr Opin Nephrol Hypertens 2012; 21: 179-182
17. Camont L, Chapman MJ, Kontush A. Biological activities of HDL-C sub-populations and their relevance to cardiovascular disease. Trends Mol Med 2011; 17: 594-603

18. Davidson WS, Silva RA, Chantepie S, Lagor WR, Chapman MJ, Kontush A. Proteomic analysis of defined HDL-C subpopulations reveals particle-specific protein clusters: Relevance to antioxidative function. Arterioscler Vasc Biol. 2009; 29: 870-876
19. Norata GD, Pirillo A, Ammirati E, Catapano AL. Emerging role of high density lipoproteins as a player in the immune system. Atherosclerosis 2012; 220: 11-21
20. Drew BG, Rye KA, Duffy SJ, Barter P, Kingwell BA. The emerging role of HDL-C in glucose metabolism. Nat Rev Endocrinol 2012; 8: 237-245
21. Turkmen K, Tonbul HZ, Toker A, Gaipov A, Erdur FM, Cicekler H, et al. The relationship between oxidative stress, inflammation, and atherosclerosis in renal transplant and end-stage renal disease. Renal Failure 2012; 34(10) 1229-1237
22. Boaz M, Matas Z, Biro A, Katzir Z, Green M, Fainaru M, Smetana S. Serum malondialdehyde and prevalent cardiovascular disease in haemodialysis. Kidney Int. 1999; 56: 1078-1083
23. Kannel WB, McGee DL. Diabetes and glucose tolerance as risk factors for cardiovascular disease. The Framingham study. Diabetes Care 1979; 2: 120-126
24. Blackburn P, Lemieux I, Lamarche B, Bergeron J, Perron P, Tremblay G. Angiographically-assessed coronary artery disease associates with HDL-C particle size in women. Atherosclerosis 2012; 223: 359-364
25. Zeljkovic A, Vekic J, Spasojevic-Kalimanovska V, Jelic-Ivanovic Z, Bogavac-Stanojevic N, Gulan B, e al. LDL-C and HDL-C subclasses in acute ischaemic stroke: prediction of risk and short-term mortality. Atherosclerosis 2010; 210: 548-554
26. Kontush A, Chapman MJ. Functionally defective high-density lipoprotein: a new therapeutic target at the crossroads of dyslipidaemia, inflammation and atherosclerosis. Pharmacol Rev 2006; 58: 342-374

CHAPTER 9

DISCUSSION

Among the main findings in this study, were the high prevalence of AsVD disease among stage 3 CKD, dialysis patients and KTRs, with a very high prevalence of AsVD among PD patients associated with increased levels of inflammatory and lipoprotein biomarkers and APOL1 risk variants in kidney disease patients.

Chronic kidney disease, a group of heterogeneous kidney disorders, is characterized by relentless decline in glomerular filtration rate (GFR) and has diverse consequences.[1] Progressive decrease in GFR has been associated with an astronomical rise in the risk of death, cardiovascular disease (CVD) and hospitalization.[1] A study reported that elderly CKD patients have a near 3-fold risk of death instead of progressing to end-stage kidney disease (ESKD).[2] Cardiovascular disease is 10-20 times more likely in ESKD patients and has been shown to be responsible for approximately 1 out of every 2 deaths. The contribution of AsVD to death among ESKD patients on maintenance haemodialysis was evaluated in a longitudinal study; it was found that AsVD was responsible for three-fifths of deaths in this group.[4] Amira et al[5] found higher prevalence of carotid plaques among black South African haemodialysis patients (38.1 %) compared to controls (7.9 %).[5] These studies underscore the enormity of the contribution of AsVD to mortality among ESKD patients.

Traditional risk factors of CVD can explain AsVD in the general population, however, the finding of a high prevalence of CVD among CKD patients necessitated the need to explore the contributions of CKD-related risk factors in the aetiopathogenesis of CVD in CKD.

Atherosclerotic vascular disease occurs in the general population and is associated with traditional risk factors for CVD including age, cigarette smoking, alcohol consumption, hypertension, elevated fasting plasma glucose and deranged serum lipids.[6] The prevalence of carotid plaques was 7.9 % among black South African healthy controls.[5] Understandably,

AsVD is common among CKD patients with a significantly higher burden of disease compared to controls.[5,7] Savage et al[7] found a prevalence of 71 % among white ESKD patients compared to 21 % in healthy controls while Amira et al found a prevalence of 38.1 % among black ESKD compared to 7.9 % in healthy controls. The prevalence of AsVD was found to be 66.7% among kidney transplant recipients and significantly higher than prevalence in CKD control group.[8]

The traditional belief is that levels of inflammation are higher among HD patients compared to PD patients; and was supported by reports of increased levels of hs-CRP among ESKD patients initiated on HD compared to PD patients.[9] Several inflammatory biomarkers have been associated with chronic inflammation and AsVD in CKD and KTRs,[10] the most widely studied inflammatory biomarker in CKD is highly-sensitive C-reactive protein (hs-CRP).[11] The role of pentraxin-3 in CVD is now seen to be anti-atherogenic, supported by two landmark studies.[12,13]

Lipoprotein (a) [Lp (a)] is ten times more atherogenic than low density lipoprotein (LDL-C) and elevated plasma levels have been associated with premature, and increasing severity of atherosclerosis.[14-16] Lipoprotein phospholipoprotein A2 (Lp-PLA2) was an independent predictor of coronary heart disease among patients with low LDL-C and apolipoprotein A1 (APO A1) and has been found to be critical in reverse cholesterol transfer.[17,18]

The association between apolipoprotein L1 (*APOL1*) risk variants and hypertension-attributed nephropathy (HAN), HIV-associated nephropathy and focal segmental glomerulosclerosis (FSGS) has been demonstrated and may account for the increased prevalence of atherosclerosis in these populations.[19, 20]

In our study, we found a significantly higher prevalence of AsVD among stage 3 CKD, dialysis (PD and HD) patients and KTRs compared to controls, ($p < 0.01$). 70 % of the PD patients, 47.5% of stage 3CKD and HD patients and 46.3 % of KTRs had AsVD compared to 17 % of controls. This shows a huge burden among PD patients with almost three-quarters having

AsVD. Almost half of the stage 3CKD and HD patients and KTRs had AsVD compared to less than one-fifth in the control group. This is consistent with previous studies among ESKD patients.[4, 21, 22] Our study is the first in Africa to assess the burden of AsVD among stage 3 CKD, PD, HD patients and KTRs in a single study. Although the finding of high prevalence of AsVD in our stage 3 CKD and HD groups can be explained by the high prevalence of cardiovascular risk factors such as hypertension and reduced renal function among the kidney disease groups, we suggest that the very high prevalence of AsVD seen in PD patients may be associated with additional PD-related factors. Firstly, we suggest a synergistic interplay between high glucose exposure and increased levels of inflammation contributing to the high prevalence of AsVD among PD patients. Exposure of PD patients to high glucose concentrations via the use of glucose-containing PD fluids, in the presence of high levels of inflammation, could result in increased glucose absorption and consequent hyperinsulinaemia, which has been associated with hypertension, obesity, dyslipidaemia and glucose intolerance.[23] In the setting of chronic hyperinsulinaemia, experimental evidence supports marked increase in lipogenesis in white adipose tissue and liver.[24] Our findings of higher prevalence of increased TC and LDL-C among PD patients compared to stage 3 CKD and HD patients further buttressed the possibility of hyperinsulinaemia among PD patients. Secondly, we found significantly higher levels of serum creatinine and urea among PD patients compared to HD and stage 3 CKD patients; the lower levels of uraemic retention solutes in HD patients seen in our study may be explained by the effectiveness of current HD procedures in the clearance of uraemic toxins using biocompatible membranes and high flux dialyzers. Thirdly, higher uraemic solute retention among PD patients may likely connote the presence of high levels of uraemic toxins such as middle molecules and protein-bound solutes among the PD patients which have been associated with CVD.[25-27] Bammens et al[25] reported that increasing the PD dose may compensate for declining residual renal function by increasing elimination of water

soluble uraemic solutes but not middle molecules like p-cresol. Several studies have demonstrated an association between uraemic toxins such as indoxyl sulphate, p-cresyl sulphate and CVD.[26,27] Both total and free indoxyl sulphate and p-cresyl sulphate were found to be independently associated with structural and functional markers of CVD.[27] Further studies on the relationship of chronic inflammation and uraemic toxins to AsVD in ESKD among black Africans will be required to establish this association.

