CHRONIC KIDNEY DISEASE IN HIV POPULATIONS: PREVALENCE, RISK FACTORS AND ROLE OF TRANSFORMING GROWTH FACTOR BETA (TGF-β1) POLYMORPHISMS

BY

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1. Chronic kidney disease in the global adult HIV-infected population: A systematic review and meta-analysis**. PLoS One**. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0195443
2. Prevalence and correlates of chronic kidney disease (CKD) among ART-naïve HIV patients in the Niger-Delta region of Nigeria. **Medicine** (Baltimore). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5916672/
3. Prevalence and correlates of traditional risk factors for cardiovascular disease in a Nigerian ART-naïve HIV population: a cross-sectional study. **BMJ Open**. https://bmjopen.bmj.com/content/8/7/e019664.long
4. Urinary Transforming Growth Factor-Beta 1 (uTGF-β1) and prevalent chronic kidney disease risk in HIV-positive patients in West Africa (Accepted for publication with Kidney International Reports).
5. Association of Genetic Polymorphisms of *TGF-β1, HMOX1* and *APOL1* with CKD in Patients with HIV-related nephropathies in Extreme Southern Nigeria [submitted to American Journal of Kidney Disease (AJKD)].

Signed by candidate

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DEDICATION

This work is dedicated to the people who made the greatest sacrifice for this work to come to fruition – my wife (Edidiong Udeme Ekrikpo) and children (Inyeneabasi, Inimfonabasi and Ifiokabasi). You will grow to fulfill your God-given call on earth.

I also dedicate this work to all the HIV patients in Nigeria with Chronic kidney disease who daily go through the anxieties of living with HIV and CKD in a developing country.

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LIST OF ACRONYMS	
AKI	ACUTE KIDNEY INJURY
APOL1	APOLIPOPROTEIN-1
ART	ANTIRETROVIRAL THERAPY
BMP	BONE MORPHOGENIC PROTEIN
CD4+	CLUSTER DESIGNATION-4 POSITIVE
CG	COCKCROFT-GAULT
CKD	CHRONIC KIDNEY DISEASE
CKF-EPI	CHRONIC KIDNEY DISEASE EPIDEMIOLOGY COLLABORATION
CVD	CARDIOVASCULAR DISEASE
DM	DIABETES MELLITUS
ESRD	END-STAGE RENAL DISEASE
eGFR	ESTIMATED GLOMERULAR FILTRATION RATE
EDTA	ETHYLENE DIAMINETETRACETIC ACID
FSGS	FOCAL SEGMENTAL GLOMERULOSCLEROSIS
GFR	GLOMERULAR FILTRATION RATE
HMOX1	HEME OXYGENASE-1
HIVAN	HIV-ASSOCIATED NEPHROPATHY
HIVTMA	HIV-ASSOCIATED THROMBOTIC MICROANGIOPATHY
HIVICK	HIV IMMUNE COMPLEX DISEASE OF THE KIDNEY
HIV	HUMAN IMMUNODEFICIENCY VIRUS
IGFBP7	INSULIN-LIKE GROWTH FACTOR-BINDING PROTEIN

KIM-1	KIDNEY INJURY MOLECULE-1
L-FABP	LIVER-TYPE FATTY ACID BINDING PROTEIN
MDRD	MODIFICATION OF DIET IN RENAL DISEASE
NAG	N-ACETYL β-D-GLUCOSAMINIDASE
NGAL	NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN
NCDs	NON-COMMUNICABLE DISEASES
REC	RENAL EPITHELIAL CELLS
RTEC	RENAL TUBULAR EPITHELIAL CELLS
TAF	TENOFOVIR ALAFENAMIDE
TDF	TENOFOVIR DISOPROXIL FUMARATE
TIMP	TISSUE INHIBITOR OF METALLOPROTEINASE
TGF-β	TRANSFORMING GROWTH FACTOR-BETA
UTI	URINARY TRACT INFECTION
WHO	WORLD HEALTH ORGANIZATION

ADDENDA	
Appendix 1	Ethical approval certificate from the University of Uyo teaching hospital institutional health research ethics committee
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ABSTRACT

Background and purpose: With the advent of antiretroviral therapy, HIV-infected individuals now live longer and are at increased risk of chronic kidney disease (CKD). Also, recent studies indicate a genetic predisposition to CKD in the African HIV population. This work investigated the prevalence of CKD (and its correlates) in the global and local HIV population and proceeded to investigate the diagnostic utility of urinary transforming growth factor-beta-1 (TGF-β1) for CKD in the HIV population and determine the association between polymorphisms of *TGF-β1* gene and prevalent CKD.

Methods: A meta-analysis was performed to document the prevalence of CKD in the global HIV population. From the local HIV population in Nigeria, the prevalence of CKD and traditional risk factors for cardiovascular disease was determined. Using ELISA, TGF-β1 levels was assayed in the urine samples of HIV patients with or without CKD to investigate the ability of urinary TGF-β1 to diagnose early CKD. SNP genotyping of rs1800469, rs1800470, rs1800471, rs121918282 in *TGF-β1,* rs60910145 (*APOL1*), rs73885319 (*APOL1*), rs71785313 (*APOL1*) and rs743811 (*HMOX1*) was performed using predesigned TaqMan genotyping assays.

Results: Using meta-analytic methods, the global pooled CKD prevalence was 6.4% (95%CI 5.2–7.7%) with MDRD, and 4.8% (2.9–7.1%) with CKD-EPI. Among the WHO regions, Africa had the highest MDRD-based prevalence, 7.9% (5.2-11.1%) with the West African subregion carrying the heaviest burden, 14.6% (9.9-20.0%). Among the local HIV population, using the CKD-EPI equation, the prevalence of CKD was 13.4% (11.6-15.4%). Hypertension prevalence was 26.7% (25.5-28.0%); diabetes 5.6% (4.5-6.7%); obesity 8.3% (7.6-9.1%) and dyslipidaemia 29.1% (26.1-32.1%). HIV-infected individuals with CKD had significantly higher levels of urinary TGF-β1-creatinine ratio (uTGFβ1Cr) after controlling for potential confounding factors in regression models. However, within the CKD-HIV group, uTGFβ1Cr reduced as CKD stage worsened. The presence of *APOL1* genetic risk independently increased the risk of CKD (OR 2.54, 95% CI 1.44-4.51) in the HIV population while the *TGF-β1* SNP, rs1800470, appeared to have a protective effect (OR 0.44 (95% CI 0.20-0.97). There was no significant association between *HMOX1* SNPs and CKD occurrence.

Conclusion: There is a high prevalence of CKD (and other cardiovascular risk factors) in the adult HIV-population. Urinary TGF-β1 may be useful in the non-invasive detection of early CKD in the HIV population. Genetic testing may be used to predict the risk of CKD in the HIV population.

CHAPTER ONE: INTRODUCTION

About thirty years ago, it was first noticed that the Human Immunodeficiency Virus (HIV) may be a risk factor for Chronic Kidney Disease (CKD) (1). At that time, the focal and segmental glomerular lesions seen in HIV-positive patients was not only associated with massive proteinuria but had a rapid decline to End Stage Renal Disease (ESRD) and high mortality rates (1, 2). Within a few years, it was agreed that an entity with distinct sociodemographic, clinical and histologic features was a predominant cause of CKD in HIV patients. This entity, called HIV–associated Nephropathy (HIVAN), was noted to be the predominant cause of CKD in HIV patients of Black African origin; regarded as an AIDS–defining illness and had strong evidence of intra-renal HIV gene expression (3-5).

With the advent of Antiretroviral Therapy (ART), individuals with HIV disease are living longer (6). We now see a wide spectrum of renal diseases in HIV patients ranging from asymptomatic changes in renal function like proteinuria (7, 8), electrolyte losses (9) and acute kidney injury (AKI) (10) occurring as a result of diarrheal diseases prevalent among HIV patients to CKD. The renal histologic types in CKD patients with HIV disease are also quite varied. HIVAN, a form of focal segmental glomerulosclerosis (FSGS) with significant tubulointerstitial pathology is the most common histologic type encountered in HIV patients, especially in Black populations (11). There is also a variety of immune complex glomerulonephritides collectively known as HIV – immune complex kidney disease (HIVICK) which could account for as much as 31% of glomerular lesions in HIV patients with CKD (11, 12). HIV disease does not preclude the presence of other aetiologies of CKD. Non-collapsing FSGS, interstitial nephritis and diabetic nephropathy (and other vascular and metabolic effects on the kidneys) have been documented in HIV patients (13-15). Arterionephrosclerosis has been documented to be the most

common histologic diagnosis in HIV patients with no clinical indication for renal biopsy in the post-ART era (16). Also, the continued use of tenofovir and ritonavir-based ART has been shown to increase risk of development of CKD (17).

Among incident ESRD patients, HIV has been implicated as the main etiologic factor in 1% (USA); 0.36% - 0.67% (France)(18, 19); 0.54% - 1.15% (Spain) (20, 21) 6.6% (Cameroon) (22); and 28.5% (South Africa)(23). Depending on the definition of CKD in different HIV populations, the prevalence of CKD has been varied. In the United States, the prevalence in a cohort of HIV–positive women at baseline (before commencing ART) was 7.2% and this increased to 14% within 21 months of commencing treatment (24). In a predominant male HIV population in the United States, the prevalence was put at 8.5% (25) but could be as high as 15.5% (26). In Brazil, analysis of about 1,900 adult HIV patients revealed a CKD prevalence of 13.3% (27). It also appears duration on treatment and type of ART influences CKD prevalence. As much as 3.3% of HIV patients with normal estimated glomerular filtration rate (eGFR) may develop CKD in a median follow up period of 3.7 years (28). About 5% of HIV infected adults in China had eGFR< 60ml/min/1.73m^2(29).

The prevalence of CKD in HIV populations across Africa is not well documented despite having the highest worldwide burden of HIV. A study in South Africa recorded a prevalence of 7.3% (7) which is not too different from data in other parts of the world. A Kenyan study had a prevalence of 11.5% (30) while a relatively small study from Nigeria had a high prevalence of 24% (31). Another Nigerian study had 38% of their HIV cohort having either elevated serum creatinine or persistent proteinuria but was only able to biopsy ten individuals because of the lack of histopathological facilities (32, 33). Other Nigerian studies (31, 34, 35) have documented a prevalence of CKD of 38% - 53.5% depending on the definition of CKD but none had a sample size exceeding 400 for a condition such as HIV that is highly prevalent. Considering that HIV patients are living

longer, and that kidney disease is becoming a major cause of morbidity and mortality in this patient population it is important to have reliable data on the burden of CKD among HIV patients. The effect of rising CKD prevalence in the HIV population may become a difficult burden for an already fragile health system in most Sub-Saharan African countries.

1.1 RATIONALE FOR THIS STUDY

The burden of CKD continues to increase in developing countries through increasing non – communicable diseases (NCDs) and the huge burden of HIV. With the rising economic challenges and poverty in low- and middle-income countries (LMICs) and with poor health care facilities, the problems associated with the treatment of CKD/ESRD will be dire. Understanding the burden of CKD as well as risk factors for CKD in the large HIV population is an initial first step towards implementing health care strategies to combat these conditions. Moreover, the role of biomarkers (such as TGF-β) and other genetic factors (besides APOL1) have not been previously studied in Sub-Saharan African population even though there is evidence that they may be useful markers and may play a role in progression of CKD, respectively. The present study is therefore important as it will provide critical answers to questions around epidemiology and pathogenesis of CKD in the HIV population in Nigeria and beyond.

1.2 PATHOGENESIS OF HIV – RELATED NEPHROPATHIES

HIVAN, the commonest HIV – related nephropathy in Blacks, is believed to require the expression of HIV- 1 gene in the kidney for its pathogenesis (36) and HIV-1 genetic material has been detected in renal epithelial cells (RECs) even when undetectable levels of viral RNA have been achieved in plasma (37). The human RECs,

renal Tubular epithelial cells (RTECs) and renovascular cells lack CD4 receptors and the co-receptors CXCR4 and CCR5 (36, 38, 39), therefore the mode of entry of HIV into the RECs and RTECs remains unclear. Some have suggested the existence of kidney-tropic strains of HIV following observation of certain strains of HIV which are capable of infecting epithelial cells independent of the CD4 pathway (39). It is possible however to have a direct transfer of viral RNA from infected CD4+ T-cells to RTECs when there is stable cell-cell adhesion in the presence of heparin sulfate proteoglycans (40). In HIVAN, infection of the podocytes results in increased proliferation, apoptosis and dedifferentiation of the podocytes. There is also upregulation of genes encoding for inflammatory proteins leading to initiation of a cascade of inflammatory events leading to tubulointerstitial fibrosis (41).

HIV – immune complex kidney disease (HIVICK), on the other hand, is less common than HIVAN. While it has been established that viral expression alone in the RECs and RTECs are able to initiate the lesions seen in HIVAN, this cannot be said to be true with HIVICK (42). In HIVICK, active viral replication and immune response to viral proteins herald the onset of the renal lesions. HIVICK is typically associated with co-infection with Hepatitis C virus and is characterized by immune complex (Complement, HIV-1 antigens and reactive antibodies) deposition (43). The commonly implicated viral proteins, *Nef, Vpr and Tat* have been shown to initiate complex inflammatory response to their presence and Vpr alone has been shown to cause kidney inflammation (43). Clinically, HIVICK is also predominantly found in Blacks, have lower HIV RNA viral loads and higher CD4 counts than found in HIVAN and are more likely to have concurrent diabetes mellitus and hypertension than HIVAN patients (44). They are also less likely to progress to ESRD than HIVAN patients.

HIV-associated thrombotic microangiopathy (HIVTMA) is the most uncommon of the HIV related kidney diseases encountered in about 0.3% (45) of HIV patients having an incidence of 0.079 per 100 person–years of

follow up. It is said to be associated with lower CD4 counts, higher viral loads and co-infection with Mycobacterium Avium-intracellulare complex (45). The characteristic histologic feature of HIVTMA is a dearth of inflammatory cells with increased evidence of apoptosis of microvascular endothelial cells (46). Clinically, patients may present with low grade thrombocytopenia and mild renal insufficiency or as severe renal failure needing renal replacement therapy associated with neurologic deficits. Therapeutic plasma exchange appears to give some benefit for patients that develop HIVTMA (46).

1.3 KIDNEY DISEASE FOLLOWING TREATMENT OF HIV

The drug treatment of HIV/AIDS is also implicated in the development of both acute and chronic kidney disease. Acute Kidney Injury (AKI) could occur in as much as 5.7-10% of patients initiated on ART and is usually caused by both traditional factors known to cause AKI and the use of Cotrimoxazole (47-49). A number of these patients with AKI do progress to CKD on follow-up. Tenofovir disoproxil fumarate (TDF) is associated with severe AKI sometimes but more commonly its effect may be completely asymptomatic (48). The diagnosis of ART–induced CKD should be a diagnosis of exclusion, seeing the spectrum of conditions in HIV patients that can lead to CKD. Though the incidence of CKD attributable to ART use is relatively low, 2.7 to 7% (50), the numbers of those affected may place an increased demand for care in large HIV care programs in Sub-Saharan Africa. The commonly implicated antiretroviral medications causing CKD include TDF (51, 52), indinavir (53) and atazanavir (54). The risk of CKD while on ART increases in the presence of traditional risk factors for CKD e.g. hypertension, diabetes mellitus and concomitant use of nephrotoxic medications. Other identified risk factors include nadir CD4 count of less than 200 cells/mm^3, opportunistic infection and longer ART use (50). The combination of Ritonavir and TDF has been found to increase the risk of renal toxicity (55) and the Fanconi syndrome occurring 0.3 to 2% of patients on ART are mostly attributable to TDF (56). Acute

interstitial nephritis is known to occur in patients taking abacavir, indinavir, atazanavir and ritonavir (57). These observations now indicate the need for more collaboration between Primary HIV–care providers and Nephrologists for the purpose of early detection and treatment of renal toxicities in these patients. Assessment of renal function before beginning ART should be made mandatory in all HIV care facilities and protocol for regular renal function check instituted. Dose adjustment should be employed in patients where the offending drug cannot be substituted because of the overarching benefits of the medication. More recently, the development of Tenofovir Alafenamide (TAF) that has shown less toxicity and reduction in eGFR compared to TDF has brought hope for patients that really need Tenofovir (58). Long term data on TAF regarding renal safety is still being awaited.

1.4 TRADITIONAL RISK FACTORS FOR CKD IN THE HIV POPULATION

Traditional risk factors for CKD like hypertension, Diabetes Mellitus (DM), Obesity and Dyslipidemia also contribute to renal function decline in HIV patients. Whether there is a difference in prevalence of these metabolic syndrome factors in the HIV population compared to the general population has been investigated in different climes. A DM prevalence of 5.6% found in Guinea Bissau among ARV–naïve HIV patients was noted to be higher than the general population and occurring in younger individuals (59). Among Ethiopians with HIV, a very high prevalence of DM, 31%, has been reported compared to 8% in non-HIV infected Ethiopians despite relatively low BMI (60). This finding did not hold true in Puerto Rico where the 13.7% recorded among HIV patients was not significantly different from the 12.8% in the general population (61). The increased prevalence/incidence of DM in the HIV population may partly be due to dysglycemia caused by use of non-nucleoside reverse transcriptase inhibitors that constitute part of first line therapy for HIV (62). ART may be implicated in the higher prevalence of cardiovascular risk factors such as hypertension and dyslipidemia. The

prevalence of hypercholesterolaemia was 8 times higher in the group of patients on ART compared to the ART–naïve group in a Cameroonian study (63). Studies have found hypertension prevalence (26.5%) twice higher in the ART group compared to the ART–naïve group (63, 64) and obesity prevalence of 11% in ART patients compared to 2% in the ART–naïve patients (65). More traditional markers of cardiovascular risk like insulin resistance has been found to be higher in HIV patients than non-HIV controls (66).

1.5 RISK OF CKD OCCURRENCE IN HIV PATIENTS

Seeing that HIV cohorts round the world are living longer and therefore accumulating many other factors that could increase the risk of CKD (including age, hypertension, Diabetes Mellitus, dyslipidemia and use of ART), it would be important to predict the risk of development of CKD in this "at-risk" population. This led to the development of the D:A:D risk score for CKD progression in HIV patients (67). Unfortunately, the population used for the development of this score had only 7.2% of the study population being Black and the population used for validation was also predominantly Caucasian making it difficult to employ this risk score in Black HIV patients. Validation of this risk score in a large Black HIV positive population would be of immense help to HIV clinicians in predicting risk of CKD progression therefore creating an opportunity to influence modifiable risk factors for high risk HIV patients. Seeing that the HIV population is a high-risk group for the development of CKD, it is therefore imperative to clearly define CKD in this group.

1.6 CKD DEFINITION IN THE HIV POPULATION

Chronic Kidney Disease, on the most part, is defined using glomerular filtration rate (GFR). GFR can be measured directly or estimated using equations based on serum creatinine or Cystatin C levels. The accuracy of creatinine based estimated GFR (eGFR) in HIV populations has been a source of debate among researchers.

The more clinically established creatinine-based equations – the Cockcroft–Gault (CG); Modification of Diet in Renal Disease (MDRD) and CKD-EPI equations were derived from Western populations and have not been appropriately validated in Black Africans, much less in HIV–infected Black Africans. An attempt to validate these creatinine-based equations in Black South Africans suggested a removal of the race factor in the equations (68). This too will require validation with a larger population of Black individuals and different Black races. The lack of reliability of serum creatinine measurements may also affect eGFR calculated from creatinine–based equations. In HIV patients, there is a tendency of muscle wasting, which may lead to spuriously low serum creatinine levels and therefore overestimation of GFR if creatinine–based equations are employed. Conversely, the weight gain that occurs following ART therapy may lead to serum creatinine increases which may falsely suggest GFR reductions. Some have suggested that Cystatin–C based equations be employed but this too requires validation and is known to be affected by ongoing inflammation and HIV replication (69). The use of proteinuria (or persistent proteinuria) in HIV patients may also be problematic because of the increased incidence of urinary tract infection (UTI), including recurrent UTI in this patient population. While this debate about GFR estimation in HIV patients continues, most researchers and clinicians will continue to use the creatinine–based equations till studies show clear evidence for the use of another formula. Equations combining Serum creatinine and Serum Cystatin C have been shown to improve accuracy of CKD diagnosis better than serum creatinine or serum Cystatin C alone (70).The concern about serum creatinine-based formulae lies majorly in the fact that it may be too late to reverse changes in the kidneys by the time creatinine begins to rise in blood therefore missing the golden time for intervention. This has led to a flurry of research on urinary biomarkers that could predict CKD earlier in HIV patients.

1.7 URINARY BIOMARKERS OF CKD IN HIV PATIENTS

Research on urinary biomarkers in HIV has aimed at finding molecules that their urinary levels will help in the diagnosis of early AKI before the onset of serum creatinine increase; determine ongoing renal tubular damage of tubulotoxic ART agents and associated antibiotics or predict progression to CKD. One of such markers is α1–microglobulin (a marker of proximal tubular dysfunction) which has been found to predict GFR decline over a 10-year period in HIV–infected women (71) and may be better than β2–microglobulin (β2m), N- acetyl- β-d-glucosaminidase (NAG) and albumin/creatinine ratio in detecting TDF-induced tubular dysfunction in HIV men (72). Other biomarkers associated with GFR decline and/or all-cause mortality in HIV cohorts include Kidney injury molecule–1 (KIM-1), Neutrophil gelatinase–associated lipocalin (NGAL), Liver fatty acid binding protein (L-FABP) and Interleukin 18 (IL-18) (73, 74). These molecules have been extensively investigated in AKI and have shown promise for CKD prediction. However, cycle arrest urinary biomarkers like Tissue inhibitor of metalloproteinase 2 (TIMP-2) and Insulin-like growth factor–binding protein 7 (IGFBP7) appear to have performed better than KIM-1 and NGAL in predicting the development of moderate to severe AKI (70). Maybe a panel of these urinary biomarkers may be more helpful in predicting CKD progression than single biomarkers (73, 75) but this would significantly increase cost of care and may not be clinically practicable in resource-poor settings of Sub-Saharan Africa where the burden of HIV disease is highest. Many of these biomarkers are increased in urine when there is injury to renal tubular or epithelial cells but other biomarkers (such as transforming growth factor–beta [TGF-β]) may measure the degree of fibrosis occurring in the renal interstitium and could therefore perform better for predicting chronic damage to the kidneys.

TGF-β is a multifunctional peptide that has three isoforms - TGF-β1, TGF-β2, TGF-β3 and is part of the TGF-β superfamily of cytokines which consists of the isoforms of TGF-β, Bone Morphogenic Protein (BMP), Inhibin

and Activin (76). TGF-β1, the first discovered in 1983, the most abundant and probably the most effective (77), is a 25KDa peptide with 112 amino acid residues (78) derived from a larger protein precursor that has 390 amino acid residues (Pre-pro TGF-β). Pre-pro- TGF-β has a hydrophobic 29 amino acid signal sequence which is cleaved to obtain Pro- TGF-β (latent form of TGF-β1). This latent form of TGF-β1 is made up of TGF-β1 and Latency–associated peptide (LAP) which binds to LTBP (Latent TGF-β binding protein) in the target tissues (10). The carboxyl terminal 112 amino acid sequence that makes up TGF-β is cleaved from Pro- TGF-β at a dibasic cleavage site to release the active TGF-β1 when exposed to a variety of stimuli (10). TGF-β is ubiquitous and produced by virtually every cell in the body. All TGF-β share the same receptor but with differing affinity (79). TGF-β1 binds to a transmembrane receptor, TGF-β receptor II but exerts its effects only in the presence of TGF-β receptor I. It has been shown that both receptors must be present for its intracellular effect to be initiated (80). The actions of TGF-β1 hereafter, acting in a paracrine and autocrine way, is pleiotropic.

1.8 Pathogenesis of renal fibrosis by TGF-B

The discussion following will focus on the profibrotic and antifibrotic pathways with which TGF-β1 exerts its effect on the renal tissues. Figures 1 and 2 show the “canonical” pathway by which TGF-β1 leads to renal fibrosis.

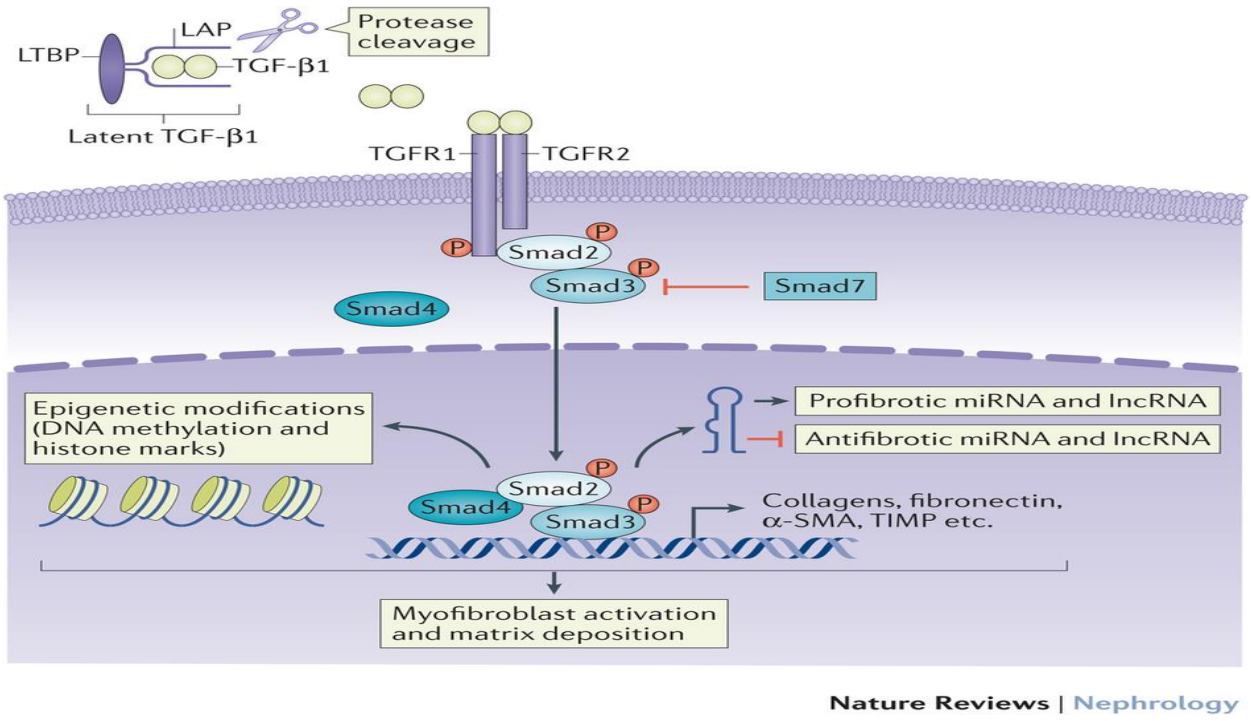

Figure 1: Meng XM et al. Nat Rev 2016 doi:10.1038/nrneph.2016.48 (Reproduced with permission)

***LTBP** – Latent TGF-β Binding Protein; **LAP** - Latency–associated peptide; **TGF-β1** – Transforming Growth factor – beta 1; **TGFR1** – Transforming Growth factor Receptor 1; **TGFR2** – Transforming Growth factor Receptor 2; **Smad** - Mothers Against Decapentaplegic, Drosophila*

Activated TGFR2 then activates TGFR1 which phosphorylates Smad2 and Smad3. These Smad proteins complex with Smad4 and exerts its influence in the nucleus by binding to gene promoters to induce production of profibrotic molecules which induce matrix deposition. Smad3 also causes transcription of profibrotic microRNA (miRNA) and long noncoding RNA (lncRNA and inhibits transcription of antifibrotic miRNAs (10).

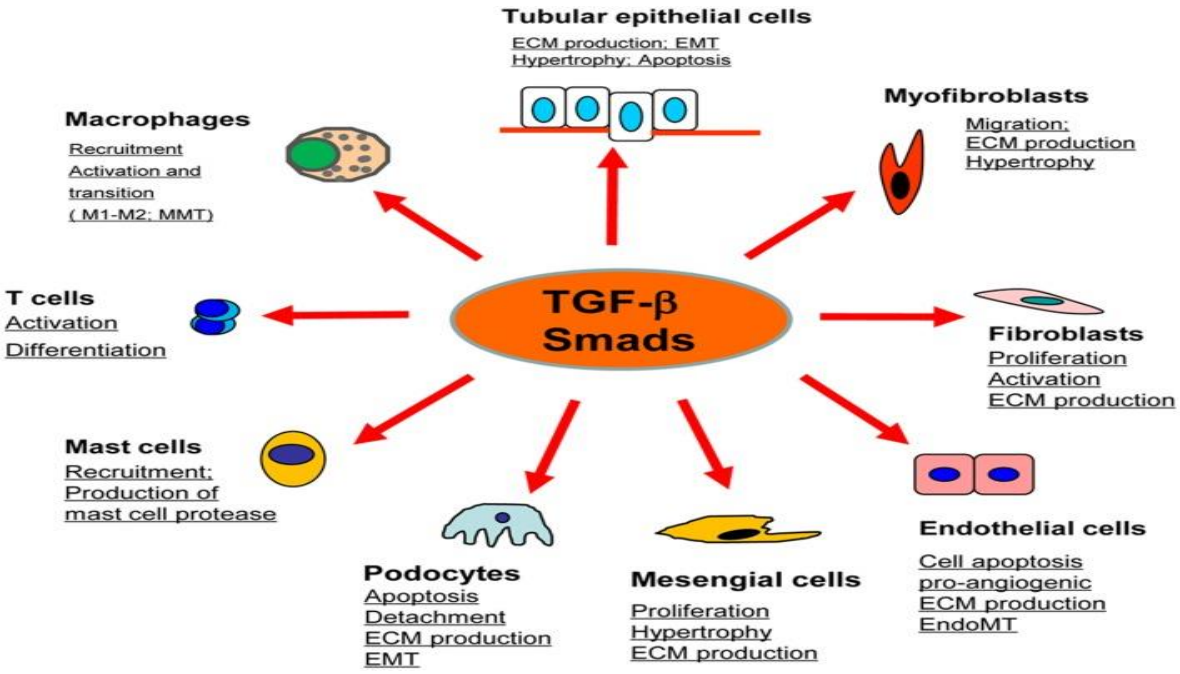

Figure 2: Meng XM, et al. Front Physiol. 2015; 6: 82. TGF-b in Renal fibrosis (Reproduced with permission)

This pathway is so important in the pathogenesis of renal fibrosis that researchers now believe that targeting the TGF-β/Smad 3 system may be one avenue of slowing down CKD progression (81).

1.9 URINARY TGF-β1 AS A POSSIBLE BIOMARKER FOR CKD IN HIV PATIENTS

Following from the above, studies have indicated increased urinary excretion of TGF-β in a variety of renal glomerular and lesions – Diabetic nephropathy (82, 83), Lupus Nephritis, IgA nephropathy, Rapidly progressive glomerulonephritis (RPGN), forms of Chronic glomerulonephritis and interstitial nephritis (84, 85). Some have been able to show correlation between serum creatinine and urinary TGF-β levels but correlation with degree of interstitial fibrosis has not been consistent.

More indirect evidence about the role of TGF-β in CKD progression abound. Individuals with IgA nephropathy treated with prednisolone have been observed to have reduced urinary TGF-β1 levels compared to pre-

treatment levels (86, 87). IgA nephropathy patients treated with Losartan also had their urinary TGF-β levels significantly reduced (88). More recently, elevated urinary TGF-β levels have also been demonstrated in HIVAN patients (89). An increased expression of TGF-β in Blacks compared to Caucasians has been well documented in end-stage renal disease and hypertensive patients (90-92). This increases our suspicion that TGF-β may be elevated in the urine of our Black patients with CKD and may play a central role in the increased burden of CKD in HIV positive Black patients. Therefore, accurate measurement of this cytokine in well collected urine samples may be useful in detecting early renal damage and predicting progression to CKD in HIV patients as it has been done in other glomerular and tubulointerstitial disease (93).

1.10 TGF-Beta POLYMORPHISMS IN AFRICANS AND THEIR ROLE IN KIDNEY DISEASES

Polymorphisms in the gene coding for TGF-β1 may also be associated with CKD. In an important study among Caucasians in the UK (94), single nucleotide polymorphisms (SNPs) in the TGFβ1 gene were found to be associated with progression of chronic kidney disease. Indeed, the study showed significant differences in the allele frequencies of C-509T (p=0.02) and Leu10Pro (p=0.038). There was also significant association between CKD progression and homozygosity for Arg25. Other studies among Diabetic patients have found association of the TGF-β1 gene polymorphisms (T869C, 915G>C, -800G>A, codon 10, T29C) with the development and/or progression of CKD in Caucasian and Asian populations (95-99). There is a dearth of TGF-β1 genetic polymorphism studies in relation to CKD in Sub-Saharan Africa.

1.11 *APOL1* and *HMOX1* GENE POLYMORPHISMS AND CHRONIC KIDNEY DISEASE PROGRESSION

APOL1 kidney disease risk alleles (*G1* and *G2*) are common throughout Sub-Saharan Africa especially West Africa and African Americans of West African descent (100). *APOL1* mediated kidney disease tends to follow a

recessive mode of inheritance with those with 2 alleles having greater risk of progression to end–stage kidney disease (100). A spectrum of CKD aetiologies (including HIV) have been associated with *APOL1* risk alleles. Indeed, the absence of these risk variants in some populations has been thought to be the reason for the low prevalence of HIVAN in Ethiopian communities (101). While this may be true, others have suggested that other genetic, viral and environmental factors should be sought as contributors to progression of CKD (102) among the HIV population as *APOL1* alone does not completely explain CKD progression in this special population. Finding an association between these *APOL1* risk variants and HIV–related kidney disease in Nigeria may be helpful in planning CKD prevention programs among HIV population in Nigeria and beyond.

Moreover, *HMOX1* forms the rate-limiting step in the catabolism of free heme (103), with two promoter polymorphisms, a GT-dinucleotide repeat (rs3074372) and a SNP (rs743811), associated with CKD (104, 105). Genetic variations in both *APOL1* and *HMOX1* have been associated with Sickle cell nephropathy among adult African Americans (104, 106, 107). In parts of Cameroon which has historical relationship with the part of Nigeria where this work was undertaken, borderline association has been found between *HMOX1* and microalbuminuria (108). The study of associations between these genomic variants, in *HMOX1* and the development of CKD has not yet been investigated in HIVAN.

Gene-gene interactions associated with CKD

It is believed that individuals with the *APOL1* genetic risk who do not develop CKD either have some protective factor or have not been exposed to additional risk that acts as a 'second hit'. These factors could either be genetic in nature or environmental.

Genetic factors documented to modify the risk of *APOL1* for ESRD include SNPs in the podocin gene, *NPHS2*, which reduce the risk of ESRD in *APOL1* associated nephropathy while the serologically defined colon cancer antigen 8 (*SDCCAG8)* increased the risk of ESRD in nondiabetic individuals (109, 110). However, no gene-gene interaction has ever been documented between *APOL1* and *TGF-β1* or *HMOX1* in any population. Indeed, a recent genome-wide association study (111) failed to identify any gene-gene interaction with *APOL1*, rather environmental factors (like HIV disease) appear to be more important as a second hit, leading to progression of CKD to ESRD.

Gene-environment interactions associated with CKD

The relationship between *APOL1* risk alleles and HIV is considered a classical example of gene-environment relationship leading to CKD. It has been shown that African Americans (112) and black South African individuals (113) with the *APOL1* genetic risk and HIV infection have significantly increased risk of CKD and that the use of appropriate ARVs blunts this risk, to the extent that HIV-positive individuals with *APOL1* genetic risk who have achieved viral replication control are unlikely to develop HIVAN. Other identified environmental factors that modify CKD risk in individuals with *APOL1* genetic risk includes the JC polyomavirus infection which has been shown to reduce the risk of CKD in HIV-positive individuals with the *APOL1* risk allele (114). There is no documented evidence of gene-environment interactions leading to CKD regarding *TGF-β1* or *HMOX1.*

1.12 Purpose of thesis:

This work investigated the prevalence of CKD (and its correlates) in the global and local HIV population and proceeded to investigate the diagnostic utility of urinary transforming growth factor-beta-1 (TGF-β1) for CKD in

the HIV population and determine the association between polymorphisms of *TGF-β1, APOL1 and HMOX1* genes and prevalent CKD.

1.13 Research questions:

The series of questions this thesis will answer using separate but related publications include:

1. What is the prevalence of CKD in the global HIV population? Do regional and sub-regional differences occur in the prevalence of CKD among the HIV population in these regions?
2. What is the prevalence of CKD in the local HIV population in Nigeria?
3. What is the prevalence of other CVD risk factors in the local HIV population?
4. Is there a relationship between urinary TGF-beta levels and prevalent CKD in the adult HIV population?
5. Is there an association between genetic polymorphisms of *TGF-β1, APOL1 and HMOX1* and prevalent CKD in the HIV population?

CHAPTER TWO: OVERVIEW OF METHODS

To meet the research objectives of this study, it has been divided into the following sub-studies which have been schematically summarized in Figure 3 below.