The finding of a high burden of AsVD among PD patients may predispose this group of patients to higher cardiovascular and all-cause mortality. A study on the baseline predictors of mortality in the two modalities of dialysis among rural-dwelling ESKD patients in Limpopo, South Africa, found PD as an independent predictor of mortality.[28] Similarly, CVD was found to be more prevalent among ESKD patients on PD (57 %) compared to 44 % among patients on HD, ($p < 0.001$); in addition, the risk of death was found to be higher among patients initiated on PD than in patients on HD, (HR 2.08, 95 % CI – 1.67-2.59; $p < 0.001$).[29]

Statistically significant differences were found in clinical and echocardiographic cardiovascular risk factors when each of the kidney disease groups was compared with controls. Of particular importance are systolic blood pressure (SBP), mean arterial blood pressure (MABP), waist-hip ratio (WHR), eGFR, non-HDL-C cholesterol (non-HDL-C), left atrial diameter (LAD), left ventricular mass index (LVMI) and carotid intima media thickness (CIMT) which showed a near consistent value of $p < 0.001$ when the kidney disease groups were compared with controls. These findings are consistent with those documented in previous studies designed to evaluate the CVD risk factors in CKD.[30, 31] In addition, when KTRs and controls were stratified based on AsVD, we found an association between some lipoprotein ratios including Castelli indices 1 and 2, non-HDL-C, atherogenic index (AI) and lipoprotein combined index (LCI) and AsVD. Castelli 2 also correlated with CIMT among KTRs. In our study population, serum fibrinogen was significantly increased among KTRs compared to

controls and was also significantly higher among participants who had AsVD compared to those without AsVD. Atherosclerotic vascular disease is currently viewed as an inflammatory disease and serum levels of fibrinogen have been influenced by several risk factors of CVD such as hypertension, diabetes and inflammation.[32,33]

Age (> 40 years) and LVH independently predicted AsVD among ESKD patients after adjusting for gender, WHR, systolic hypertension, LDL-C and TG. We found that LVH confers a 49-fold risk of AsVD among our ESKD patients. This highlights the robust relationship between LVH and AsVD among our ESKD group and also underscores the importance of LVH as a CVD risk factor and the need to direct treatment strategies towards LVH reduction among CKD patients.

Age and KTR status were independent predictors of AsVD after correcting for WHR, proteinuria, GFR, Castelli index 2 and LVH. Among KTRs, only age > 40 years predicted AsVD even when adjusted for LVH, duration of dialysis and post-transplant duration. Increasing age has been demonstrated to be an important predictor of vascular injury[34] and atherosclerosis has been shown to be associated with changes in CIMT early in the post-kidney transplant period.[35]

We evaluated the relationship of inflammatory markers to AsVD in our study and found significantly higher levels of pentraxin-3 among stage 3 CKD, PD and HD patients, and KTRs compared to controls. Similarly, the levels of hsCRP were significantly increased in PD, HD and in stage 3 CKD patients; higher levels were also demonstrated among KTRs compared to controls but was not statistically significant. In addition, levels of TNF-α and EN-RAGE were higher in all the kidney groups compared to controls but were significantly higher in PD and KTRs respectively. Our findings suggest that chronic inflammation in kidney disease starts

long before CKD patients became dialysis-requiring, and persists with ESKD and in the post-transplant period. Similar findings were reported in a cross-sectional study in HD and PD.[36,37]

When levels of pentraxin-3 were compared between PD and HD patients, we found significantly higher levels among PD patients; higher levels of other inflammatory markers (hsCRP, TNF-α, EN-RAGE) were documented in PD compared to HD patients. This contradicts findings from a previous study which found higher levels of pentraxin-3 among HD compared with PD patients.[10] It also contradicts the traditional dogma of inflammation being more severe in HD compared to PD.[9] The lower levels of inflammatory markers found among HD patients in our study could be as a result of effectiveness of haemodialysis in removing toxic molecules using high-flux biocompatible membranes. This is a plausible explanation for the lower levels of inflammatory markers found in HD but may not explain the significantly higher levels of inflammatory markers among PD which may be due to some PD-related factors; firstly, insertion of peritoneal catheter and filling of peritoneum with PD fluid triggers inflammatory responses in the peritoneum.[38] A study found an increase in the levels of inflammatory markers after initiation of PD, with persistent rise in levels of inflammatory markers with increasing duration of exposure to PD.[38] Secondly, persistent exposure of peritoneal mesothelial cells to glucose-based peritoneal dialysis fluid triggers inflammatory responses which can self-persist by continuous activation of inflammatory markers.[39,40] Similarly, formation of glucose degradation product (GDP) which occurs during heat sterilization of glucose-based PD fluid has been implicated in the morphological changes seen in the peritoneum of patients treated with glucose-based PD fluids.[41] Therefore, the use of bioincompatible dialysate combination of low P^H, high lactate, glucose-induced high osmolarity which can precipitate GDP in our glucose-based PD fluid can explain the high level of inflammation among our PD patients.

The finding of a high prevalence of AsVD among the kidney disease patients (CKD stage 3, PD, HD and KTRs) compared to controls and the significantly higher levels of inflammatory markers demonstrated among the kidney disease patients in our study suggest a possible central role for inflammation in the pathogenesis of AsVD among CKD patients.

When the association between pentraxin-3 levels and kidney function was evaluated, we found an inverse correlation. This is similar to result from previous studies.[10, 42] A study among stage 3 and 4 CKD and ESKD patients demonstrated that increase in pentraxin-3 levels was related to decreased kidney function.[42]

Our study demonstrated a correlation between pentraxin-3 and CIMT among KTRs but not among stage 3 CKD, PD or HD. This is similar to findings in previous studies.[10,42]

Lipoprotein biomarkers (Lp (a) and Lp-PLA2) were elevated in all kidney disease groups compared to controls in our study; significant increase was seen among PD, HD and the combined kidney disease group while lower levels of APO A1 were seen in all kidney disease groups. This study found an inverse correlation between Lp (a) levels and eGFR and an increasing strength of association with worsening eGFR, which suggests defective clearance of Lp (a) with deteriorating renal function.

Predictors of AsVD were age (> 40 years), male gender, low HDL-C cholesterol and elevated Lp (a) in this study; this is consistent with findings from several other studies.[43,44,45] The residence time of Lp (a) protein components (apo(a) and Apo B) was evaluated among haemodialysis patients; prolonged residence time was observed among haemodialysis patients compared to controls, this finding was explained by defective clearance of Lp (a) from the kidney because the kidney is involved in the catabolism of Lp (a), though via yet to be unravelled mechanism.[45] This prolonged residence time was thought to contribute to the atherogenic risk of Lp (a).[45] Similarly, the relationship between low HDL-C and AsVDin our

study is consistent with findings in previous studies.[46,47] In a large study among more than 45,000 haemodialysis patients without history of CVD, incident MI was inversely associated with HDL-C.[46] Statins have lipid lowering effect on TC and LDL-C and at the same time increases the HDL-C; the effect of statins on HDL-C may be beneficial in our study population. The SHARP (Study of Heart and Renal Protection) study, which recruited over 9000 of predialytic CKD and ESKD on dialysis, found that combination of statin and ezetimibe was associated with significant reduction of the relative risk of major atherosclerotic events, (HR 0.83, 95 % CI 0.74 – 0.94).[48] Although the 4D study found no benefit in the use of atorvastatin for preventing cardiovascular events; the cohort recruited into the 4D study were diabetic patients on HD however our study excluded diabetes patients.[48]

Limitations of the study

The exclusion of the elderly and diabetic patients from this study could have reduced the overall prevalence of AsVD in the different patient groups investigated.

We did not assess serum concentrations of insulin and insulin resistance in these patients; these could have helped to confirm hyperinsulinaemia as a risk factor for AsVD among CKD and especially in PD patients.

The cross-sectional nature of this study allowed for measurements of the various parameters at a single point; a longitudinal study will provide data on the evolution of atherosclerosis over the period of CKD and dialysis.