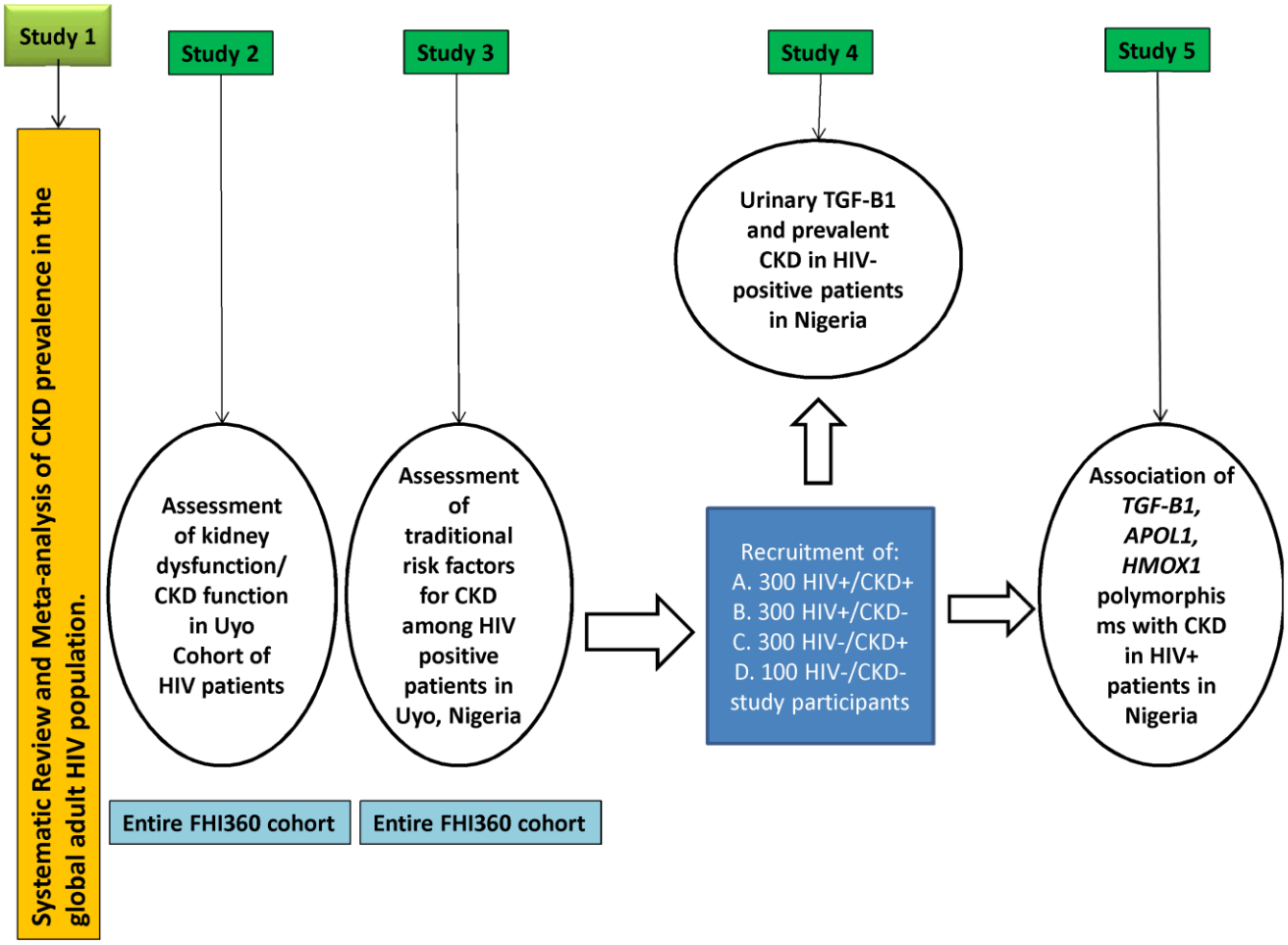

Figure 3: Outlay of Planned activities

In presenting the overall study methodology, I have taken advantage of the sub-studies shown in Figure 3 (above) to describe the methods used in each sub-study to achieve the aims of this study. Therefore, although the study location and patient population are the same, the methods used in each sub-study is separately presented in sections 2.1 (sub-study 1); 2.2 (sub-study 2); 2.3 (sub-study 3); 2.4 (sub-study 4) and 2.5 (sub-study 5).

THE STUDY POPULATION

Uyo, the Capital City of Crude Oil rich Akwa Ibom State of Nigeria is located 5°2'N 7°55'E. Akwa Ibom is a rapidly growing state with its southern limits extending into the Atlantic Ocean of West Africa. The state has a population of 4.6 million according to latest United Nations estimates (115) with the predominant occupation being farming for those living in the hinterlands and fishing for those in communities near the Ocean and public service for individuals in the Urban areas. It has only one tertiary hospital, the University of Uyo Teaching Hospital, a 520 – bed hospital affiliated to the University of Uyo, Nigeria.

STUDY LOCATION

This study was undertaken at the HIV Clinic of the University of Uyo Teaching Hospital which started operations in 2006 and has registered a little over twelve thousand patients since inception. About four thousand patients are on ART while the loss to follow up rate has been computed to be 15 – 20% depending on duration since enrollment. At entry, after confirmation of patients' HIV status, patients have their CD4 count, viral load, Complete blood count, serum electrolytes, urea and creatinine, Liver function test done. Patients who meet criteria are commenced on ART after ensuring they have treatment partners who will help ensure adherence to therapy and clinic visits. Follow up visits are every 3 months; repeat CD4 count 6–

monthly and viral load annually. There has been an increase in the number of patients with kidney disease referred from this clinic to the renal clinic of the hospital in the last four years (116).

2.1 SUB-STUDY 1: SYSTEMATIC REVIEW/META ANALYSIS OF PREVALENCE OF CKD IN THE GLOBAL ADULT HIV POPULATION

A systematic review/meta-analysis was undertaken to determine the prevalence of CKD in other HIV population worldwide using eGFR of ≤ 60ml/min/1.73m^2 as definition of CKD.

SEARCH STRATEGY

A comprehensive electronic search was done to identify all studies that are deemed relevant to this review, with no language restriction, regardless of publication status (published, unpublished, *in press* and *in progress).* We will search the

1. PUBMED 1982 till date
2. Web of Science
3. EBSCO host
4. Africa Journals online (AJOL)

We also did a handsearch of the reference lists of identified articles and relevant review articles using search terms: HIV AND (“CKD” or Chronic Kidney failure or Chronic renal failure or chronic renal insufficiency or Chronic Kidney insufficiency) AND “Prevalence” OR “Incidence” OR “eGFR<60ml/min” OR “Proteinuria”.

ASSESSMENT OF METHODOLOGICAL QUALITY OF ARTICLES

High quality

For studies of the highest quality, assessors answered yes or no to the following questions

1. Subject sampling and precision

A. Are the included people representative of the HIV population? (Comment: if people were included on the basis of use of ART, gender or insurance claims then they should not be considered representative of the HIV population.)

B. Is the sample size adequate to address the question of prevalence in the studied population? A minimum sample size of 100 will be the threshold.

2 Sampling technique : Were the people recruited at random?

3 Response rate

A. Does the article report a response rate in total sample?

B. Is that response rate 70% or higher?

4 Exclusion rate

A. Does the article report an exclusion rate in total sample?

B. Is the exclusion rate 10% or less?

5. Measurement and method of determination of kidney disease

A. Does the study report the method used for determination of CKD status?

B. Does the study use a consistent method for determination of CKD status?

5. Study type – should be a Cohort study

Medium quality

Assessors should answer yes to the following questions:

1. Participants are not representative of the HIV population, but are representative of a particular group within the HIV population (e.g. male/female HIV population, ART – naïve, paediatric population, HIV population with no other established risk factor for CKD – Diabetes, Hypertension, obstructive uropathy).
2. If participants were not recruited at random, then were they recruited in a random non-health-care convenience method from the entire HIV population?
3. Is the study sample size adequate to answer the question of prevalence in the studied population?
4. Does the study use a consistent method for determination of CKD?
5. Is it a cross-sectional study?

Low quality

For studies of the lowest quality:

Assessors would be unable to answer yes to all of the above questions.

Only high and medium quality studies will be included in the analysis.

STATISTICAL ANALYSIS

The statistical software, Stata 15.1 was used to determine the pooled prevalence of CKD in HIV populations both globally and for Sub-Saharan Africa using the random effects model. The results were presented using the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines.

This systematic review protocol was registered with PROSPERO with ID number CRD42016036246.

2.2 SUB-STUDY 2: Determine the prevalence of baseline abnormal kidney function among the HIV patients presenting at the HIV clinic in Uyo between January 2002 and December 2016.

This was a retrospective cohort analysis of data from more than 12,000 incident HIV patients recruited into the HIV clinic. Data from the case records of these patients was transferred into Microsoft Excel 2010 and anonymized. This data contained the patients' clinic number; our study number, mobile number, age at first presentation, age at the time of data analysis, sex, weight at first presentation, weight at last clinic visit, height, BMI at first presentation, serum creatinine at first presentation; other serum creatinine measurements and time of measurement, packed cell volume, dipstick urinalysis at first presentation, electrolytes at first presentation, HBsAg status, Anti–HCV status, CD4 count at presentation, viral load at presentation, date of commencement of ART, ART combination used and any changes in combination, antibiotics and antifungals used during follow up, blood pressure at entry and follow up and blood sugar at entry and follow up. Data was then transferred into STATA 15.1, StataCorp, College Station, Texas, USA and analyzed.

2.3 SUB-STUDY 3: Elucidate the prevalence of traditional risk factors for CKD in the Uyo HIV cohort.

The aim of this aspect of the study was to determine the prevalence of other traditional CKD risk factors like hypertension, diabetes Mellitus, obesity, and dyslipidaemia among the HIV-infected patients. The case definitions of these risk factors are as stated below:

Hypertension: 2 or more recordings of blood pressure with systolic blood pressure at least 140mmHg and/or diastolic blood pressure of at least 90mmHg OR patient who is on antihypertensive medication.

Diabetes Mellitus: Fasting plasma glucose of at least 7.0mmol/L and/or Random/2-hour post meal plasma glucose of at least 11.1 mmol/l OR patient taking oral hypoglycemic agents or insulin

Obesity: BMI of at least 30kg/m2 in a patient without peripheral oedema.

Hepatitis B positive: If the surface antigen for Hepatitis B virus is reactive using standard of care kits.

Hepatitis C positive: If Antibody to Hepatitis C virus is detectable in serum using standard of care laboratory techniques.

Identification and recruitment of study participants for urinary TGF-beta and genetic polymorphism study

The next task was to identify individuals with or without CKD among the cohort of HIV patients attending clinic regularly. The sociodemographic, clinical and anthropometric data of the patients was collected at first encounter. Ten milliliters of venous blood was drawn for serum creatinine measurement at first encounter,

and spot urine collection taken for dipstick urinalysis and urinary protein/creatinine ratios (uPCR). This process was repeated 3 months after the initial visit. Every participant with eGFR (as calculated by the Cockcroft-Gault, 4-variable MDRD and CKD-EPI equations) ≤ 60ml/min/1.73m^2 and/or uPCR of ≥ 0.05g/mmol creatinine at baseline and by month 3 was deemed to have CKD. At the second clinic visit, venous blood was also drawn for DNA extraction and urine sample collected for urinary TGF-beta-1 assay.

2.4 SUB-STUDY 4: Investigate the association between Urinary TGF-β1 levels and CKD in the HIV population.

All the incident HIV patients seen at the second clinic visit had 20 mls of spot urine samples collected for assessment of urinary TGF-β1 levels using ELISA. The samples were centrifuged for 5 minutes to remove cells and particulate matter and thereafter stored at -70 degrees centigrade in 2 aliquots. At the time of laboratory analysis, gentle thawing was performed on one of the urine samples per patient. ELISA was then run on this sample and urinary TGF-β1 levels obtained. Urinary creatinine of the same sample will be obtained and urinary TGF-β1/creatinine ratio calculated and presented in ng/g creatinine. The urinary TGF-β1/creatinine levels in the HIV positive/CKD positive group was compared to that of the HIV positive/ CKD negative group.

2.5 SUB-STUDY 5: Investigate the association between *TGF-β1*, *APOL1* and *HMOX1* gene polymorphisms and CKD in the Uyo HIV cohort.

For those included in the genetic study, patients signed a DNA consent form to allow blood drawn and stored for genetic analysis. Approximately, 10mls of blood was drawn and the DNA extracted using standard methods. The DNA samples were stored at -20° C in Nigeria and later transported to the Division of Human Genetics, University of Cape Town where genetic sequencing was undertaken to determine the allelic and

genotype frequencies of the SNPs to be studied. Controls were selected from the same cohort - HIV positive individuals without CKD, who are appropriately matched for duration since presentation at the clinic, age and sex with the subjects above. Another control group included HIV-uninfected individuals with CKD from other aetiology while the population control will consist of individuals from the community without HIV or CKD.

2.5.1 DNA extraction

DNA was extracted from whole blood collected into dipotassium EDTA bottles and stored at -20 degrees centigrade using the method described in the Quick-DNA^{TM} Miniprep Plus kit (Inqaba Biotech). The DNA extraction was performed at the University of Niger-Delta PCR laboratory.

The DNA concentration and purity of each sample was determined using NanoDrop Lite spectrophotometer. This spectrophotometer measures optical density to determine DNA concentration, while the absorbance ratio (260nm/280nm) is a measure of DNA purity.

All universal health and safety precautions were followed. The material safety data sheets (MSDS), available from the product supplier was strictly adhered to. Genetic sequencing for *TGF-β1, APOL1 and HMOX1* risk variants was performed at the Human Genetics Laboratory at the University of Cape Town.

Targeted SNPs in *TGF-β1*, *APOL1* and *HMOX1* genes

SNP genotyping of rs1800469, rs1800471, rs1982073, rs121918282 in *TGF-β1,* rs60910145 (*APOL1 G1*), rs73885319 (*APOL1 G1*) and rs743811 (*HMOX1*) was performed using predesigned TaqMan genotyping assays (Applied Biosystems, CA, USA). The PCR protocols was performed on the Bio-Rad CFX96 real time PCR system

(Bio-Rad laboratories, CA, USA). The rs3074372 (*HMOX1*) and rs71785313 (*APOL1 G2*) variants was genotyped using fragment analysis, incorporating fluorescently labelled forward primers. The *HMOX1* repeats was classified as short (</= 25 repeats) or long (>25 repeats) (117). PCR protocols was performed on the Bio-Rad thermal cycler T100^{TM}, and analysis of the genotype was achieved using the ABI Prism 3130*xl* Genetic Analyzer (Applied Biosystems, CA, USA). Thereafter, Direct Cycle Sequencing using the ABI Prism 3130*xl* was performed on a subset (10%) of the samples and in order to validate the genotyping results.

2.5.2 Statistical Analysis

Descriptive statistical analysis of the prospectively collected patient data was performed using STATA Software version 15.1 for Windows^{TM} (StataCorp, TX, USA). Moreover, this software was utilized to investigate associations between clinical variables. A χ^2 test with one degree of freedom was used to perform the Hardy-Weinberg equilibrium (HWE). General logistic regression frameworks, adjusted for age and sex, was performed to investigate the relationship between genotype results and clinical. *P* values <0.05 were considered statistically significant.

2.6 ETHICAL CONSIDERATIONS

Ethical approval for this work was obtained from the University of Uyo teaching Hospital human research ethics committee and the UCT Research Ethics Committee (Appendix 2 and 3).

A written informed consent was obtained from each of the participants for objectives 4 and 5 of this work. Participation was **voluntary** and no patient suffered any penalty for declining to be part of the study. No names were recorded on the extracted dataset and questionnaire and a study identification number was assigned to each of the participants to ensure proper identification without breaching **confidentiality**. Data was stored on

my personal computer and backed up on my external hard drive and using Cloud storage, both data stores were encrypted to ensure no other person except myself and my supervisor has access to the data. This also enhanced **confidentiality**.

Beneficience was addressed as all results obtained were relayed to the patients and their doctors immediately employed these findings to ensure better care for the patients. Also, health education campaigns were organized for the participants to help them understand the symptoms of CKD and lifestyle modifications (including adherence to ART) that will help reduce the risk of AKI/CKD. Apart from the blood samples taken by routine venepuncture, no other invasive procedure was undertaken; therefore, the principle of **non-maleficience** was not violated.

CHAPTER 3: CHRONIC KIDNEY DISEASE IN THE GLOBAL HIV-INFECTED POPULATION: A SYSTEMATIC REVIEW AND META-ANALYSIS

Chapter 3 is a systematic review and meta-analysis of CKD prevalence in the global HIV-infected population. Comparison of prevalence across the World Health Organization (WHO) regions were undertaken with special emphasis on sub-Saharan Africa – the epicenter of the HIV pandemic. The paper titled **"CHRONIC KIDNEY DISEASE IN THE GLOBAL HIV-INFECTED POPULATION: A SYSTEMATIC REVIEW AND META-ANALYSIS"** is presented as published in PLOSone and available using the link below:

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0195443

PLOS ONE

RESEARCH ARTICLE

Chronic kidney disease in the global adult HIV-infected population: A systematic review and meta-analysis

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Abstract

Introduction

The widespread use of antiretroviral therapies (ART) has increased life expectancy in HIV patients, predisposing them to chronic non-communicable diseases including Chronic Kidney Disease (CKD). We performed a systematic review and meta-analysis (PROSPERO registration number CRD42016036246) to determine the global and regional prevalence of CKD in HIV patients.

Methods

We searched PubMed, Web of Science, EBSCO and AJOL for articles published between January 1982 and May 2016. CKD was defined as estimated glomerular filtration rate (eGFR) <60ml/min using the MDRD, Cockcroft-Gault or CKD-EPI equations. Random effects model was used to combine prevalence estimates from across studies after variance stabilization via Freeman–Tukey transformation.

Result

Sixty-one eligible articles (n = 209,078 HIV patients) in 60 countries were selected. The overall CKD prevalence was 6.4% (95%CI 5.2–7.7%) with MDRD, 4.8% (95%CI 2.9–7.1%) with CKD-EPI and 12.3% (95%CI 8.4–16.7%) with Cockcroft–Gault; p = 0.003 for difference across estimators. Sub-group analysis identified differences in prevalence by WHO region

with Africa having the highest MDRD-based prevalence at 7.9% (95%CI 5.2–11.1%). Within Africa, the pooled MDRD-based prevalence was highest in West Africa [14.6% (95%CI 9.9–20.0%)] and lowest in Southern Africa (3.2%, 95%CI 3.0–3.4%). The heterogeneity observed could be explained by WHO region, comorbid hypertension and diabetes mellitus, but not by gender, hepatitis B or C coinfection, CD4 count or antiretroviral status.

Conclusion

CKD is common in HIV-infected people, particularly in Africa. HIV treatment programs need to intensify screening for CKD with added need to introduce global guidelines for CKD identification and treatment in HIV positive patients.

Introduction

Chronic Kidney Disease (CKD) is a worldwide public health problem; moving from 27th to the 18th most important global cause of death within the last 2 decades [1]. This degree of shift was second only to HIV/AIDS [1], suggesting a significant relationship between HIV and CKD as an important intersection between chronic non-communicable diseases (NCDs) and communicable diseases.

With the roll-out of antiretroviral therapies (ARTs), individuals with HIV are now living longer. As a consequence, the spectrum of kidney diseases in HIV patients has broadened, ranging from asymptomatic changes in renal function like proteinuria, [2, 3] electrolyte losses [4] and acute kidney injury [5] occurring from diarrheal illnesses to various degrees of CKD occurring as a result of renal damage from chronic non-communicable diseases or HIV-associated nephropathy (HIVAN). Furthermore, the use of certain medications included in some ART regimens such as tenofovir and ritonavir, has been shown to increase the risk of CKD[6]. Among incident end-stage renal disease (ESRD) patients, HIV has been implicated as the etiologic factor in 0.4%–0.7% of patients in France [7, 8]; 0.5%–1.1% in Spain [9, 10]; 6.6% in Cameroon [11]; and 28.5% in South Africa [12]. One large study has shown that as much as 3.3% of HIV positive patients with normal baseline estimated glomerular filtration rate (eGFR) developed CKD over a relatively short follow up period of 3.7 years, highlighting the burden of kidney disease in HIV patients [13]. The prevalence of CKD in HIV-infected individuals varies widely between geographic regions and depends on the reporting methods and the definition of CKD used, ranging from 2% to 38% [14, 15]. Although there is an increasing number of individual reports on the prevalence of CKD in the HIV population, the data have not been appropriately synthesized to date.

In this analysis, we synthesized available data on CKD prevalence in the adult HIV population at both regional and global levels. The overarching goal was to provide an essential basis to guide contextualized effective prevention and control strategies to tackle the burden of CKD in this population.

Methods

Selection of studies for inclusion in the review

The Preferred Reporting Items for Systematic Reviews and Meta–Analysis (PRISMA) 2009 guidelines [16] served as the template for reporting the present review (S1 Table, Fig 1). The study protocol was published at the International Prospective Register of systematic reviews,

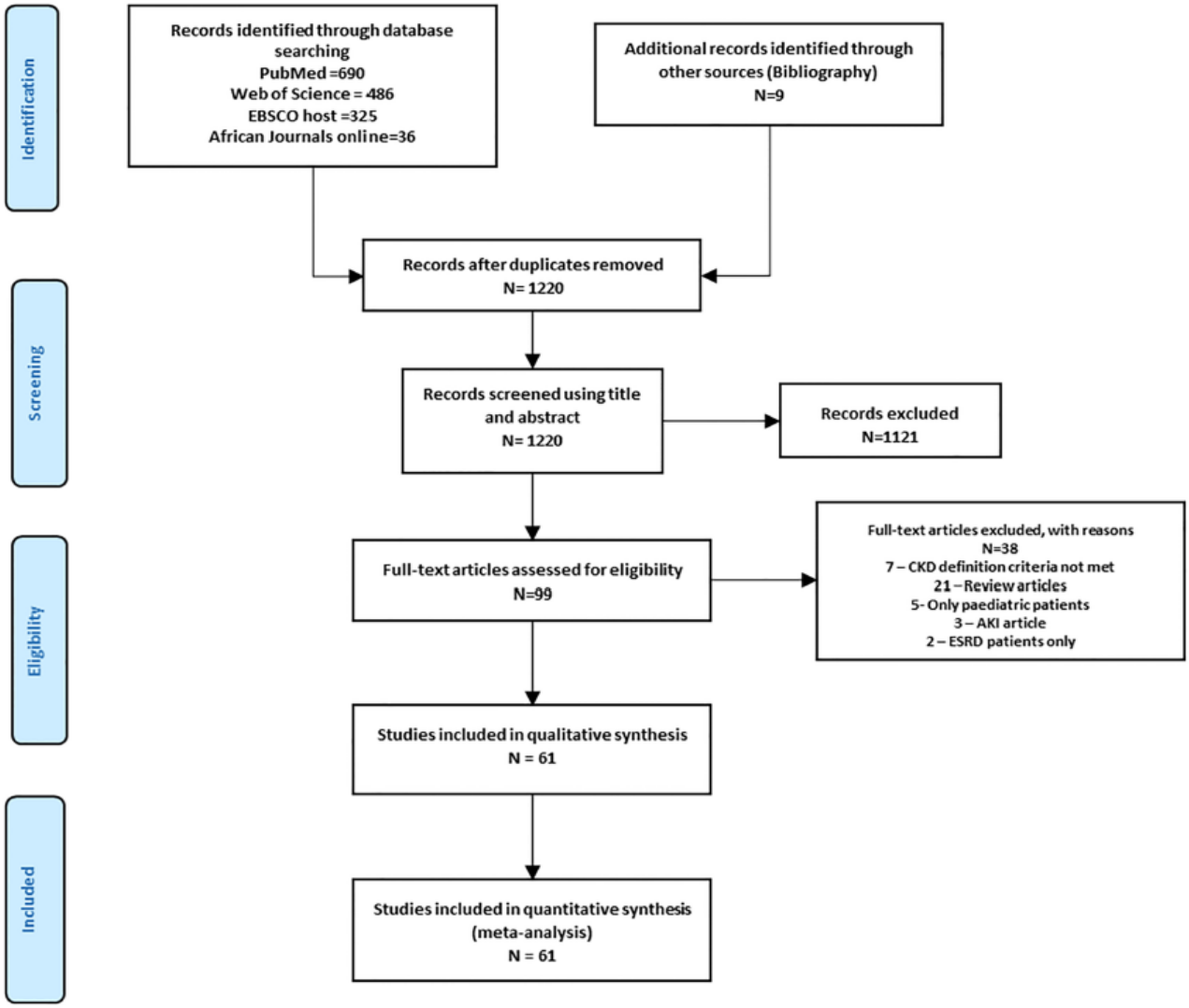

Fig 1. Flow diagram for the selection of studies.

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(PROSPERO registration number CRD42016036246). All observational studies and clinical trials reporting on the prevalence of CKD in HIV-infected adults (≥ 18 years) or providing enough data to compute it, using established creatinine-based equations [Modification of Diet in Renal Disease (MDRD) [17], Cockcroft–Gault (CG) [18], Chronic Kidney Disease Epidemiology (CKD-EPI) [19]] to estimate GFR were included. CKD was defined as eGFR <60ml/min/1.73m^2 irrespective of proteinuria status. Studies that reported CKD as eGFR <60ml/min/1.73m^2 and/or persistent proteinuria were only included if we could compute the frequency of those with eGFR <60 ml/min/1.73m^2 from available data in the article. We also included studies that reported CKD prevalence using a single estimated eGFR in order to accommodate studies from low-income countries where repeated serum creatinine measurement might not have been performed. A comparison of the pooled prevalence from studies with a single eGFR estimate and that with multiple estimates was also undertaken. We

excluded studies with small sample size (<100 participants) and those including both adult and pediatric populations in which it was not possible to disaggregate data for adults. For studies published in more than one report (duplicates), the most comprehensive reporting the largest sample size was considered.

Identification of studies

We searched PubMed/MEDLINE, EBSCO, Web of Science and African Journals Online to identify all relevant articles reporting data on the prevalence of CKD in HIV-infected adults published from January 1, 1982 (when the HIV epidemic started) to September 30, 2016. We conceived and applied a search strategy based on the combination of relevant terms relating to HIV and CKD. The search strategy for Pubmed, web of science, EBSCO and AJOL is shown in S2 Table. No language restrictions were applied. References of all relevant research articles and reviews were also scrutinized to identify additional potential data sources.

Assessment of methodological quality of included articles

The methodological quality of included studies was evaluated using the 9-point rating system developed by Stanifer et al [20] and modified for the purposes of this study. The scoring criteria for quality of studies is shown in S3 Table while S4 Table shows the methodological quality of the included articles. The scoring criteria assessed factors related to representativeness of the study participants, sampling, sample size and assessment of possible confounders to the relationship between HIV and CKD. Studies were rated as having a high, medium or low methodological quality when they were assigned a score higher than 6, 5 and 6; or 4 and below respectively.

Study selection and data extraction

Two investigators (UEE and IGO) independently screened the titles and abstracts of articles retrieved from literature search, and the full-texts of articles found potentially eligible were obtained and further assessed for final inclusion (Fig 1). Disagreements were resolved by consensus or consultation of a third investigator (APK). For clinical trials, we used baseline data. A World Health Organization (WHO) region [21] was assigned to each study depending on the country of recruitment. All studies from Africa were subsequently seperated from the rest and further sub-divided into the different African Union (AU) sub-regions [22] for the purpose of statistical comparisons. Two investigators (UEE and EEE) independently extracted data; discrepancies between investigators were resolved through discussion until consensus was achieved. In one instance [23], an author was contacted for clarification where data was uncertain. Data extracted included first author name, year of publication, country of study origin, WHO region, African sub-region (if study was from Africa), gender proportions in the study population, median age, Body mass index (BMI), CD4 count and viral load of the study population, prevalence of hepatitis B, C co-infection; and the prevalence of hypertension and diabetes mellitus in the study population.

Statistical analyses

A meta-analysis was used to summarize prevalence data. We pooled the study-specific estimates using a random-effects meta-analysis model (DerSimonian-Laird) to obtain an overall summary estimate of the prevalence of CKD according to the different eGFR equations across studies [24], after stabilizing the variance of individual studies with the use of the Freeman-Tukey double arcsine transformation to minimize the effect of extreme prevalence on the

overall estimate [25]. Heterogeneity was assessed using the χ^2 test on Cochrane's Q statistic [26] and quantified by calculating the I^2 (with values of 25%, 50% and 75% representing low, medium and high heterogeneity respectively)[27]. Subgroups analysis was also performed using the Q-test based on ANOVA. We assessed the presence of publication bias using funnel plots and the Egger's test [28]. We assessed inter-rater agreement for study inclusion and data extraction using Cohen's kappa (κ) coefficient [29]. A p-value <0.05 was considered indicative of statistically significant difference between subgroups. Data was analyzed using the statistical software Open Meta Analyst [30] and the *metaprop* [31] *p*ackage in STATA version 14.0 for Windows (Stata Corp. 2015. Stata Statistical Software: Release 14. College Station, Tx: Stata Corp USA).

Results

The initial literature search retrieved 1220 articles of which 99 were selected after title and abstract screening for full-text review. Finally, 61 articles [23, 32–91] were eligible and included in this systematic review (Fig 1). There was a high agreement between investigators for study inclusion ($\kappa = 0.81$).

Included studies reported on 209,078 HIV-infected adults from 60 countries. There were 46,295 participants (26 studies) from Africa; 52,785 (9 studies) from Europe; 52,305 (11 studies) from North America; 3,661 (4 studies) from South America; 49,147 (9 studies) from Western Pacific and 248 (1 study) from the Eastern Mediterranean. One study [76] from multiple countries in more than two continents had 4,637 HIV–infected adults. MDRD, CG and CKD-EPI equations were used to estimate GFR in 45 studies (n = 167,011 participants), 19 studies (n = 59,414 participants) and 14 studies (n = 41,791 participants) respectively. Thirty-one studies (n = 111,415 participants) used MDRD [32–35, 37–45, 48, 51–54, 57, 59, 62, 65–69, 82, 84, 85, 89] equation only; 7 (n = 16,756 participants) used CKD–EPI [76–80, 83, 86] and 9 (n = 24,622 participants) used CG only [23, 70–75, 81]. Seven articles (n = 31,268 participants) applied MDRD and CG [36, 50, 55, 56, 60, 64, 87]; 4 (n = 20,742 participants) applied MDRD and CKD-EPI [46, 47, 49, 58] while 3 (n = 4,275 participants) applied all 3 equations [61, 63, 88]. Most of the articles were cross-sectional (75.4%); followed by cohort studies (18.0%); then case-control 2 (3.3%); clinical trials 2 (3.3%).

The component studies had a sample size range of 163 [58] to 41,862 [89] participants with the proportion of women ranging from 0% [79] to 100% [88]. The mean age of participants ranged from 31.4 [86] to 48.7 [39] years, and median CD4 count from 147 cells/ul [70] to 651 cells/ul [76]. Some studies [23, 34, 35, 42, 55, 59, 73–77, 80, 89] consisted exclusively of ART–naïve individuals while the rest had varying proportions on ARTs. The prevalence of hepatitis B and C co-infection ranged from 1.6% [33] to 15.1% [70] and from 3.3% [69] to 50.3% [47] respectively. Most of the studies had medium methodological quality (63.9%, n = 39) (S4 Table); 11 studies (18.0%) were of high quality, including 2 (7.7%) studies from Africa, 3 (33.3%) from Europe and 3 (27.3%) from North America. Table 1 provides a summary of data extracted.

The overall prevalence of CKD was 6.4% (95%CI 5.2–7.7%, N = 45 studies, 167,011 participants, $I^2 = 98.9\%$, heterogeneity-$p<0.001$) using the MDRD equation, 4.8% (95%CI 2.9–7.1%, N = 14 studies, 41,791 participants, $I^2 = 98.7\%$; $p<0.001$) with CKD-EPI and 12.3% (95%CI 8.4–16.7%; N = 19 studies, 59,414 participants, $I^2 = 99.4\%$, $p<0.001$) with the CG equation ($p = 0.003$ for difference across GFR estimators) (Fig 2). There was no evidence of publication bias (Fig 3) all $p \geq 0.147$ for the Egger test).

Using the MDRD equation, the African region had the highest prevalence estimate at 7.9% (95%CI 5.2%-11.2%) while the European region had the lowest estimate at 3.7% (95%CI 2.5–

Table 1. Summary of extracted data from all included studies.