The short exposure time on dialysis might have also contributed to the disparities seen between the PD and HD cohorts

The small number of patients who had hypertension-attributed CKD in our study may have reduced the power of the analysis.

Conclusions

AsVD was common among all kidney disease patients and most prevalent among PD patients compared to pre-dialysis CKD, KTRs and HD patients.

Inflammation was present in all the kidney disease groups: CKD stage 3, PD, HD and persists in the post transplant period. Pentraxin-3 levels may increase with worsening renal function. Pentraxin-3 correlated positively with other inflammatory markers (except for hsCRP) and negatively with eGFR ($r = -0.171$, $p = 0.030$) and serum albumin levels ($r = -0.168$, $p = 0.033$) when the kidney disease groups (stage 3 CKD and ESKD and KTRs) were combined. Pentraxin-3 correlated positively with CIMT among KTRs ($r = 0.336$, $p = 0.032$), but there was no correlation between hsCRP and CIMT in any of the kidney disease groups. Predictors of high pentraxin-3 levels were hypertension, elevated total cholesterol, TNF-α and EN-RAGE levels

The levels of Lp (a) and Lp-PLA2 were increased in all the kidney disease groups with the strongest association seen among PD patients. The levels of APO A1 were lower in all kidney disease patients. The burden of abnormal lipid biomarkers was worse among ESKD patients with the highest burden in PD patients. Kidney transplant recipients had a better lipid profile compared to ESKD patients. Lipoprotein (a) independently predicted AsVD so conscious therapeutic effort directed at lowering Lp (a) levels may be beneficial in CKD patients.

Age (> 40years), male gender, low HDL-C and elevated Lp (a) independently predicted AsVD. In addition, the presence of *APOL1* 2 risk alleles predicted AsVD among patients with hypertension-attributed kidney disease.

Recommendations

We recommend routine evaluation of CKD patients, HD and PD patients and KTRs for cardiovascular disease including the assessment of carotid intima media thickness. We also

recommend a more frequent assessment of cardiovascular disease among male CKD patients who are older than 40 years.

We recommend that the use of more biocompatible PD fluid could be beneficial in reducing the prevalence and magnitude of inflammation among PD patients.

We also recommend calculation of the lipoprotein ratios, particularly Castelli index 2 and lipoprotein combined index because they correlate better with cardiovascular disease than traditional lipid profile parameters among KTRs.

In view of the findings from our study and the outcomes from the landmark trials on th usefulness of statins in preventing cardiovascular events in CKD and ESKD cited above, we recommend that use of statins in the non-dialysis group may be beneficial.

Similarly, we suggest that a periodic assay of Lp (a) levels among CKD patients, probably starting from KDOQI stage 2 but with more emphasis in ESKD patients, particularly those on PD.

We recommend incorporation of *APOL1* genotyping into routine CKD and pre-transplant screening of patients with HAN for targeted follow-up of the high risk group may be beneficial.

We recommend further research in a longitudional cohort, to study the evolution of lipid disorders and inflammation and their impact on AsVD, and to evaluate whether therapy may modulate the burden of AsVD.

References

1. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events and hospitalization. N Engl J Med 2004; 351: 1296-1305

2. Santos J, Fonseca I, Malheiro J, Beirao I, Lobato L, Oliveira P, et al. End-stage renal disease versus death in a Portuguese cohort of elderly patients: an approach using competing event analysis. J Investig Med. 2017; 65(7): 1041-1048
3. Amann K, Tyralla K, Gross ML, Eifert T, AdamczakM, Ritz E. Special characteristics of atherosclerosis in chronic renal failure. Clin Nephrol. 2003; 60(1): 13-20
4. Lindner A, Charra B, Sherrard DJ Scribner BH. Accelerated atherosclerosis in prolonged maintenance hemodialysis. N Engl. J Med. 1974; 290: 697-701
5. Amira OC, Naicker S, Manga P, Sliwa K, Mia A, Raal F, et al. Adiponectin and atherosclerosis risk factors in African haemodialysis patients: A population at low risk for atherosclerotic cardiovascular disease. Hemodialysis Int. 2012; 16(1): 59-68
6. Guan Y, Yu C, Shi M, Ni J, Wu Y, Gu H, et al. The associations between elevated plasma glucose levels and carotid intima media thickness in non-diabetic adults: A population-based cross-sectional study. Oncotarget 2017; 8(67): 111053-111063
7. Savage T, Clarke AL, Giles M, Tomson CR, Raine AE. Calcified plaques is common in carotid and femoral arteries of dialysis patients without clinical vascular disease. Nephrol Dial Transplant. 1998; 13: 2004-20
8. Cader RA, Zakaria NI, Yaacob Y, Shah SA. Carotid intima-media thickness in kidney transplant recipients. Hong Kong Journal of Nephrology 2016; 19: 36-41
9. Yong K, Dogra G, Boudville N, Lim W. Increased inflammatory response in association with initiation of haemodialysis compared with peritoneal dialysis in a prospective study of end-stage kidney disease patients. Perit Dial Int. 2018; 38(1): 18-23
10. Turkmen K, Tonbul HZ, Toker A, Gaipov A, Erdur FM, Cicekler H, et al. The relationship between oxidative stress, inflammation, and atherosclerosis in renal transplant and end-stage renal disease. Ren Fail 2012; 34 (10)1229-1237

11. Stuveling EM, Hilege HL, Bakker SJ, Gans RO, De Jong PE, De Zeeuw D. C-reactive protein is associated with renal function abnormalities in a non-diabetic population. Kidney Int 2003; 63:654-661
12. Salio M, Chimenti S, De Angelis N, Molia F, Maina V, Nebuloni M, et al. Cardioprotective function of the long pentraxin PTX3 in Acute myocardial infarction. Circulation. 2008; 117: 1055-1064
13. Norata GD, Marchesi P, Pulakazhi venu VK, Pasqualini F, Anselmo A, Moalli F, et al. Deficiency of the long pentraxin PTX3 promotes vascular inflammation and atherosclerosis. Circulation. 2009; 120: 699-708
14. Lawn RM. Lipoprotein(a) in heart disease. Sci Am. 1992; 266(6): 54-60
15. Scanu AM, Fless GM. Lipoprotein(a); heterogeneity and biological relevance. J Clin invest. 1990; 85(6): 1709-1715
16. Rosengren A, Wilhelmsen L, Eriksson E, Risberg B, Wedel H. Lipoprotein(a) and coronary heart disease: a propective case-control study in a general population sample of middle aged men. BMJ. 1990; 301(6763): 1248-1251
17. Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Heiss G, et al. Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein and risk for incident coronary heart disease in middle-aged men and women in the Atherosclerotic Risk in Communities (ARIC) study. Circulation 2004; 109: 837-842
18. Mukhamedova N, Escher G, D'Souza W, Tchoua U, Grant A, Krozowski Z, et al. Enhancing apolipoprotein A1-dependent cholesterol efflux elevates cholesterol export from macrophages in vivo. J Lipid Res. 2008; 49(11):2312-2322
19. Papeta N, Kiryluk K, Patel A, Sterken R, Kacak N, Snyder HJ, et al. ApoL1 variants increase risk for FSGS and HIVAN but not IgA nephropathy. J Am Soc Nephrol 2011; 22: 1991-1996