Author	Year	Country	WHO Region	Sample Size	No. of CKD Patients	Age	Male (%)	Female (%)	CD4	VL (log)	ARV naïve (%)	ARV Use (%)	HBV (%)	HCV (%)	HTN (%)	DM (%)	BMI (kg/m^2)
MDRD																	
Adedeji et al [32]	2015	Nigeria	AFRO	183	44	37.9	42.6	57.4	201								
Al-Sheikh et al [33]	2013	Saudi Arabia	EMRO	248	2	39	66.5	33.5	305	4.8	3	97	1.6	8.6	13.3	16.1	
Anyabolu et al [34]	2016	Nigeria	AFRO	375	32	38.8	28	72			100						
Cao et al [35]	2013	China	WPRO	538	13	36.5	74.2	25.8	173	4.6	100		14.4	14.9	3.2	3	21.4
Caihol et al [36]	2011	Burundi	AFRO	300	5	40.1	29.7	70.3	325	1.65	30.2	69.8	5	5.3	2.7	2	21.8
Calza et al [37]	2013	Italy	EURO	894	44	44.2	70.9	29.1	508	2.2	22.5	77.5	5.9	35.9	25.7	6	24.6
Campbell et al [38]	2009	UK	EURO	3439	81	42	72.1	27.9	135				3.9	5.1	29.6	22.2	
Cheung et al [85]	2007	China	WPRO	322	18	45.2	82	18	50	4.89	6.5	93.5	14.9	4.3	7.4	10.6	
Choi et al [39]	2007	USA	AMRO1	12315	1041	48.7	97.7	2.3			86	14			38.9	15.8	
Cianflone et al [40]	2010	USA	AMRO1	717	22	41	92	8	515		27	73	5.2	3.9	33	8	
Colson et al [41]	2010	Belgium	EURO	2275	68	42.6	70.5	29.5			18.6	81.4					
Ekat et al [42]	2012	Congo	AFRO	562	48	38.8	33.9	66.1	192		100						20.3
Fernando et al [43]	2008	USA	AMRO1	421	41	43.6	60.2	39.8	422	4.5	11	89	5	32	27.5	2.5	
Fischer et al [44]	2010	USA	AMRO1	23155	2833	44	98	2	336	3	83	17	4	40	17	15	
Flandre et al [45]	2011	USA	AMRO1	7378	349	31.2	70.3	29.7	365		10.7	89.3	7.4	21.4	16	4	
Fulop et al [82]	2010	USA	AMRO1	941	23	40.3	60.7	39.3	335		24.3	75.7	10.2	9.5	30	7	
George et al [46]	2011	USA	AMRO1	252	22	49.5	63.5	36.5	375	2.88	50.8	49.2			36.5	11.1	
Gonzalez et al [47]	2014	Brazil	AMRO2	195	4	47.6	78.5	21.5	676		7.7	92.3	6.7	50.3	32.6	10.3	
Gracey et al [48]	2012	Australia	WPRO	733	45	45.6	93	7			16	84		6	28	5	
Hsieh et al [90]	2013	Taiwan	WPRO	512	4	43.2	92	8	206	4.93	45.4	54.5	16	33.4	6.1	0.51	
Ibrahim et al [49]	2011	UK	EURO	20132	463	34	78	22	350	3.8	20	80	5.3	7.7			
Longo et al [50]	2011	Congo	AFRO	300	9	43	23	77	231		12	88			13		24
Lucas et al [51]	2010	Uganda	AFRO	1202	8	30	35.4	64.6									
Lucas et al [52]	2008	USA	AFRO	4259	284	38	68	32	180		0		8	50		8	
Mayor et al [53]	2010	Puerto Rico	AMRO2	1283	116	40.8	69	31	277	5.7	51.7	48.3		20.1	18.9	9.9	
Menezes et al [54]	2011	Brazil	AMRO2	213	18	45.6	51.6	48.4	569			100			20.7	14.1	
Msango et al [55]	2013	Tanzania	AFRO	355	61	36.1					100						19.7
Mulenga et al [56]	2008	Zambia	AFRO	25779	812	38.5	39.8	60.2	144			100					
Nakamura et al [57]	2008	Japan	WPRO	748	121	44.9											
Obirikorang et al [58]	2014	Ghana	AFRO	163	16	39.9	22.1	77.9	523		31.9	68.1					
Okafor et al [59]	2011	Nigeria	AFRO	383	121	36			239			100					
Overton et al [60]	2009	USA	AMRO	845	63	40.3	63.7	36.3	433		63.9	36.1	4.9	12	34.3	6.1	
Owiredu et al [61]	2013	Ghana	AFRO	479	46	35.8	28.3	71.1	290		62.4	37.6					
Peck et al [62]	2014	Tanzania	AFRO	301	35	38.5	32.2	67.8	297		50	50			16.9	0.7	
Sarfo et al [63]	2013	Ghana	AFRO	3137	429	38	33	67	133		39.5	60.5					20.3
Stohr et al [64]	2008	Uganda/ Zim	AFRO	3316	102	36.8	35	65	86								21.1

Table 1. (Continued)

Author	Year	Country	WHO Region	Sample Size	No. of CKD Patients	Age	Male (%)	Female (%)	CD4	VL (log)	ARV naïve (%)	ARV Use (%)	HBV (%)	HCV (%)	HTN (%)	DM (%)	BMI (kg/m^2)
Sorli et al [65]	2008	Spain	EURO	854	65						12.5	87.5					
Umeizudike et al [66]	2012	Nigeria	AFRO	402	38	35	37.8	62.2	223	5.4							22.1
Wools-Kaloustian [87]	2007	Kenya	AFRO	373	7	35	32.1	67.9	391								
Wyatt et al [67]	2007	USA	AMRO1	1239	73	47.1	57	43	397		16	84	6.3	41			
Wyatt et al [88]	2011	Rwanda	AFRO	659	97	34		100	256						4.8	0.5	20.9
Yanigasawa et al [69]	2011	Japan	WPRO	732	71	46.7	93.9	6.1	416	1.98	9.3	90.7	7.1	3.3	30.3	7.9	
Yanigasawa et al [68]	2014	Japan	WPRO	1447	96	44.4	93.3	6.7	487								
Zhao et al [89]	2015	China	WPRO	41862	1377	38	68.5	31.5	220		100			11			
Muramatsu et al [84]	2013	Japan	WPRO	1482	99	44.2	93.4	6.6	487								
CG																	
Agbaji et al [70]	2011	Nigeria	AFRO	491	117	38.8	40.1	59.9	147		100		15.1	11.9			
Brennan et al [71]	2011	South Africa	AFRO	890	46	37.1	26.5	73.5	245		21.3	78.7					
Caihol et al [36]	2011	Burundi	AFRO	300	15	40.1	29.7	70.3	325	5.3	30.2	69.8	5	5.3	2.7	2	
Kamkuemah et al [72]	2015	South Africa	AFRO	1092	18	34	38	62			100						
Longo et al [50]	2011	Congo	AFRO	300	30	43	23	77	397		12	88					
Mizushima et al [91]	2013	Vietnam	WPRO	771	74	36.4	61.6	38.4	349	1.79	65.2	34.8				4.2	
Msango et al [55]	2013	Tanzania	AFRO	355	89	36.1											
Mulenga et al [56]	2008	Zambia	AFRO	25779	2240	38.5	39.8	60.2	144		100					19.7	
Onodugo et al [73]	2014	Nigeria	AFRO	300	73	38.1	34.7	65.3	273	5.46	100						21.8
Overton et al [60]	2009	USA	AMRO1	845	63	39.8	64	36	37	12	37	63	4.9	12	34.3	6.1	
Owiredu et al [61]	2013	Ghana	AFRO	479	48	35.2	24	76	57.6	42.4							
Reid et al [74]	2007	Uganda/ Zim	AFRO	3316	242	37	35	65			100						
Sakajiki et al [23]	2014	Nigeria	AFRO	400	64	34	40	60			100						22
Sarfo et al [63]	2013	Ghana	AFRO	3137	1186	38	33	67	133		39.5	60.5					20.3
Schoffelen et al [81]	2015	Netherland	EURO	16836	460	42.4	83.2	16.8	440		46.6	53.4	6.4	7.6	9.7	3.2	23.4
Stohr et al [64]	2008	Uganda	AFRO	3316	242	36.8	35	65	86		100						21.1
Struik et al [75]	2011	Malawi	AFRO	526	111	34	33.5	66.5	305		100				6.1	0.6	
Wools-Kaloustian [87]	2007	Kenya	AFRO	373	43	35	32.1	67.9	391		100						
Wyatt et al [88]	2011	Rwanda	AFRO	659	166	34		100	256		100				4.8	0.5	20.9
CKD-EPI																	
Acchra et al [76]	2015	START*	-	4637	286	36.8	73.1	26.9	651		100		2.9	3.7	19.2	3.5	
Bandera et al [77]	2015	Italy	EURO	7385	206	36	73.4	26.6			100		4.4	24.5	3.1	2.1	
Bonjoch et al [78]	2014	Spain	EURO	970	29	48	75.6	24.4	567						19	3	23.8
Estrella et al [79]	2011	USA	AMRO1	783	39	47	100		511		27	73			37	13	24.9
George et al [46]	2011	USA	AMRO1	252	20	49.5	63.5	36.5	375	2.88	50.8	49.2			36.5	11.1	

(*Continued*)

Table 1. (Continued)

Author	Year	Country	WHO Region	Sample Size	No. of CKD Patients	Age	Male (%)	Female (%)	CD4	VL (log)	ARV naïve (%)	ARV Use (%)	HBV (%)	HCV (%)	HTN (%)	DM (%)	BMI (kg/m^2)
Gonzalez et al [47]	2014	Brazil	AMRO2	195	3	47.6	78.5	21.5	676		7.7	92.3	6.7	50.3	32.6	10.3	
Ibrahim et al [49]	2012	UK	EURO	20132	403	34	78	22	350		20	80	5.3	7.7			
Obirikorang et al [58]	2014	Ghana	AFRO	163	6	39.9	22.1	77.9	523		31.9	68.1					
Odongo et al [86]	2015	Uganda	AFRO	361	52	31.4	36.3	63.7			100						20
Owiredu et al [61]	2013	Ghana	AFRO	479	51	35.8	28.3	71.7	290		62.4	37.6					
Santiago et al [83]	2014	Brazil	AMRO2	1970	74	41.6	63.6	36.4	184		17.1	82.9	2.9	6	26.6	9.3	
Sarfo et al [63]	2013	Ghana	AFRO	3137	434	38	33	67	133		39.5	60.5					20.3
Wyatt et al [88]	2011	Rwanda	AFRO	659	52	34		100	256		100						
Zachor et al [80]	2016	South Africa	AFRO	650	15	37.9	34.5	65.5	186		100		13.7		7.8	2.2	24.9

VL = Viral load (in $\log_{10}$) BMI = Body Mass index HTN = Hypertension DM = Diabetes Mellitus

*START trials in 35 countries AFRO = African region EURO = Europe EMRO = Eastern Mediterranean WPRO = Western Pacific AMRO1 = North AmericaAMRO2 = South America/Carribean

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5.1%); p = 0.004 for difference across regions. Summaries of pooled prevalence by region and GFR estimators are presented in Fig 4 and Table 2; summary statistics from meta-analyses of prevalence studies on CKD in people with HIV using random effects model and arcsine transformations are shown in S5 Table.

CKD prevalence was also high in Africa using the CKD-EPI equation: 7.0% (95%CI 2.8–12.9%). Studies using CG equation were mostly from Africa (84% of the studies), precluding sound regional analysis. The pooled prevalence of CKD in Africa from CG estimator was 13.7% (95%CI 9.1–19.0%); Table 2.

Of the studies performed in Africa, studies originating from West Africa had the highest pooled prevalence estimate using the MDRD equation: 14.6% (95%CI 9.9–20.0%) while the estimates from Southern Africa (3.2%, 95%CI 3.0–3.4%) were the lowest; p<0.001 for difference across African sub-regions, (Fig 5, Table 2). With the CG equation, West Africa still had the highest estimate, 22.0% (95%CI 11.8–34.3%); East Africa's estimate was 20.2% (95% CI 12.0–29.9) while Southern Africa had 7.5% (95%CI 5.4–9.9%) (Table 1); p<0.001 for difference across the regions.

One study [88] reported CKD prevalence for only women and another [79] for only men; the pooled prevalence for men compared to women (MDRD) was 4.9% (95%CI 3.1–7.0%) versus 4.5% (95%CI 3.3–5.8%), p = 0.93 for difference by gender. The pooled prevalence (CG equation) for men was 8.3% (95%CI 1.1–20.8%) while that for women was 15.2% (95%CI 4.9–29.7%); p-value = 0.41 for difference by gender, (S5 Table).

Older (≥median age 38.5 years) compared with younger participants (<38.5 years) had lower but non-significant difference in CKD prevalence: MDRD: 6.1% (95%CI 4.6–7.9%) vs. 6.9% (95%CI 5.2–8.8%), p = 0.54; CKD-EPI: 4.8% (95%CI 2.6–7.5%) vs. 4.8% (95%CI 2.3–8.2%), p = 0.98; CG: 8.5% (95%CI 4.5–13.6%) vs. 14.2% (95%CI 7.9–21.9%), p = 0.17. Substantial heterogeneity was apparent within age-groups regardless of the criteria (all p-heterogeneity <0.001). The CKD prevalence rates for patients with co-infection with hepatitis B and C, by level of CD4 count, by ART status and for those with comorbid systemic hypertension and

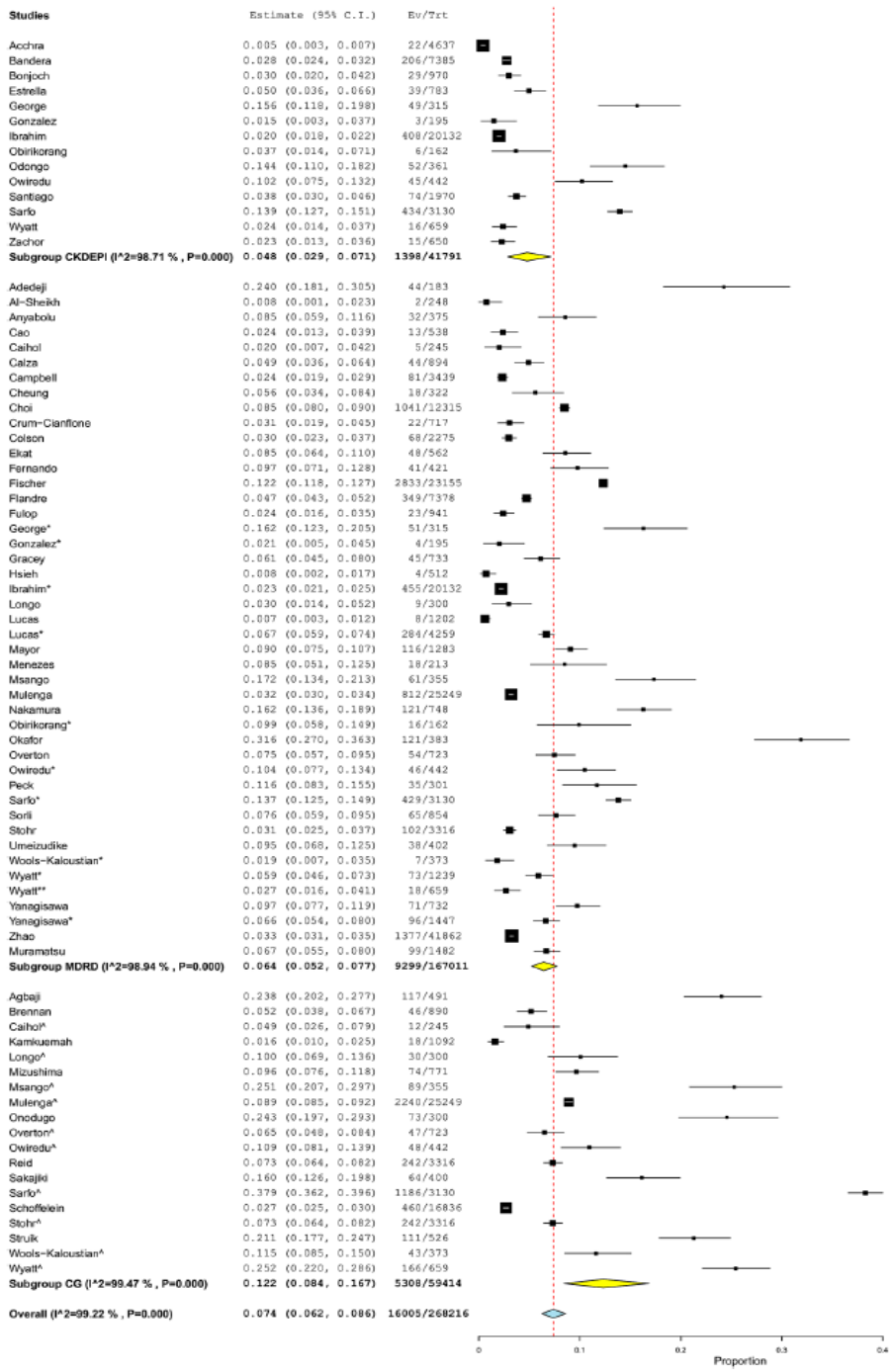

Fig 2. Forest plot showing the overall CKD prevalence in the HIV-infected using the MDRD, CKD-EPI and CG equations. For each study the black box represents the study estimate (prevalence of CKD) and the horizontal bar represents the 95% confidence intervals (95%CI). The yellow diamond at the lower tail for each equation is the pooled effect estimates from random effects models.

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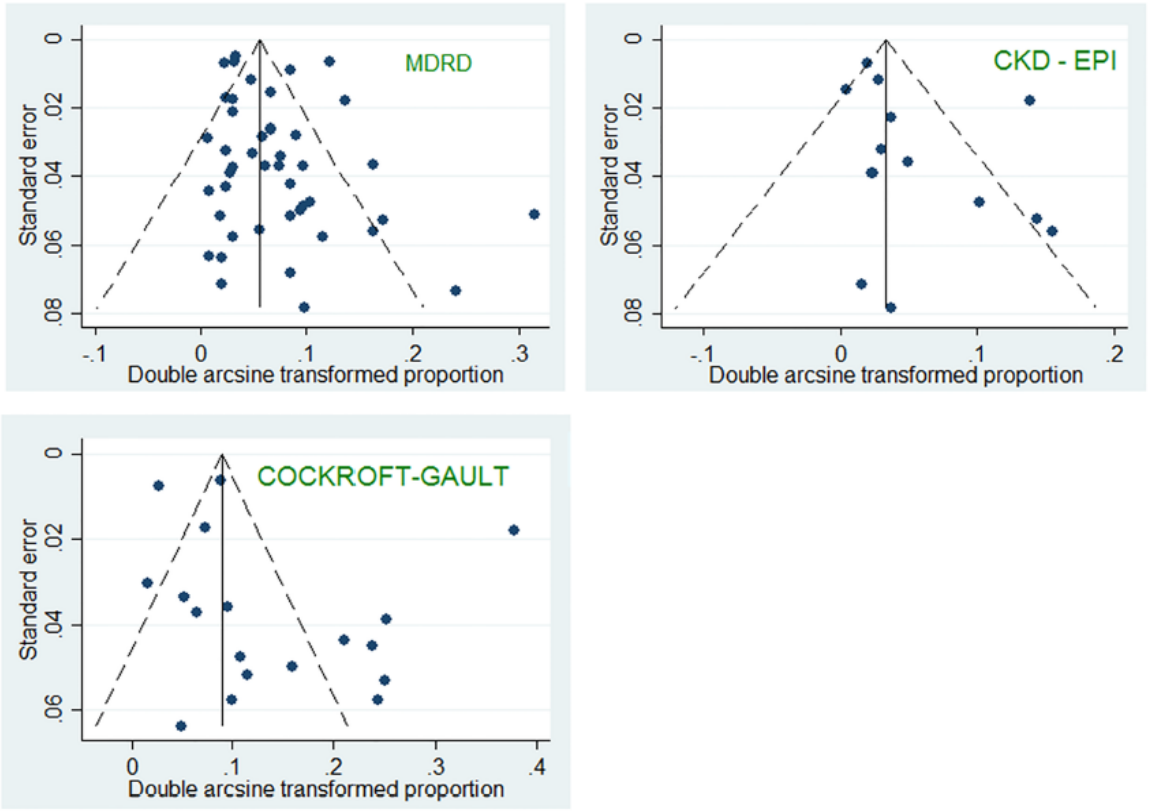

Fig 3. Funnel plots for included studies across different serum creatinine-based GFR equations. For each estimation equation, the arcsine transformed proportion of participants with CKD (relative to the total sample) for each relevant study (horizontal axis) is plotted against its standard error (vertical axis), and represented by the dots. When the dots distribute symmetrically in a funnel shape, this implies an absence of bias. All p-values were >0.05 (Egger test) indicating no evidence of significant publication bias.

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diabetes mellitus are summarized in S5 Table. Importantly, we found that co-infection with hepatitis B or C, level of CD4 count and use of ART did not have a significant effect on CKD prevalence. However, CKD prevalence was significantly increased with comorbid hypertension (MDRD: 20.7% [95%CI 14.3–27.8%] vs 5.4% [95%CI 3.4–7.9%]; p<0.001) or diabetes mellitus (MDRD: 19.4% [95%CI 13.5–26.0%] vs 8.4% [95%CI 5.5–11.8%]; p<0.001) (S5 Table).

Twenty-one studies [35–38, 41, 42, 45, 47, 52, 54, 60, 64, 65, 68, 69, 72, 77, 81, 85, 90, 91] had serum creatinine measured at least twice (four from sub-Saharan Africa [SSA]), a minimum of three months apart. The pooled prevalence for MDRD equation-based studies was 4.7% (95%CI 3.7–5.9%); CKD-EPI 2.6% (95%CI 2.3–3.0%) and CG 5.1% (95%CI 2.8–8.0%), with a significant difference across estimators (p<0.001). The forest plot showing the pooled prevalence of CKD in HIV populations for studies with at least two eGFR estimates using MDRD, CKD-EPI and CG equations is given in S1 Fig. There was no evidence of publication bias as shown in the funnel plots for studies with two or more eGFR values (S2 Fig), p-value = 0.07 for the Egger test).

Using the MDRD equation, the pooled prevalence of CKD reported from African studies that used two eGFR measurements was 4.2% (95%CI 1.4–8.3%) versus 8.9% (95%CI 5.3–13.3)

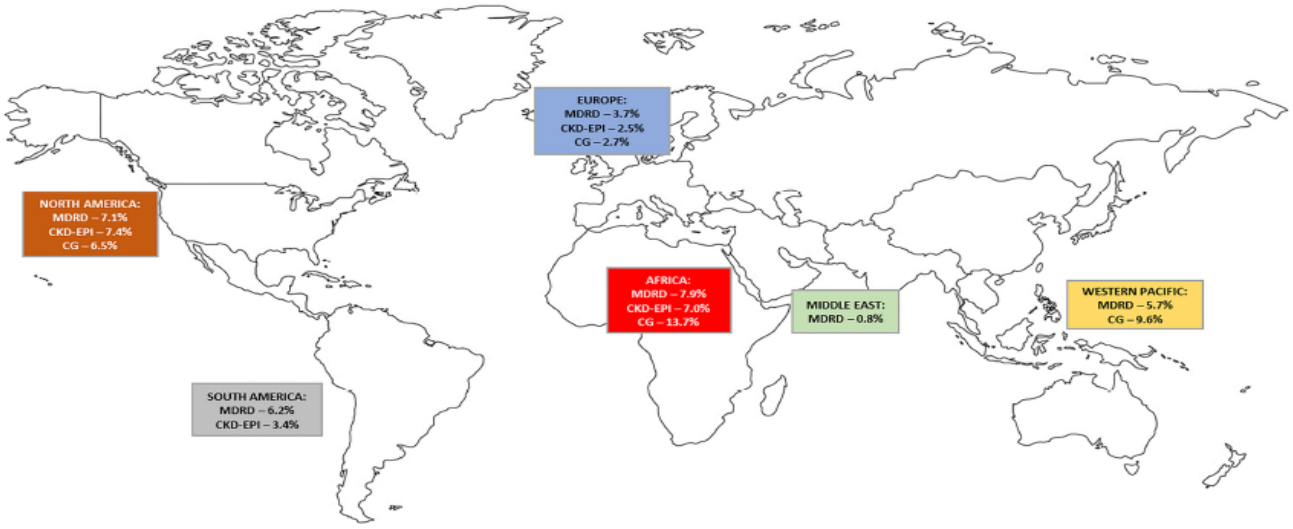

Fig 4. Summary of pooled prevalence of CKD in HIV populations across WHO regions.

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(p-value 0.09) for those that used one eGFR measurement. For studies from North America, the pooled CKD prevalence for studies with at least 2 eGFR measures was 6.1% (95%CI 4.5–7.9%) compared to the pooled prevalence of studies with one eGFR measure of 7.6% (95%CI 5.2–10.4%), p = 0.34. The forest plot showing pooled prevalence for studies with two or more MDRD-based eGFR estimates across the WHO regions is shown in S3 Fig.

Discussion

To our knowledge, this is the first attempt to provide prevalence estimates for CKD in HIV populations across various WHO regions. Prevalence was highest in Africa and lowest in Europe although the data shows substantial heterogeneity. Despite this, sociodemographic and clinical factors such as gender, age, coinfections with HBV and HCV did not significantly affect the estimates while coincident hypertension and diabetes mellitus had significant effect on the estimates. Paradoxically, our analysis did not reveal significant contribution to CKD prevalence of HIV related factors such as CD4 counts and ART status.

The overall prevalence of CKD in HIV populations is high, regardless of estimator used. This is more so in Africa where the prevalence of CKD in the general population is already high [20]. The high CKD prevalence in HIV patients presents an enormous challenge to health care systems in low to middle income countries (LMICs) with high prevalence of HIV and where access to CKD care is significantly lacking [92]. The clinical and economic implication of a high CKD burden has effects on the functioning of health systems. In higher income countries, high CKD burden may represent remarkable increase in healthcare costs for managing HIV related CKD whereas in LMICs, it may mean enormous pressure on an already weakened and poorly funded health system. The interplay between HIV and CKD also presents an opportunity for integration of chronic non-communicable disease care with communicable disease treatment as this may enhance more effective use of health resources and improve long term outcomes for HIV patients. It is important to determine if there is a higher CKD prevalence among HIV populations than the general population. In Africa, the prevalence of

Table 2. Summary statistics from meta-analyses of prevalence studies on CKD in people with HIV using random effects model and arcsine transformations.

Group	Subgroup	eGFR formula	Number of studies	Number of participants	Number of cases	Prevalence (95% CI)	I^2 (%)	P—heterogeneity	p-different formulae	p-diff sub-groups	p-Egger test
WHO Region	Overall								0.003		
		MDRD	45	167,011	9,299	6.4 (5.2–7.7)	98.9	<0.001		0.004	0.16
		CKD-EPI	14	41,791	1,398	4.8 (2.9–7.1)	98.7	<0.001		<0.001	0.14
		CG	19	59,414	5,308	12.3 (8.4–16.7)	99.4	<0.001		<0.001	0.15
	Africa								0.08		
		MDRD	17	37,639	1,831	7.9 (5.2–11.2)	98.4	<0.001			0.04
		CKD-EPI	6	5,404	568	7.0 (2.8–12.9)	97.5	<0.001			0.21
		CG	16	41,084	4,727	13.7 (9.1–19.0)	99.3	<0.001			0.29
	Europe								<0.001		
		MDRD	5	27,594	713	3.7 (2.5–5.1)	94.6	<0.001			0.09
		CKD-EPI	3	28,487	643	2.5 (1.9–3.2)	87.5	<0.001			0.47
		CG	1	16,836	460	2.7 (2.5–3.0)	-	-			-
	N. America								<0.001		
		MDRD	10	51,463	4,771	7.1 (5.1–9.5)	98.6	<0.001			0.18
		CKD-EPI	2	1,098	88	7.4 (6.0–9.1)	99.8	<0.001			-
		CG	1	723	47	6.5 (4.9–8.5)	-	-			-
	S. America								0.16		
		MDRD	3	1,691	138	6.2 (2.6–11.3)	87.7	<0.001			0.48
		CKD-EPI	2	2,165	77	3.4 (2.7–4.3)	98.2	<0.001			-
		CG	-	-	-	-	-	-			-
	E. Mediterranean										
		MDRD	1	248	2	0.8 (0.2–2.9)	-	-	-	-	-
	W. Pacific										
		MDRD	9	48,376	1,844	5.7 (3.5–8.4)	97.4	<0.001			0.08
		CKD-EPI	-	-	-	-					-
		CG	1	771	74	9.6 (7.7–11.9)	-	-			-
Africa											
	Overall								0.09		
		MDRD	17	37,639	1,831	7.9 (5.2–11.2)	98.4	<0.001		<0.001	0.04
		CKD-EPI	6	5,404	568	7.0 (2.8–12.9)	97.5	<0.001		<0.001	0.21
		CG	16	41,084	4,727	13.7 (9.1–19.0)	99.2	<0.001		<0.001	0.29
	West Africa								0.08		
		MDRD	7	5,055	726	14.6 (9.9–20.0)	94.5	<0.001			0.72
		CKD-EPI	3	3,734	485	9.2 (4.8–14.8)	91.5	<0.001			0.09
		CG	5	4,763	1,488	22.0 (11.8–34.3)	98.4	<0.001			0.03
	Southern Africa								<0.001		
		MDRD	2	28,565	914	3.2 (3.0–3.4)	99.9	<0.001			-
		CKD-EPI	1	650	15	2.3 (1.4–3.8)	-	-			-
		CG	6	34,389	2,899	7.6 (5.2–10.4)	97.7	<0.001			0.77
	East Africa								<0.001		
		MDRD	5	2,890	129	5.3 (1.1–12.2)	97.6	<0.001			0.14
		CKD-EPI	2	1,020	68	5.6 (4.3–7.1)	97.1	<0.001			-
		CG	3	1,387	298	20.2 (12.0–29.9)	94.2	<0.001			0.65
	Central Africa								0.19		
		MDRD	3	1107	62	4.2 (1.2–9.0)	89.9	<0.001			0.08
		CKD-EPI	-	-	-	-	-	-			-

(*Continued*)

Table 2. (Continued)

Group	Subgroup	eGFR formula	Number of studies	Number of participants	Number of cases	Prevalence (95% CI)	I^2 (%)	P—heterogeneity	p-different formulae	p-diff sub-groups	p-Egger test
		CG	2	545	42	7.5 (5.4–9.9)	94.9	<0.001			-

eGFR—estimated glomerular filtration rate; MDRD—Modification of diet in renal disease, CKD-EPI—Chronic kidney disease Epidemiology collaboration; CG—Cockroft-Gault

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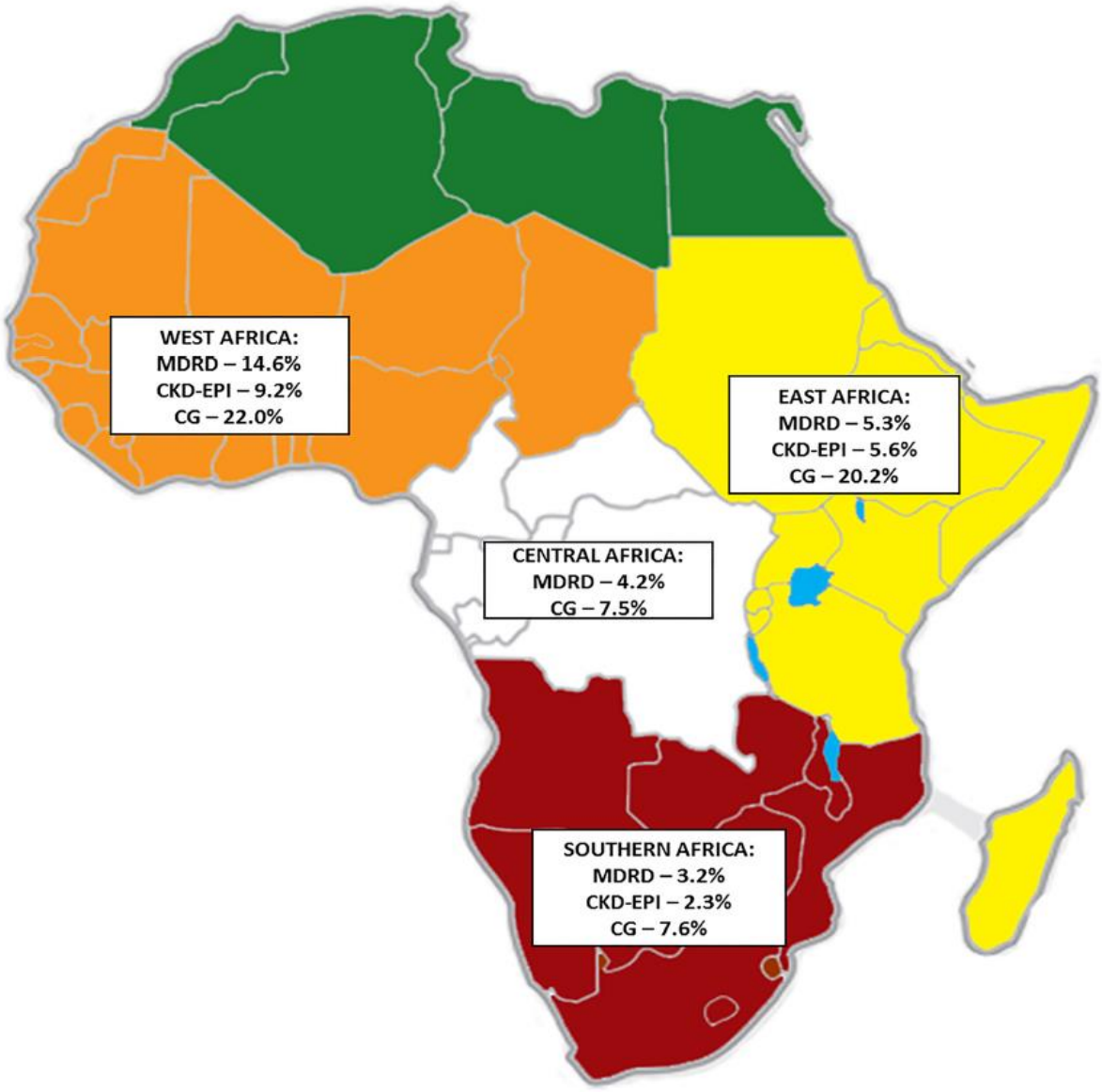

Fig 5. Summary of pooled prevalence of CKD in HIV populations of the African sub-regions.

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individuals with eGFR less than 60ml/min/1.73m^2 in the general population is not clear but Stanifer et al[20] reported a pooled prevalence of 13.9% using both eGFR and proteinuria in the definition of CKD. Studies in Sub-saharan Africa have reported a prevalence of eGFR less than 60ml/min/1.73m^2 of 1.6%[93] to 8.0%[94] using the MDRD formula. In this analysis, the pooled prevalence (using MDRD) was 7.9% for Sub-Saharan Africa. However, studies undertaking head-to-head comparison of CKD prevalence in the HIV–infected population and the general population [95, 96] in climes with better data collection suggest higher CKD prevalence in the HIV population than the general population.

Consistently, there was significant difference in the prevalence reported across the three estimators. Prevalence estimates obtained using the CG equation were generally higher than those obtained from MDRD and CKD-EPI with CKD-EPI being the most conservative of the three. In the general population, the CKD-EPI equation appears to outperform the MDRD and CG equations [97–99]; however, the best equation for GFR estimation and cut-off for definition of CKD in HIV patients remains controversial [100]. Some authors have suggested that existing equations do not take into account the lean muscle mass of malnourished HIV patients and the lipodystrophy associated with ART use [101]. One study report suggests that the CKD-EPI equation may underestimate CKD prevalence in the HIV population in Africans [102]. Whether this is also applicable to European or North American HIV populations, is uncertain. Other studies have supported the idea that eGFR values obtained from CG do not have clinically significant difference from those obtained from CKD-EPI equation in HIV patients and so could be used interchangeably [103], while MDRD is thought to be less sensitive to moderate GFR reductions and thus not useful in HIV patients with early CKD [64]. Noteworthy is the observation that most of the studies from Africa (where patients present with advanced HIV disease) used the Cockroft-Gault equation to estimate GFR either alone or in combination with other creatinine-based formulae. This may be responsible for the relatively higher CKD prevalence obtained from the Cockroft-Gault equation. There have been attempts at validating these creatinine-based estimators in the HIV population [104–106] but there is yet no consensus on the best creatinine-based GFR estimator in this special population.

Although not statistically significant in most of the comparisons, there was clearly a trend towards lower CKD prevalence estimates in the studies with more than one GFR estimate compared with those with only one estimate. This validates the KDIGO position of demonstration of GFR <60ml/min/1.73m^2 for at least 3 months [107] before a firm diagnosis of CKD is made. This may provide evidence of significant risk of overestimation of CKD prevalence in single eGFR studies because of the possibility of undiagnosed acute kidney injury (AKI) especially in patients with HIV who tend to have higher risk of AKI than the general population [108].

Hypertension and diabetes mellitus remains significant risk factors for CKD in the HIV population as seen in this analysis when head-to-head comparison was performed between HIV only cohorts and HIV/hypertension or DM co-morbidities. Both hypertension and diabetes mellitus are age-related conditions and with the increasing age of HIV patients, a higher prevalence of CKD might be predicted in future in HIV positive patients. Both conditions, however, did not appear to explain some of the heterogeneities in CKD prevalence estimates, when comparison was made based on median hypertension or DM prevalence (S5 Table). This may not be unconnected with the lack of uniformity in the definition or method of assessment of these factors among the constituent studies. For example, one study [38] defined hypertension as blood pressure of at least 160/90mmHg while others [33, 43] used a cut-off of 140/90mmHg. Also, some studies [35, 44, 85] did not provide definition of hypertension while others [36] used patient-reported history of hypertension. Similarly diabetes mellitus had

varying definitions ranging from self-reported history of diabetes mellitus [36] to a combination of fasting plasma glucose, random plasma glucose, related symptoms and current use of antidiabetic medication [37, 90] or inadequate information about criteria for diagnosis [82]. However, multivariate regression in some of the component studies [38, 82, 109] identified significant association between diabetes and hypertension with CKD in HIV patients.

The effect of hepatitis B and/or hepatitis C on CKD occurrence in HIV patients has not been consistent. In this study, we found no significant difference in the pooled prevalence of studies with high hepatitis B or C co-infection compared with those with low prevalence of these viral co-infections. Some observational studies have found a higher risk of CKD [35, 90, 110] among hepatitis B or C co-infected HIV patients while others found no significant effect with hepatitis B or C co-infection [36, 37]. A meta-analysis investigating the effect of hepatitis C co-infection on CKD occurrence and progression in HIV patients [111] found significantly increased risk of CKD, proteinuria and AKI in co-infected individuals compared to those with only HIV infection. We are unaware of any published meta-analysis comparing CKD prevalence or progression in HIV-hepatitis B co-infected individuals with HIV only patients though observational studies [110, 112] suggest increased CKD risk with hepatitis B co-infection. Aggregation of data from high-income countries (high HCV co-infection and relatively low CKD prevalence) with LMIC (low HCV co-infection and high CKD-HIV prevalence) may have led to a loss of significant difference in CKD prevalence in the HIV-HCV co-infected compared to those without the co-infection.

One possible reason for the relatively high prevalence of CKD in African patients is late presentation to HIV care clinics at advanced stages of disease. This is evidenced by the significantly lower CD4 counts in African patients compared to the other regions. This may be compounded by late initiation of anti-retroviral medications giving adequate time for HIV–induced or related damage to the kidneys. In North America, ARTs are given to all HIV–infected individuals regardless of CD4 count to reduce morbidity and mortality associated with HIV infection [113]. This is has not been the case in most SSA countries where cut-offs of CD4 counts were used for initiation of ART [South Africa (2013), < 350cells/μl [114]; South Africa (2015), <500 cells/μl [115]; Nigeria (2007), <200/μl [116]; Nigeria (2010), <350 cells/μl [117]]. It was only in 2016 that ART initiation was done regardless of CD4 count in some SSA countries. The effect of this policy change on CKD prevalence among HIV patients may only become apparent in the future. Early initiation of ARTs, especially in blacks, has been proposed as one of the measures for preventing CKD progression among HIV patients [118]. As more patients in SSA access ARTs it is possible that the incidence of CKD may not be too different between individuals of SSA origin compared with Caucasians [81]. There is also the problem of poor and inadequate facilities for long term monitoring of HIV patients on ARTs in Africa which makes early diagnosis of CKD difficult.

Furthermore, CKD in HIV patients may occur because of repeated episodes of undocumented AKI. AKI is common among HIV patients and is an important cause of morbidity and mortality in this patient group with sepsis and hypovolemia from diarrhea being the commonest causes [119–121]. AKI has also been documented as an independent risk factor for future ESRD with increasing ESRD risk associated with worsening AKI stage in HIV patients [109, 122].

The higher prevalence of HIV–related kidney disease in African Americans compared to Caucasian Americans [52] and very high CKD prevalence among HIV patients in West Africa suggests a possible genetic role in the increased CKD prevalence in SSA. This hypothesis is further strengthened by the observation that most African Americans are of West African origin and this study has shown the highest prevalence of CKD in HIV among West Africans. APOL1 and MYH9 polymorphisms have been implicated in conferring possible increased risk

of CKD in Africans [123–127] but there may be more, yet to be identified, genetic risk factors. There also may be confounding environmental factors in Africa contributing to the increased CKD risk among HIV patients.

The global HIV population is quite heterogeneous; male preponderance in North America and Europe whereas females constitute 60–70% of the HIV patients in the African studies reviewed. The influence, if any, of gender difference on the CKD prevalence remains unclear. The prevalence of traditional risk factors for CKD like hypertension, diabetes mellitus and Hepatitis C is also higher in North America and Europe than in Africa. The high prevalence of these modifiable CKD risk factors present a window of opportunity for sustaining therapies that may ultimately slow down CKD progression. The experience garnered from chronic care management of HIV could be leveraged as a platform for integration of non-communicable disease services into HIV populations. The different dimensions of HIV care–prevention, diagnosis, enrollment into care, disease management and palliative care—could also be useful for NCDs. The integrated care model appears to have achieved good results in parts of SSA [128] and emphasis on CKD preventive services among the HIV population may reduce the burden of CKD in LMICs.