20. Lipkowitz MS, Freedman BI, Langefeld CD, Comeau ME, Bowden DW, Kao WHL, et al. Apolipoprotein L1 gene variants associate with hypertension-attributed nephropathy and the rate of kidney function decline in African Americans. Kidney Int. 2013; 83(1): 114-120
21. Freercks R, Swanepoel C, Carrara H, Moosa S, Lachman A, Rayner B. Vascular calcification in South African dialysis patients: Ethnic variation, prevalence, detection and haemodynamic correlates. Nephrology 2012; 17: 607-615
22. Japichino GG, Bonati L, Rubini P, Capocasale E. Prevalence of atherosclerosis in renal transplant recipients. Minerva Cardioangiol. 2001; 49(4) 229-238
23. Modan M, Halkin H, Almog S, Lusky A, Eshkol A, Shefi M, et al. Hyperinsulinaemia: A link between hypertension obesity and glucose intolerance. J. Clin. Invest. 1985; 75: 809-817
24. Assimacopoulos-Jeannet F, Brichard S, Rencurel F, Cusin I, Jeanrenaud B. In-vivo effects of hyperinsulinaemia on lipogenic enzymes and glucose transporter expression in rat liver and adipose tissue. Metabolism 1995; 44(2): 228-233
25. Bammens B, Evenepoel P, Verbeke K, Vanrenterghem Y. Time profiles of peritoneal and renal clearances of different uraemic solutes in incident peritoneal dialysis patients. Am J Kid. Dis. 2005; 46(3): 512-519
26. Schmidt S, Westhoff TH, Krauser P, Zidek W, Van der Giet M. The uraemic toxin phenylacetic acid increases the formation of reactive oxygen species in vascular smooth muscle cells. Nephrology Dialysis Transplantation. 2008; 23(1): 65-71
27. Rossi M, Campbell K, Johnson D, Stanton T, Pascoe E, Hawley C, et al. Uraemic toxins and cardiovascular disease across the chronic kidney spectrum: An observational study. Nutrition, Metabolism and cardiovascular diseases 2014; 24: 1035-1042

28. Tamayo-Isla RA, Ameh O, Mapiye D, Swanepoel CR, Bello AK, Ratsela AR, et al. Baseline Predictors of mortality among predominantly rural dwelling end-stage renal disease patients on chronic dialysis therapies in Limpopo, South Africa. PLoS ONE 2016; 11(6): e0156642. Doi: 10.1371/journal.pone.0156642

29. Yang F, Khin L, Lau T, Chua H, Vathsala A, Lee E. Haemodialysis versus peritoneal dialysis: a comparison of survival outcomes in South-East Asian patients with end-stage renal disease. PLOS ONE 2015; doi: 10.1317/journal.pone.0140195

30. Parikh NI, Hwang S, Larson MG. Cardiovascular disease risk factors in chronic kidney disease. Overall burden and rates of treatment and control. Arch Intern Med. 2006; 166(17): 1884-1891

31. Burmeister JE, Mosmann CB, Costa VB, Saraiva RT, Grandi RR, Bastos JP. Prevalence of cardiovascular risk factors in haemodialysis patients- The CORDIAL Study. Arq. Bras. Cardiol. 2014; 102(5): 473-480

32 Lee AJ, Lowe GD, Woodward M, Tunstall-Pedoe H. Fibrinogen in relation to personal history of prevalent hypertension, diabetes, stroke, intermittent claudication, coronary heart disease and family history: The Scottish Heart Health Study. Br Heart J 1993; 69: 338-342.

33 Ridker PM. Inflammation, atherosclerosis and cardiovascular risk: an epidemiologic view. Blood Coagul Fibrinolysis 1999; 10 Suppl 1: S9-S12

34 Lyons D, Roy S, Patel M, Benjamin N, Swift CG. Impaired nitric oxide-mediated vasodilatation and total body nitric oxide production in healthy old age. Clinical Science. 1997; 93: 519-525

35 Nafar M, Khatami F, Kardavani B, Farjad R, Pour-Reza-Gholi F, Firoozan A. et al. Atherosclerosis after kidney transplantation: changes of intima media thickness of carotids during early post-transplant period. Urol J. 2007; 4(2): 105-110

36 Xu Y, Ding X, Zou J, Liu Z, Jiang Z, Xu S, et al. Plasma Pentraxin-3 is associated with cardiovascular disease in haemodialysis patients. Ren Fail, 2011; 33(10): 998-1004

37 Palomar-Fontanet R, Lavin-Gomez BA, Quintanar-Lartundo JA, Garcia-Unzueta MT, Gago-Fraile M, Torrealba-Rodriguez MI, et al. Markers of inflammation before and during peritoneal dialysis. Adv Perit Dial. 2011; 27: 28-32

38 Farhat K, Van Ittersum FJ, Ter Wee PM, Paauw NJ, Beelen RHJ, Douma CE. Initiation of peritoneal dialysis in the first weeks after catheter insertion: A comparison of a neutral P^H, low GDP PD fluid and a conventional PD fluid. Clin Nephrol 2018; 89(2): 75-82

39 Li XY, Wu J, Luo D, Chen WX, Zhu GL, Zhang YX, et al. Effect of high glucose-based peritoneal dialysis fluid on NLRP3-IL-1β in peritoneal mesothelial cells. Beijing Da Xue Xue Bao Yi Xue Ban 2017; 49(6): 954-960

40 Chu Y, Wang Y, Zheng Z, Lin Y, He R, Liu J, et al. Pro-inflammatory effect of high glucose concentrations on HMrSV5 cells via the autocrine effects of HMGB1. Front. Physiol. 2017; 8:762. Doi: 10.3389/fphys.2017.00762

41 Johnson DW, Clarke M, Wilson V, Woods F, Brown FG. Rationale and design of the balANZ trial. A randomised controlled trial of low GDP, neutral P^H versus standard peritoneal dialysis solution for the preservation of residual renal function. BMC Nephrol. 2010; 11:25 doi: 10.1186/1471.2369-11-25

42 Tong M, Carrero JJ, Qureshi AR, Anderstam B, Heimburger O, Barany P, et al. Plasma pentraxin-3 in patients with chronic kidney disease: Associations with renal function, protein-energy wasting, cardiovascular disease and mortality. Clin J Am Soc Nephrol 2007; 2:889-897

43 Bajaj A, Damrauer SM, Anderson AH, Xie D, Budoff MJ, Go AS, et al. Lipoprotein (a) and risk of myocardial infarction and death in chronic kidney disease. Findings from

the Chronic Renal Insufficiency Cohort (CRIC) Study. Atherosclerosis, Thrombosis and Vascular Biology. 2017 doi:10.1161/ATVBAHA.117.309920

44 Rofieian-Kopaei M, Nasri H. Serum lipoprotein (a) and atherosclerotic changes in haemodialysis patients. J Renal Inj Prev.2013; 2(2): 47-50

45 Frischmann ME, Kronenberg F, Trenkwalder E, Schaefer JR, Schweer H, Dieplinger B. In vivo turn over study demonstrates diminished clearance of Lp (a) in haemodialysis patients. Kidney Int. 2007; 71: 1036-1043

46 Shoji T, Masakane I, Watanabe Y, Iseki K, Tsubakihara Y, Committee of Renal Data Registry, Japanese Society for Dialysis Therapy. Elevated non-High-density lipoprotein cholesterol (non-HDL-C-C) predicts atherosclerotic cardiovascular events in haemodialysis patients. Clin J Am Soc Nephrol. 2011; 6(5): 1112-1120

47 Lamprea-Montealegre JA, Astor BC, McClelland RL, De Boer IH, Burke GL, Sibley CT, et al. CKD, plasma lipids and common carotid intima-media thickness: results from the Multi-Ethnic Study of Atherosclerosis (MESA). Clin J Am Soc Nephrol. 2012; 7(11): 1777-1785

48 Wanner C, Schmidt K, Krane V. Results of the 4D study: ten years of follow-up. Clin Exp Nephrol 2014; 18: 274-277

APPENDIX 1

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APPENDIX 2

Ethics Clearance certificate

R14/49 Dr Oguntola Stephen Olawale et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M160614

NAME: (Principal Investigator)	Dr Oguntola Stephen Olawale et al
DEPARTMENT:	Medicine Division of Nephrology Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Relationship of Chronic Inflammatory Markers and Dyslipidemia to Atherosclerotic Vascular Disease in Different Categories of Chronic Kidney Disease Patients
DATE CONSIDERED:	24/06/2016
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Saraladevi Naicker
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	17/08/2016

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004, 10th floor, Senate House/3rd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in June and will therefore be due in the month of June each year.

Principal Investigator Signature

Date 18th September, 2016

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX 3

University of the Witwatersrand

The Subject Information Sheet and informed consent form

Part A

STUDY NUMBER: M160614

TITLE: The relationship of chronic inflammatory markers and dyslipidemia to atherosclerotic vascular disease in different categories of chronic kidney disease

INVESTIGATOR: Dr. Stephen Olawale Oguntola

INSTITUTION: University of the Witwatersrand, Internal Mmedicine

TELEPHONE NUMBER: Mobile – 0781489295

Landline (Departmental) – 011-488-3672

Part B

Introduction:

Good day, My name is Stephen Oguntola. I am a medical doctor, training as a kidney doctor at the Johannesburg Hospital in the department of Internal Medicine. I am also a PhD student with the University of Witwatersrand. I am the principal investigator of this inflammation and lipid study research. However, before you participate in this study, I would like to make sure you read all the information about the study and fully understand what the study is all about. If you do not read or understand English, an interpreter will be provided for you.

Purpose of the study

Patients with chronic kidney disease (CKD) are at high risk of developing cardiovascular (heart) disease. Heart disease is the commonest cause of death in CKD patients. Traditional and non-traditional factors have been implicated in the cause of vascular disease in chronic kidney disease patients. Heart disease also causes significant health problems in kidney disease patients. Systemic inflammation and lipid abnormalities play a major role in the development of vascular diseases (atherosclerosis) among CKD patients. This study is designed to investigate the relationship of inflammation and dyslipidemia to the development of cardiovascular disease in different categories of CKD patients and also to evaluate the possibility of a surrogate biomarker for heart disease in CKD patients.

Participation:

Your participation in this study is entirely voluntary. Before you agree to take part, it is important that you read and understand the explanation of the purpose of this study and the procedures that will be performed. This information sheet will help you decide if you would like to take part or not. If you have any questions, do not hesitate to ask me. If you do agree to take part, you are still free to withdraw from the study at any time and this will not be held against you. If you decide to take part, you will be asked to sign this document to confirm that you understand the nature of the study and agree to take part and you will be given a copy to keep. Should you decide to take part in this study; each study visit will not take more than 3 hours of your time.

Procedures:

If you agree to take part in the study; you will be asked to sign the informed consent form, you will be examined, and your weight, height, waist circumference and blood pressure will be taken. You will require imaging studies of your neck blood vessels (carotid arteries) and your

heart. These are painless procedures. You will also have some blood drawn which will be about 2 teaspoons.

Benefits:

The benefit of this study is that a full lipid study will be carried out on your blood, this can help to determine patients that are at high risk of heart disease. The ultrasound (sonar) of your neck blood vessels could also help identify patient at risk of more sinister disease like stroke.

Possible risks:

Drawing blood is normally done as a part of routine medical care. It will cause some pain at the site and some physical discomfort.

Confidentiality:

All the information we will get during the course of this study, including your clinic records, will be kept strictly confidential. The information may be reported in the research report and scientific journals. Anonymity will be strictly ensured by using codes to represent the participants so that no result can be traced to you. Only the researcher will have access to your information.

Ethical consideration:

This study has been approved by the Human Research Ethics Committee (HREC). It is supported by the Division of Nephrology. There will not be any conflict of interest on my part.

Source of additional information

All the study participants will be under the care of Dr. Stephen Oguntola who will be reachable 24 hours on this number; 0781489295.

Part C

Informed Consent:

I hereby confirm that I have been informed by the study investigator, Dr. Stephen Oguntola, about the nature, conduct, benefits and possible risks of the study. I have also received, read and understood the participant information sheet regarding the study. I am aware that the results of the study will be anonymously processed in a study report and that I may, at any stage without prejudice withdraw my consent and participation in the study. I had sufficient opportunity to ask questions and of my own free will have declared myself prepared to participate in the study.

PARTICIPANT (Printed name): …….

..... **.....**

Signature **Date**

Witness:..... **.....** **.....**

(Study nurse)Printed name **Signature** **Date**

I, Dr. Stephen O. Oguntola, herewith confirm that the above participant has been fully informed about the nature and conduct of the above study.

STUDY INVESTIGATOR

....

Printed name **Signature** **Date**

Part D

Study information sheet and consent for storage of biological specimens for future testing

Hello again. As discussed with you earlier during the study informed consent process, we asked for your permission to take some of your blood. This will be for preparing serum and extracting DNA. We plan to store these samples for future testing and will keep these samples for a maximum of 15 years.

We plan to use the DNA for future genetic studies to see how changes in your genes could be linked to the development of heart disease when one has CKD. One gene of interest is the APOL1 gene. There are versions of this gene that have been linked to increased severity of the kidney disease and onset of associated heart problem.

We will use the serum to look at different molecules in the blood that could increase the bad lipids in the body and cause one to develop heart disease.

Your biological samples will be processed and stored at the Department of Internal Medicine research laboratory, area 553, Blue block , CMJAH, University of the Witwatersrand, At the conclusion of the study any remaining samples will continue to be stored in a secured research laboratory within the Department of Internal Medicine, University of the Witwatersrand. The samples will be stored catalogued in ultra low temperature freezers. These freezers are under automated temperature control and network linked. Any changes in temperature will be fed to several members of the laboratory staff for immediate attention. The freezers have CO2 back and are linked to an electrical circuit that has generator backup. The freezers are housed in a restricted access laboratory. Before we use any of your stored samples we will get permission of the University of the Witwatersrand Ethics Committee to do research and make sure all your

information is kept confidential. The biological samples will be used for no other purposes than those that have been described to you. In the future if there may be other tests that we would like to do on your samples we will only do those if we are given the permission by the Human Research Ethics Committee of the University of the Witwatersrand.

It is optional to consent to the storage of your biological specimens and if you don't want us to do so you may still take part in the study without being prejudiced.

If you have any questions, please do not hesitate to ask me.

We remind you that your participation in this study is entirely voluntary and you can refuse or withdraw at any time. Your withdrawal will not affect your access to medical care in any way. This study has been approved by the Human Research Ethics Committee (HREC). It is supported by the Division of Nephrology. There will not be any conflict of interest on my part.

Informed Consent:

I hereby confirm that I have been informed by the study investigator, Dr. Stephen Oguntola, about the nature, conduct, benefits and possible risks of the study. I have also received, read and understood the participant information sheet regarding the study. I am aware that the results of the study will be anonymously processed in a study report and that I may, at any stage without prejudice withdraw my consent and participation in the study. I had sufficient opportunity to ask questions and of my own free will have declared myself prepared to participate in the study.

PARTICIPANT (Printed name): …….

..... **.....**

Signature **Date**

Witness:..... **.....** **.....**

(Study nurse)Printed name **Signature** **Date**

I, Dr. Stephen O. Oguntola, herewith confirm that the above participant has been fully informed about the nature and conduct of the above study.

STUDY INVESTIGATOR

..... **.....** **.....**

Printed name **Signature** **Date**

APPENDIX 4

University of the Witwatersrand

The Control Information Sheet and informed consent form

Part A

STUDY NUMBER: M160614

TITLE: The relationship of chronic inflammatory markers and dyslipidemia to atherosclerotic vascular disease in different categories of chronic kidney disease

INVESTIGATOR: Dr. Stephen Olawale Oguntola

INSTITUTION: University of the Witwatersrand, Internal Mmedicine

TELEPHONE NUMBER: Mobile – 0781489295

Landline (Departmental) – 011-488-3672

Part B

Introduction:

Good day. My name is Stephen Oguntola. I am a medical doctor, training as a kidney doctor at the Johannesburg Hospital in the department of Internal Medicine. I am also a PhD student with the University of Witwatersrand. I am the principal investigator of this lipid study research. However, before you participate in this study, I would like to make sure you read all the information about the study and fully understand what the study is all about. If you do not read or understand English, an interpreter will be provided for you.

Purpose of the study

Patients with chronic kidney disease (CKD) are at high risk of developing cardiovascular (heart) disease. Heart disease is the commonest cause of death in CKD patients. Traditional and non-traditional factors have been implicated in the cause of vascular disease in chronic kidney disease patients. Heart disease also causes significant health problems in kidney disease patients. Systemic inflammation and lipid abnormalities play a major role in the development of vascular diseases (atherosclerosis) among CKD patients. This study is designed to investigate the relationship of inflammation and lipid disorders to the development of cardiovascular disease in different categories of CKD patients and also to evaluate the possibility of a surrogate biomarker for heart disease in CKD patients.

Why are you needed to participate in the study:

Whenever scientific research is conducted, it is important to compare the patients with the disease being evaluated (in this case CKD) with normal healthy people. This is the reason why you have been approached to be part of this study.