There is still inadequate information about the best creatinine–based eGFR formula for Africa in general [93] and the HIV population specifically and as our study has not been able to address this, it is a limitation. Some have suggested that non-inclusion of race to the MDRD equation may improve eGFR estimation in Africans [129] but this has not been validated in the HIV population. The use of Cystatin C is not yet widespread in Africa and may not be sustainable in Africa because of the cost. It is important to determine the best measure of CKD in this special population. We did not include individuals with eGFR greater than 60mls/min/1.73m^2 and persistent proteinuria in this study. If the definition of CKD was made to include persistent proteinuria, then the prevalence of CKD among HIV patients may be much higher than reported in this study. The lack of information on specific antiretroviral drugs and their potential contribution to the burden of CKD in this work is a limitation.

The burden of CKD in HIV positive patients is high globally, particularly in African patients. HIV treatment programs need to intensify routine screening for CKD at baseline and ART follow up clinics using relatively cheap and simple test for urinary proteins. There is now a great need to produce global guidelines for CKD identification and treatment in HIV patients and integrate treatment for chronic non-communicable disease with HIV patient care.

Supporting information

S1 Table. PRISMA 2009 checklist.
(DOCX)

S2 Table. Search strategy for Pubmed, web of science, EBSCO host and AJOL.
(DOCX)

S3 Table. Scoring criteria for quality of studies.
(DOCX)

S4 Table. Assessment of methodological quality of included articles.
(DOCX)

S5 Table. Summary statistics from meta-analyses of prevalence studies on CKD in people with HIV random effects model and arcsine transformations (subgroup analyses of

gender, ARV status, CD4 count levels, Age groups, and co-morbid hypertension, diabetes mellitus, hepatitis B and C infection).
(DOCX)

S1 Fig. Forest plot showing the pooled prevalence of CKD in HIV populations for studies with at least two eGFR estimates using MDRD, CKD-EPI and CG equations.
(TIF)

S2 Fig. Funnel plots for studies with two or more eGFR values.
(TIF)

S3 Fig. Forest plot showing pooled prevalence for studies with two or more MDRD-based eGFR estimates across the WHO regions.
(TIF)

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References

1. Liyanage T, Ninomiya T, Jha V, Neal B, Patrice HM, Okpechi I, et al. Worldwide access to treatment for end-stage kidney disease: a systematic review. The Lancet. 2015; 385(9981):1975–82.
2. Han TM, Naicker S, Ramdial PK, Assounga AG. A cross-sectional study of HIV-seropositive patients with varying degrees of proteinuria in South Africa. Kidney international. 2006; 69(12):2243–50. https://doi.org/10.1038/sj.ki.5000339 PMID: 16672914
3. Szczech LA, Menezes P, Byrd Quinlivan E, van der Horst C, Bartlett JA, Svetkey LP. Microalbuminuria predicts overt proteinuria among patients with HIV infection. HIV medicine. 2010; 11(7):419–26. https://doi.org/10.1111/j.1468-1293.2009.00805.x PMID: 20059571
4. Musso CG, Belloso WH, Glassock RJ. Water, electrolytes, and acid-base alterations in human immunodeficiency virus infected patients. World journal of nephrology. 2016; 5(1):33–42. https://doi.org/10.5527/wjn.v5.i1.33 PMID: 26788462
5. Li Y, Shlipak MG, Grunfeld C, Choi AI. Incidence and risk factors for acute kidney injury in HIV Infection. American journal of nephrology. 2012; 35(4):327–34. https://doi.org/10.1159/000337151 PMID: 22456100
6. Mocroft A, Lundgren JD, Ross M, Fux CA, Reiss P, Moranne O, et al. Cumulative and current exposure to potentially nephrotoxic antiretrovirals and development of chronic kidney disease in HIV-positive individuals with a normal baseline estimated glomerular filtration rate: a prospective international cohort study. The lancet HIV. 2016; 3(1):e23–32. https://doi.org/10.1016/S2352-3018(15)00211-8 PMID: 26762990

7. Poignet JL, Desassis JF, Chanton N, Litchinko MB, Zins B, Kolko A, et al. [Prevalence of HIV infection in dialysis patients: results of a national multicenter study]. Nephrologie. 1999; 20(3):159–63. PMID: 10418006
8. Vigneau C, Guiard-Schmid JB, Tourret J, Flahault A, Rozenbaum W, Pialoux G, et al. The clinical characteristics of HIV-infected patients receiving dialysis in France between 1997 and 2002. Kidney international. 2005; 67(4):1509–14. https://doi.org/10.1111/j.1523-1755.2005.00229.x PMID: 15780104
9. Barril G, Trullas JC, Gonzalez-Parra E, Moreno A, Bergada E, Jofre R, et al. [Prevalence of HIV-1-infection in dialysis units in Spain and potential candidates for renal transplantation: results of a Spanish survey]. Enfermedades infecciosas y microbiologia clinica. 2005; 23(6):335–9.
10. Trullas JC, Barril G, Cofan F, Moreno A, Cases A, Fernandez-Lucas M, et al. Prevalence and clinical characteristics of HIV type 1-infected patients receiving dialysis in Spain: results of a Spanish survey in 2006: GESIDA 48/05 study. AIDS research and human retroviruses. 2008; 24(10):1229–35. https://doi.org/10.1089/aid.2008.0158 PMID: 18834322
11. Halle MP, Takongue C, Kengne AP, Kaze FF, Ngu KB. Epidemiological profile of patients with end stage renal disease in a referral hospital in Cameroon. BMC nephrology. 2015; 16:59. https://doi.org/10.1186/s12882-015-0044-2 PMID: 25896605
12. Madala ND, Thusi GP, Assounga AG, Naicker S. Characteristics of South African patients presenting with kidney disease in rural KwaZulu-Natal: a cross sectional study. BMC nephrology. 2014; 15:61. https://doi.org/10.1186/1471-2369-15-61 PMID: 24731300
13. Mocroft A, Kirk O, Reiss P, De Wit S, Sedlacek D, Beniowski M, et al. Estimated glomerular filtration rate, chronic kidney disease and antiretroviral drug use in HIV-positive patients. AIDS (London, England). 2010; 24(11):1667–78.
14. Rosenberg AZ, Naicker S, Winkler CA, Kopp JB. HIV-associated nephropathies: epidemiology, pathology, mechanisms and treatment. Nature Reviews Nephrology. 2015; 11(3):150–60. https://doi.org/10.1038/nrneph.2015.9 PMID: 25686569
15. Winston JA. HIV and CKD epidemiology. Advances in chronic kidney disease. 2010; 17(1):19–25. https://doi.org/10.1053/j.ackd.2009.08.006 PMID: 20005485
16. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS med. 2009; 6(7):e1000097. https://doi.org/10.1371/journal.pmed.1000097 PMID: 19621072
17. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Annals of internal medicine. 1999; 130(6):461–70. PMID: 10075613
18. Cockcroft DW, Gault H. Prediction of creatinine clearance from serum creatinine. Nephron. 1976; 16 (1):31–41. https://doi.org/10.1159/000180580 PMID: 1244564
19. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Annals of internal medicine. 2009; 150(9):604–12. PMID: 19414839
20. Stanifer JW, Jing B, Tolan S, Helmke N, Mukerjee R, Naicker S, et al. The epidemiology of chronic kidney disease in sub-Saharan Africa: a systematic review and meta-analysis. The Lancet Global health. 2014; 2(3):e174–81. https://doi.org/10.1016/S2214-109X(14)70002-6 PMID: 25102850
21. WHO. WHO Regional Offices Geneva: World Health Organization; 2017 [http://www.who.int/about/regions/en/.
22. AU. Strengthening popular participation in the African Union—a guide to AU structures and processes. South Africa: Open Society Initiative for Southern Africa; 2009. p. 11, 62–3.
23. Sakajiki AM, Adamu B, Arogundade FA, Abdu A, Atanda AT, Garba BI. Prevalence, risk factors, and histological pattern of kidney disease in patients with Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome at Aminu Kano Teaching Hospital: A clinicopathologic study. Annals of Nigerian Medicine. 2014; 8(2):69–75.
24. Higgins J, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses [journal article as teaching resource, deposited by John Flynn]. British medical journal. 2003; 327:557–60. https://doi.org/10.1136/bmj.327.7414.557 PMID: 12958120
25. Barendregt JJ, Doi SA, Lee YY, Norman RE, Vos T. Meta-analysis of prevalence. Journal of epidemiology and community health. 2013; 67(11):974–8. https://doi.org/10.1136/jech-2013-203104 PMID: 23963506
26. Cochran WG. The combination of estimates from different experiments. Biometrics. 1954; 10(1):101–29.
27. Higgins J, Thompson SG. Quantifying heterogeneity in a meta-analysis. Statistics in medicine. 2002; 21(11):1539–58. https://doi.org/10.1002/sim.1186 PMID: 12111919

28. Egger M, Smith GD, Minder C. Bias in meta-analysis detected by a simple, graphical test. British Medical Journal. 1998; 316(7129):470–1.
29. Viera AJ, Garrett JM. Understanding interobserver agreement: the kappa statistic. Fam Med. 2005; 37 (5):360–3. PMID: 15883903
30. Wallace BC, Schmid CH, Lau J, Trikalinos TA. Meta-Analyst: software for meta-analysis of binary, continuous and diagnostic data. BMC medical research methodology. 2009; 9(1):80.
31. Nyaga VN, Arbyn M, Aerts M. Metaprop: a Stata command to perform meta-analysis of binomial data. Archives of public health = Archives belges de sante publique. 2014; 72(1):39. https://doi.org/10.1186/2049-3258-72-39 PMID: 25810908
32. Adedeji TA, Adedeji NO, Adebisi SA, Idowu AA, Fawale MB, Jimoh KA. Prevalence and Pattern of Chronic Kidney Disease in Antiretroviral-Naive Patients with HIV/AIDS. Journal of the International Association of Providers of AIDS Care. 2015; 14(5):434–40. https://doi.org/10.1177/2325957415587570 PMID: 26013249
33. Al-Sheikh H, Al-Sunaid M, Alrajhi AA. HIV-associated nephropathy in Saudi Arabia. Annals of Saudi Medicine. 2013; 33(4):347–50. https://doi.org/10.5144/0256-4947.2013.347 PMID: 24060712
34. Anyabolu EN, Chukwuonye II, Arodiwe E, Ijoma CK, Ulasi I. Prevalence and predictors of chronic kidney disease in newly diagnosed human immunodeficiency virus patients in Owerri, Nigeria. Indian Journal of Nephrology. 2016; 26(1):10–5. https://doi.org/10.4103/0971-4065.156115 PMID: 26937072
35. Cao Y, Gong MC, Han Y, Xie J, Li XM, Zhang LX, et al. Prevalence and risk factors for chronic kidney disease among HIV-infected antiretroviral therapy-naive patients in Mainland China: A multicenter cross-sectional study. Nephrology. 2013; 18(4):307–12. https://doi.org/10.1111/nep.12031 PMID: 23311442
36. Cailhol J, Nkurunziza B, Izzedine H, Nindagiye E, Munyana L, Baramperanye E, et al. Prevalence of chronic kidney disease among people living with HIV/AIDS in Burundi: a cross-sectional study. BMC nephrology. 2011; 12.
37. Calza L, Vanino E, Magistrelli E, Salvadori C, Cascavilla A, Colangeli V, et al. Prevalence of renal disease within an urban HIV-infected cohort in northern Italy. Clinical and experimental nephrology. 2014; 18(1):104–12. https://doi.org/10.1007/s10157-013-0817-5 PMID: 23712539
38. Campbell LJ, Ibrahim F, Fisher M, Holt SG, Hendry BM, Post FA. Spectrum of chronic kidney disease in HIV-infected patients. HIV medicine. 2009; 10(6):329–36. https://doi.org/10.1111/j.1468-1293.2008.00691.x PMID: 19226409
39. Choi AI, Rodriguez RA, Bacchetti P, Volberding PA, Havlir D, Bertenthal D, et al. Low rates of antiretroviral therapy among HIV-infected patients with chronic kidney disease. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2007; 45(12):1633–9.
40. Crum-Cianflone N, Ganesan A, Teneza-Mora N, Riddle M, Medina S, Barahona I, et al. Prevalence and factors associated with renal dysfunction among HIV-infected patients. AIDS patient care and STDs. 2010; 24(6):353–60. https://doi.org/10.1089/apc.2009.0326 PMID: 20515419
41. Colson AW, Florence E, Augustijn H, Verpooten GA, Lynen L, Gheuens E. Prevalence of chronic renal failure stage 3 or more in HIV-infected patients in Antwerp: an observational study. Acta clinica Belgica. 2010; 65(6):392–8. https://doi.org/10.1179/acb.2010.65.6.004 PMID: 21268952
42. Ekat MH, Courpotin C, Diafouka M, Akolbout M, Mahambou-Nsonde D, Bitsindou PR, et al. [Prevalence and factors associated with renal disease among patients with newly diagnoses of HIV in Brazzaville, Republic of Congo]. Medecine et sante tropicales. 2013; 23(2):176–80. https://doi.org/10.1684/mst.2013.0170 PMID: 23787222
43. Fernando SK, Finkelstein FO, Moore BA, Weissman S. Prevalence of chronic kidney disease in an urban HIV infected population. American Journal of the Medical Sciences. 2008; 335(2):89–94.
44. Fischer MJ, Wyatt CM, Gordon K, Gibert CL, Brown ST, Rimland D, et al. Hepatitis C and the risk of kidney disease and mortality in veterans with HIV. Journal of acquired immune deficiency syndromes (1999). 2010; 53(2):222–6.
45. Flandre P, Pugliese P, Cuzin L, Bagnis CI, Tack I, Cabie A, et al. Risk Factors of Chronic Kidney Disease in HIV-infected Patients. Clinical Journal of the American Society of Nephrology. 2011; 6 (7):1700–7. https://doi.org/10.2215/CJN.09191010 PMID: 21566114
46. George E, Lucas GM, Nadkarni GN, Fine DM, Moore R, Atta MG. Kidney function and the risk of cardiovascular events in HIV-1-infected patients. AIDS (London, England). 2010; 24(3):387–94.
47. Gonzalez-Lopez A, Chocarro-Martinez A, Alvarez-Navia F, Alvarez-Tundidor S, Andres-Martin B, Nava-Rebollo A, et al. Prevalence of chronic kidney disease in patients infected by the human immunodeficiency virus. Nefrologia. 2014; 34(1):126–9.

48. Gracey D, Chan D, Bailey M, Richards D, Dalton B. Screening and management of renal disease in human immunodeficiency virus-infected patients in Australia. Internal medicine journal. 2013; 43 (4):410–6. https://doi.org/10.1111/j.1445-5994.2012.02933.x PMID: 22931386

49. Ibrahim F, Hamzah L, Jones R, Nitsch D, Sabin C, Post FA. Comparison of CKD-EPI and MDRD to estimate baseline renal function in HIV-positive patients. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association—European Renal Association. 2012; 27(6):2291–7.

50. Longo AL, Lepira FB, Sumaili EK, Makulo JRR, Mukumbi H, Bukabau JB, et al. Prevalence of Low Estimated Glomerular Filtration Rate, Proteinuria, and Associated Risk Factors Among HIV-Infected Black Patients Using Cockroft-Gault and Modification of Diet in Renal Disease Study Equations. Jaids-Journal of Acquired Immune Deficiency Syndromes. 2012; 59(1):59–64.

51. Lucas GM, Clarke W, Kagaayi J, Atta MG, Fine DM, Laeyendecker O, et al. Decreased Kidney Function in a Community-based Cohort of HIV-Infected and HIV-Negative Individuals in Rakai, Uganda. Jaids-Journal of Acquired Immune Deficiency Syndromes. 2010; 55(4):491–4.

52. Lucas GM, Lau B, Atta MG, Fine DM, Keruly J, Moore RD. Chronic kidney disease incidence, and progression to end-stage renal disease, in HIV-Infected individuals: A tale of two races. Journal of Infectious Diseases. 2008; 197(11):1548–57. https://doi.org/10.1086/587994 PMID: 18422458

53. Mayor AM, Dworkin M, Quesada L, Rios-Olivares E, Hunter-Mellado RF. The morbidity and mortality associated with kidney disease in an HIV-infected cohort in Puerto Rico. Ethnicity & disease. 2010; 20 (1 Suppl 1):S1-163-7.

54. Menezes AM, Torelly J, Real L, Bay M, Poeta J, Sprinz E. Prevalence and Risk Factors Associated to Chronic Kidney Disease in HIV-Infected Patients on HAART and Undetectable Viral Load in Brazil. Plos One. 2011; 6(10).

55. Msango L, Downs JA, Kalluvya SE, Kidenya BR, Kabangila R, Johnson WD, et al. Renal dysfunction among HIV-infected patients starting antiretroviral therapy. AIDS (London, England). 2011; 25 (11):1421–5.

56. Mulenga LB, Kruse G, Lakhi S, Cantrell RA, Reid SE, Zulu I, et al. Baseline renal insufficiency and risk of death among HIV-infected adults on antiretroviral therapy in Lusaka, Zambia. AIDS (London, England). 2008; 22(14):1821–7.

57. Nakamura Y, Shibuya A, Suzuki H, Ando M. [Prevalence of chronic kidney disease (CKD) and significant contributors to CKD in HIV-infected patients]. Nihon Jinzo Gakkai shi. 2008; 50(4):499–505. PMID: 18546881

58. Obirikorang C, Osakunor DNM, Ntaadu B, Adarkwa OK. Renal Function in Ghanaian HIV-Infected Patients on Highly Active Antiretroviral Therapy: A Case-Control Study. Plos One. 2014; 9(6).

59. Okafor UH, Unuigbe EI, Wokoma FS. Spectrum of Clinical Presentations in Human Immunodeficiency Virus (HIV) Infected Patients with Renal Disease. African journal of infectious diseases. 2011; 5 (2):28–32. PMID: 23878704

60. Overton ET, Nurutdinova D, Freeman J, Seyfried W, Mondy KE. Factors associated with renal dysfunction within an urban HIV-infected cohort in the era of highly active antiretroviral therapy. HIV medicine. 2009; 10(6):343–50. https://doi.org/10.1111/j.1468-1293.2009.00693.x PMID: 19490182

61. Owiredu WK, Quaye L, Amidu N, Addai-Mensah O. Renal insufficiency in Ghanaian HIV infected patients: need for dose adjustment. African health sciences. 2013; 13(1):101–11. https://doi.org/10.4314/ahs.v13i1.14 PMID: 23658575

62. Peck RN, Shedafa R, Kalluvya S, Downs JA, Todd J, Suthanthiran M, et al. Hypertension, kidney disease, HIV and antiretroviral therapy among Tanzanian adults: a cross-sectional study. Bmc Medicine. 2014; 12.

63. Sarfo FS, Keegan R, Appiah L, Shakoor S, Phillips R, Norman B, et al. High prevalence of renal dysfunction and association with risk of death amongst HIV-infected Ghanaians. Journal of Infection. 2013; 67(1):43–50. https://doi.org/10.1016/j.jinf.2013.03.008 PMID: 23542785

64. Stohr W, Walker AS, Munderi P, Tugume S, Gilks CF, Darbyshire JH, et al. Estimating glomerular filtration rate in HIV-infected adults in Africa: comparison of Cockcroft-Gault and Modification of Diet in Renal Disease formulae. Antiviral therapy. 2008; 13(6):761–70. PMID: 18839777

65. Sorli ML, Guelar A, Montero M, Gonzalez A, Rodriguez E, Knobel H. Chronic kidney disease prevalence and risk factors among HIV-infected patients. Jaids-Journal of Acquired Immune Deficiency Syndromes. 2008; 48(4):506–8.

66. Umeizudike T, Mabayoje M, Okany C, Adeyomoye A, Okubadejo N. Prevalence of chronic kidney disease in HIV positive patients in Lagos, south-west Nigeria. Nephrology Rev. 2012; 4:22–6.

67. Wyatt CM, Winston JA, Malvestutto CD, Fishbein DA, Barash I, Cohen AJ, et al. Chronic kidney disease in HIV infection: an urban epidemic. AIDS (London, England). 2007; 21(15):2101–3.

68. Yanagisawa N, Ando M, Suganuma A, Akifumi I, Ajisawa A. [Prevalence of kidney disease in HIV-infected patients in Japan—A single center study]. Kansenshogaku zasshi The Journal of the Japanese Association for Infectious Diseases. 2010; 84(1):28–32. PMID: 20170011

69. Yanagisawa N, Ando M, Ajisawa A, Imamura A, Suganuma A, Tsuchiya K, et al. Clinical characteristics of kidney disease in Japanese HIV-infected patients. Nephron Clinical practice. 2011; 118(3): c285–91. https://doi.org/10.1159/000322278 PMID: 21212692

70. Agbaji OO, Onu A, Agaba PE, Muazu MA, Falang KD, Idoko JA. Predictors of impaired renal function among HIV infected patients commencing highly active antiretroviral therapy in Jos, Nigeria. Nigerian medical journal: journal of the Nigeria Medical Association. 2011; 52(3):182–5.

71. Brennan A, Evans D, Maskew M, Naicker S, Ive P, Sanne I, et al. Relationship between renal dysfunction, nephrotoxicity and death among HIV adults on tenofovir. AIDS (London, England). 2011; 25 (13):1603–9.

72. Kamkuemah M, Kaplan R, Bekker LG, Little F, Myer L. Renal impairment in HIV-infected patients initiating tenofovir-containing antiretroviral therapy regimens in a Primary Healthcare Setting in South Africa. Tropical medicine & international health: TM & IH. 2015; 20(4):518–26.

73. Onodugo OD, Chukwuka C, Onyedum C, Ejim E, Mbah A, Nkwo P, et al. Baseline Renal Function among Antiretroviral Therapy-Naive, HIV-Infected Patients in South East Nigeria. Journal of the International Association of Providers of AIDS Care. 2013.

74. Reid A, Stohr W, Walker AS, Williams IG, Kityo C, Hughes P, et al. Severe renal dysfunction and risk factors associated with renal impairment in HIV-infected adults in Africa initiating antiretroviral therapy. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2008; 46(8):1271–81.

75. Struik GM, den Exter RA, Munthali C, Chipeta D, van Oosterhout JJ, Nouwen JL, et al. The prevalence of renal impairment among adults with early HIV disease in Blantyre, Malawi. International journal of STD & AIDS. 2011; 22(8):457–62.

76. Achhra AC, Mocroft A, Ross MJ, Ryom L, Lucas GM, Furrer H, et al. Kidney disease in antiretroviral-naive HIV-positive adults with high CD4 counts: prevalence and predictors of kidney disease at enrolment in the INSIGHT Strategic Timing of AntiRetroviral Treatment (START) trial. HIV medicine. 2015; 16:55–63. https://doi.org/10.1111/hiv.12234 PMID: 25711324

77. Bandera A, Gori A, Sabbatini F, Madeddu G, Bonora S, Libertone R, et al. Evaluation of the Prognostic Value of Impaired Renal Function on Clinical Progression in a Large Cohort of HIV-Infected People Seen for Care in Italy. PLoS One. 2015; 10(5):e0124252. https://doi.org/10.1371/journal.pone.0124252 PMID: 25933346

78. Bonjoch A, Juega J, Puig J, Perez-Alvarez N, Aiestaran A, Echeverria P, et al. High prevalence of signs of renal damage despite normal renal function in a cohort of HIV-infected patients: evaluation of associated factors. AIDS patient care and STDs. 2014; 28(10):524–9. https://doi.org/10.1089/apc.2014.0172 PMID: 25238104

79. Estrella MM, Parekh RS, Astor BC, Bolan R, Evans RW, Palella FJ Jr., et al. Chronic kidney disease and estimates of kidney function in HIV infection: a cross-sectional study in the multicenter AIDS cohort study. Journal of acquired immune deficiency syndromes (1999). 2011; 57(5):380–6.

80. Zachor H, Machekano R, Estrella MM, Veldkamp PJ, Zeier MD, Uthman OA, et al. Incidence of stage 3 chronic kidney disease and progression on tenofovir-based regimens. AIDS (London, England). 2016; 30(8):1221–8.

81. Schoffelen AF, Smit C, van Lelyveld SFL, Vogt L, Bauer MP, Reiss P, et al. Diminished Impact of Ethnicity as a Risk Factor for Chronic Kidney Disease in the Current HIV Treatment Era. Journal of Infectious Diseases. 2015; 212(2):264–74. https://doi.org/10.1093/infdis/jiv026 PMID: 25601941

82. Fulop T, Olivier J, Meador RS, Hall J, Islam N, Mena L, et al. Screening for chronic kidney disease in the ambulatory HIV population. Clinical Nephrology. 2010; 73(3):190–6. PMID: 20178717

83. Santiago P, Grinsztejn B, Friedman RK, Cunha CB, Coelho LE, Luz PM, et al. Screening for decreased glomerular filtration rate and associated risk factors in a cohort of HIV-infected patients in a middle-income country. PLoS One. 2014; 9(4):e93748. https://doi.org/10.1371/journal.pone.0093748 PMID: 24699873

84. Muramatsu T, Yanagisawa N, Chikasawa Y, Seita I, Yotsumoto M, Otaki M, et al. [Prevalence of chronic kidney disease among HIV-infected individuals in Japan—a report from two tertiary hospitals]. Kansenshogaku zasshi The Journal of the Japanese Association for Infectious Diseases. 2013; 87 (1):14–21. PMID: 23484373

85. Cheung CY, Wong KM, Lee MP, Liu YL, Kwok H, Chung R, et al. Prevalence of chronic kidney disease in Chinese HIV-infected patients. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association—European Renal Association. 2007; 22(11):3186–90.

86. Odongo P, Wanyama R, Obol JH, Apiyo P, Byakika-Kibwika P. Impaired renal function and associated risk factors in newly diagnosed HIV-infected adults in Gulu Hospital, Northern Uganda. BMC nephrology. 2015; 16:43. https://doi.org/10.1186/s12882-015-0035-3 PMID: 25881003

87. Wools-Kaloustian K, Gupta SK, Muloma E, Owino-Ong'or W, Sidle J, Aubrey RW, et al. Renal disease in an antiretroviral-naive HIV-infected outpatient population in Western Kenya. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association—European Renal Association. 2007; 22(8):2208–12.

88. Wyatt CM, Shi Q, Novak JE, Hoover DR, Szczech L, Mugabo JS, et al. Prevalence of kidney disease in HIV-infected and uninfected Rwandan women. PLoS One. 2011; 6(3):e18352. https://doi.org/10.1371/journal.pone.0018352 PMID: 21464937

89. Zhao Y, Zhang M, Shi CX, Zhang Y, Cai W, Zhao Q, et al. Renal Function in Chinese HIV-Positive Individuals following Initiation of Antiretroviral Therapy. PLoS One. 2015; 10(8):e0135462. https://doi.org/10.1371/journal.pone.0135462 PMID: 26317657

90. Hsieh M-H, Lu P-L, Kuo M-C, Lin W-R, Lin C-Y, Lai C-C, et al. Prevalence of and associated factors with chronic kidney disease in human immunodeficiency virus-infected patients in Taiwan. Journal of Microbiology, Immunology and Infection. 2015; 48(3):256–62.

91. Mizushima D, Tanuma J, Kanaya F, Nishijima T, Gatanaga H, Lam NT, et al. WHO antiretroviral therapy guidelines 2010 and impact of tenofovir on chronic kidney disease in Vietnamese HIV-infected patients. PloS one. 2013; 8(11):e79885. https://doi.org/10.1371/journal.pone.0079885 PMID: 24223203

92. Liyanage T, Ninomiya T, Jha V, Neal B, Patrice HM, Okpechi I, et al. Worldwide access to treatment for end-stage kidney disease: a systematic review. Lancet. 2015; 385(9981):1975–82. https://doi.org/10.1016/S0140-6736(14)61601-9 PMID: 25777665

93. Eastwood JB, Kerry SM, Plange-Rhule J, Micah FB, Antwi S, Boa FG, et al. Assessment of GFR by four methods in adults in Ashanti, Ghana: the need for an eGFR equation for lean African populations. Nephrology Dialysis Transplantation. 2010; 25(7):2178–87.

94. Sumaili EK, Krzesinski JM, Zinga CV, Cohen EP, Delanaye P, Munyanga SM, et al. Prevalence of chronic kidney disease in Kinshasa: results of a pilot study from the Democratic Republic of Congo. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association—European Renal Association. 2009; 24(1):117–22.

95. Goulet JL, Fultz SL, Rimland D, Butt A, Gibert C, Rodriguez-Barradas M, et al. Do patterns of comorbidity vary by HIV status, age, and HIV severity? Clinical Infectious Diseases. 2007; 45(12):1593–601. https://doi.org/10.1086/523577 PMID: 18190322

96. Guaraldi G, Orlando G, Zona S, Menozzi M, Carli F, Garlassi E, et al. Premature age-related comorbidities among HIV-infected persons compared with the general population. Clinical Infectious Diseases. 2011; 53(11):1120–6. https://doi.org/10.1093/cid/cir627 PMID: 21998278

97. Mulay AV, Gokhale SM. Comparison of serum creatinine-based estimating equations with gates protocol for predicting glomerular filtration rate in indian population. Indian J Nephrol. 2017; 27(2):124–8. https://doi.org/10.4103/0971-4065.200515 PMID: 28356664

98. Xie P, Huang JM, Lin HY, Wu WJ, Pan LP. CDK-EPI equation may be the most proper formula based on creatinine in determining glomerular filtration rate in Chinese patients with chronic kidney disease. Int Urol Nephrol. 2013; 45(4):1057–64. https://doi.org/10.1007/s11255-012-0325-7 PMID: 23136033

99. Al-Wakeel JS. Accuracy and precision of the CKD-EPI and MDRD predictive equations compared with glomerular filtration rate measured by inulin clearance in a Saudi population. Ann Saudi Med. 2016; 36 (2):128–34. https://doi.org/10.5144/0256-4947.2016.28.3.1715 PMID: 27018810

100. Mocroft A. The difficulties of classifying renal disease in HIV-infected patients. HIV medicine. 2011; 12 (1):1–3. https://doi.org/10.1111/j.1468-1293.2010.00854.x PMID: 21129144

101. Barraclough K, Er L, Ng F, Harris M, Montaner J, Levin A. A Comparison of the Predictive Performance of Different Methods of Kidney Function Estimation in a Well-Characterized HIV-Infected Population. Nephron Clinical Practice. 2009; 111(1):c39–c48. https://doi.org/10.1159/000178978 PMID: 19052469

102. Glaser N, Deckert A, Phiri S, Rothenbacher D, Neuhann F. Comparison of Various Equations for Estimating GFR in Malawi: How to Determine Renal Function in Resource Limited Settings? PLoS ONE. 2015; 10(6):e0130453. https://doi.org/10.1371/journal.pone.0130453 PMID: 26083345

103. Mocroft A, Ryom L, Reiss P, Furrer H, D'Arminio Monforte A, Gatell J, et al. A comparison of estimated glomerular filtration rates using Cockcroft–Gault and the Chronic Kidney Disease Epidemiology Collaboration estimating equations in HIV infection. HIV medicine. 2014; 15(3):144–52. https://doi.org/10.1111/hiv.12095 PMID: 24118916

104. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al. Estimating Glomerular Filtration Rate from Serum Creatinine and Cystatin C. New England Journal of Medicine. 2012; 367 (1):20–9. https://doi.org/10.1056/NEJMoa1114248 PMID: 22762315

105. Bhasin B, Lau B, Atta MG, Fine DM, Estrella MM, Schwartz GJ, et al. HIV viremia and T-cell activation differentially affect the performance of glomerular filtration rate equations based on creatinine and cystatin C. PLoS One. 2013; 8(12):e82028. https://doi.org/10.1371/journal.pone.0082028 PMID: 24376511

106. Wyatt CM, Schwartz GJ, Owino Ong'or W, Abuya J, Abraham AG, Mboku C, et al. Estimating kidney function in HIV-infected adults in Kenya: comparison to a direct measure of glomerular filtration rate by iohexol clearance. PLoS One. 2013; 8(8):e69601. https://doi.org/10.1371/journal.pone.0069601 PMID: 23950899

107. Levey AS, de Jong PE, Coresh J, El Nahas M, Astor BC, Matsushita K, et al. The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. Kidney international. 2011; 80(1):17–28. https://doi.org/10.1038/ki.2010.483 PMID: 21150873

108. Evans RDR, Hemmilä U, Craik A, Mtekateka M, Hamilton F, Kawale Z, et al. Incidence, aetiology and outcome of community-acquired acute kidney injury in medical admissions in Malawi. BMC nephrology. 2017; 18(1):21. https://doi.org/10.1186/s12882-017-0446-4 PMID: 28088183

109. Choi AI, Li Y, Parikh C, Volberding PA, Shlipak MG. Long-term clinical consequences of acute kidney injury in the HIV-infected. Kidney international. 2010; 78(5):478–85. https://doi.org/10.1038/ki.2010.171 PMID: 20520594

110. Mocroft A, Neuhaus J, Peters L, Ryom L, Bickel M, Grint D, et al. Hepatitis B and C co-infection are independent predictors of progressive kidney disease in HIV-positive, antiretroviral-treated adults. PLoS One. 2012; 7(7):e40245. https://doi.org/10.1371/journal.pone.0040245 PMID: 22911697

111. Wyatt CM, Malvestutto C, Coca SG, Klotman PE, Parikh CR. The Impact of Hepatitis C Virus Co-infection on HIV-Related Kidney Disease: A Systematic Review and Meta-analysis. AIDS (London, England). 2008; 22(14):1799–807.

112. Ganesan A, Krantz EM, Huppler Hullsiek K, Riddle MS, Weintrob AC, Lalani T, et al. Determinants of incident chronic kidney disease and progression in a cohort of HIV-infected persons with unrestricted access to health care. HIV medicine. 2013; 14(2):65–76. https://doi.org/10.1111/j.1468-1293.2012.01036.x PMID: 22808988

113. AIDSinfo. Guidelines for the use of Antiretroviral Agents in HIV-1 infected Adults and Adolescents2016 16th November 2016 https://aidsinfo.nih.gov/contentfiles/lvguidelines/AA_Recommendations.pdf.

114. Department of Health RoSA. The South African Antiretroviral treatment guidelines 20132013 17th November 2016]. http://www.sahivsoc.org/Files/2013%20ART%20Treatment%20Guidelines%20Final%2025%20March%202013%20corrected.pdf.

115. Health NDo. National Consolidated Guidelines for the Prevention of Mother-to-child transmission of HIV (PMTCT) and the Management of HIV in Children, adolesecnts and adults2015. http://www.sahivsoc.org/Files/ART%20Guidelines%2015052015.pdf.

116. Nigeria FMoH. National Guidelines for HIV and AIDS treatment and care in adolescents and adults2007 17th November 2016]. http://www.who.int/hiv/amds/Nigeria_adult_2007.pdf.

117. Nigeria FMoH. 2010 17th November 2016 17th November 2016]. https://aidsfree.usaid.gov/sites/default/files/tx_nigeria_adults_ado_2010.pdf.