Participation:

Your participation in this study is entirely voluntary. Before you agree to take part, it is important that you read and understand the explanation of the purpose of this study and the procedures that will be performed. This information sheet will help you decide if you would like to take part or not. If you have any questions, do not hesitate to ask me. If you do agree to take part, you are still free to withdraw from the study at any time and this will not be held against you. If you decide to take part, you will be asked to sign this document to confirm that you understand the nature of the study and agree to take part and you will be given a copy to keep. Should you decide to take part in this study; each study visit will not take more than 3 hours of your time.

Procedures:

If you agree to take part in the study; you will be asked to sign the informed consent form, you will be examined, and your weight, height, waist circumference and blood pressure will be taken. You will require imaging studies of your neck blood vessels (carotid arteries) and your heart. These are painless procedures. You will also have some blood drawn which will be about 2 teaspoons.

Benefits:

The benefit of this study is that a full lipid study will be carried out on your blood sample this can help to determine patients that are at high risk of heart disease. The ultrasound (sonar) of your neck blood vessels could also help identify patient at risk of more sinister disease like stroke.

Possible risks:

Drawing blood is normally done as a part of routine medical care. It will cause some pain at the site and some physical discomfort.

Confidentiality:

All the information we will get during the course of this study, including your clinic records, will be kept strictly confidential. The information may be reported in the research report and scientific journals. Anonymity will be strictly ensured by using codes to represent the participants so that no result can be traced to you. Only the researcher will have access to your information.

Ethical consideration:

This study has been approved by the Human Research Ethics Committee (HREC). It is supported by the Division of Nephrology. There will not be any conflict of interest on my part.

Source of additional information

All the study participants will be under the care of Dr. Stephen Oguntola who will be reachable 24 hours everyday on this number; 0781489295.

Part C

Informed Consent:

I hereby confirm that I have been informed by the study investigator, Dr. Stephen Oguntola, about the nature, conduct, benefits and possible risks of the study. I have also received, read and understood the participant information sheet regarding the study. I am aware that the results of the study will be anonymously processed in a study report and that I may, at any stage without prejudice withdraw my consent and participation in the study. I had sufficient opportunity to ask questions and of my own free will have declared myself prepared to participate in the study.

PARTICIPANT (Printed name): …….

....

Signature Date

Witness:..... ....

(Study nurse)Printed name Signature Date

I, Dr. Stephen O. Oguntola, herewith confirm that the above participant has been fully informed about the nature and conduct of the above study.

STUDY INVESTIGATOR

..... **.....** **.....**

Printed name **Signature** **Date**

Part D

Study information sheet and consent for storage of biological specimens for future testing

Hello again. As discussed with you earlier during the study informed consent process, we asked for your permission to take some of your blood. This will be for preparing serum and extracting DNA. We plan to store these samples for future testing and will keep these samples for a maximum of 15 years.

We plan to use the DNA for future genetic studies to see how changes in your genes could be linked to the development of heart disease when one has CKD. One gene of interest is the APOL1 gene. There are versions of this gene that have been linked to increased severity of the kidney disease and onset of associated heart problem.

We will use the serum to look at different molecules in the blood that could increase the bad lipids in the body and cause one to develop heart disease.

Your biological samples will be processed and stored at Department of Internal Medicine research laboratory, area 553, Blue block, CMJAH, University of the Witwatersrand. At the conclusion of the study any remaining samples will continue to be stored in a secured laboratory within the Department of Internal Medicine, University of the Witwatersrand. The samples will be stored catalogued in ultra low temperature freezers. These freezers are under automated temperature control and network linked. Any changes in temperature will be fed to

several members of the laboratory staff for immediate attention. The freezers have CO2 back and are linked to an electrical circuit that has generator backup. The freezers are housed in a restricted access laboratory. Before we use any of your stored samples we will get permission of the University of the Witwatersrand Ethics Committee to do research and make sure all your information is kept confidential. The biological samples will be used for no other purposes than those that have been described to you. In the future if there may be other tests that we would like to do on your samples we will only do those if we are given the permission by the Human Research Ethics Committee of the University of the Witwatersrand.

It is optional to consent to the storage of your biological specimens and if you don't want us to do so you may still take part in the study without being prejudiced.

If you have any questions, please do not hesitate to ask me.

We remind you that your participation in this study is entirely voluntary and you can refuse or withdraw at any time. Your withdrawal will not affect your access to medical care in any way. This study has been approved by the Human Research Ethics Committee (HREC). It is supported by the Division of Nephrology. There will not be any conflict of interest on my part.

Informed Consent:

I hereby confirm that I have been informed by the study investigator, Dr. Stephen Oguntola, about the nature, conduct, benefits and possible risks of the study. I have also received, read and understood the participant information sheet regarding the study. I am aware that the results of the study will be anonymously processed in a study report and that I may, at any stage without prejudice withdraw my consent and participation in the study. I had sufficient opportunity to ask questions and of my own free will have declared myself prepared to participate in the study.

PARTICIPANT (Printed name): …….

....

Signature Date

Witness:..... ....

(Study nurse)Printed name Signature Date

I, Dr. Stephen O. Oguntola, herewith confirm that the above participant has been fully informed about the nature and conduct of the above study.

STUDY INVESTIGATOR

....

Printed name Signature Date

APPENDIX 5

DATA COLLECTION SHEET

Serial number …….

BIODATA

Name: Hosp no:

Age: Phone no:

Sex: Religion:

Occupation: Educational status:

Weight: Height: BMI:

Waist Circ.: Hip Circ.:

Marital status: Date:

Address:

Predialysis Yes / No How many years……..

HD Yes / No How many years.....

PD Yes / No How many years……..

Post-kidney transplant Yes / No How many years? **……**

Cause of CKD……..

Post-transplant duration…….

HISTORY

Intermittent claudication Yes/No

Recurrent precordial chest pain Yes/No

(Radiates to the jaw/shoulder tip Yes/No

Fatigue Yes/No

orthopnea Yes/No

PND Yes/No

PAST MEDICAL HISTORY **DURATION (**if yes**)**

Hypertension Yes/No

Diabetes Yes/No

Heart disease Yes/No

Peripheral arterial disease Yes/No

Cancer Yes /No

PAST SURGICAL INTERVENTION FOR THE CONDITIONS BELOW

Stroke Yes / No

AMI/IHD Yes / No

PAD Yes / No

CARDIOVASCULAR EXAMINATION

Pulse rate : Volume Rhythm

Blood pressure: (sitting) (standing)

Jugular venous pressure: Elevated/Not elevated

Apex beat: (location/character)

Left parasternal heave:

Palpable sounds/Thrills:

Heart sounds: murmurs Pericardial friction rub

LAB INVESTIGATIONS:

Blood

Hb/ WCC

U&E, Cr:

Ca^{2+}: Phos:

Total Protein: Alb:

TC: TG: LDL-C: HDL-C:

Lp (a): Apo A1: Lp-PLA2:

Pentraxin-3: hs-CRP: TNF-α: EN-RAGE: IL-6:

Fibrinogen:

ApoL1 genotype:

Urine

UACR:

APPENDIX 6

HycultBiotech Human hsCRP ELISA procedure

1. Standard sera were diluted 1:1000 by pipetting 10µl of each standard into separate dilution tubes and 990µl of diluted sample dilution buffer was added and mixed carefully.
2. The patients' samples were diluted in two consecutive steps
3. 100µl of the diluted calibrators and samples were pipette into each of a pair of adjacent wells
4. The plate was covered and the microtitre strips was incubated for 30 minutes at room temperature
5. The microtitrestrips was washed three times with washing solution.
6. 100µl of conjugate solution was added to the micrititrestrips and incubated for30 minutes at room temperature
7. Microtitrestrips washing was repeated three times with washing solution
8. 100µl of chromogen solution was added to each well
9. It was incubated for 10 minutes at room temperature and covered with a foil to avoid light exposure
10. 50µl of stopping solution was added to each well.
11. Absorbance of each well was determined at 450nm immediately following the addition of acid

APPENDIX 7

Magnetic Luminex Assay: Human premixed multi-analyte kit

PRINCIPLE OF THE ASSAY

Magnetic luminex assay multiplex kits were designed for use with the luminex MAGPIX CCD imager. Alternatively, kits can be used with the luminex 200 or Bio-Rad, Bio-Plex, dual laser, flow-based sorting and detection platform.

Analyte-specific antibodies were pre-coated onto colour-coded magnetic microparticles. Microparticles, standards and samples were pippetted into wells and the immobilized antibodies bind the analytes of interest. After washing away any unbound substances, a biotinylated antibody cocktail specific to the analytes of interest was added to each well. Following a wash to remove any unbound biotinylated antibody, streptavidin-phycoerythrin conjugate (Streptavidin-PE), which binds to the biotinylated antibody was added to each well. A final wash removes unbound streptavidin-PE, the microparticles were re-suspended in a buffer and read using the BIORAD Bioplex 200 analyzer. A magnet in the analyzer captured and held the super paramagnetic microparticles in a monolayer. Two spectrally distinct light emitting diodes (LEDs) illuminate the beads. One LED identifies the analyte that is being detected and the second LED determines the magnitude of the PE-derived signal, which is in direct proportion to the amount of analyte bound. Each well is imaged with a CCD camera.

Appropriate dilution for each analyte was determined using the on-line link http;//www.RnDSystems.com/Products/LXSAHM.

APPENDIX 8

Salting out Procedure for extracting DNA from Human Nucleated Cells in Whole Blood.

Solutions Used:

1. Sucrose-Triton-X Lysis Buffer

	1 000 ml	500 ml	100 ml
1M Tris-HCL pH 8.0	10 ml	5 ml	1 ml
1M $MgCl_2$	5 ml	2.5 ml	0.5 ml
Triton X-100	10 ml	5 ml	1 ml
distilled water	1 litre	500 ml	100 ml
Autoclave and store at 4°C.			
Sucrose - *just before use*	109.5g.	54.75 g	10.95 g

   Once the sucrose has been added, the solution does not keep, so only make sufficient each batch. Need ± 70ml/ 10ml sample. Work on ice.

   [Sucrose: 7.7g sucrose per 70ml (i.e. one sample)]

2. T 20 E 5 (20mM Tris-HCl, 5mM EDTA)

   20 ml 1M Tris-HCL

   10 ml 0.5M EDTA

   Make up to 1 litre with dH_2O

   pH 8.0

   Autoclave and store at room temperature.

3. Saturated NaCl.

   Slowly add 40g NaCl to 100ml sterile water until completely saturated.

   Filter sterilize. Store at room temperature.

4. Proteinase K Master Mix.

   Stock solutions;

   - 10% SDS made in sterile H_2O
   - 0.5M EDTA made in sterile H_2O
   - 20mg/ml Proteinase K

Stock Solution	**[Final]**	**500µl** 1 sample	**8 mL** 16 samples	**10 mL** 20 samples
10% SDS	1% SDS	50 µl	800 µl	1 000 µl
0.5M EDTA	2mM EDTA	2 µl	32 µl	40 µl
20 mg/mL Proteinase K	2mg/mL Proteinase K	50 µl	800 µl	1 000 µl
H_2O	H_2O	400 µl	6 400 µl	8 000 µl

Make fresh each time. You need 500µl mix /blood sample. (make up in 15 ml tube)

5. TBE Stock Solution (10x). 10X TBE

108g Tris Base 54g Tris (Powder)

55g Boric Acid 27.5g Boric acid (Powder)

40ml 0.5 EDTA pH 8 4.65g EDTA (Powder)

Make up to 1 litre with H_2O Make up to 500 ml with distilled Water

6. TE Buffer (10 mM Tris-Cl, 1 mM EDTA)

10 ml 1M Tris-Cl, pH 7.5

2 ml 0.5M EDTA, pH 8.0

Make up to 1 litre with H_2O

7. Plastic ware:

2-3X 50 ml tubes per sample

2X 15 ml tubes per run

6X Blue tips per sample

4X yellow tips per sample

1-2X white tips per sample

1-2X Eppendorfs/sample

8. Other things needed

Autoclaved beaker for measuring out sucrose

Sterile magnetic flea for mixing sucrose in lysis buffer.

Autoclaved beaker for pouring lysis buffer

METHODS

Day 1

1. Pre-cool the centrifuge to 4°C.

2. Blood must be collected into EDTA or ACD tubes.
3. Use ±10ml blood, place in a 50ml tube and mix with ±40ml lysing solution.
   NB: Lysing solution must be kept cold during the procedure. Mix well. Work on ice.
4. Spin for 10 min at ± 2300 rpm (setting 5 on the Sorvall Centrifuge) at 4°C.
5. Decant supernatant and re-suspend pellet in ± 20ml lysing buffer. Work on ice.
6. Place on ice or at –20°C for 5 min.
7. Spin for a further 10 min at 2300 rpm (setting 5) at 4°C.
8. Decant supernatant and add 3 ml T20E5 to each tube. Mix well.
9. Add 200µl of 10% SDS to each and mix.
   Then, add 495 µl proteinase K mix, mix and place samples at 42°C -50°C overnight.

Day 2

10. Add 1 ml saturated NaCl solution to each tube. Mix well and place on ice for 5 min.
11. Spin at ± 2800 rpm (setting 5 ½ on the Sorvall Centrifuge) for 30 min, 4°C.
    If the supernatant is cloudy, decant into a clean tube and spin for a further 15- 20 min. The pellet consists of proteins precipitated by the salt and the DNA should be present in the supernatant.
12. Transfer the supernatant to a clean 50ml tube and add 20ml absolute ethanol at room temperature.
13. DNA should be visible as a white aggregate.
    If none is visible, place samples at *– 20°C overnight, or at -70°C for 3 hours. (See 13b below).
14. Otherwise, fish or spool the DNA out using a P200 gilson, wash gently in ice cold 70% ethanol (approx. 200ul). Do this twice.
15. Place in a clean eppendorf tube and allow ethanol to evaporate off at room temperature. Do not allow to over dry.
16. Dissolve DNA in an appropriate volume (~100ul) of TE buffer (or water) to a maximum of 1 ml. Put at 37°C to dissolve completely (can leave at 37°C/room temperature overnight to ensure DNA has dissolved.
17. Check DNA concentration on Nanodrop. Mix well before measuring concentration.

Day 3 (if DNA samples did not aggregate)

13b. Centrifuge samples in absolute ethanol at 2800 rpm (setting 5 ½ on the Sorvall Centrifuge), 4°C, 15 mins

13c. Pour off absolute alcohol. Add 1 ml of ice-cold 70% ethanol.

13d. Centrifuge at 2800 rpm, 4°C, 15 mins.

13e. Remove 70% Ethanol.

13f. Wash a second time with 1 ml ice-cold 70% ethanol.

13g. Centrifuge at 2800 rpm, 4°C, 15 mins. Remove ethanol (Blot excess ethanol on tissue paper). Allow pellet to dry (but not over dry).

13h. Add 60 µl TE buffer. Close lid. Allow DNA pellet to dissolve in TE overnight, RT°, shaker.

13i. Transfer to a labelled 1.5ml Eppendorf.

13j. Measure DNA concentration.

APPENDIX 9

***APOL1* DNA sequence**

>gi|568815576:36253071-36267531 Homo sapiens chromosome 22, GRCh38 Primary Assembly

ACCAACTCACACGAGGCATTGGGAAGGACATCCGTGCCCTCAGACGAGCCAGAGCC
AATCTTCAGTCAGTACCGCATGCCTCAGCCTCACGCCCCCGGGTCACTGAGCCAAT
CTCAGCTGAAAGCGGTGAACAGGTGGAGAGGGTTAATGAACCCAGCATCCTGGAAA
TGAGCAGAGGAGTCAAGCTCACGGATGTGGCCCCTGTA(A/G)GCTTCTTTCTTGTGC
TGGATGTAGTCTACCTCGTGTACGAATCAAAGCACTTACATGAGGGGGCAAAGTCA
GAGACAGCTGAGGAGCTGAAGAAGGTGGCTCAGGAGCTGGAGGAGAAGCTAAACA
T(T/G)CTCAACAATAATTATAAGATTCTGCAGGCGGACCAAGAACTGTGACCACAGG
GCAGGGCAGCCACCAGGAGAGATATGCCTGGCAG

Key:

area shaded pink – forward primer

area shaded blue backward primer

area shaded grey – G1 rs73885319 mutation,

area shaded green – G1 rs6091014 mutation

area shaded yellow – G2 insertion/deletion (rs71785313)

APPENDIX 10

RESTRICTION FRAGMENT LENGTH POLYMORPHISM PROCEDURE

Day 1

1.5µl tubes appropriately labeled, then a master mix was prepared with H_2O (3.75µl/sample), 10X buffer (1µl/sample) and restriction enzyme, NSPI and mLuCI, (0.25µl/sample) respectively. The master mix was properly mixed by up and down pipetting. Appropriate volume of the master mix was aliquoted into the labeled tubes, then 5µl of PCR product was added and mixed well. Then 15µl of mineral oil was added and centrifuged (quick spin), the solution is incubated on dry hot block overnight at 37^{o}C.