118. Schwartz EJ, Szczech LA, Ross MJ, Klotman ME, Winston JA, Klotman PE. Highly Active Antiretroviral Therapy and the Epidemic of HIV+ End-Stage Renal Disease. Journal of the American Society of Nephrology. 2005; 16(8):2412–20. https://doi.org/10.1681/ASN.2005040340 PMID: 15987747

119. Prakash J, Gupta T, Prakash S, Rathore SS, Usha, Sunder S. Acute kidney injury in patients with human immunodeficiency virus infection. Indian J Nephrol. 2015; 25(2):86–90. https://doi.org/10.4103/0971-4065.138696 PMID: 25838645

120. Vachiat AI, Musenge E, Wadee S, Naicker S. Renal failure in HIV-positive patients-a South African experience. Clinical kidney journal. 2013; 6(6):584–9. https://doi.org/10.1093/ckj/sft128 PMID: 26069826

121. Naicker S, Aboud O, Gharbi MB. Epidemiology of acute kidney injury in Africa. Seminars in nephrology. 2008; 28(4):348–53. https://doi.org/10.1016/j.semnephrol.2008.04.003 PMID: 18620957

122. Chawla LS, Eggers PW, Star RA, Kimmel PL. Acute Kidney Injury and Chronic Kidney Disease as Interconnected Syndromes. New England Journal of Medicine. 2014; 371(1):58–66. https://doi.org/10.1056/NEJMra1214243 PMID: 24988558

123. Genovese G, Friedman DJ, Ross MD, Lecordier L, Uzureau P, Freedman BI, et al. Association of Trypanolytic ApoL1 Variants with Kidney Disease in African Americans. Science. 2010; 329(5993):841–5. https://doi.org/10.1126/science.1193032 PMID: 20647424

124. 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. Journal of the American Society of Nephrology. 2011; 22(11):2129–37. https://doi.org/10.1681/ASN.2011040388 PMID: 21997394

125. 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. Journal of the American Society of Nephrology. 2011; 22 (11):1991–6. https://doi.org/10.1681/ASN.2011040434 PMID: 21997397

126. Tayo BO, Kramer H, Salako BL, Gottesman O, McKenzie CA, Ogunniyi A, et al. Genetic variation in APOL1 and MYH9 genes is associated with chronic kidney disease among Nigerians. International Urology and Nephrology. 2013; 45(2):485–94. https://doi.org/10.1007/s11255-012-0263-4 PMID: 22956460

127. Ulasi II, Tzur S, Wasser WG, Shemer R, Kruzel E, Feigin E, et al. High Population Frequencies of APOL1 Risk Variants Are Associated with Increased Prevalence of Non-Diabetic Chronic Kidney Disease in the Igbo People from South-Eastern Nigeria. Nephron Clinical Practice. 2013; 123(1–2):123–8. https://doi.org/10.1159/000353223 PMID: 23860441

128. Edwards JK, Bygrave H, Van den Bergh R, Kizito W, Cheti E, Kosgei RJ, et al. HIV with non-communicable diseases in primary care in Kibera, Nairobi, Kenya: characteristics and outcomes 2010–2013. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2015; 109(7):440–6. https://doi.org/10.1093/trstmh/trv038 PMID: 25997923

129. Madala ND, Nkwanyana N, Dubula T, Naiker IP. Predictive performance of eGFR equations in South Africans of African and Indian ancestry compared with (9)(9)mTc-DTPA imaging. Int Urol Nephrol. 2012; 44(3):847–55. https://doi.org/10.1007/s11255-011-9928-7 PMID: 21373844

S1 Table: PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	4
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	S2 Table
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5 – 6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6

S1 Table: PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6/7, Fig 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	8, S4
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Fig 2
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Fig 2, S2 Fig, S4 Fig
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	S2 Fig, S3 Fig
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	10
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	15
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	15
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	15

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

S2 Table

Pubmed

Search	Add to Builder	Query
#1		Search ((((((Chronic kidney disease) OR CKD) OR Chronic renal insufficiency) OR Chronic kidney failure) OR Chronic renal failure) OR Chronic kidney insufficiency) OR GFR<60ml/min
#2		Search (((HIV) OR Human immunodeficiency virus) OR AIDS) OR Acquired immune deficiency syndrome
#3		Search (Prevalence) OR incidence
#4		Search ("1982/01/01"[Date - Publication] : "2016/09/30"[Date - Publication])

Web of Science

Set	Web of Science Core Collection Search History -
#1	TS=(Chronic Kidney Disease OR Chronic Renal insufficiency OR Chronic OR chronic Renal failure OR kidney dysfunction) *DocType=All document types; Language=All languages;*
#2	TS=(Human Immunodeficiency Virus OR HIV) *DocType=All document types; Language=All languages;*
#3	#2 AND #1 *DocType=All document types; Language=All languages;*
#4	TS=(Prevalence OR frequency OR incidence) *DocType=All document types; Language=All languages;*
#5	#4 AND #3 *DocType=All document types; Language=All languages;*

EBSCOhost

S2 ("Chronic Kidney Disease+")

S3 prevalence OR epidemiology OR frequency

S4 HIV OR human immunodeficiency virus

S5 AIDS OR acquired immunodeficiency syndrome

S6 S4 OR S5 ((HIV OR human immunodeficiency virus) OR (AIDS OR acquired immunodeficiency syndrome))

S7 S2 OR Chronic Kidney failure (Chronic Kidney insufficiency OR Renal failure)

S8 S6 AND S7 (((HIV OR human immunodeficiency virus) OR (AIDS OR acquired immunodeficiency syndrome)) AND (Chronic Kidney insufficiency OR Renal failure))

S9 S3 AND S8 (prevalence OR epidemiology OR frequency) AND (((HIV OR human immunodeficiency virus) OR (AIDS OR acquired immunodeficiency syndrome)) AND (Chronic Kidney insufficiency OR Renal failure))

AJOL

Chronic kidney disease) OR CKD) OR Chronic renal insufficiency) OR Chronic kidney failure) OR Chronic renal failure) OR Chronic kidney insufficiency) AND renal disease)) AND ((HIV) OR Human Immunodeficiency virus)) AND ((prevalence) OR incidence

S3 Table: SCORING CRITERIA FOR QUALITY OF STUDIES (Adapted from Stanifer et al. Lancet Glob Health 2014; 2 e174 – 181.

	CRITERIA QUESTION	YES(1)	NO (0)
1	Are the study participants representative of the HIV population in the country of study?		
2	Did the study exclude individuals with other established risk factors for CKD? (These factors may include Hypertension, obesity, underweight and Diabetes)		
3	Is the sample size adequate (1,000 will be regarded as adequate)		
4	Were the study participants recruited at random? (Non-probability sampling methods will be considered inadequate)		
5	Was the response rate at least 60% of the initial sample size?		
6	Was the exclusion rate less than 10% of the total sample?		
7	Was eGFR defined as being ≤ 60ml/min/1.73m^2 using the MDRD/CKD-EPI or Cockroft-Gault formulae?		
8	Was prevalence reported by ARV status? (A differentiation of prevalence in ARV – naïve patients and those on ARV).		
9	Were the sociodemographic characteristics of the study participants adequately characterized?		

High quality – 7 – 9; Medium quality – 4-6; Low quality – less than 4

S4 Table: Assessment of methodological quality of included articles

AUTHOR NAME	STUDY DESIGN	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	TOTAL	QUALITY
Adedeji et al	cross-sectional	0	1	0	0	1	1	1	0	1	5	MEDIUM
Al-Sheikh et al	cross-sectional	0	0	0	1	1	0	1	0	1	4	LOW
Anyabolu et al	cross-sectional	1	1	0	1	1	1	1	0	1	7	MEDIUM
Cao et al	cross-sectional	1	0	0	1	1	1	1	0	1	6	MEDIUM
Caihol et al	cross-sectional	1	0	0	1	1	1	1	0	1	6	MEDIUM
Calza et al	cross-sectional	1	0	0	1	1	1	1	0	1	6	MEDIUM
Campbell et al	cross-sectional	1	0	1	1	1	1	1	0	0	6	MEDIUM
Cheung et al	cross-sectional	1	0	0	1	1	1	1	0	1	6	MEDIUM
Choi et al	cross-sectional	1	0	1	1	1	1	1	1	1	8	HIGH
Cianflone et al	cross-sectional	0	0	0	1	1	1	1	1	1	6	MEDIUM
Colson et al	cross-sectional	1	1	0	1	1	1	1	1	1	8	MEDIUM
Ekat et al	cross-sectional	1	0	0	0	0	1	1	0	1	4	LOW
Fernando et al	cross-sectional	1	0	0	1	1	0	1	0	1	5	MEDIUM
Fischer et al	cross-sectional	0	0	1	1	1	1	1	0	1	6	MEDIUM
Flandre et al	retrospective cohort	0	0	1	1	1	1	1	0	1	6	HIGH

George et al	nested case-control	1	0	0	1	1	1	1	0	1	6	MEDIUM
Gonzalez et al	cross-sectional	0	0	0	0	1	1	1	0	0	3	LOW
Gracey et al	cross-sectional	1	0	0	1	1	1	1	0	1	6	MEDIUM
Ibrahim et al	retrospective cohort	1	1	1	1	1	1	1	0	1	8	HIGH
Longo et al	cross-sectional	0	0	0	0	1	1	1	1	1	5	MEDIUM
Lucas et al	retrospective cohort	1	0	1	1	1	1	1	0	0	6	MEDIUM
Lucas et al2	retrospective cohort	1	1	1	1	1	1	1	0	1	8	HIGH
Mayor et al	retrospective cohort	1	0	1	1	1	1	1	0	1	7	HIGH
Menezes et al	cross-sectional	1	0	0	1	1	1	1	0	1	6	MEDIUM
Mizushima et al	cross-sectional	1	0	0	1	0	0	1	0	1	4	MEDIUM
Msango et al	cross-sectional	1	0	0	0	1	1	1	1	1	6	MEDIUM
Mulenga LB	cross-sectional	1	1	1	1	1	1	1	0	0	7	HIGH
Nakamura et al	retrospective cohort	1	0	0	1	1	1	1	0	1	6	MEDIUM
Obirikorang et al	case-control	1	0	0	1	0	1	1	0	0	4	LOW
Odongo et al	cross-sectional	1	1	0	0	0	0	1	0	1	4	LOW
Okafor et al	cross-sectional	0	1	0	1	1	1	1	0	0	5	MEDIUM
Overton et al	cross-sectional	1	0	0	1	1	0	1	0	1	5	MEDIUM
Owiredu et al	cross-sectional	1	1	0	1	1	1	1	1	0	7	MEDIUM

Peck et al	cross-sectional	1	0	0	1	0	0	1	1	1	5	MEDIUM
Sarfo et al	retrospective cohort	1	0	1	1	1	1	1	1	1	8	HIGH
Stohr W et al	Cohort	1	0	1	1	0	0	1	1	1	6	MEDIUM
Sorli et al	cross-sectional	1	0	1	1	0	0	1	1	0	5	MEDIUM
Umezudike et al	cross-sectional	1	1	0	1	1	1	1	0	0	6	MEDIUM
Wools-Kaloustian etal	cross-sectional	1	1	0	1	0	0	1	0	1	5	MEDIUM
Wyatt et al	cross-sectional	1	0	1	1	0	1	1	0	1	6	MEDIUM
Wyatt et al (Rwanda)	cross-sectional	0	0	0	1	1	1	1	0	1	5	MEDIUM
Yanigasawa et al	cross-sectional	1	0	0	1	1	1	0	0	1	5	MEDIUM
Yanigasawa et al	cross-sectional	1	0	1	1	1	1	0	0	1	6	MEDIUM
Zhao et al	cross-sectional	1	0	1	1	1	0	1	0	1	6	MEDIUM
Agbaji et al	cross-sectional	0	0	0	1	0	0	1	0	0	2	LOW
Brennan et al	retrospective cohort	0	0	0	1	1	0	1	0	1	4	LOW
Kamkuemah et al	prospective cohort	0	0	1	0	1	0	1	0	0	3	LOW
Onodugo et al	cross-sectional	1	1	0	1	0	0	1	0	0	4	LOW
Reid et al	cross-sectional	0	0	1	1	1	1	1	0	0	5	MEDIUM

Struik et al	cross-sectional	1	0	0	1	1	1	1	0	0	5	MEDIUM
Acchra et al	prospective cohort	0	0	1	1	1	1	1	0	1	6	MEDIUM
Bandera et al	prospective cohort	1	0	1	1	1	1	1	1	1	8	HIGH
Bonjoch et al	cross-sectional	1	0	0	1	1	1	1	0	1	6	MEDIUM
Estrella et al	Cross-sectional	1	0	0	0	1	1	1	0	1	5	MEDIUM
Zachor et al	retrospective cohort	0	0	0	1	1	1	1	0	1	5	MEDIUM
Santiago et al	Cross-sectional	1	0	1	1	1	1	1	1	1	8	MEDIUM
Sakajiki et al	cross-sectional	0	0	0	1	1	1	1	0	0	4	LOW
Schoffelen et al	cross-sectional	1	0	1	1	1	1	1	0	1	7	HIGH
Fulop et al	cross-sectional	1	0	0	1	1	1	1	1	1	7	HIGH
Hsieh et al	cross-sectional	1	0	0	1	0	0	1	1	0	4	LOW
Muramatsu et al	cross-sectional	1	0	1	1	1	1	1	0	1	7	HIGH

S5 Table: Summary statistics from meta-analyses of prevalence studies on CKD in people with HIV using random effects model and arcsine transformations

Group	Subgroup	eGFR formula	Number of studies	Number of participants	Number of cases	Prevalence (95%CI)	I^2 (%)	P - heterogeneity	p-different formulae	p-diff sub-groups	p-Egger test
Gender											
	Overall								0.007		
		MDRD	19	147,637	6,612	4.7 (3.6 – 6.0)	97.6	<0.001		0.93	0.48
		CKD-EPI	3	4,599	164	3.3 (2.5-4.3)	32.7	<0.001		<0.001	0.36
		CG	9	6,205	674	12.0 (5.0-21.3)	98.1	<0.001		0.41	0.44
	Male								<0.001		
		MDRD	9	73,489	3,297	4.9 (3.1-7.0)	98.8	<0.001			0.55
		CKD-EPI	1	1,970	74	3.9 (3.0-5.1)	-	<0.001			-
		CG	4	2,773	254	8.3 (1.1-20.8)	96.6	<0.001			0.12
	Female								0.005		
		MDRD	10	74,148	3,315	4.5 (3.3-5.8)	85.9	<0.001			0.72
		CKD-EPI	2	2,629	90	3.0 (2.1-3.9)	98.4	<0.001			-
		CG	5	3,432	420	15.2 (4.9-29.7)	98.7	<0.001			0.34
ARV status											
	Overall								<0.001		
		MDRD	21	105,846	3,688	5.4 (4.5-6.4)	96.8	<0.001		0.19	0.02
		CKD-EPI	7	35,330	780	3.0 (1.8-4.5)	97.4	<0.001		0.52	0.30
		CG	8	32,073	2,824	10.4 (7.3-14.1)	97.6	<0.001		0.27	0.48
	ARV – naïve								0.06		
		MDRD	5	43,720	1,591	9.0 (3.0-17.7)	98.7	<0.001			0.22
		CKD-EPI	4	13,033	295	3.6 (1.1-7.4)	98.5	<0.001			0.41
		CG	5	5,634	508	12.4 (5.4-21.8)	98.5	<0.001			0.18
	ARV - exposed								0.002		
		MDRD	16	62,126	2,097	4.6 (3.7-5.6)	95.1	<0.001			0.02
		CKD-EPI	3	22,297	485	2.5 (1.3-4.0)	90.1	<0.001			0.67
		CG	3	26,439	2,316	7.8 (5.2-10.8)	89.2	<0.001			0.65
CD4 COUNT											
	Overall								0.007		
		MDRD	37	147,185	7,799	6.2 (4.9-7.7)	98.8	<0.001		0.37	0.22
		CKD-EPI	12	34,045	1,140	4.4 (2.2 – 7.3)	98.9	<0.001		0.59	0.27

		CG	15	54,251	4,895	12.7 (8.1 – 18.1)	99.3	<0.001		0.33	0.19
	<200 cells/ul								0.09		
		MDRD	8	40,815	1,787	6.2 (4.9-7.7)	98.8	<0.001			0.37
		CKD-EPI	3	5,750	523	4.4 (2.2-7.3)	97.1	<0.001			0.48
		CG	4	32,186	3,785	17.9 (6.5-33.4)	98.7	<0.001			0.44
	≥ 200 cells/ul								0.02		
		MDRD	29	107,339	2,708	6.5 (4.9 – 8.4)	99.1	<0.001			0.34
		CKD-EPI	9	28,295	617	3.9 (2.2-6.1)	99.1	<0.001			0.11
		CG	11	22,065	1,110	11.0 (6.1-17.0)	99.8	<0.001			0.007
	<350 cells/ul								0.02		
		MDRD	24	139,241	7,262	6.0 (4.4-7.3)	88.6	<0.001			0.37
		CKD-EPI	6	26,983	992	5.0 (1.6-10.1)	97.5	<0.001			0.40
		CG	12	36,319	4,345	14.6 (8.8-21.4)	97.2	<0.001			0.31
	≥350 cells/ul								0.16		
		MDRD	13	8,913	596	6.0 (4.4-7.8)	99.3	<0.001			0.51
		CKD-EPI	6	7,062	148	3.9 (1.0-8.3)	99.2	<0.001			0.15
		CG	3	17,932	550	6.3 (2.2-12.3)	99.4	<0.001			0.14
Age, median 38.5 years											
	Overall								0.003		
		MDRD	44	166,157	9,234	6.4 (5.2-7.7)	98.9	<0.001		0.54	0.16
		CKD-EPI	14	41,791	1,398	4.8 (2.9-7.1)	98.7	<0.001		0.98	0.15
		CG	19	59,414	5,308	12.3 (8.4-16.7)	99.5	<0.001		0.17	0.15
	Above median								0.33		
		MDRD	27	78,099	5,799	6.1 (4.6-7.9)	98.7	<0.001			0.66
		CKD-EPI	6	4,395	200	4.8 (2.3-8.2)	91.8	<0.001			0.52
		CG	6	43,844	2,906	8.5 (4.5-13.6)	99.4	<0.001			0.69
	Below Median								0.03		
		MDRD	17	87,190	3,455	6.9 (5.2-8.8)	98.7	<0.001			0.02
		CKD-EPI	8	37,396	1,198	4.8 (2.6-7.5)	99.2	<0.001			0.26
		CG	13	15,570	2,402	14.2 (7.9-21.9)	99.3	<0.001			0.95
Hypertension											
	HIV/No HTN								-		
		MDRD	10	22,649	870	5.4 (3.4-7.9)	97.9	<0.001	-	<0.001	0.15

		CKD-EPI	1	3,746	198	5.3 (4.6-6.0)	-	-		-
		CG	-	-	-	-	-	-	-	-
	HIV/HTN									
		MDRD	10	7,012	1,118	20.7 (14.3-27.8)	95.6	<0.001	-	0.29
		CKD-EPI	1	891	88	9.9 (8.1-12.0)	-	-		-
		CG	-	-	-	-	-	-	-	-
Hypertension prevalence (median 19%)										
	Overall							0.08		
		MDRD	23	55,920	4,897	5.3 (3.8-7.0)	98.2	<0.001	0.30	0.02
		CKD-EPI	9	17,564	453	3.3 (1.8-5.2)	96.8	<0.001	0.42	0.19
		CG	6	19,289	826	10.4 (3.5-20.4)	99.1	<0.001	<0.001	0.10
	Above median							<0.001		
		MDRD	12	21,638	1,495	6.2 (4.2-8.5)	96.7	<0.001		0.60
		CKD-EPI	6	8,870	216	3.9 (1.3-7.6)	97.9	<0.001		0.11
		CG	1	723	47	6.5 (4.9-8.5)	-	-		-
	Below median							0.05		
		MDRD	11	34,941	3,402	4.3 (2.2-7.2)	98.7	<0.001		0.04
		CKD-EPI	3	8,694	237	2.7 (2.4-3.0)	0.0	<0.001		0.09
		CG	5	18,566	779	11.3 (2.6-24.8)	99.1	<0.001		0.14
Diabetes										
	HIV/ No DM									
		MDRD	10	22,620	1644	8.4 (5.5-11.8)	98.2	<0.001	-	<0.001	0.38
		CKD-EPI	2	11,702	456	3.7 (3.4-4.1)	99.1	<0.001		-
		CG	-	-	-	-	-	-		-
	HIV/DM									
		MDRD	10	2403	339	19.4 (13.5-26.0)	83.6	<0.001	-	-	0.08
		CKD-EPI	2	320	36	11.2 (8.0-15.0)	99.1	<0.001			-
		CG	-	-	-	-	-	-			-

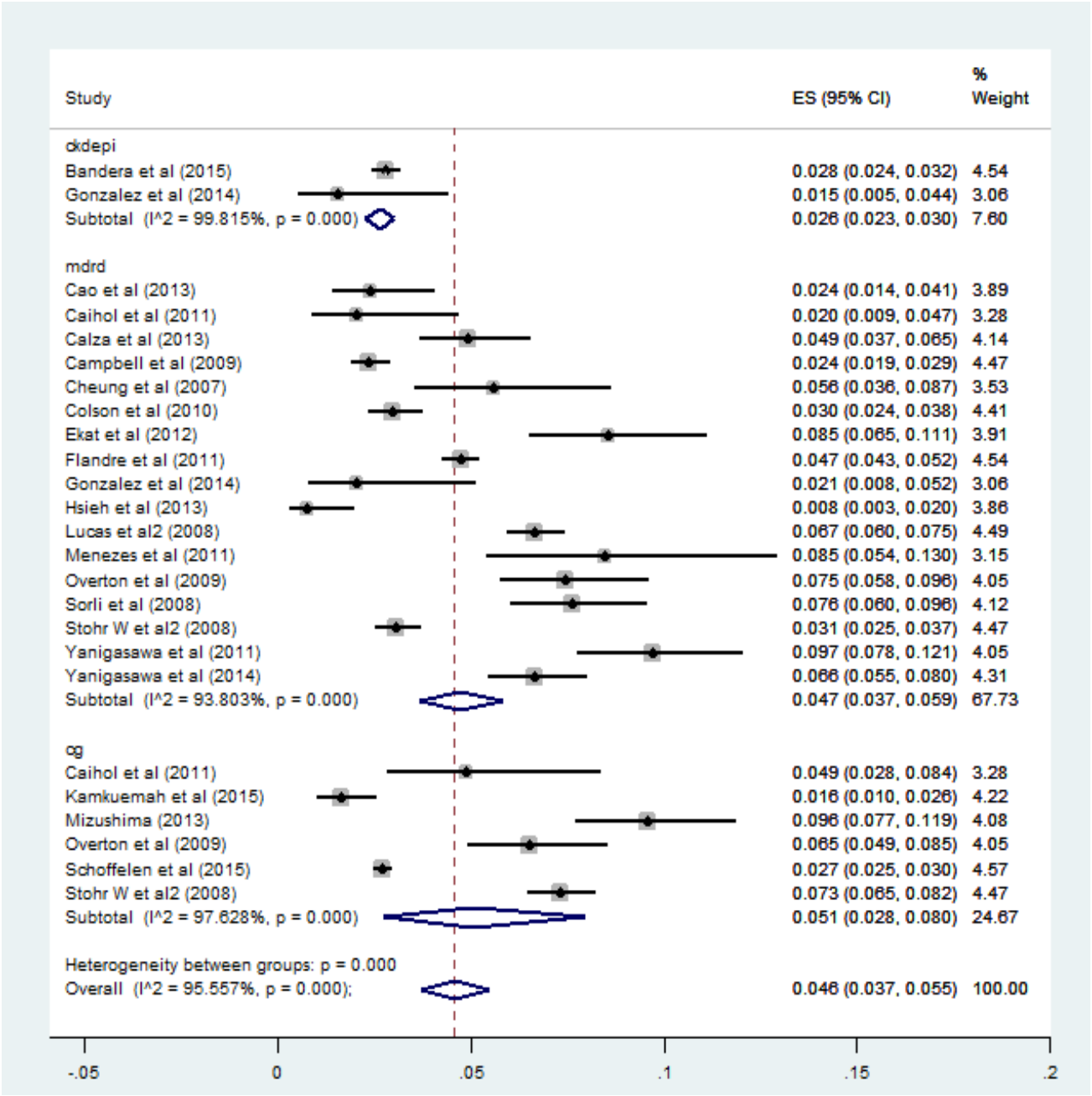
Diabetes prevalence (median 6.1%)										
	Overall							0.06		
		MDRD	23	60,538	5,172	5.5 (4.0-7.1)	98.2	<0.001	0.19	0.02
		CKD-EPI	9	17,564	453	3.3 (1.8-5.2)	96.8	<0.001	0.06	
		CG	6	19,760	870	10.4 (3.8-19.7)	99.1	<0.001	<0.001	
	Above median							<0.001		
		MDRD	14	48,857	4,618	6.1 (4.3-8.3)	98.4	<0.001		0.06
		CKD-EPI	4	3,263	165	5.6 (2.3-10.2)	94.5	<0.001		0.53
		CG	1	723	47	6.5 (4.9-8.5)	-	-		-
	Below median							0.02		
		MDRD	9	11,681	554	4.4 (3.0-6.2)	91.3	<0.001		0.84
		CKD-EPI	5	14,301	288	2.0 (0.8-3.8)	96.7	<0.001		0.90
		CG	5	19,037	823	11.2 (3.1-23.4)	99.3	<0.001		0.11
Hepatitis B										
	HIV/ No HBV							<0.001		
		MDRD	13	82,428	2,747	7.5 (5.5-9.8)	98.8	<0.001	0.89	0.02
		CKD-EPI	4	31,008	912	3.6 (2.0-5.6)	98.3	<0.001		0.33
		CG	1	419	101	24.1 (20.3-28.4)				-
	HIV/HBV							0.004		
		MDRD	13	5,741	236	7.2 (4.4-10.5)	90.2	<0.001		0.01
		CKD-EPI	4	1,574	30	2.3 (0.8-4.3)	64.5	0.04	0.44	0.21
		CG	1	72	16	22.2 (14.2-33.1)	-	-		-
Hepatitis B co-infection prevalence, median 5.6%										
	Overall							0.03		
		MDRD	18	66,090	4,376	4.3 (2.5-6.5)	99.2	<0.001	0.98	0.33
		CKD-EPI	6	34,969	728	2.0 (1.1-3.1)	96.4	<0.001	0.81	0.88
		CG	4	18,295	636	8.1 (1.9-17.9)	98.8	<0.001	<0.001	0.29

	Above median							0.03			
		MDRD	10	17,010	883	4.3 (3.1-5.6)	92.3	<0.001			0.49
		CKD-EPI	2	845	18	2.1 (1.2-3.2)	99.3	<0.001			-
		CG	2	17,327	577	3.0 (2.8-3.3)	99.6	<0.001			-
	Below median							<0.001			
		MDRD	8	49,080	3,493	4.3 (1.3-8.9)	99.7	<0.001			0.64
		CKD-EPI	4	34,124	710	2.0 (1.0-3.4)	97.8	<0.001			0.84
		CG	2	968	59	6.0 (4.6-7.7)	99.6	<0.001			-
Hepatitis C											
	HIV/No HCV							<0.001			
		MDRD	14	71,799	2,695	7.3 (5.6-9.3)	98.3	<0.001		0.74	0.01
		CKD-EPI	4	29,286	842	3.5 (1.9-5.5)	98.2	<0.001			0.36
		CG	1	433	101	23.3 (19.6-27.5)	-				
	HIV/HCV							0.06			
		MDRD	14	11,183	598	7.6 (4.5-11.4)	97.1	<0.001			0.10
		CKD-EPI	4	3,649	93	3.7 (1.8-6.2)	87.3	<0.001		0.78	0.08
		CG	1	58	16	27.6 (17.8-40.2)	-				
Hepatitis C co-infection prevalence, median – 11%											
								0.03			
	Overall	MDRD	21	109,968	5,914	4.5 (3.0-6.2)	99.2	<0.001		0.18	0.96
		CKD-EPI	6	35,102	752	2.3 (1.4-3.6)	96.9	<0.001		0.72	0.56
		CG	4	18,295	636	8.1 (1,9-17.9)	98.8	<0.001			0.29
	Above median							<0.001			
		MDRD	12	82,459	5,192	5.3 (3.1-8.1)	99.4	<0.001			0.96

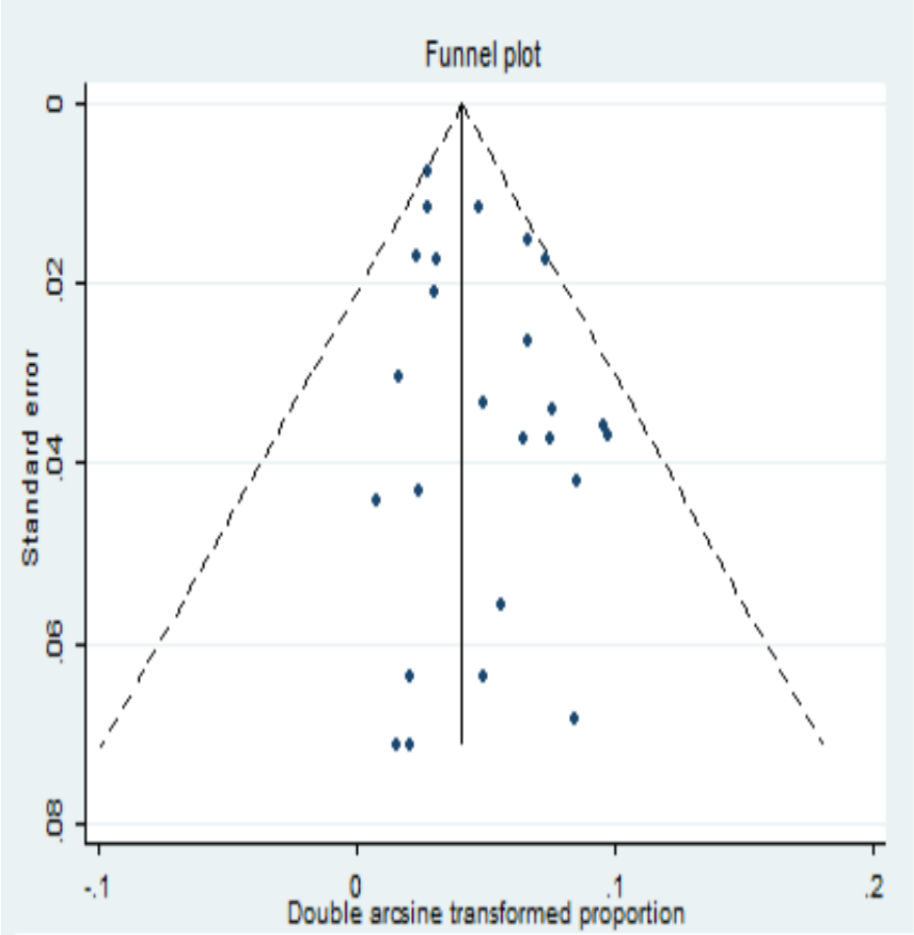
	CKD-EPI	2	7,580	209	2.6 (2.3-3.0)	99.3	<0.001		-
	CG	2	1,214	164	12.4 (10.6-14.3)	99.6	<0.001		-
Below median								0.39	
	MDRD	9	27,509	722	3.5 (2.3-4.8)	93.2	<0.001		0.16
	CKD-EPI	4	27,522	543	2.4 (1.0-4.3)	97.8	<0.001		0.58
	CG	2	17,081	472	2.7 (2.4-2.9)	99.6	<0.001		-

HTN=Hypertension; DM=Diabetes Mellitus; HBV=Hepatitis B; HCV=Hepatitis C

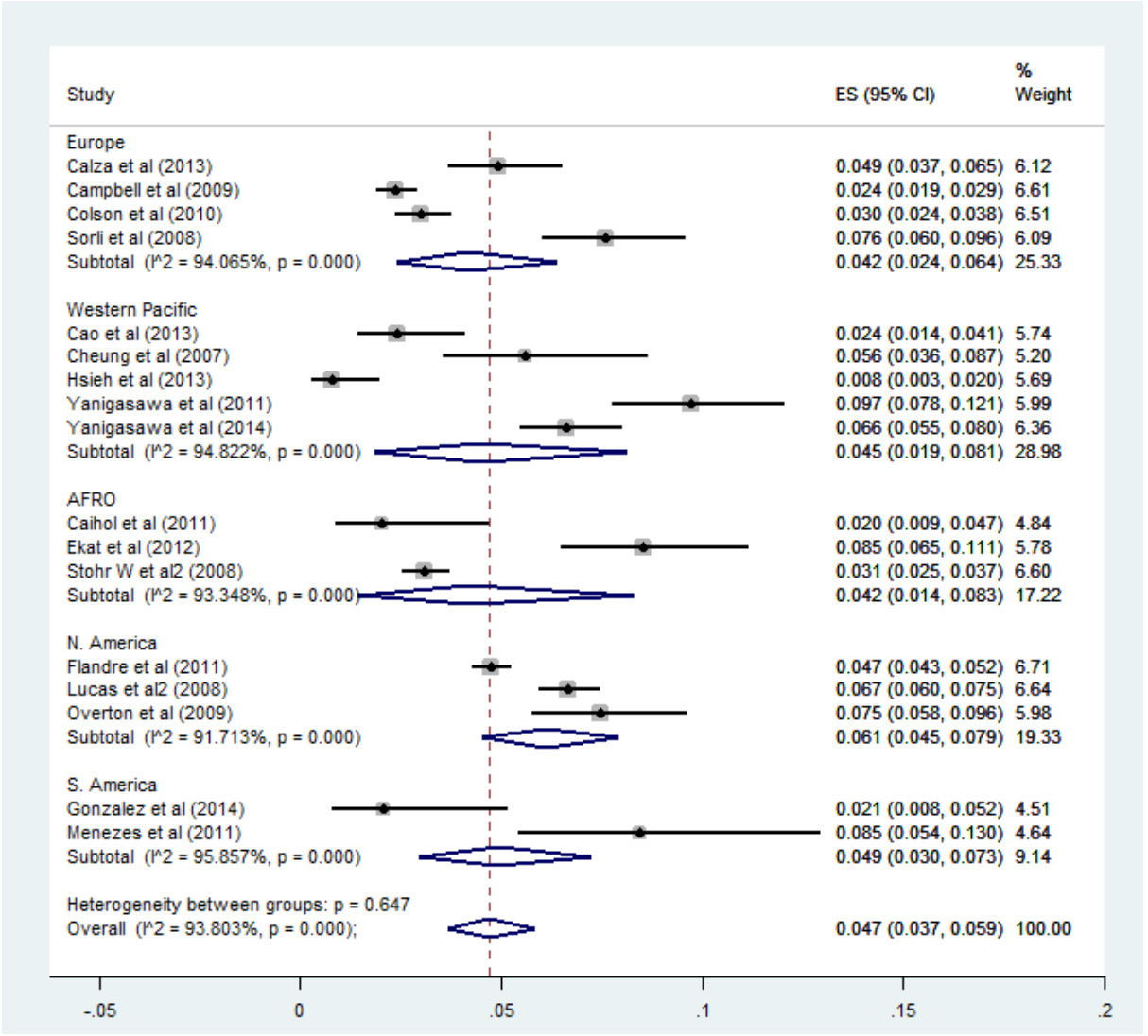
S1 Fig. Forest plot showing the pooled prevalence of CKD in HIV populations for studies with at least two eGFR estimates using MDRD, CKD-EPI and CG equations.



S2 Fig. Funnel plots for studies with two or more eGFR values.



S3 Fig. Forest plot showing pooled prevalence for studies with two or more MDRD-based eGFR estimates across the WHO regions.



Chapter 3 showed a higher prevalence of CKD in Sub-Saharan Africa compared with other WHO regions. Within Africa, West Africa had the highest recorded prevalence. There was high heterogeneity in the prevalence across the regions which could be explained by WHO region, co-morbid hypertension and diabetes mellitus levels but not by gender, hepatitis B, hepatitis C, CD4 count or antiretroviral use status.

Having seen the global picture and documented the burden of CKD in the global HIV-infected population, it became imperative to document the CKD prevalence in the HIV population from which data for this work will be drawn from.

CHAPTER 4: PREVALENCE AND CORRELATES OF CHRONIC KIDNEY (CKD) AMONG ART-NAÏVE HIV PATIENTS IN THE NIGER-DELTA REGION OF NIGERIA

Chapter 4 is a 15-year analysis among ART-naïve patients presenting at the University of Uyo Teaching Hospital HIV clinic. The prevalence of CKD in this population was documented and factors associated with CKD occurrence elucidated. This is presented in a manuscript format entitled **"Prevalence and correlates of Chronic kidney disease (CKD) among ART-naïve patients in the Niger-Delta region of Nigeria"** as published by ***Medicine (Baltimore)*** and available using the link below:

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Prevalence and correlates of chronic kidney disease (CKD) among ART-naive HIV patients in the Niger-Delta region of Nigeria

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Abstract

Widespread use of antiretroviral therapy (ART) in human immunodeficiency virus (HIV) patients has led to improved longevity with the attendant increase in noncommunicable disease prevalence including chronic kidney disease (CKD). This study documents the prevalence of CKD in a large HIV population in Southern Nigeria.

This is a single center, 15-year analysis in ART-naïve patients. CKD was defined as the occurrence of estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 on 2 consecutive occasions 3 to 12 months apart using the chronic kidney disease epidemiology collaboration (CKD-EPI) equation. The Cochran-Armitage and Cuzick tests were employed to assess for trend across the years for CKD prevalence and CD4 count, respectively. Multivariable logistic regression models were used to identify independent associations with CKD.

In all, 1317 patients (62.2% females) with mean age of 34.5 years and median CD4 count of 194 cells/μL were included. CKD prevalence was 13.4% (95%CI 11.6%–15.4%) using the CKD-EPI equation (without the race factor). Multivariable analysis identified increasing age and CD4 count <200 cells/μL as being independently associated with CKD occurrence.

This study reports a high prevalence of CKD in ART-naïve HIV-infected patients. Measures to improve diagnosis of kidney disease and ensure early initiation of treatment should be integrated in HIV treatment programmes in this setting.

Abbreviations: APOL = apolipoprotein, ART = antiretroviral therapy, BMI = body mass index, CKD = chronic kidney disease, CKD-EPI = chronic kidney disease epidemiology collaboration, DBP = diastolic blood pressure, DM = diabetes mellitus, eGFR = estimated glomerular filtration rate, HIV = human immunodeficiency virus, HIVAN = HIV associated nephropathy, SSA = Sub-Saharan Africa.

Keywords: CKD, HIV, Nigeria, prevalence

1. Introduction

As at the end of 2015, about 36.7 million people were living with the human immunodeficiency virus (HIV) globally with 23.5 million living in Sub-Saharan Africa (SSA).[1] Of those affected, 3.2 million individuals live in Nigeria with the highest prevalence reported from the Niger-Delta region.[2] Nigeria is estimated to have the 2nd highest HIV population in the world, with South Africa ranking 1st with 7 million affected individuals.[3] The increased availability of antiretroviral therapies (ARTs) in many SSA countries has led to increased life expectancy with an attendant increase of noncommunicable diseases among HIV populations.[1,4]

Kidney disease is a common complication of HIV infection[5,6] and HIV is a common cause of chronic kidney disease (CKD) in SSA.[7,8] Despite the rationing of dialysis in South Africa where being HIV positive adversely impacts on patients' chances of acceptance to dialysis, the South African Renal registry has recently reported an increase from 8.3% in 2012 to 9.3% in 2014 of HIV positive end-stage renal disease patients receiving dialysis.[9] One study from the US showed that the annual number of patients with incident end-stage renal disease secondary to HIV-associated nephropathy (HIVAN) increased steadily from 1989 to 1995 and then remained stable until 2006.[10] Also, some studies in Nigeria have documented high prevalence of kidney disease among HIV patients ranging from 22.9% to 51.8% depending on the geographic location and definition of kidney disease utilized.[11–14] Such data continue to highlight the impact of HIV on kidney disease. However, most of the studies have been underpowered and may therefore not report accurate estimates of CKD prevalence. There is therefore a need to assess kidney disease prevalence in SSA using a large HIV study

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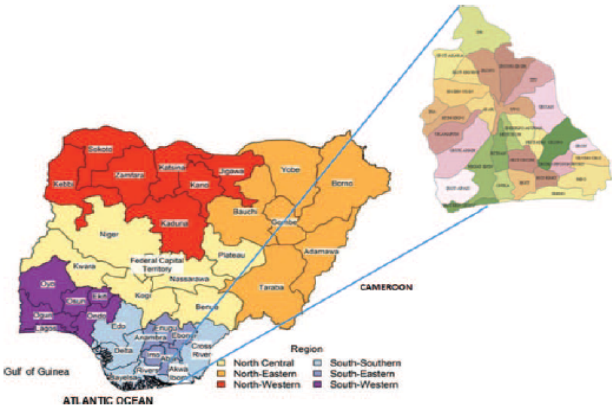

Figure 1. Map of Nigeria showing Akwa Ibom State.

population to provide a more accurate estimate of the disease burden. This will assist the planning for health services delivery in the region.