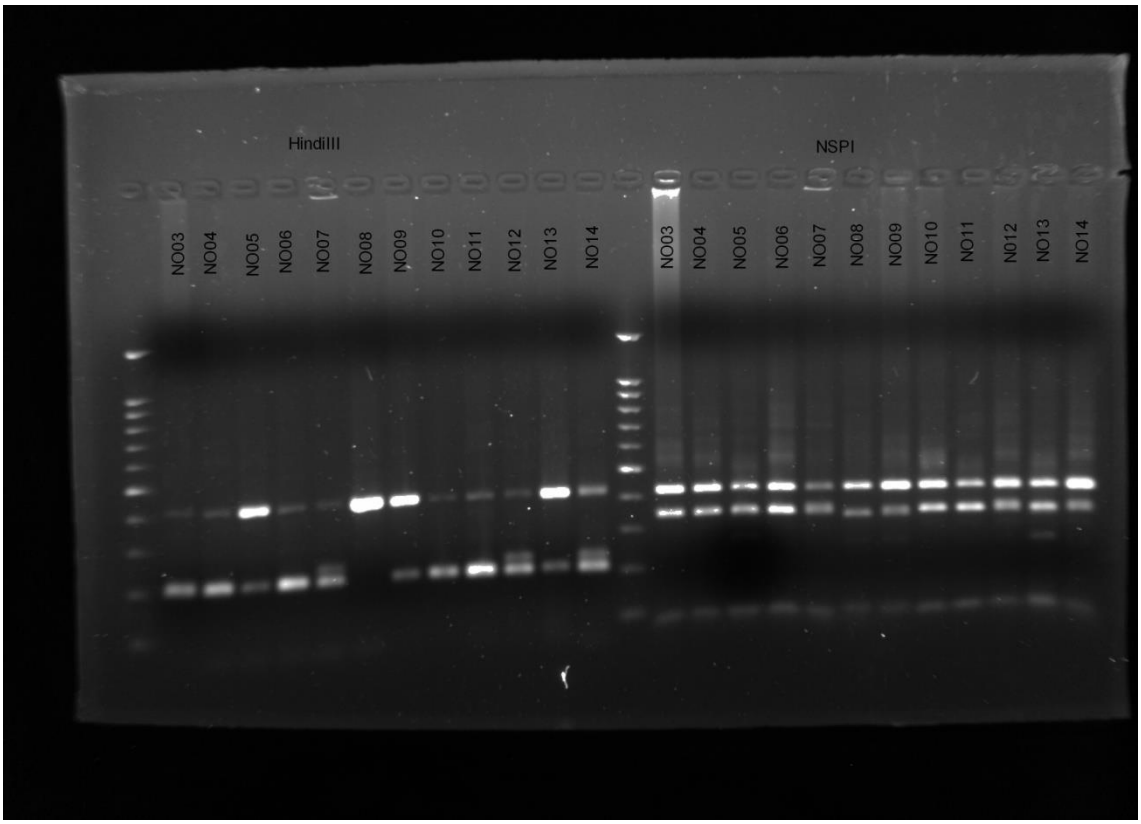
Day 2

The restriction was terminated by the addition of 2µl of ethylene diamine tetraacetic acid.

The above protocol was repeated with HindiIII as the restriction enzyme and incubated on hot block at 37^{o}C for 3 hours. Restriction was terminated by the addition of 2µl of ethylene diamine tetraacetic acid.

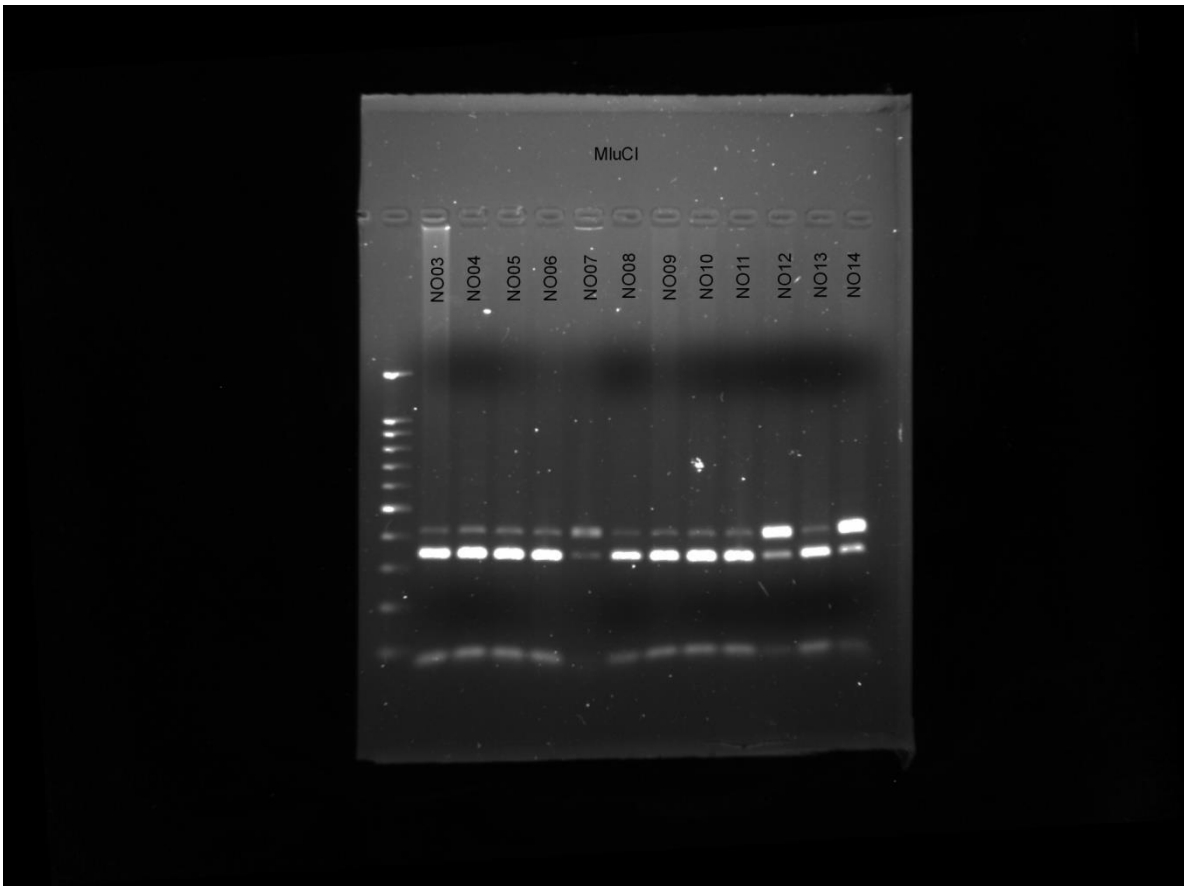
APPENDIX 11

Restriction fragment length polymorphism gel for HindI 111 and NspI



APPENDIX 12

Restriction fragment length polymorphism gel for mLUC1



APPENDIX 13

Dove Medical Press: Submission accepted for publication

1 March 2018 at 01:49

Ms Sandi McIver

<sandi@dovepress.com>
To: Dr Oguntola <olawaleogun1@gmail.com>
Reply | Reply to all | Forward | Print | Delete | Show original

Dear Dr Oguntola,

I am pleased to inform you that your submission, "Atherosclerotic vascular disease and its correlates in stable black South African kidney transplant recipients", has been accepted for publication in "International Journal of Nephrology and Renovascular Disease". The publication processing fee is now payable before your paper can be progressed any further and an invoice is accessible here:
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