2. Methods

This study is a 15-year (2002–2016) assessment of renal function in ART-naïve HIV-infected patients at the University of Uyo Teaching Hospital (UUTH), Uyo, Nigeria (Human Research Ethics number: UUTH/AD/S/vol. XIX/15. August 9, 2016). The UUTH is the only tertiary health facility serving a population of over 4 million people in the extreme Southern (Niger-Delta) region of Nigeria (Fig. 1). The UUTH HIV clinic, funded by the United States Agency for International Development, is involved in voluntary counseling and testing for HIV, provision of ART, identification and treatment of opportunistic infections, and follow-up care for HIV positive patients. Clinical and demographic features such as age, gender, weight, height, body mass index (BMI), blood pressure, hypertension and diabetes mellitus (DM) status, and date of commencement of ART were extracted from the records. Records for CD4 count, viral load, electrolytes, urea, creatinine (measured using an isotope dilution mass spectrophotometry-traceable Jaffe kinetic reaction), hepatitis B surface antigen, and antibody to hepatitis C virus were also extracted. Serum creatinine is routinely done at first contact with the patient but programmatic deficiencies do not often allow repeat serum creatinine except in those who can afford out-of-pocket payment for the test. Information on proteinuria (dipstick assessment) was also recorded where available, because this was not routinely done. Hypertension was defined as 2 or more recordings of blood pressure with systolic blood pressure (SBP) at least 140 mm Hg and/or diastolic blood pressure (DBP) of at least 90 mm Hg or patients on antihypertensive medication.[15] Mean arterial blood pressure was calculated as [DBP + (SBP − DBP)/3]. DM was defined as fasting plasma glucose of at least 7.0 mmol/L and/or random/2 hour postmeal plasma glucose of at least 11.1 mmol/L[16] or in patients taking antidiabetic agents. Obesity was defined as BMI of at least 30 kg/m^2 in a patient without peripheral edema[17]; overweight as BMI of 25 to 29.9 kg/m^2 while BMI of 18.0 to 24.9 and less than 18.0 kg/m^2 were defined as normal and underweight, respectively. Dyslipidemia was defined using the National Cholesterol Education program adult treatment panel III criteria[18] – total cholesterol greater than 200 mg/dL or low density lipoprotein cholesterol greater than 150 mg/dL or high density lipoprotein cholesterol less than 40 mg/dL or triglycerides greater than 150 mg/dL.

For the purposes of this study, data were extracted from patient's physical case records and transferred into STATA 14 (StataCorp, TX) for analysis. The Student t test (or its nonparametric equivalent, the Mann–Whitney U test, where necessary) was used to compare continuous variables while the Chi-square test was used to compare categorical variables. CKD was defined as 2 consecutive values of estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 recorded within 3 to 12 months apart. We estimated GFR using the chronic kidney disease epidemiology collaboration (CKD-EPI) equation.[19] The CKD-EPI equation without the race factor was utilized for estimating GFR in this study given that recent studies[20–22] have suggested that the inclusion of race may generate less precise estimates in SSA. Participants' kidney function was staged using the Kidney Disease outcome quality initiative classification.[23] The prevalence (and 95% confidence interval) of CKD in the overall population and subgroups among the HIV patients was computed. To increase the power to generate stable estimates over time, the entire time of observation was divided into 5 time periods of 3 years each. The Cochran-Armitage trend test was used to assess for the presence of linear trends in CKD prevalence while the Cuzick trend test was used to determine linear trend in median CD4 count over the study period. Multivariable logistic regression models (using a threshold significance of P-value <.25 in univariable analyses and known CKD risk factors) were used to identify independent predictors of CKD in the study population. Three sets of sensitivity analyses were performed using eGFR estimates with the race factor for the CKD-EPI equation; eGFR estimates using the 4-variable Modification of Diet in Renal Disease equation with and without the race factor; and eGFR estimates using the CKD-EPI equation without the race factor for the population with at least 1 serum creatinine record.

3. Results

3.1. Demographic and clinical features

A total of 6676 patients had at least 1 GFR estimate. Of these, 1317 had 2 GFR estimates performed 3 or more months apart.

Table 1 summarizes the demographic and clinical characteristics of included patients. From the sample of 1317 patients, 62.2% were female. The mean age at enrolment was 35.4 ± 9.5 years; median CD4 count was 194 (interquartile range 95–343) cells/μL; and 51.1% had CD4 count lower than 200 cells/μL. Hypertension was present in 43.6% (95% CI: 41.5%–49.9%) and DM in 8.8% (95% CI: 5.6%–12.9%). Obesity (BMI > 30 kg/m^2) was seen in 8.3% (95% CI: 6.7%–10.1%) of the study population, and overweight (BMI 25.0–29.9 kg/m^2) in 21.9%. Dyslipidemia was present in 28.8% (95% CI 26.3%–38.8%), hepatitis B in 5.7%, and hepatitis C infection in 2.2%.

3.2. Prevalence of CKD

Of the 1317 patients who had at least 2 creatinine measures (at initiation of care and 3 months or more apart), the prevalence of CKD using the CKD-EPI equation without race was 13.4% (95% CI: 11.6%–15.4%). There was no significant change in CKD prevalence between 2002 and 2016, P = .62 (Fig. 2) while the median CD4 count at presentation progressively increased over the same period (P = .01).

Table 1

Demographic and clinical characteristics of HIV positive patients with at least 2 measurements of glomerular filtration rate.

Variable	Value (N=1317)
Age, years	35.4±9.5
Female sex	819 (62.2)
Systolic BP, mm Hg	122.5±24.8
Diastolic BP, mm Hg	79.9±18.2
Mean arterial blood pressure, mm Hg	92.2±17.2
Hypertension (n=565)	258 (45.7)
Fasting plasma glucose, mmol/L	4.8±2.2
Diabetes mellitus (n=251)	22 (8.8)
BMI, kg/m^2 (n=1081)	23.3±4.5
Underweight	113 (10.5)
Normal BMI	641 (59.3)
Overweight	237 (21.9)
Obese	90 [8.3% (95% CI 6.7%–10.1%)]
Hemoglobin, g/dL	11.3±2.2
Hepatitis C infection (n=497)	17 [3.4% (95% CI 2.0%–5.2%)]
Hepatitis B infection (n=829)	58 [7.0% (95% CI 5.3%–8.9%)]
Serum albumin, g/dL	39.0±11.4
CD4 count, cells/μL	194 (95–343)
CD4 count (<200) (n=1237)	632 (51.1)
CD4 count (<350)	939 (75.9)
Log viral load (n=586)	3.9±1.2
Total cholesterol, mg/dL (n=783)	166.2±54.1
Triglyceride, mg/dL	141.7±70.9
LDL-c, mmol/L	85.1±42.5
HDL-c, mmol/L	50.3±30.9
Dyslipidemia (n=232)	75 (32.3)
Dipstick proteinuria (yes) (n=32)	18 (56.3)

BMI=body mass index, BP=blood pressure, CD4=cluster of differentiation 4, CI=confidence interval, HDL-c=high density lipoprotein cholesterol, HIV=human immunodeficiency virus, LDL-c=low density lipoprotein cholesterol.

The prevalence of CKD (stages 3, 4, and 5) was 8.8%, 2.2%, and 2.4%, respectively. There was no significant gender difference in the CKD prevalence (male vs female prevalence of 7.8% vs 9.4% for stage 3; 2.0% vs 2.3% for stage 4; and 2.6% vs 2.3% for stage 5, $P=.75$). Those with advanced CKD (stages 4 and 5) constituted 4.6% of the total population.

3.3. Sensitivity analyses of GFR equation estimates

Using the CKD-EPI equation with the race factor, the prevalence of CKD was 8.9% (95% CI: 7.5%–10.6%). The proportion of patients with stages 3, 4, and 5 CKD was 4.9%, 1.9%, and 2.1%, respectively. The prevalence of advanced CKD (stages 4 and 5) was 4.0% for CKD-EPI equation (with race factor).

The modification of diet in renal disease equation without the race factor yielded a CKD prevalence of 15.9% (95% CI: 13.9%–17.9%) with stages 3, 4, and 5 constituting 11.1%, 2.4%, and 2.4%, respectively. With the race factor, stages 3, 4, and 5 accounted for 5.5%, 1.9%, and 1.97%, respectively, making up 9.3% of the study population.

Using the single eGFR at initiation of care, a CKD prevalence of 26.6% (95% CI 25.6%–27.7%) with stages 3, 4, and 5 being 20.3%, 3.1%, and 3.2%, respectively, using the CKD-EPI equation without the race factor. Using the CKD-EPI equation with the race factor, stages 3, 4, and 5 had prevalence of 12.9%, 2.5%, and 2.9% respectively summing up to a CKD prevalence of 18.3%.

3.4. Factors associated with CKD

On multivariable analysis, increasing age was associated with increased risk of developing CKD – odds ratio (OR) 1.07 (95% CI: 1.05–1.10; $P<.001$) (Table 2). HIV patients with CD4 count less than 200 cells/μL were also at increased risk of developing CKD (Table 2). The presence of DM, hepatitis B and C coinfection, and hypertension did not increase CKD risk at the multivariable level. Comparison of sociodemographic and clinical characteristics of those with 1 and 2 GFR estimates (Supplementary Table 1, http://links.lww.com/MD/C194) showed statistical (but not clinically relevant) difference in age; higher proportion of hypertension and DM among those with 2 GFR estimates and similar BMI, hemoglobin, and hepatitis B coinfection prevalence.

4. Discussion

This study is one of the few attempts, in a large HIV population in SSA, to report the prevalence of CKD at initiation of care. The main findings and therefore importance of this study are: showing a high prevalence of CKD in a population of HIV positive patients; identification of increasing age and low CD4 count as independent CKD risk factors in our HIV population.

The prevalence of CKD in our population of HIV positive patients is high. Several studies among HIV patients in Southern Nigeria[11,12,24,25] with similar sociodemographic characteristics have also shown high prevalence of HIV individuals with

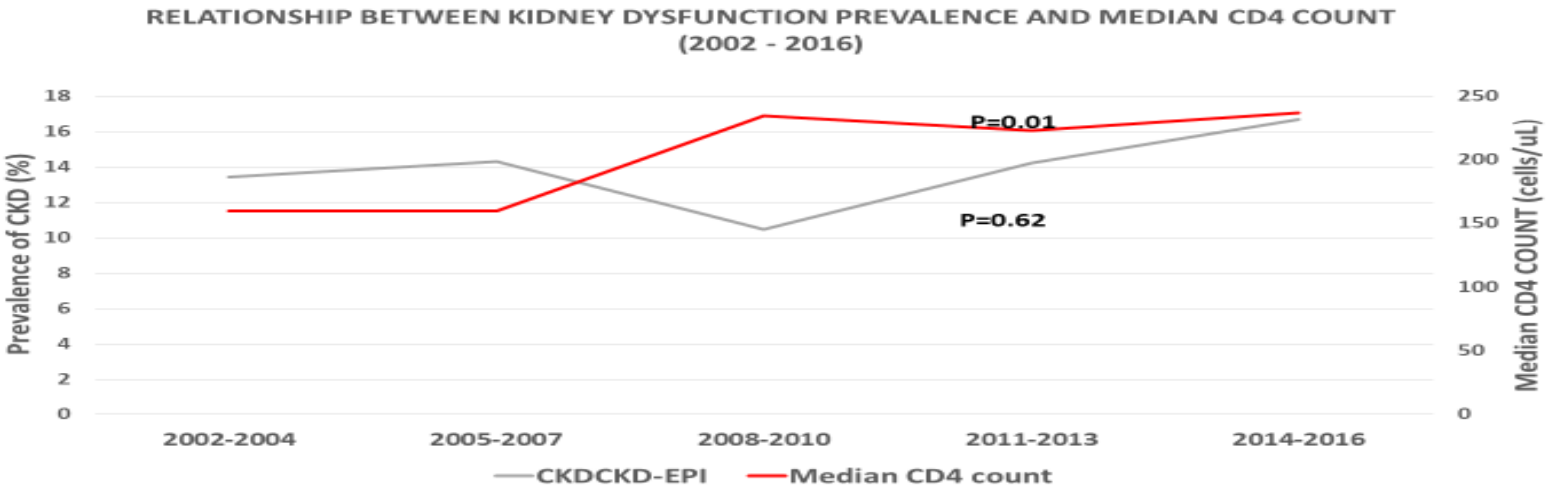

Figure 2. Relationship between CKD prevalence and median CD4 count (2002–2016). CD4 = cluster of differentiation 4, CKD = chronic kidney disease.

Table 2

Multivariate analysis of the predictors of CKD in HIV positive patients in the Niger-Delta region of Southern Nigeria.

	Univariable Odds ratio (95%CI) *P*-value	Multivariable Odds ratio (95%CI) *P*-value
Age, y	1.06 (1.04–1.08) **<.001**	1.07 (1.05–1.10) **<.001**
Female gender	1.15 (0.82–1.60) .41	1.44 (0.94–2.20) .09
CD4 count		
≥200 cells/μL	1	1
<200 cells/μL	1.65 (1.17–2.31) **.004**	1.51 (1.00–2.29) **.04**
Hypertension	1.68 (1.05–2.68) **.03**	1.72 (0.95–3.11) .07
Diabetes mellitus	1.74 (0.54–5.52) .35	2.68 (0.59–12.10) .20
BMI, kg/m^2	0.97 (0.93–1.02) .22	0.97 (0.93–1.02) .27
Positive hepatitis B	0.56 (0.22–1.43) .23	0.64 (0.21–1.91) .43
Positive hepatitis C	0.77 (0.17–3.43) .73	1.46 (0.29–7.39) .65
Time period		
2002–2004	1	1
2005–2007	1.08 (0.64–1.82) .78	1.05 (0.50–2.18) .94
2008–2010	0.76 (0.43–1.32) .33	0.47 (0.22–1.02) .06
2011–2013	1.07 (0.63–1.80) .80	0.97 (0.50–1.92) .95
2014–2016	1.29 (0.67–2.48) .44	1.13 (0.48–2.63) .78
Area under ROC*		**0.70**

BMI = body mass index, CI = confidence interval, CKD = chronic kidney disease, ROC = receiver operator characteristic.

* ROC curve of final multivariate logistic regression model.

eGFR < 60 mL/min/1.73 m^2 in ART-naïve patients. Some have reported prevalence as high as 47.6%[12] to 53%[24] when proteinuria was also considered, even though persistent proteinuria was not demonstrated in those studies and single eGFR measures were used which cannot discriminate between CKD and AKI patients. Other studies have equally reported a high prevalence of CKD among ART-naïve individuals in the West African sub-region such as Ghana (38.8%)[26] and Cameroon (54%).[27] In other African regions, the prevalence of CKD in HIV positive patients is variable with South Africa recording 2%[28,29] and Kenya 12%.[30] Overall, it has been shown that blacks (in SSA or other parts of the world) have higher risk of CKD when they are HIV-infected.[31]

A number of factors complicate the HIV-CKD interplay in Africa. First is the suboptimal implementation of available guidelines[32] for the screening, diagnosis, and management of CKD in HIV populations, especially in Africa. In our study population, less than 2% had dipstick proteinuria performed at initiation of care and none had proteinuria quantified either by spot or 24-hour urine collection. A recent report of the Global Kidney Health Atlas exploring global access of patients to health technologies and medications corroborates our finding by showing that African countries, of all world regions, had the lowest capacity for identification, monitoring, and management of CKD.[33] Secondly, there is a need to develop CKD risk assessment tools that aid the identification of high risk HIV patients for screening and initiation of appropriate therapies. Of the few available CKD risk assessment tools, one was derived from a predominantly Caucasian HIV population which differs significantly according to demographics from the HIV populations in SSA.[34] This tool has also not been validated in SSA HIV populations. Thirdly, GFR estimation formulae have not been properly validated in the HIV population. The few studies that have attempted doing this[35,36] either had very small sample sizes or were performed in a restricted population, making the results not generalizable. This leaves room for inconsistencies and great variations in the diagnosis of CKD among HIV-infected individuals. Recently, some workers in West Africa[20] have proposed the use of the full age spectrum serum creatinine-based equations constructed and validated by Pottel et al[37] in Caucasian populations. The best creatinine-based eGFR equation is yet to be determined for the African HIV population and considerable variations exist in the proportion of CKD among the HIV population depending on the equation used.

Late presentation of patients to the HIV clinics may also have contributed to the high prevalence of CKD in our study population. Many patients do not routinely go to hospital for treatment in Nigeria due to the cost (out-of-pocket payment) associated with health care. In this study, late presentation is supported by the relatively low CD4 counts of these patients at time of their first visit. A low CD4 count has also been documented as a risk factor for CKD in other HIV populations[38,39] probably mirroring more severe HIV disease, longer duration of exposure to HIV or presence of opportunistic infections all of which may predispose to both AKI and CKD. Increasing age and low CD4 counts were independent predictors of occurrence of CKD. Increasing age is a known risk factor for CKD in the general population and in the HIV population.[39] Recurrent diarrheal disease in HIV patients not having adequate care may also lead to repeated episodes of undiagnosed acute kidney injury which may ultimately lead to CKD. Other cultural factors like use of herbal remedies with unproven efficacy for HIV cure but with known nephrotoxic potential may also contribute to the increased CKD occurrence in HIV patients in West Africa. These factors were however not assessed by this study.

Although our study did not assess genetic factors, the increased prevalence of CKD in people of West African descent with HIV compared to other parts of the world may suggest a genetic predisposition.[40] Studies have reported that the presence of G1 and G2 high risk alleles of the apolipoprotein (APOL)1 gene is associated with HIVAN in South Africa.[41,42] High risk alleles of APOL1 have been documented as risk factors among nondiabetic CKD patients in South East Nigeria,[43] including a small group of HIVAN patients. Individuals with both APOL1 risk alleles have an estimated 4% lifetime risk for developing focal segmental glomerulosclerosis (FSGS), and untreated HIV-infected individuals have a 50% risk for developing HIVAN.[44] On the other hand, HIV positive patients without the risk variants have negligible risk of developing HIVAN as documented in the Ethiopian population.[45] A gene–environment interaction mediated by interferons and other cytokines (elaborated by the HIV infection) has been suggested as one of the factors leading to high CKD risk.[46] There is also the possibility of gene–gene interactions increasing the risk of CKD progression in HIV patients of West African origin suggesting the need for investigation of other genetic risk factors for CKD progression among HIV patients.

The large proportion of patients for whom urine protein was not assessed nor serum creatinine repeated suggests significant gaps in both the evaluation and care offered to HIV patients. It may be necessary to set up prospective studies in a more controlled setting to evaluate the real estimates of chronic kidney dysfunction.

Most of the patients with CKD in our study population were in stage 3 disease where there is still a window of opportunity to slow down or stop progression to end-stage kidney disease by early initiation of ART and other renal-specific interventions such as the use of ACE-inhibitors (or angiotensin receptor blockers) to reduce proteinuria and control of blood pressure. Early detection

and retarding of CKD progression through initiation of appropriate therapies may be more cost effective at retarding mortality given that access to treatment of advanced CKD is limited and expensive.[47]

4.1. Limitations

A limitation of this study includes the retrospective design as medical records of some patients who were registered at the HIV clinic were unavailable for assessment and inclusion into this study. However, given the large sample size of our study, we believe patients included are an adequate representation of the enrolled patients. Another study limitation relates to the unavailability of albuminuria. Our definition of CKD therefore depended only on serum creatinine and eGFR which could underestimate CKD prevalence. Studies reporting CKD prevalence using eGFR and albuminuria[5,6] generally tend to have higher prevalence rates than if CKD was diagnosed with eGFR only. This study is however strengthened by the large sample size and by utilizing various GFR estimation equations for assessing CKD. The large sample size increases confidence in the reported prevalence rates. The inclusion of patients across many years of activities has provided the opportunity of assessing the time trends.

5. Conclusion

This study reports a high prevalence of CKD in HIV positive patients in Nigeria. Earlier presentation of patients and earlier initiation of treatment could contribute to further reduction in the prevalence of disease. Strategies toward earlier identification of HIV positive patients at risk of CKD are therefore needed to reduce CKD burden among HIV patients in SSA. It is also important to determine the best serum creatinine-based GFR estimating equation for HIV patients in SSA.

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References

[1] UNAIDS. Global AIDS Update 2016. Geneva: UNAIDS; 2016.
[2] National Agency for the Control of AIDS (NACA). Global AIDS response; country progress report Nigeria 2014. Abuja: NACA; 2014.
[3] UNAIDS. The GAP Report. Geneva: UNAIDS; 2014.
[4] Samji H, Cescon A, Hogg RS, et al. Closing the gap: increases in life expectancy among treated HIV-positive individuals in the United States and Canada. PloS One 2013;8:e81355.
[5] Gupta SK, Mamlin BW, Johnson CS, et al. Prevalence of proteinuria and the development of chronic kidney disease in HIV-infected patients. Clin Nephrol 2004;61:1–6.
[6] Wyatt CM, Winston JA, Malvestutto CD, et al. Chronic kidney disease in HIV infection: an urban epidemic. AIDS (Lond, Engl) 2007;21: 2101–3.
[7] Okpechi IG, Rayner BL, Swanepoel CR. Nephrotic syndrome in adult black South Africans: HIV-associated nephropathy as the main culprit. J Natl Med Assoc 2010;102:1193–7.
[8] Akpan EE, Ekrikpo UE, Udo AIA. Changing aetiogies of end stage kidney disease in a resource poor enivornment: what is the way forward? World J Biomed Res 2014;1:1–5.
[9] Davids M, Marais N, Jacobs J. South African Renal Registry Annual Report 2012;2012.
[10] Razzak Chaudhary S, Workeneh BT, Montez-Rath ME, et al. Trends in the outcomes of end-stage renal disease secondary to human immunodeficiency virus-associated nephropathy. Nephrol Dial Transplant 2015;30:1734–40.
[11] Anyabolu EN, Chukwuonye II, Arodiwe E, et al. Prevalence and predictors of chronic kidney disease in newly diagnosed human immunodeficiency virus patients in Owerri, Nigeria. Indian J Nephrol 2016;26:10–5.
[12] Ayokunle DS, Olusegun OT, Ademola A, et al. Prevalence of chronic kidney disease in newly diagnosed patients with Human immunodeficiency virus in Ilorin, Nigeria. J Bras Nefrol 2015;37:177–84.
[13] Umeizudike T, Mabayoje M, Okany C, et al. Prevalence of chronic kidney disease in HIV positive patients in Lagos, south-west Nigeria. Nephrol Rev 2012;4:22–6.
[14] Agaba EI, Agaba PA, Sirisena ND, et al. Renal disease in the acquired immunodeficiency syndrome in north central Nigeria. Niger J Med 2003;12:120–5.
[15] Chobanian AV, Bakris GL, Black HR, et al. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. JAMA 2003;289:2560–72.
[16] ADA. Diagnosis and classification of diabetes mellitus. Diabetes Care 2010;33(Supplement 1):S62–9.
[17] World Health OrganizationObesity: Preventing and Managing the Global Epidemic. Report of a WHO consultation. Geneva: WHO; 2000.
[18] Williams L. Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. Circulation 2002;106:3143–13143.
[19] Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604–12.
[20] Yayo E, Ayé M, Yao C, et al. Measured (and estimated) glomerular filtration rate: reference values in West Africa. Nephrol Dial Transplant 2017;doi: 10.1093/ndt/gfx244.
[21] Anker N, Scherzer R, Peralta C, et al. Racial disparities in creatinine-based kidney function estimates among HIV-infected adults. Ethn Dis 2016;26:213–20.
[22] van Deventer HE, George JA, Paiker JE, et al. Estimating glomerular filtration rate in black South Africans by use of the modification of diet in renal disease and Cockcroft-Gault equations. Clin Chem 2008; 54:1197–202.
[23] Levey AS, Coresh J, Bolton K, et al. K/DOQI clinical practice guidelines for chronic kidney disease. Am J Kidney Dis 2002;39(2 Suppl 1):
[24] Okafor UH, Unuigbe EI, Chukwuonye E. Prevalence and clinical and laboratory characteristics of kidney disease in anti-retroviral-naive human immunodeficiency virus-infected patients in South-South Nigeria. Saudi J Kidney Dis Transpl 2016;27:129–34.
[25] Emem CP, Arogundade F, Sanusi A, et al. Renal disease in HIV-seropositive patients in Nigeria: an assessment of prevalence, clinical features and risk factors. Nephrol Dial Transplant 2008;23:741–6.
[26] Sarfo FS, Keegan R, Appiah L, et al. High prevalence of renal dysfunction and association with risk of death amongst HIV-infected Ghanaians. J Infect 2013;67:43–50.

[27] FolefackKaze F, Kengne AP, Pefura Yone EW, et al. Renal function, urinalysis abnormalities and correlates among HIV-infected Cameroonians naive to antiretroviral therapy. Saudi J Kidney Dis Transpl 2013;24:1291–7.
[28] Wensink GE, Schoffelen AF, Tempelman HA, et al. Albuminuria is associated with traditional cardiovascular risk factors and viral load in HIV-infected patients in rural South Africa. PLoS One 2015;10: e0136529.
[29] Kamkuemah M, Kaplan R, Bekker LG, et al. Renal impairment in HIV-infected patients initiating tenofovir-containing antiretroviral therapy regimens in a primary healthcare setting in South Africa. Trop Med Int Health 2015;20:518–26.
[30] Edwards JK, Bygrave H, Van den Bergh R, et al. HIV with non-communicable diseases in primary care in Kibera, Nairobi, Kenya: characteristics and outcomes 2010–2013. Trans R Soc Trop Med Hyg 2015;109:440–6.
[31] Lucas GM, Lau B, Atta MG, et al. Chronic kidney disease incidence, and progression to end-stage renal disease, in HIV-infected individuals: a tale of two races. J Infect Dis 2008;197:1548–57.
[32] Lucas GM, Ross MJ, Stock PG, et al. Clinical practice guideline for the management of chronic kidney disease in patients infected with HIV: 2014 update by the HIV Medicine Association of the Infectious Diseases Society of America. Clin Infect Dis 2014;59:e96–38.
[33] Htay H, Alrukhaimi M, Ashuntantang GE, et al. Global access of patients with kidney disease to health technologies and medications: findings from the Global Kidney Health Atlas project. Kidney Int Suppl 2018;8:64–73.
[34] Mocroft A, Lundgren JD, Ross M, et al. Development and validation of a risk score for chronic kidney disease in HIV infection using prospective cohort data from the D:A:D study. PLoS Med 2015;12:e1001809.
[35] Barraclough K, Er L, Ng F, et al. A comparison of the predictive performance of different methods of kidney function estimation in a well-characterized HIV-infected population. Nephron Clin Pract 2009;111: c39–48.
[36] Praditpornsilpa K, Avihingsanon A, Chaiwatanarat T, et al. Comparisons between validated estimated glomerular filtration rate (GFR) equations and isotopic GFR in HIV patients. AIDS (Lond, Engl) 2012;26:1781–8.
[37] Pottel H, Hoste L, Dubourg L, et al. An estimated glomerular filtration rate equation for the full age spectrum. Nephrol Dial Transpl 2016;31:798–806.
[38] da Silva DR, Gluz IC, Kurz J, et al. Multiple facets of HIV-associated renal disease. Braz J Med Biol Res 2016;49:e5176.
[39] Cheung CY, Wong KM, Lee MP, et al. Prevalence of chronic kidney disease in Chinese HIV-infected patients. Nephrol Dial Transpl 2007;22:3186–90.
[40] Mallipattu SK, Salem F, Wyatt CM. The changing epidemiology of HIV-related chronic kidney disease in the era of antiretroviral therapy. Kidney Int 2014;86:259–65.
[41] Kasembeli AN, Duarte R, Ramsay M, et al. APOL1 risk variants are strongly associated with HIV-associated nephropathy in Black South Africans. J Am Soc Nephrol 2015;26:2882–90.
[42] Kasembeli AN, Duarte R, Ramsay M, et al. African origins and chronic kidney disease susceptibility in the human immunodeficiency virus era. World J Nephrol 2015;4:295–306.
[43] Ulasi II, Tzur S, Wasser WG, et al. High population frequencies of APOL1 risk variants are associated with increased prevalence of non-diabetic chronic kidney disease in the Igbo people from south-eastern Nigeria. Nephron Clin Pract 2013;123:123–8.
[44] Kopp JB, Nelson GW, Sampath K, et al. APOL1 genetic variants in focal segmental glomerulosclerosis and HIV-associated nephropathy. J Am Soc Nephrol 2011;22:2129–37.
[45] Behar DM, Shlush LI, Maor C, et al. Absence of HIV-associated nephropathy in Ethiopians. Am J Kidney Dis 2006;47:88–94.
[46] Nichols B, Jog P, Lee JH, et al. Innate immunity pathways regulate the nephropathy gene apolipoprotein L1. Kidney Int 2015;87:332–42.
[47] Okpechi IG. ESKD in sub-Saharan Africa: will governments now listen? Lancet Glob Health 2017;5:e373–4.

The prevalence of CKD in this HIV population was high – 13.4% (95%CI 11.6-15.4%). Increasing age and CD4 count less than 200 cells/µL were independently associated with CKD occurrence among this patient populace. This high CKD prevalence may partly be due to co-morbidities like hypertension and diabetes mellitus among these patients though at multivariable level, they did not show statistical significance. Therefore, the next chapter deals with the prevalence of traditional CVD risk factors in this HIV population.

CHAPTER 5: PREVALENCE AND CORRELATES OF TRADITIONAL RISK FACTORS FOR CARDIOVASCULAR DISEASE IN A NIGERIAN ART-NAÏVE HIV POPULATION: A CROSS-SECTIONAL STUDY

Chapter 5 explores important co-morbidities that have been established as traditional risk factors for cardiovascular disease – hypertension, diabetes mellitus, obesity and dyslipidaemia. Their prevalence is documented and associations with these risk factors sought. The findings have been published by BMJ open with the title "Prevalence and correlates for cardiovascular disease in a Nigerian ART-naïve HIV population: A cross-sectional study". This publication can be accessed suing the link below:

https://bmjopen.bmj.com/content/bmjopen/8/7/e019664.full.pdf

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Prevalence and correlates of traditional risk factors for cardiovascular disease in a Nigerian ART-naive HIV population: a cross-sectional study

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ABSTRACT

Objectives HIV infection environment presents a classic example of the interplay between infectious diseases and non-communicable diseases (NCDs). Traditional cardiovascular disease (CVD) risk factors abound in the HIV population even before initiation of antiretrovirals (ARVs) and predispose them to the development of stroke and myocardial infarction. This work focuses on determining the prevalence of traditional CVD risk factors among ARV-naive HIV individuals in southern Nigeria.

Methods This was a cross-sectional study of ARV-naive patients initiating care at the University of Uyo Teaching Hospital HIV clinic cohort to determine the prevalence and correlates of hypertension, diabetes mellitus (DM), obesity and dyslipidaemia.

Results The sample consisted of 4925 assessed for hypertension, 5223 for obesity, 1818 for DM and 926 for dyslipidaemia. Hypertension prevalence was 26.7% (95% CI 25.5% to 28.0%) with a male preponderance (p=0.02). DM was found in 5.6% (95% CI 4.5% to 6.7%), obesity in 8.3% (95% CI 7.6% to 9.1%) and dyslipidaemia in 29.1% (95% CI 26.1% to 32.1%) with a high prevalence of low high-density lipoprotein-c (42.6%). Hypertension was independently associated with age (OR 1.04 (95% CI 1.03 to 1.05), p<0.001) and body mass index (BMI) (OR 1.06 (95% CI 1.03 to 1.08), p<0.001), obesity with age (OR 1.02 (95% CI 1.01 to 1.03), p<0.001), male gender (OR 0.38 (95% CI 0.29 to 0.49), p<0.001) and CD4 count (OR 2.63 (95% CI 1.96 to 3.53), p<0.001) while dyslipidaemia was associated with BMI (OR 1.05 (95% CI 1.01 to 1.10), p=0.03).

Conclusion The prevalence of traditional CVD risk factors is high in this ART-naive HIV population. An integrated approach of HIV and NCD screening/treatment may be relevant for centres in sub-Saharan Africa.

Strengths and limitations of this study

- This study documents the prevalence of hitherto neglected cardiovascular disease (CVD) risk factors—hypertension, diabetes mellitus, obesity and dyslipidaemia—in a large HIV population in sub-Saharan Africa.
- The findings from this work will help HIV caregivers and health policy makers develop long-term intervention plan for HIV patients with CVD risk factors.
- Missing data regarding some of the cardiovascular risk factors (including tobacco smoking) made it difficult to assess the degree of clustering of risk factors in this HIV population.

INTRODUCTION

HIV infection and the treatment thereof have been identified as being associated with increased frequencies of traditional cardiovascular disease (CVD) risk factors.[1 2] HIV infection environment presents a classic example of the interplay between infectious disease and non-communicable diseases (NCDs). While people with HIV infection now live longer, there may be an increased risk of stroke, myocardial infarction and chronic kidney disease because of the abundance of traditional CVD risk factors. Indeed, the use of antiretroviral (ARV) medications may in the long run increase prevalence of CVD risk factors like dyslipidaemia, hypertension and dysglycaemia.[3–5]

Modelling studies have indicated that 84% of HIV-infected patients will have at least one NCD by 2030 with about one-third of HIV patients having three or more NCDs.[6] We are already seeing a higher incidence of NCDs among HIV-infected patients than the general population[7] with its attendant economic and health system implications, especially in sub-Saharan Africa—the epicentre of HIV infection. It is therefore important to determine the burden of these risk factors among the HIV population before initiation of ARV for the purpose of healthcare delivery planning. We document in this study the prevalence of CVD risk factors and correlates in a large ARV-naive HIV population in Southern Nigeria.

METHODS

The University of Uyo Teaching Hospital (UUTH) HIV clinic is a US Agency for International Development-funded HIV care programme in Southern Nigeria that offers voluntary counselling and testing for HIV, provision of ARVs, identification and treatment of opportunistic infections and follow-up care for HIV patients. Patients enrolled into care at this facility have their sociodemographic characteristics collected; their weight, height, blood pressure are also measured according to the WHO STEPwise approach to surveillance (STEPS) protocol[8] and body mass index (BMI) calculated in kg/m^2 from the weight and height measurements. Blood samples are drawn for CD4 count, viral load, electrolytes, urea, alanine transaminase, hepatitis B surface antigen, antibody to hepatitis C virus and lipid profile at entry. This analysis was done using baseline characteristics not taking into cognisance changes occurring during follow-up of patients. We excluded paediatric patients (age less than 18 years) and individuals transferred in from other HIV treatment centres.

Hypertension was defined as two or more recordings of blood pressure with systolic blood pressure (SBP) at least 140 mm Hg and/or diastolic blood pressure (DBP) of at least 90 mm Hg measured within 1 month of initiating care at the clinic or patient who is on antihypertensive medication. Individuals with hypertension were further classified into stage 1 and stage 2 hypertension as per the seventh report of the Joint National Committee on prevention, detection, evaluation and treatment of high blood pressure (JNC 7) classification.[9] Prehypertension was defined as SBP 120–139 mm Hg and/or DBP of 80–89 mm Hg;[9] isolated systolic hypertension (ISH) if SBP ≥140 mm Hg and DBP <90 mm Hg; isolated diastolic hypertension if SBP <140 mm Hg and DBP ≥90 mm Hg; mixed hypertension (MH) if SBP ≥140 mm Hg and DBP ≥90 mm Hg[10]. Mean arterial blood pressure was computed using the formula [DBP+(SBP–DBP)/3].

Diabetes mellitus (DM) was defined as fasting plasma glucose of at least 7.0 mmol/L and/or random/2-hour post meal plasma glucose of at least 11.1 mmol/L or individuals taking antidiabetic agents.[11] Obesity was defined as BMI of at least 30 kg/m^2 in a patient without peripheral oedema.[12] Unfortunately, waist circumference and related anthropometry were not collected routinely at baseline and therefore not included in this study. Dyslipidaemia was defined as deviation from reference values of the hospital chemical pathology laboratory (>6.5 mmol/L for total cholesterol; >3.5 mmol/L for low-density lipoprotein (LDL)-c,<0.9 mmol/L for high-density lipoprotein (HDL)-c and >2.0 mmol/L for serum triglyceride). Other dyslipidaemic characteristics including the Castelli Risk Index 1 (total cholesterol/HDL-c), Castelli Risk Index 2 (LDL-c/HDL-c) and atherogenic index of plasma (logarithm of triglyceride/HDL-c) were calculated where adequate data were available and considered to be elevated when greater than 4.4, 2.5 and 0.5, respectively.[13 14] Unfortunately, data on alcohol and tobacco use were not routinely collected and therefore not available. The proportion of the study population who were aware of their hypertension and diabetic status was also determined.

Due to programmatic constraints, data were not available for all the parameters measured. Blood pressure data were available for 4925 (40.5%); BMI (5223, 42.9%); DM (1818, 14.9%) and dyslipidaemia (926, 7.6%) of the study population. Analysis was therefore performed on a different subset of the HIV population for each of the risk factors. Data were extracted from patient's physical case records and transferred into STATA V.15 (StataCorp, Texas, USA) for analysis. Graphs were drawn using Microsoft Excel. The 15-year period was divided into five 3-year categories for the purpose of assessment of time trends. The Student's t-test (or its non-parametric equivalent, where necessary) was used to compare continuous variables while a χ^2 test was employed to compare categorical variables. The Cochran-Armitage trend test across ordered groups was performed to determine significant trends in prevalence of traditional risk factors during the period of the study and across different age groups. Four multivariable regression models were used to identify sociodemographic and clinical factors independently associated with each of the considered CVD risk factors (hypertension, DM, obesity and dyslipidaemia). The independent variables included in the models were important demographic factors like age (in 1-year increments), sex (with women being the reference category) and clinical factors like CD4 count and BMI at initiation of care. The years of study were included in the models to adjust for unmeasured confounders that can vary by year, and to explore if the trend across years is significant after adjustment for confounders. Collinearity was assessed using the condition number test. The goodness of fit of the models was assessed using the Hosmer-Lemeshow test while the predictive performance of the models was determined using receiver operating characteristic curves. P values less than 0.05 were deemed statistically significant. This cross-sectional study was reported using the Strengthening the Reporting of Observational Studies in Epidemiology guidelines[15] (see online supplementary 1).

Patient and public involvement

The patients in our HIV clinics have expressed concern about having concomitant high blood pressure/elevated blood glucose and HIV disease, especially because of their inability to procure hypertension and DM medications. However, the patients were not involved in the design, recruitment and conduct of this study. The results from this work will be disseminated to patients during the monthly clinic health talk.

RESULTS

Two thousand two hundred and seventy-five HIV-positive patients were seen between 2002 and 2004, 3725 in the 2005–2007 year category, 2628 for 2008–2010, 1962

Table 1 Sociodemographic and clinical characteristics of HIV patients

	HIV population	Women	Men	P value
Age (years)	34.3±9.9	32.3±9.6	37.4±9.4	<0.001
SBP (mm Hg)	114.4±23.3	113.5±23.4	115.5±23.0	0.01
DBP (mm Hg)	76.5±17.7	75.7±17.2	77.4±18.3	0.001
MABP (mm Hg)	87.1±16.3	86.5±16.3	87.9±16.2	0.01
Hypertension (n=4925)	1315 (26.7% (95% CI 25.5% to 28.0%))	717 (25.4 (23.8–27.1))	598 (28.4 (26.5–30.4))	0.02
BMI (kg/m^2) (n=5223)	23.1±4.6	23.5±5.0	22.4±3.9	<0.001
Underweight	619 (11.9 (11.0–12.8))	407 (12.0 11.0–13.2))	212 (11.5 (10.1–13.0))	0.84
Normal BMI	3022 (57.9 (95% CI 56.5 to 59.2))	1827 (54.1 (52.4–55.8))	1195 (64.8 (62.6–67.0))	<0.001
Overweight	1147 (22.0 (95% CI 20.8 to 23.1))	790 (23.4 (21.9–24.8))	357 (19.4 (17.6–21.2))	0.13
Obese	435 (8.3% (95% CI 7.6% to 9.1%))	355 (10.5 (9.5–11.6))	80 (4.3 (3.5–5.4))	0.08
Haemoglobin (g/dL) (n=1510)	10.9±2.4	10.6±2.3	11.4±2.6	<0.001
Hepatitis C infection (n=3056)	68 (2.2% (95% CI 1.7% to 2.8%))	46 (2.5 (1.8–3.3))	22 (1.8 (1.1–2.7))	0.2
Hepatitis B infection (n=4909)	314 (6.4% (95% CI 5.7% to 7.1%))	140 (4.8 (4.0–5.6))	174 (8.8 (7.6–10.1))	<0.001
FPG (mmol/L)	4.6±2.1	4.5±1.8	4.8±2.4	0.02
Diabetes mellitus (n=1818)	101 (5.6% (95% CI 4.5% to 6.7%))	49 (4.7 (3.5–6.2))	52 (6.7 (5.1–8.7))	0.06
Serum albumin (g/L)	39.9±13.9	39.5±13.0	40.3±15.0	0.31
CD4 count (cells/µL) (n=8811)	197 (91–353)	209 (100–387)	180 (80–304)	<0.001
CD4 count (<200)	4435 (50.3% (95% CI 49.3% to 51.4%))	2557 (47.7 (46.3–49.0))	1878 (54.5 (52.8–56.1))	<0.001
CD4 count (<350)	6565 (74.5% (95% CI (73.6 to 75.4))	3810 (71.0 (69.8–72.2))	2755 (79.9 (78.5–81.3))	<0.001
Log viral load (n=741)	8.56 (5.97–10.61)	8.44 (6.00–10.41)	8.62 (5.82–10.91)	0.78
TC (mmol/L) (n=877)	4.1±1.3	4.3±1.3	3.9±1.2	<0.001
Triglyceride (mmol/L)	1.6±0.8	1.6±0.9	1.6±0.8	0.95
LDL-c (mmol/L)	2.1±0.9	2.0±0.9	2.1±0.8	0.74
HDL-c (mmol/L)	1.3±0.8	1.3±0.7	1.2±0.8	0.65
Dyslipidaemia (n=926)	269 (29.1% (95% CI 26.1% to 32.1%))	163 (29.2 (25.5–33.2))	106 (28.8 (24.2–33.7))	0.89
ALT n=5186 (median, IQR)	14 (7–28)	13 (7–27)	15 (8–30)	<0.001
AST n=3427 (median, IQR)	19 (12–34)	18 (11–32)	20 (12–36)	0.002

ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL-c, high-density lipoprotein; LDL-c, low-density lipoprotein; MABP, mean arterial blood pressure; SBP, systolic blood pressure; TC, total cholesterol.

for 2011–2013 and 1577 for 2014–2016. Women constituted 60.3% of the total HIV population. The mean age of the study sample was 34.3±9.9 years (32.3±9.6 years for women versus 37.4±9.4 years for men; p<0.001). Only 247 (2.0%) participants were aged 60 years and above and 1099 (9.0%) aged 50 years and above. The median CD4 count was 197 cells/µL (25th–75th percentiles 91–353). Only 1.3% and 0.6% of the study population knew about their hypertension and diabetic status, respectively. Table 1 summarises the characteristics of the study population. Men were older (p<0.001), had higher blood pressure (SBP (p=0.01), DBP (p=0.001), mean arterial blood pressure (p=0.01)), haemoglobin (p<0.001), hepatitis B prevalence (p<0.001), fasting plasma glucose (p=0.02) and lower CD4 count at initiation of care (p<0.001), BMI (p<0.001) and total cholesterol (p<0.001) than the women. Figure 1A,B shows the time trend and age group trend for the CVD risk factors.

Hypertension

The overall prevalence of hypertension was 26.7% (95% CI 25.5% to 28.0%). Male patients were more likely to be hypertensive at initiation of care (31.8% vs 28.5%, p=0.01). There was progressive increase in frequency of hypertension as age and CD4 count increased for both men and women (table 2, figure 2). This was also true for stage 1 and 2 hypertension, ISH and MH. Individuals with hypertension were older and had higher levels of BMI, fasting blood sugar and CD4 count than the non-hypertensive patients (figure 3). The overall prevalence of prehypertension was 26.5% (95% CI 25.3% to 27.8%).

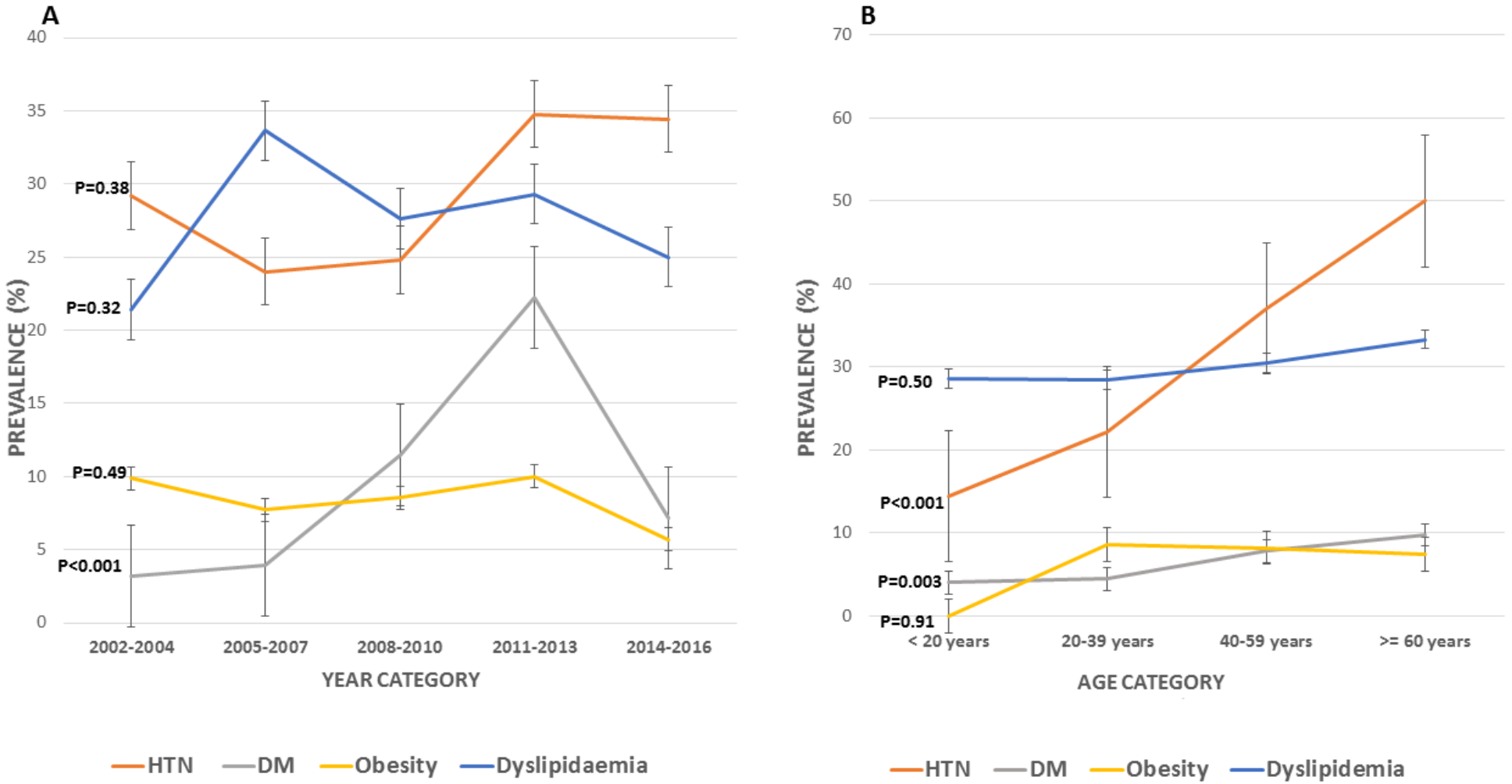

Figure 1: Trends of traditional CVD risk factors prevalence (A) over the study period (B) across age categories

The prevalence of hypertension remained unchanged through the period of study (p=0.38) but increased with age (p<0.001) (figure 1A-B).

Diabetes mellitus

The prevalence of DM was 5.6% (95% CI 4.5% to 6.7%). There was no significant gender difference in prevalence (4.7% in women versus 6.7% in men, p=0.06). There was significant increase in DM prevalence across the years (p<0.001) and as patient age increased (figure 1A,B, table 2). The prevalence of DM increased with CD4 count increase (figure 2).

Obesity

Obesity was found in 8.3% (95% CI 7.6% to 9.1%) with a strong female predilection (10.5% vs 4.3%, p<0.001). There was also a female preponderance of overweight individuals (23.4% vs 19.4%, p<0.001). The total proportion of overweight individuals was 21.9%. Overall, 30.3% of the cohort was either overweight or obese and only 11.9% were underweight. Normal BMI was found in 57.9% of the cohort. There was no significant change in the prevalence of obesity across the years (p=0.49) or by age categories (p=0.91) (figure 1A,B) but increased with increase in CD4 count (figure 2).

Dyslipidaemia

Of the 926 individuals who had at least one component of the lipid profile done at baseline, dyslipidaemia occurred in 29.1% (95% CI 26.1% to 32.1%) with hypercholesterolaemia in 4.1% (95% CI 2.9% to 5.6%), elevated LDL-c in 7.8% (95% CI 3.8% to 14.0%), elevated triglyceride in 21.1% (95% CI 18.3% to 24.1%) and low HDL-c in 42.6% (95% CI 34.9% to 50.6%). There was increasing prevalence of high total cholesterol with increasing age (p=0.02), though this relationship was not evident with the other components of the cholesterol panel. There was also no gender difference in all the components of the lipid profile or their derivatives (p=0.16, 0.79, 0.80, 0.16 for total cholesterol, LDL-c, HDL-c and triglycerides, respectively). There was no significant change in the prevalence of dyslipidaemia across the years (p=0.32) or by age categories (p=0.50) (figure 1A,B).

Independent associations with CVD risk factors

Table 3 summarises the sociodemographic and clinical attributes independently associated with prevalent hypertension, DM, obesity and dyslipidaemia in our sample. Age (p<0.001), BMI (p<0.001) and year of the study were independently associated with hypertension. Age, gender, CD4 category and year category were associated with obesity while the risk of dyslipidaemia increased with increasing BMI (p=0.03).

DISCUSSION

This study has shown a high prevalence of hypertension and dyslipidaemia in a large African ARV-naive HIV population with a relatively low prevalence of obesity. Hypertension was independently associated with increasing age and BMI, obesity with age and CD4 count, while dyslipidaemia was associated with increasing BMI.

The risk of CVD is at least 50% higher in people with HIV compared with the general population in some populations.[16] The prevalence of hypertension in this

Table 2: CVD risk factor prevalence across age groups and by gender

	Male					Female					All				
Age (years)	**< 20**	**20-39**	**40-59**	**≥ 60**	**p**	**< 20**	**20-39**	**40-59**	**≥ 60**	**p**	**< 20**	**20-39**	**40-59**	**≥ 60**	**p**
Normal n (%)	5 (62.5)	622 (48.2)	294 (38.9)	16 (32.0)	0.08	52 (63.4)	1081 (51.5)	219 (37.5)	15 (26.8)	0.08	57 (63.3)	1703 (50.3)	513 (38.3)	31 (29.3)	<0.001
Pre-HTN n (%)	2 (25.0)	371 (28.8)	185 (24.5)	11 (22.0)	0.17	18 (21.9)	563 (26.8)	145 (24.8)	11 (19.6)	0.49	20 (22.2)	934 (27.6)	330 (24.6)	22 (20.8)	0.08
Stage 1 HTN n (%)	0 (0.0)	136 (10.5)	114 (15.1)	8 (16.0)	0.08	7 (8.5)	204 (9.7)	89 (15.2)	12 (21.4)	0.08	7 (7.8)	340 (10.0)	203 (15.2)	20 (18.8)	<0.001
Stage 2 HTN n (%)	1 (12.5)	161 (12.5)	163 (21.6)	15 (30.0)	0.1	5 (6.1)	251 (11.9)	131 (22.4)	18 (32.1)	0.08	6 (6.7)	412 (12.2)	294 (21.9)	33 (31.1)	<0.001
ISH n (%)	0 (0.0)	137 (2.9)	41 (5.4)	5 (10.0)	<0.001	0 (0.0)	46 (2.2)	28 (4.8)	10 (17.9)	<0.001	0 (0.0)	83 (2.5)	69 (5.2)	15 (14.2)	<0.001
IDH n (%)	0 (0.0)	73 (5.7)	64 (8.5)	3 (6.0)	0.08	6 (7.3)	103 (4.9)	39 (6.7)	3 (5.4)	0.32	6 (6.7)	176 (5.2)	103 (7.7)	6 (5.7)	0.01
Mixed HTN n (%)	1 (12.5)	187 (14.5)	172 (22.8)	15 (30.0)	<0.001	6 (7.3)	306 (14.6)	153 (26.2)	17 (30.4)	<0.001	7 (7.8)	493 (14.6)	325 (24.3)	32 (30.2)	<0.001
HTN n (%)	1 (12.5)	297 (23.0)	277 (36.6)	23 (46.0)	<0.001	12 (14.6)	455 (21.7)	220 (37.7)	30 (53.6)	<0.001	13 (14.4)	752 (22.2)	497 (37.1)	53 (50.0)	<0.001
DM	0 (0.0)	27 (6.1)	22 (7.1)	3 (13.6)	0.27	1 (4.2)	27 (3.5)	20 (8.8)	1 (5.3)	0.01	1 (4.0)	54 (4.5)	42 (7.8)	4 (9.8)	0.003
Underweight	1 (14.3)	129 (12.4)	80 (10.7)	2 (3.7)	0.06	15 (28.9)	288 (11.3)	89 (12.5)	15 (22.1)	0.39	16(27.1)	417 (11.6)	169 (11.6)	17 (13.9)	0.54
Normal	5 (71.4)	702 (67.7)	452 (60.6)	36 (66.7)	0.03	33 (63.5)	1373 (53.9)	382 (53.7)	39 (57.4)	0.82	38 (64.4)	2075 (57.9)	834 (57.2)	75 (61.5)	0.81
Overweight	1 (14.3)	175 (16.9)	172 (23.1)	9 (16.7)	0.01	4 (7.7)	611 (24.0)	163 (22.9)	12 (17.7)	0.80	5 (8.47)	786(21.9)	335(23.0)	21(17.2)	0.48
Obesity	0 (0.0)	31 (3.0)	42 (5.6)	7 (12.9)	0.001	0 (0.0)	275 (10.8)	78 (10.9)	2 (2.9)	0.86	0 (0.0)	306(8.5)	120(8.2)	9 (7.4)	0.91
High TC	0(0.0)	2(0.99)	7(5.3)	1(12.5)	0.005	0 (0.0)	18 (4.3)	8 (8.1)	0 (0.0)	0.17	0 (0.0)	20 (3.2)	15 (6.5)	1 (7.1)	0.02
High LDL-c	0(0.0)	2(5.9)	2(10.0)	0 (0.0)	0.79	0 (0.0)	4 (7.1)	2 (15.4)	0 (0.0)	0.45	0 (0.0)	6 (6.7)	4 (12.1)	0 (0.0)	0.52
Low HDL-c	0(0.0)	17(40.5)	14(66.7)	1 (50.0)	0.09	1(50.0)	27(37.0)	9 (42.9)	0 (0.0)	0.99	1 (50.0)	44 (38.3)	23 (54.8)	1 (33.3)	0.17
High CI 1	0 (0.0)	14(37.8)	11(68.8)	1 (50.0)	0.08	1(50.0)	22(34.9)	10(52.6)	0 (0.0)	0.46	1 (50.0)	36(36.0)	21 (60.0)	1 (33.3)	0.07
High CI 2	0 (0.0)	4 (11.8)	7 (36.8)	1 (50.0)	0.02	0 (0.0)	7(12.7)	5 (38.4)	0 (0.0)	0.08	0 (0.0)	11 (12.4)	12 (37.5)	1 (33.3)	0.003
High AIP	0 (0.0)	3 (8.8)	1 (5.6)	0 (0.0)	0.56	1(50.0)	7(11.3)	3 (17.6)	0 (0.0)	0.85	1 (50.0)	10 (10.4)	4 (11.4)	1 (33.3)	0.50
Dyslipidemia	0(0.0)	55(25.5)	47(33.1)	4(44.4)	0.05	4(30.8)	131(30)	27(26.7)	1(16)	0.38	4 (28.6)	186 (28.4)	74 (30.4)	5 (33.3)	0.50

HTN-Hypertension; ISH-Isolated Systolic Hypertension; IDH-Isolated Diastolic Hypertension; DM-Diabetes Mellitus; TC-Total Cholesterol; LDL-c=Low density lipoprotein; HDL-c = High density lipoprotein; CI 1- Castelli index 1; CI 2 = Castelli index 2; AIP = Atherogenic index of plasma

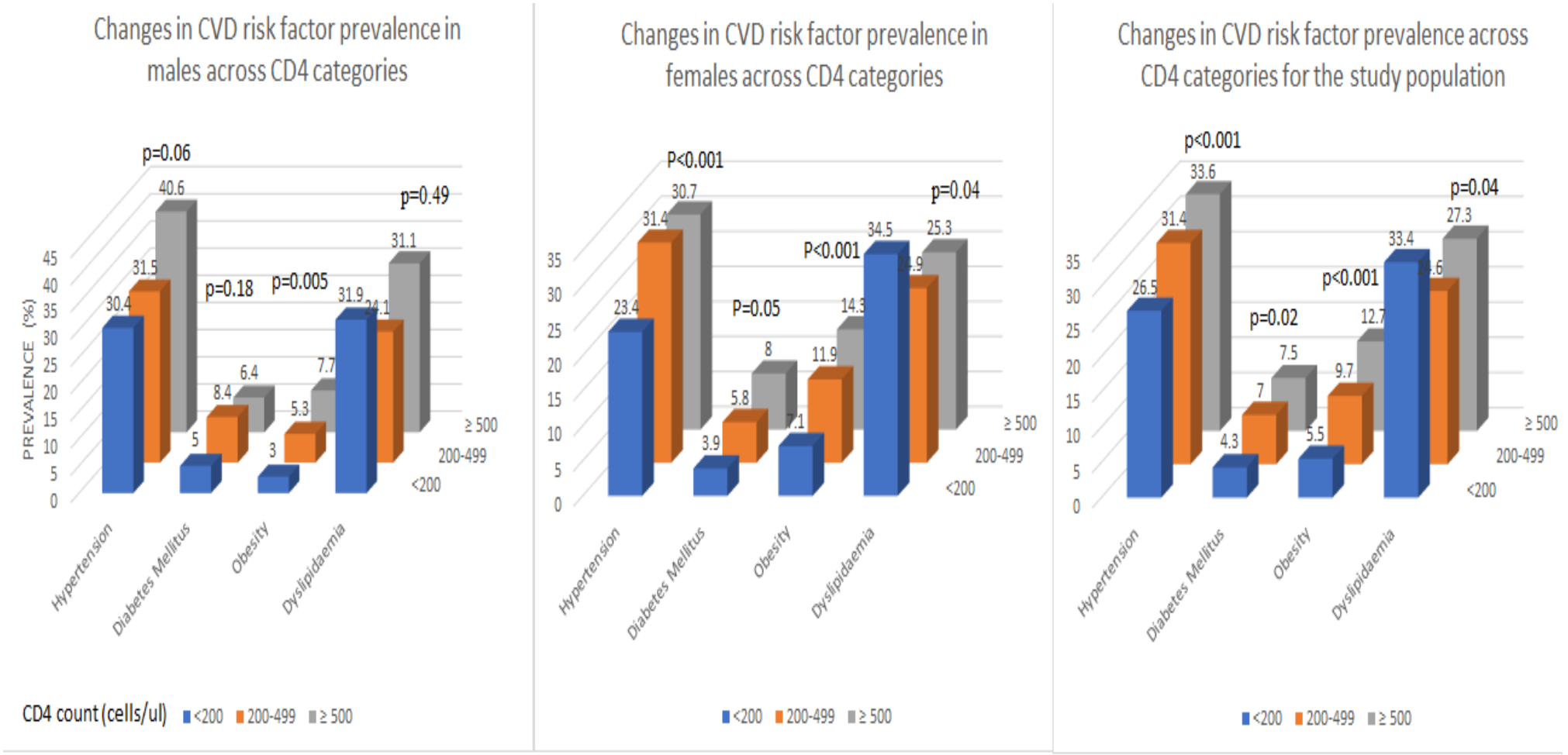

Figure 2: Changes in CVD risk factor prevalence across CD4 categories

relatively young HIV population is high but this may be a reflection of the hypertension prevalence in the general population in this region which ranged from 24.5% to 44.3% in recent community-based studies.[17–19] The similarity in prevalence of hypertension in the HIV population compared with the general population has been observed in other studies.[20–23] HIV infection, however, has been shown to be associated with low-grade inflammation and elevated levels of inflammatory markers despite virological control.[24] This may lead to accelerated atherosclerosis and ultimately hypertension earlier in life. Indeed, this has been shown to be the case in other climes where hypertension prevalence is significantly higher in the HIV population compared with the general population.[20] However, in sub-Saharan Africa, a lower prevalence of hypertension has been documented among HIV patients than those non-infected,[25 26] despite having a relatively higher age than that recorded in this study. We did not attempt a head-to-head comparison of hypertension prevalence between age-matched HIV positive and negative individuals in this study. The prevalence of prehypertension of 26.5% suggests a likelihood of even higher hypertension prevalence as these patients live longer with the infection. An important point to note is that most of the HIV patients in low-income countries are either unaware of their hypertension status (as documented in this study) or unable to afford blood pressure medications in the long term because of out-of-pocket payment for medications. This may contribute to increased occurrence of target organ damage leading to stroke, heart disease

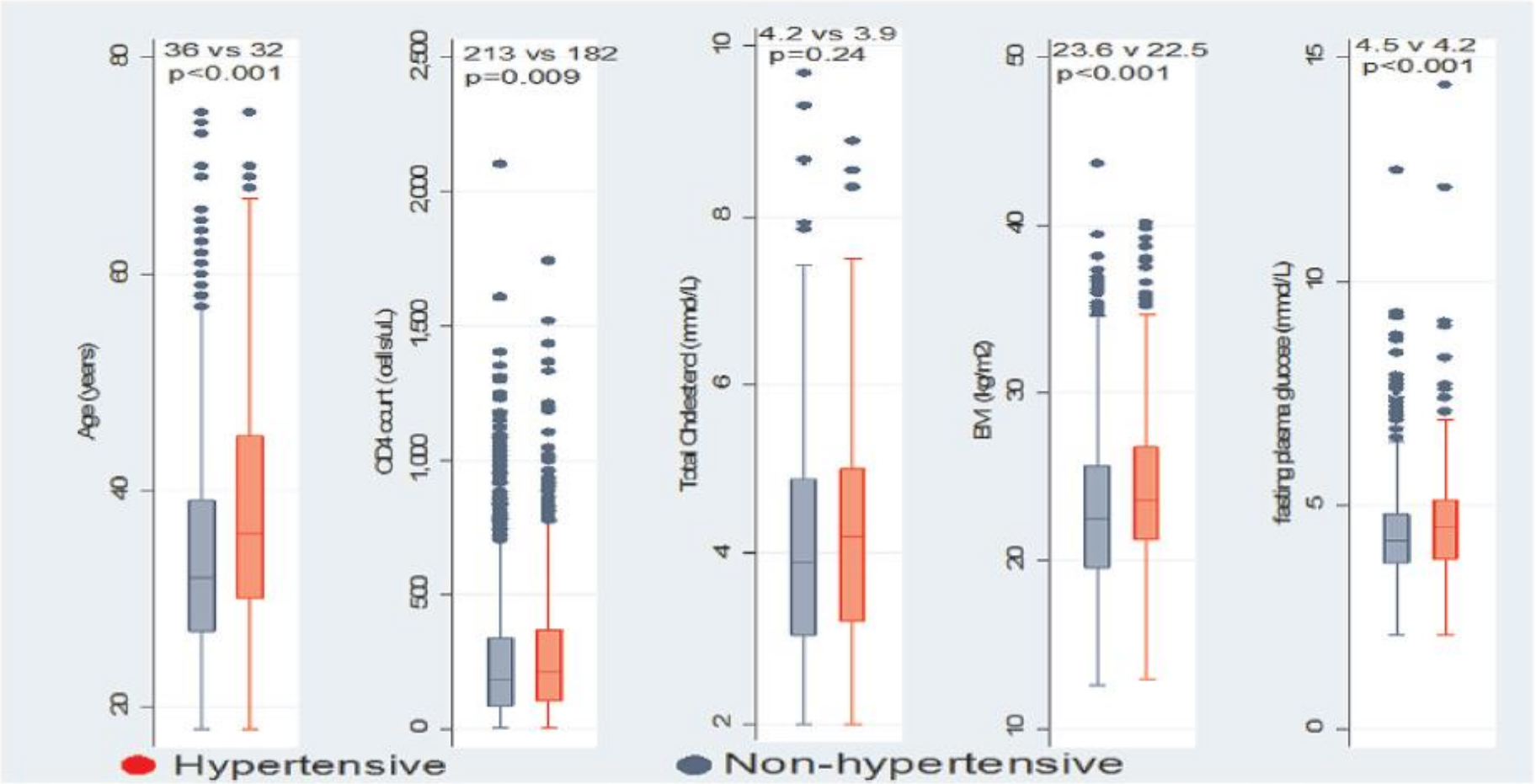

Figure 3 Relationship of other cardiovascular disease risk factors with hypertension among HIV patients.

Table 3 Multivariable logistic regression models showing independent associations with CVD risk factors

	Hypertension (n=1729)	Obesity (n=4946)	Diabetes mellitus (n=587)	Dyslipidaemia (n=478)
	OR (95% CI) P value	OR (95% CI) P value	OR (95% CI) P value	OR (95% CI) P value
Age (years)	1.04 (1.03 to 1.05)<0.001	1.02 (1.01 to 1.03)<0.001	1.03 (0.99 to 1.06) 0.13	1.01 (0.99 to 1.03) 0.51
Sex				
Women	1	1	1	1
Men	1.21 (0.97 to 1.50) 0.09	0.38 (0.29 to 0.49)<0.001	1.74 (0.80 to 3.79) 0.16	1.24 (0.81 to 1.89) 0.33
BMI (kg/m^2)	1.06 (1.03 to 1.08)<0.001	–	1.07 (0.99 to 1.15) 0.10	1.05 (1.01 to 1.10) 0.03
CD4 category				
<200	1	1	1	1
200–499	1.17 (0.94 to 1.47) 0.16	1.90 (1.50 to 2.42)<0.001	1.94 (0.87 to 4.32) 0.10	0.85 (0.55 to 1.32) 0.47
≥500	1.13 (0.83 to 1.54) 0.44	2.63 (1.96 to 3.53)<0.001	3.07 (0.97–9.65) 0.06	0.80 (0.43 to 1.49) 0.48
Year category				
2002–2004	1	1	1	1
2005–2007	0.65 (0.47 to 0.91) 0.01	0.59 (0.41 to 0.85) 0.005	1.22 (0.64 to 2.35) 0.54	1.86 (0.50 to 6.87) 0.35
2008–2010	0.65 (0.46 to 0.93) 0.02	0.59 (0.42 to 0.84) 0.004	3.91 (1.96 to 7.82)<0.001	1.40 (0.39 to 5.08) 0.61
2011–2013	0.63 (0.44 to 0.90) 0.01	0.76 (0.55 to 1.06) 0.11	8.60 (3.76 to 19.64)<0.001	1.52 (0.38 to 6.14) 0.56
2014–2016	0.57 (0.39 to 0.85) 0.006	0.38 (0.26 to 0.56)<0.001	2.31 (0.72 to 7.44) 0.16	1.22 (0.16 to 9.47) 0.85
AUROC	0.64	0.68	0.69	0.59

AUROC, area under the Receiver operating characteristic curve; BMI, body mass index.

and kidney failure among HIV patients in the developing world. Patient education regarding these NCDs may help bridge the gap between high prevalence of hypertension and knowledge of hypertension status but not necessarily lead to control because of the relatively high cost of blood pressure medications.

DM prevalence in this study was not different from that of the general population (2.2%–7.0%).[27–30] This is not unexpected as this cohort was yet to commence ARVs. This seems to be corroborated by a meta-analysis of several studies from sub–Saharan Africa showing no relationship between HIV and glycated haemoglobin levels.[26] DM incidence among HIV patients have been shown to increase with long-term use of ARVs, especially protease inhibitors[31 32] presumably from increased insulin resistance associated with their use. Lower prevalence (1.8%–2.9%) from other African HIV cohorts have been documented.[33 34]

People with HIV in our setting tend to present late for treatment (as indicated by the low median CD4 count at initiation of care) when HIV wasting syndrome may have been established as seen in the pre-ARV era.[35] This may account for the relatively low prevalence of obesity and high frequency of underweight individuals in our newly presenting patients and the increased likelihood of being obese with increasing CD4 count seen with the multivariable analysis. Indeed, it has been documented that there is increasing tendency of HIV patients being obese/overweight in the ARV era approaching proportions seen in the general population.[36] Cachexia in untreated HIV patients is believed to be mediated by cytokines like interleukin 1 and 6.[37] Despite the low frequency of obesity, there was a high prevalence of lipid abnormalities, especially low HDL-c and high serum triglyceride. Low HDL–c was seen in 42.6% of the sample which was comparable to the 51.3% observed in the ARV-naive patients in Kenya.[38] High serum triglycerides in HIV patients may be initiated and perpetuated by the elaboration of cytokines, especially tumour necrosis factor (TNF) and interferon alpha, which are known to be elevated in infections including HIV infection.[39 40] It is believed that TNF rapidly increases very low density lipoprotein (VLDL) production and mobilises fatty acids from the peripheral tissues leading to elevated serum triglycerides.[39] This high frequency of dyslipidaemia may increase the risk of cardiovascular events as life expectancy increases among HIV patients in sub-Saharan Africa.

An integrated approach to care of HIV patients with CVD risk factors has been recommended.[41 42] Integration may involve one of these models—introduction of NCD care into existing HIV care clinics, integration of HIV care into primary healthcare programmes already caring for NCD patients and simultaneous integration of NCD and HIV care programmes.[43] In our clime, donor-funded HIV programmes have become well established in longitudinal care, promotion of healthy lifestyle and routine monitoring, therefore providing an opportunity for long-term care of HIV patients with cardiovascular risk factors. Unfortunately, this integration is yet to be implemented except for cervical cancer screening[44 45] because of shortage of manpower, unavailability of medications for treatment of hypertension, diabetes and dyslipidaemia

unlike ARVs, lack of facilities for diagnosis and monitoring of cardiovascular risk factors and funding constraints. Integrated care of patients with HIV and NCDs has shown promise in reduction of blood pressures and improvement in CD4 count in South Africa,[46] similar blood pressure control in HIV patients compared with HIV-negative controls in Kenya[47] and maximised healthcare delivery efficiency in a resource-limited community in Malawi.[48] Proper management of an integrated programme is expected to yield benefits in the prevention of the occurrence of CVD risk factors, increase detection rates of these NCDs and ensure good treatment outcomes. The timely and adequate prevention and treatment of these conditions may help to reduce the burden of target organ damage in HIV patients. An integrated approach may also reduce cost and increase efficiency in sub-Saharan Africa where financial and manpower resources are scarce.[49]

Strengths and limitations

The current study used routinely collected data in the process of providing care to people with HIV, and therefore has some limitations. Some key CVD risk factors of interest not routinely collected (including tobacco smoking) were missing in the database, and therefore limited our capacity to explore all major CVD risk factors singly and in combination in this sample. The unavailability of complete CVD risk factor data has highlighted the low premium placed on the importance of these factors by HIV caregivers in our centre and provided an avenue for improvement of the current programme. Again, the relatively large patient number may help address this problem in part. We also believe these findings are important, coming from a high HIV prevalence area with small number of published data on CVD risk factors in the HIV population.

CONCLUSION

The high prevalence of traditional CVD risk factors makes it imperative to ensure detailed screening for cardiovascular risk factors in HIV patients at initiation of care and at regular intervals during follow-up. An integrated approach to NCD/HIV care may be the answer to this double burden of disease.

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REFERENCES

1. McDonald CL, Kaltman JR. Cardiovascular disease in adult and pediatric HIV/AIDS. *J Am Coll Cardiol* 2009;54:1185–8.
2. Grunfeld C, Delaney JA, Wanke C, *et al*. Preclinical atherosclerosis due to HIV infection: carotid intima-medial thickness measurements from the FRAM study. *AIDS* 2009;23:1841–9.
3. Sabin CA, Worm SW, Weber R, *et al*. Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients enrolled in the D:A:D study: a multi-cohort collaboration. *Lancet* 2008;371:1417–26.
4. Holmberg SD, Moorman AC, Williamson JM, *et al*. Protease inhibitors and cardiovascular outcomes in patients with HIV-1. *Lancet* 2002;360:1747–8.
5. Rakotoambinina B, Médioni J, Rabian C, *et al*. Lipodystrophic syndromes and hyperlipidemia in a cohort of HIV-1-infected patients receiving triple combination antiretroviral therapy with a protease inhibitor. *J Acquir Immune Defic Syndr* 2001;27:443–9.
6. Smit M, Brinkman K, Geerlings S, *et al*. Future challenges for clinical care of an ageing population infected with HIV: a modelling study. *Lancet Infect Dis* 2015;15:810–8.
7. Schouten J, Wit FW, Stolte IG, *et al*. Cross-sectional comparison of the prevalence of age-associated comorbidities and their risk factors between HIV-infected and uninfected individuals: the AGEhIV cohort study. *Clin Infect Dis* 2014;59:1787–97.
8. WHO. *WHO STEPwise approach to surveillance (STEPS)*. Geneva: World Health Organization, 2003.
9. Chobanian AV, Bakris GL, Black HR, *et al*. The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure: the JNC 7 report. *JAMA* 2003;289:2560–72.
10. Franklin SS, Jacobs MJ, Wong ND, *et al*. Predominance of isolated systolic hypertension among middle-aged and elderly US hypertensives. *Hypertension* 2001;37:869–74.
11. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2014;37(Suppl 1):S81–S90.
12. WHO. *Obesity: preventing and managing the global epidemic: World Health Organization*, 2000.
13. Barbalho SM, Tofano RJ, Bechara MD, *et al*. Castelli Index and estimative of LDL-c particle size may still help in the clinical practice? *Journal of Cardiovascular Disease Research* 2016;7:86–89.
14. Dobiásová M, Frohlich J. The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein-depleted plasma (FER(HDL)). *Clin Biochem* 2001;34:583–8.
15. von Elm E, Altman DG, Egger M, *et al*. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *BMJ* 2007;335:806–8.
16. Islam FM, Wu J, Jansson J, *et al*. Relative risk of cardiovascular disease among people living with HIV: a systematic review and meta-analysis. *HIV Med* 2012;13:453–68.
17. Akpan EE, Ekrikpo UE, Udo AI, *et al*. Prevalence of Hypertension in Akwa Ibom State, South-South Nigeria: Rural versus Urban Communities Study. *Int J Hypertens* 2015;2015:1–5.
18. Andy JJ, Peters EJ, Ekrikpo UE, *et al*. Prevalence and correlates of hypertension among the Ibibio/Annangs, Efiks and Obolos: a cross

sectional community survey in rural South-South Nigeria. *Ethn Dis* 2012;22:335–9.
19. Akpan EE, Ekrikpo UE, Udo AI. Risk factors for chronic kidney disease in Urban Uyo, South-South, Nigeria. *Saudi J Kidney Dis Transpl* 2016;27:1011–7.
20. Kwarisiima D, Balzer L, Heller D, *et al*. Population-based assessment of hypertension epidemiology and risk factors among HIV-positive and general populations in rural Uganda. *PLoS One* 2016;11:e0156309.
21. Bergersen BM, Sandvik L, Dunlop O, *et al*. Prevalence of hypertension in HIV-positive patients on highly active retroviral therapy (HAART) compared with HAART-naïve and HIV-negative controls: results from a Norwegian study of 721 patients. *Eur J Clin Microbiol Infect Dis* 2003;22:731–6.
22. Baekken M, Os I, Sandvik L, *et al*. Hypertension in an urban HIV-positive population compared with the general population: influence of combination antiretroviral therapy. *J Hypertens* 2008;26:2126–33.
23. Jericó C, Knobel H, Montero M, *et al*. Hypertension in HIV-infected patients: prevalence and related factors. *Am J Hypertens* 2005;18:1396–401.
24. Mayne ES, George JA. Mortal allies: human immunodeficiency virus and noncommunicable diseases. *Curr Opin HIV AIDS* 2017;12:148–56.
25. Vos A, Devillé W, Barth R, *et al*. HIV infection and cardiovascular risk profile in a rural South African population: the Ndlovu Cohort Study. *BMJ Global Health* 2017;2(Suppl 2):A10.1–A10.
26. Dillon DG, Gurdasani D, Riha J, *et al*. Association of HIV and ART with cardiometabolic traits in sub-Saharan Africa: a systematic review and meta-analysis. *Int J Epidemiol* 2013;42:1754–71.
27. Enang OE, Otu AA, Essien OE, *et al*. Prevalence of dysglycemia in Calabar: a cross-sectional observational study among residents of Calabar, Nigeria. *BMJ Open Diabetes Res Care* 2014;2:e000032.
28. Nwatu CB, Ofoegbu EN, Unachukwu CN, *et al*. Prevalence of prediabetes and associated risk factors in a rural Nigerian community. *International Journal of Diabetes in Developing Countries* 2016;36:197–203.
29. Sabir AA, Isezuo SA, Ohwovoriole AE. Dysglycaemia and its risk factors in an urban Fulani population of northern Nigeria. *West Afr J Med* 2011;30:325-30.
30. Alebiosu OC, Familoni OB, Ogunsemi OO, *et al*. Community based diabetes risk assessment in Ogun state, Nigeria (World Diabetes Foundation project 08-321). *Indian J Endocrinol Metab* 2013;17:653.
31. Butt AA, Fultz SL, Kwoh CK, *et al*. Risk of diabetes in HIV infected veterans pre- and post-HAART and the role of HCV coinfection. *Hepatology* 2004;40:115–9.
32. Tien PC, Schneider MF, Cole SR, *et al*. Antiretroviral therapy exposure and incidence of diabetes mellitus in the Women's Interagency HIV Study. *AIDS* 2007;21:1739–45.
33. Magodoro IM, Esterhuizen TM, Chivese T. A cross-sectional, facility based study of comorbid non-communicable diseases among adults living with HIV infection in Zimbabwe. *BMC Res Notes* 2016;9:379.
34. Isa SE, Oche AO, Kang'ombe AR, *et al*. Human Immunodeficiency Virus and Risk of Type 2 Diabetes in a Large Adult Cohort in Jos, Nigeria. *Clin Infect Dis* 2016;63:830–5.
35. Kotler DP, Tierney AR, Wang J, *et al*. Magnitude of body-cell-mass depletion and the timing of death from wasting in AIDS. *Am J Clin Nutr* 1989;50:444–7.
36. Crum-Cianflone N, Tejidor R, Medina S, *et al*. Obesity among patients with HIV: the latest epidemic. *AIDS Patient Care STDS* 2008;22:925–30.
37. Bélec L, Meillet D, Hernvann A, *et al*. Differential elevation of circulating interleukin-1 beta, tumor necrosis factor alpha, and interleukin-6 in AIDS-associated cachectic states. *Clin Diagn Lab Immunol* 1994;1:117–20.
38. Manuthu EM, Joshi MD, Lule GN, *et al*. Prevalence of dyslipidemia and dysglycaemia in HIV infected patients. *East Afr Med J* 2008;85:10–17.
39. Grunfeld C, Feingold KR. The role of the cytokines, interferon alpha and tumor necrosis factor in the hypertriglyceridemia and wasting of AIDs. *J Nutr* 1992;122(3 Suppl):749–53.
40. Mildvan D, Machado SG, Wilets I, *et al*. Endogenous interferon and triglyceride concentrations to assess response to zidovudine in AIDS and advanced AIDS-related complex. *Lancet* 1992;339:453–6.
41. Lundgren JD, Battegay M, Behrens G, *et al*. European AIDS Clinical Society (EACS) guidelines on the prevention and management of metabolic diseases in HIV. *HIV Med* 2008;9:72–81.
42. WHO. *WHO global strategy on integrated people-centred health services 2016-2026. Executive summary. Placing people and communities at the centre of health services*. Geneva: WHO, 2015.
43. Duffy M, Ojikutu B, Andrian S, *et al*. Non-communicable diseases and HIV care and treatment: models of integrated service delivery. *Trop Med Int Health* 2017;22:926–37.
44. Odafe S, Torpey K, Khamofu H, *et al*. Integrating cervical cancer screening with HIV care in a district hospital in Abuja, Nigeria. *Niger Med J* 2013;54:176–84.
45. Ezechi OC, Gab-Okafor CV, Ostergren PO, *et al*. Willingness and acceptability of cervical cancer screening among HIV positive Nigerian women. *BMC Public Health* 2013;13:46.
46. Ameh S, Klipstein-Grobusch K, Musenge E, *et al*. Effectiveness of an integrated approach to HIV and hypertension care in rural South Africa: controlled interrupted time-series analysis. *J Acquir Immune Defic Syndr* 2017;75:472–9.
47. Edwards JK, Bygrave H, Van den Bergh R, *et al*. HIV with non-communicable diseases in primary care in Kibera, Nairobi, Kenya: characteristics and outcomes 2010-2013. *Trans R Soc Trop Med Hyg* 2015;109:440–6.
48. Wroe EB, Kalanga N, Mailosi B, *et al*. Leveraging HIV platforms to work toward comprehensive primary care in rural Malawi: the Integrated Chronic Care Clinic. *Healthc* 2015;3:270–6.
49. Levitt NS, Steyn K, Dave J, *et al*. Chronic noncommunicable diseases and HIV-AIDS on a collision course: relevance for health care delivery, particularly in low-resource settings-insights from South Africa. *Am J Clin Nutr* 2011;94:1690S–6.

Chapter 5 had shown a high prevalence of hypertension, diabetes mellitus and dyslipidaemia in a relatively young HIV-infected population. This, in addition to the HIV disease, puts these patients at a high risk of CKD and/or other target organ damage. Early diagnosis of CKD in the HIV population is critical, especially in the setting of poor health infrastructure, to prevent progression to ESRD.

CHAPTER 6: Urinary Transforming Growth Factor-Beta 1 (uTGF-β1) and prevalent chronic kidney disease risk in HIV-positive patients in West Africa

The early identification of CKD among adults with HIV disease, especially in Sub-Saharan Africa, may be one of the important public health interventions to prevent the rising incidence of ESRD in the HIV population. At present, clinicians depend on urine albumin-creatinine ratio with the occurrence of persistent microalbuminuria being the earliest manifestation of CKD. In nephropathies associated with expansion of the intracellular matrix (like HIV-related nephropathy), increased expression of TGF-β1 is known to occur in the proximal tubular cells. This may be useful for the early diagnosis of CKD, either alone or in combination with microalbuminuria.

Chapter 6 investigates the association between urinary TGF-β1 and prevalent CKD in the adult HIV population. This manuscript has been submitted and is currently undergoing second round review in Kidney International Reports.

Urinary Transforming Growth Factor-Beta 1 (uTGF-β1) and prevalent chronic kidney disease risk in HIV-positive patients in West Africa

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Running Title: Urinary TGF-β1 in West African HIV patients with CKD

Keywords: urinary TGF-β1, HIV, CKD, Nigeria

ABSTRACT

INTRODUCTION

This study investigated the association of urinary transforming growth factor-β1 (uTGF-β1) with prevalent chronic kidney disease (CKD) in the HIV-infected population.

METHODS

HIV-positive patients without CKD (HIV^+CKD^-, n=194) and 114 with CKD (HIV^+CKD^+) who did not have hypertension, diabetes mellitus, Hepatitis B or C, had their urinary protein-creatinine ratio (uPCR), serum TGF-β1 and uTGF-β1 measured. Urinary TGF-beta-creatinine ratios (uTGF-β1Cr) were calculated. Spearman correlation was employed to determine the association between uTGF-β1Cr and various attributes while the Cuzick trend test was used to assess the presence of a linear trend in median uTGF-β1Cr levels across the stages of CKD. Multivariable robust linear regression models were used to assess independent association with variability in uTGF-β1Cr and eGFR levels.

RESULTS

The age of the participants was 38.3±10.3 years with 73.4% being female. The median uTGF-β1Cr was higher among HIV^+CKD^+ [4.85 ng/mmol (25th-75th percentile 1.96-12.35) versus 2.95 (1.02-5.84); p=0.001)]. There was significant positive correlation between uTGF-β1Cr and age (p=0.02), negative correlation with eGFR (p=0.001) and positive correlation with uPCR (p<0.001) in the HIV^+CKD^+ group. Among the HIV^+CKD^+ patients, there was gradual reduction in the median level of uTGF-β1Cr with CKD severity (p=0.04).

$HIV^{+}CKD^{+}$ patients had significantly higher levels of uTGF-β1Cr after controlling for potential confounders. Using eGFR as dependent variable, proteinuria explained the changes associated with uTGF-β1Cr levels.

CONCLUSION

$HIV^{+}CKD^{+}$ patients express higher levels of uTGF-β1 especially in the early stages of CKD which is strongly associated with proteinuria.

INTRODUCTION

The early diagnosis and treatment of chronic kidney disease (CKD) presents a definite opportunity for reducing the incidence of end-stage renal disease (ESRD). This is especially important in regions of high HIV prevalence which unfortunately, tend to have dysfunctional health care systems. Transforming growth factor beta-1 (TGF-β1) is a 112-amino acid, 25KDa ubiquitous protein believed to play a central role in renal fibrosis[1] both as a proliferative, and in some conditions anti-proliferative factor.[2]

In diabetic nephropathy, overexpression of TGF-β1 has been found to occur in the kidneys and in-vivo studies have documented increased expression of TGF-β1 in proximal tubular cells and mesangial cells cultured in high glucose concentrations.[3] Also, in glomerulosclerosis, TGF-β1 has been observed to be centrally involved in extracellular matrix expansion.[4]

Indeed, renal disease associated with expansion of extracellular matrix (diabetic nephropathy, lupus nephritis, focal and segmental glomerulonephritis and IgA nephropathy) have all been associated with increased expression of TGF-β1 while renal conditions that do not have extracellular matrix accumulation (thin basement membrane disease, minimal change disease) seem not to have increased expression of TGF-β1.[5] The former may be the case with HIV-related nephropathy where expansion of the extracellular matrix occurs. Studies have shown that the administration of anti-TGF-β1 substances attenuate extracellular matrix production [6,7] and may lead to retardation of CKD progression in renal diseases associated with increased extracellular matrix production.

Some studies in HIV-positive patients with CKD have demonstrated elevated levels of TGF-β1 in renal tissue,[8] serum [9] and in the urine [10] of HIV-associated nephropathy (HIVAN) patients. Our study aim was to investigate the association of uTGF-β1 with prevalent CKD in HIV-positive patients in Nigeria.

METHODS

Study Participants

This was a cross-sectional study involving 194 HIV-positive patients without CKD (HIV^+CKD^-) and 114 HIV-positive patients with CKD (HIV^+CKD^+) seen at the HIV clinic and the renal unit of the University of Uyo Teaching Hospital, Nigeria (Ethical review number: *UUTH/AD/S/96/VOL.XIX/15*). The facility's HIV clinic offers care to about 50 ambulant HIV-positive patients daily with about three thousand five hundred patients on active enrollment.

Screening for renal disease is not routinely done because patients pay out-of-pocket. Ambulant HIV-positive patients on antiretroviral (ARV) treatment at the HIV clinic who gave informed consent were recruited into the study. CKD was defined as eGFR of < 60ml/min/1.73m^2 persisting in two measurements at least 3 months apart and/or urine protein-creatinine ratio (uPCR) of ≥ 0.05 g/mmol creatinine.[11] The patients' sociodemographic (age, gender, ethnicity) and clinical characteristics (e.g. BMI and blood pressures) were collected using standard techniques. Data regarding the patients' CD4 count at initiation of care and current CD4 count levels, viral load at initiation of care and current levels and antiretroviral (ARV) regimen were also collected. All patients were on ARVs. The ARV regimen used in this program included zidovudine-based regimens (AZT-3TC-EFV or AZT/3TC/NVP); tenofovir-based regimen (TDF-3TC-EFV or TDF-FTC-EFV); abacavir-based regimen (ABC-3TC-EFV) and less frequently, protease-inhibitor regimen. Patients with hypertension, diabetes mellitus; hepatitis B or C co-infection were excluded from the study.

Spot urine samples for uTGF-β1 assay were collected, processed and stored using the manufacturer's instructions. All samples were centrifuged with the supernatant stored in 2 aliquots at -20 degrees centigrade within 2 hours of collection. Blood samples (4 ml) were collected, serum separated and stored in 2 aliquots (for serum creatinine and TGF-β1 assay). Serum and urine creatinine were measured with RANDOX creatinine kits (RANDOX, UK) using the modified Jaffe's reaction. Serum and urinary TGF-β1 were measured using Biovision (California, USA) TGF-β1 (human) ELISA kit (catalog # K4342-100) in duplicates. The intra-assay and inter-assay coefficient of variation was 8.6% and 7.0% respectively. The sensitivity of the assay was < 1pg/ml.

To ameliorate the effect of varying urine concentrations, urinary TGF-β1/creatinine ratio (uTGF-β1Cr, pg/mmol) was derived by dividing urinary TGF-β1 (in pg/L) by urinary creatinine (in mmol/L) and later converted to ng/mmol. We estimated GFR using the 4-variable MDRD [12] and the CKD-EPI [13] equations. Participants' kidney function was staged using the Kidney Disease Outcome Quality Initiative (K/DOQI) classification.[11]

All data analysis was performed with Stata 15.1 (StataCorp, Texas, USA). Numerical variables were reported as means (± standard deviation) or median (25^{th}-75^{th} percentile). Categorical variables are reported as frequencies (percentages). Comparison of quantitative variables was performed using students t-test (or its non-parametric equivalent, the Mann-Whitney U test). Comparison of categorical variables was performed using the Chi-square test. The spearman rank correlation test was used to investigate the continuous association between uTGF-β1Cr and certain demographic and clinical variables (age, serum creatinine, serum TGF-β1, uPCR and CD4 count) and a formal comparison of the correlation coefficients undertaken using the Stata module for comparison of correlation coefficients based on the Steiger's test.[14] The Cuzick trend test was used to assess the presence of a linear trend in median uTGF-β1Cr levels across the stages (1 – 5) of CKD. Univariable and multivariable robust linear regression models were built to determine factors independently associated with variability in uTGF-β1Cr and eGFR levels. The Akaike information criteria (AIC) was used to determine the best multivariable model that explains the variability in uTGF-β1Cr levels. p-value less than 0.05 was deemed statistically significant.

RESULTS

Demographic and clinical characteristics

One hundred and ninety-four $HIV^{+}CKD^{-}$ and 114 $HIV^{+}CKD^{+}$ were recruited into the study. The mean age of the participants was 38.3 ± 10.3 years with statistically significant difference between the groups (p=0.02). The gender distribution showed a female preponderance (73% versus 27%). Table 1 summarizes the demographic and clinical characteristics of the study participants by CKD status. The level of serum TGF-β1 and urinary TGF-β1 was higher among the $HIV^{+}CKD^{+}$ patients than in $HIV^{+}CKD^{-}$ patients, though this difference did not achieve statistical significance. However, when urinary TGF-β1 level was standardized by using the urinary TGF-β1/creatinine ratio, those with $HIV^{+}CKD^{+}$ had significantly higher levels (Table 1).

Factors associated with uTGF-ß1Cr

Age and uPCR had significant correlation with uTGF-β1Cr while no significant correlation was found with mABP, BMI, CD4 count and serum TGF-β1 for the total study population (Table 2). However, in the $HIV^{+}CKD^{+}$ group there was significant correlation with age, serum creatinine, uPCR and eGFR. There was significant positive correlation between uPCR and uTGF-β1Cr (rho=0.32; p<0.001). A significantly higher correlation was found between uTGF-β1Cr and eGFR in the $HIV^{+}CKD^{+}$ group than the $HIV^{+}CKD^{-}$ group. For the $HIV^{+}CKD^{+}$ group, median uTGF-β1Cr significantly decreased with severity of CKD (Figure 1).

There was no correlation between serum TGF-β1 and eGFR (Rho = -0.04; p=0.52) nor between serum TGF-β1 and serum creatinine (Rho = 0.09; p=0.19).

Univariable and multivariable linear regression models

After adjusting for the effect of age at the time of data collection, gender differences, mean arterial blood pressure, waist circumference, eGFR, ARV regimen and CD4 count at enrollment, patients with $HIV^{+}CKD^{+}$ persistently had higher levels of uTGF-β1Cr compared with $HIV^{+}CKD^{-}$ (Table 3). The magnitude of the effect of uPCR on uTGF-β1Cr levels was noted to be much higher than that of eGFR. The type of ARV regimen administered, differences in gender, BMI, CD4 count and serum TGF-β1 were not significantly associated with uTGF-β1Cr at the multivariable level (Table 3). Older age was significantly associated with higher uTGF-β1Cr levels. A little more than a quarter [88 (28.6%)] of the sample population were on TDF. Sensitivity analysis done (without patients using TDF) did not show any significant qualitative difference. All independent associations seen in the initial model were maintained.

Table 4 shows univariable and multivariable robust linear regression models with $eGFR_{CKD\text{-}EPI}$ as dependent variable. The relationship between uTGF-β1Cr and renal function seen in previous analysis appears to be dependent on proteinuria levels. In both multivariable models, serum TGF-beta was not independently associated with uTGF-β1Cr or $eGFR_{CKD\text{-}EPI}$, Tables 3 and 4.

DISCUSSION

This study found significantly higher levels of uTGF-β1Cr in $HIV^{+}CKD^{+}$ patients compared to $HIV^{+}CKD^{-}$ patients even after controlling for other potential confounders. This difference also occurred despite both groups having similar levels of serum TGF-β1. There was significant positive correlation between uTGF-β1Cr and uPCR. Older age and eGFR were also independently associated with differences in uTGF-β1Cr levels. Among the $HIV^{+}CKD^{+}$ individuals, the levels of uTGF-β1Cr progressively decreased across CKD stages 1 to 5. Ultimately, uTGF-β1Cr levels were fully dependent on uPCR levels.

TGF-β1 is known to induce renal fibrosis through multiple pathways including direct action on fibroblasts and other cells that cause extracellular matrix synthesis; inhibition of anti-fibrotic pathways and induction of cell loss through apoptosis [15]. In the "canonical" pathway of TGF-β1-induced renal fibrosis, the binding of TGF-β1 to its twin transmembrane receptors leads to the activation (phosphorylation) of smad2 and smad3 which is then translocated to the nucleus with the help of smad4 protein. The activation of smad2 and smad3 is usually associated with the inhibition of smad7 which is known to have anti-fibrotic activity. The binding of smad3 to gene promoters leads to transcription of profibrotic molecules. This leads to increased laying down of extracellular matrix in the kidneys and subsequent fibrosis.[15] In the kidneys, TGF-β1 is expressed on the renal tubular epithelial cells and glomerular basement membrane [16] and also in the myofibroblasts occurring in the interstitium during chronic kidney injury.[5] The increased expression of TGF-β1 in persistent renal injury [17] manifests as increased urinary excretion of TGF-β1.[18]

This has been documented in renal diseases associated with increase in extracellular matrix production [6,7] including HIV-related nephropathy.[10] The finding of increased urinary excretion of TGF-β1 in HIV patients with CKD was corroborated by our study. Others have also found a positive correlation between urinary albumin excretion and uTGF-β1 [19] which was also corroborated by our study. Indeed, we observed a stronger effect of uPCR (compared to eGFR) on uTGF-β1Cr levels in this study. This is probably due to the activation of proximal tubular cells by persistent proteinuria leading to increased production of TGF-β1.[20] In the multivariable model with eGFR as the dependent variable, uPCR was shown to clearly explain the changes associated with uTGF-β1Cr.

We also noted a step-wise reduction in urinary TGF-β1 levels in individuals with more severe CKD (stages 4 and 5). In advanced CKD where tubular atrophy and severe tubulointerstitial fibrosis has set in, there is reduced numbers of myofibroblasts and functional proximal tubular epithelial cells (and thus TGF-beta expression) in the tubulointerstitium which may lead to reduced excretion of urinary TGF-β1. This explanation suggests a type of "burn-out" of TGF-β1 activity as CKD progresses. Some investigators have reported lower interstitial density measurements for TGF-β1 in advanced CKD from HIV-associated nephropathy (HIVAN) and diabetic nephropathy patients compared with HIV-positive and HIV-negative controls.[21] Similarly, some studies have shown a reduction in circulating TGF-β1 and TGF-β1 mRNA in end-stage kidney disease patients.[22-24] A 12-month follow up study [25] among CKD patients with a wide range of aetiology (not including HIV), found significantly higher levels of tubulointerstitial TGF-β1 mRNA in renal disease patients who did not have progressive CKD compared to those who had progressive CKD.

This suggested a protective effect of TGF-β1 but a 12-month follow up period may not be adequate to assess long term renal outcomes and serial TGF-β mRNA measures were not available in this study. Studies with contrary findings [26,27] (higher TGF-β1 immunohistochemical and mRNA expression in the renal tubulo-interstitium with increased fibrosis) were noted to have been done in patients with stages 1 to 3 CKD and not very advanced disease. Indeed, one study [26] used only patients with serum creatinine less than 2.0 mg/dl (about 177 µmol/L), understandably because of the risk involved in the biopsy of fibrotic kidneys. It appears that serial measurements of urinary TGF-β1 in progressive $HIV^{+}CKD^{+}$ patients, being a non-invasive procedure, may more succinctly, document the temporal profile of uTGF-β1 in progressive CKD.

The markedly elevated uTGF-β1Cr found in patients in stages 1 and 2 CKD present a window of opportunity to reverse or slow down progression if early diagnosis is made and available intervention like angiotensin converting enzyme inhibitors (ACE-I) or angiotensin receptor blockers (ARBs) are employed. Studies have shown reduction in uTGFβ1 levels attributable to administration of ACE-I [28] or prednisolone [29] in IgA nephropathy. The actions of these medications may not be unconnected with the fact that persistent proteinuria is known to activate complements in the renal tubular epithelial cells leading to a cascade of events that eventuate in renal fibrosis.[30,31] Reducing proteinuria using ARBs or ACE-I may indirectly reduce TGF-β1 profibrotic activity in the kidneys and slow down CKD progression. However, it is yet to be shown, in a properly conducted clinical trial, that this will be the case among HIV patients with early CKD. The type of ARV regimen used did not appear to have any effect on variability in uTGF-β1Cr levels in this study.

This includes tenofovir use, which has been known to be associated with proximal tubular dysfunction. We are not aware of any direct link between tenofovir-related renal fibrosis and TGF-β1 levels. It may also have been more informative to compare uTGF-β1Cr levels between ARV-naïve and ARV-exposed patients. Unfortunately, all patients in this study had been commenced on ARV.

A major limitation of this study is the lack of renal histopathology among the patients with CKD. This could have determined if the degree of fibrosis (glomerular, tubular and interstitial) correlated with uTGFB1 in the CKD population. Another limitation of our study is the cross-sectional design as this design does not allow for serial assessment of this marker as a measure of progressive deterioration of kidney function. Therefore, it is difficult to ascertain from this work if uTGF-β1Cr increase predates the occurrence of persistent proteinuria. Again, because the levels of uTGF-β1Cr appear to wane with advanced CKD, it is possible to miss cases of advanced CKD if this test is used as a stand-alone. Also, the lack of HIV negative controls limited comparison of uTGF-β1Cr levels in the HIV patients and the general population. Despite the limitations, the result of our study is an important addition to current body of literature and suggest that uTGF-β1Cr is an important marker of CKD in HIV positive patients. Whether this biomarker can be useful for monitoring kidney response to treatment still needs to be studied.

CONCLUSION

HIV patients with CKD express higher levels of TGF-beta-1 activity in urine especially in the early stages of CKD explained by proteinuria levels. Persistent proteinuria remains a veritable tool for early CKD detection in the HIV population.

DISCLOSURES: The authors declare no competing interests.

Table 1: Demographic and clinical characteristics

Variables	Total (N=308)	HIV^+CKD^+ (n=114)	HIV^+CKD^- (n=194)	p
Age (years)	38.3±10.3	39.9±10.4	37.1±10.1	0.02
Female gender [n (%)]	226 (73.4)	85 (74.6)	141 (72.7)	0.72
Duration on ARV (25^{th}-75^{th} percentile)	5 (2-8)	5 (2-8)	5 (2-8)	0.27
Systolic blood pressure (mmHg)	129.0±26.3	137.0±26.9	124.5±24.8	<0.001
Diastolic blood pressure (mmHg)	77.7±14.5	80.8±14.5	75.2±14.1	0.001
Mean arterial pressure (mmHg)	91.2±13.9	93.0±13.5	90.1±14.1	0.07
Weight (kg)	61.4 ±11.9	59.1±11.5	62.7±12.0	0.01
Height (m)	1.63 ± 0.09	1.63±0.08	1.63±1.0	0.38
Body mass index (kg/m^2)	23.2±4.4	22.5±4.4	23.6±4.4	0.03
Waist circumference (cm)	80.1±9.5	79.3±9.2	81.3±9.6	0.07
Serum creatinine (µmol/L)	85.3 (67.6-108.8)	110.8 (90-144)	74 (61-88)	<0.001
$eGFR_{CKD-EPI}$ ($ml/min/1.73m^2$)	80.3 (58.6-105.1)	56.1 (41.9-75.1)	97.6 (78.3-113.3)	<0.001
$eGFR_{MDRD}$($ml/min/1.73m^2$)	74.7 (55.8-97.9)	53.9 (39.9-69.5)	90.2 (72.5-111.2)	<0.001
uPCR (g/mmol creatinine)	0.04 (0.02-0.09)	0.11 (0.03-0.20)	0.03 (0.02-0.04)	<0.001
CD4 count at enrollment (cells/µL)	207 (80-380)	226 (69-384.5)	197 (89-372)	0.84
Current CD4 count (cells/µl)	499 (308-693)	472.5 (274-675)	500 (329-714)	0.32
Current Log viral load (copies/ml)	2.9±1.5	2.8±1.4	3.0±1.6	0.49
Chronic kidney disease stage [n (%)]				
1		18 (15.8)		
2		30 (26.3)		
3		48 (42.1)		
4		8 (7.0)		
5		10 (8.8)		
Serum TGF-β1 (ng/L))	13.7 (3.7-42.5)	20.1 (3.7-47.8)	13.0 (3.7-37.1)	0.22
uTGF-β1 (ng/L)	32.5 (19.2-46.6)	33.2 (21.4-45.5)	32.5 (18.2-47.9)	0.64
uTGF-β1Cr (ng/mmol)	3.7 (1.7-7.5)	4.8 (2.0-11.5)	2.9 (1.2-5.7)	<0.001
Antiretroviral regimens n (%)				
• **Zidovudine-based**	205 (66.5)	83 (72.8)	122 (62.9)	
• **Tenofovir-based**	88 (28.6)	26 (22.8)	62 (31.9)	0.09
• **Abacavir-based**	11 (3.6)	4 (3.5)	7 (3.6)	
• **Protease-inhibitor-based**	4 (1.3)	1 (0.9)	3 (1.6)	

HIV^+CKD^+ (HIV positive patients with CKD); HIV^+CKD^- (HIV positive patients without CKD); **$eGFR_{CKD-EPI}$** ***= estimated glomerular filtration rate using CKD-EPI equation;*** **$eGFR_{MDRD}$** ***=estimated glomerular filtration rate using the 4-variable MDRD formula; uPCR=urine protein-creatinine ratio; TGF- β1=transforming growth factor-beta1; uTGF-β1=urinary transforming growth factor-beta 1; uTGF-β1Cr=urinary transforming growth factor beta-1 – Creatinine ratio TDF=Tenofovir disoproxil fumarate CKD=Chronic kidney disease***

Table 2: Correlations of uTGF-β1Cr with demographic and biochemical parameters

	HIV^+CKD^+ group (n=114)		HIV^+CKD^- group (n=194)			Total (N=308)	
	Rho	**p**	**Rho**	**P**	**p-for difference**	**Rho**	**p**
Age (years)	0.20	0.02	0.09	0.22	0.35	0.19	0.001
Serum creatinine (µmol/L)	-0.35	<0.001	-0.01	0.85	0.003	0.01	0.98
Serum TGF-β1 (pg/ml)	-0.17	0.11	0.03	0.75	0.09	-0.03	0.66
mABP (mmHg)	0.04	0.62	0.04	0.64	0.99	0.07	0.22
BMI (kg/m^2)	0.02	0.85	-0.14	0.07	0.18	-0.11	0.06
Waist circumference (cm)	-0.03	0.73	-0.07	0.32	0.74	-0.06	0.26
$eGFR_{MDRD}$ ($ml/min/1.73m^2$)	0.35	<0.001	0.03	0.70	0.01	0.003	0.95
$eGFR_{CKD-EPI}$ ($ml/min/1.73m^2$)	0.35	<0.001	-0.005	0.99	0.003	-0.02	0.81
CD4 count (cells/µL)	-0.05	0.68	-0.15	0.13	0.40	-0.09	0.20
uPCR (g/mmol)	0.31	<0.001	0.14	0.05	0.13	0.32	<0.001

HIV^+CKD^+= *(HIV positive patients with CKD)****; HIV^+CKD^-=*** *(HIV positive patients without CKD)****; TGF-β1=****transforming growth factor-beta-1****; mABP=****mean arterial blood pressure****; eGFR=****estimated glomerular filtration rate****; uPCR=****urine protein-creatinine ratio****; BMI=****Body mass index*

Table 3: Linear regression models for uTGF-β1Cr prediction

	Univariable β (95% CI) p-value	Multivariable β (95% CI) p-value
Study groups		
HIV^+CKD^-	1	1
HIV^+CKD^+	2.66 (1.85 to 3.47) <0.001	3.97 (2.49 to 5.45) <0.001
Age (years)	0.04 (0.004 to 0.08) 0.03	0.07 (0.02 to 0.12) 0.01
Gender		
Female	1	1
Male	-0.98 (-1.13 to 0.94) 0.85	-0.51 (-1.78 to 0.76) 0.43
mABP (mmHg)	0.04 (0.01 to 0.06) 0.01	0.01 (-0.02 to 0.05) 0.44
BMI (kg/m^2)	-0.13 (-0.23 to -0.03) 0.02	0.03 (-0.19 to 0.25) 0.83
Waist circumference (cm)	-0.03 (-0.74 to 0.02) 0.32	-0.05 (-0.15 to 0.06) 0.39
ARV regimen		
Non TDF-based	1	1
TDF-based	-0.76 (-1.81 to 0.29) 0.15	-2.57 (-6.07to 0.93) 0.15
CD4 count (cells/µL)	-0.01 (-0.01 to 0.006) 0.81	-0.0006 (-0.007 to 0.006) 0.91
eGFR (CKD-EPI) ml/min/1.73m^2	-0.01 (-0.02 to 0.01) 0.24	0.03 (0.01 to 0.05) 0.01
uPCR (g/mmol)	12.10 (10.58 to 13.62) <0.001	6.62 (2.04 to 11.20) 0.01
Serum TGF-β1 (pg/ml)	0.002 (-0.001 to 0.002) 0.99	0.0001 (-0.001 to 0.003) 0.93

HIV^+CKD^+= *HIV positive patients with CKD;* ***HIV^+CKD^-=*** *HIV positive patients without CKD;* ***ARV****=Antiretrovirals;* ***mABP****=mean arterial blood pressure;* ***TDF****=tenofovir disoproxil fumarate;* ***CKD****=Chronic kidney disease;* ***BMI****=Body mass index;* ***eGFR (CKD-EPI)*** *= estimated glomerular filtration rate using the chronic kidney disease epidemiology collaboration equation;* ***uPCR*** *= urine protein-creatinine ratio****; TGF-β1*** *= Transforming growth factor-beta-1*

Table 4: Linear regression models for eGFR prediction

	Univariate β(95% CI) p-value	Multivariate β(95% CI) p-value
Age (years)	-0.87 (-1.16 to -0.57) <0.001	-0.86 (-1.15 to -0.56) <0.001
Sex		
Female	1	1
Male	-0.58 (-8.28 to 7.13) 0.88	4.00 (-3.41 to 11.41) 0.29
uPCR (g/mmol creatinine)	-24.27 (-36.18 to -12.36) <0.001	-29.57 (-42.68 to -16.46) <0.001
uTGF-β1Cr (ng/mmol)	-0.12 (-0.36 to 0.11) 0.31	0.18 (-0.09 to 0.44) 0.19
Serum TGF-β1 (pg/ml)	-0.001 (-0.01 to 0.01) 0.89	-0.001 (-0.01 to 0.01) 0.91
BMI (kg/m^2)	0.24 (-0.54 to 1.02) 0.55	0.09 (-0.64 to 0.82) 0.81
CD4 count (cells/µL)	-0.01 (-0.03 to 0.01) 0.24	-0.01 (-0.03 to 0.01) 0.28
ARV Regimen		
Non-TDF regimen	1	1
TDF regimen	8.88 (1.57 – 16.19) 0.02	6.10 (-1.22 to 13.42) 0.10

The essential findings in Chapter 5 was that of higher uTGF-β1Cr in HIV$^+$CKD$^+$ patients compared to HIV$^+$CKD$^-$; progressive reduction in uTGF-β1Cr levels as CKD worsens and that the changes seen with uTGF-β1Cr is dependent on proteinuria levels.

REFERENCES

1. Sporn MB, Roberts AB, Wakefield LM, de Crombrugghe B. Some recent advances in the chemistry and biology of transforming growth factor-beta. *The Journal of cell biology.* 1987;105(3):1039-1045.
2. Lan HY. Diverse roles of TGF-beta/Smads in renal fibrosis and inflammation. *International journal of biological sciences.* 2011;7(7):1056-1067.
3. Sharma K, Ziyadeh FN. Hyperglycemia and diabetic kidney disease. The case for transforming growth factor-beta as a key mediator. *Diabetes.* 1995;44(10):1139-1146.
4. Isaka Y, Fujiwara Y, Ueda N, et al. Glomerulosclerosis induced by in vivo transfection of transforming growth factor-beta or platelet-derived growth factor gene into the rat kidney. *The Journal of clinical investigation.* 1993;92(6):2597-2601.
5. Yamamoto T, Noble NA, Cohen AH, et al. Expression of transforming growth factor-beta isoforms in human glomerular diseases. *Kidney international.* 1996;49(2):461-469.
6. Border WA, Okuda S, Languino LR, et al. Suppression of experimental glomerulonephritis by antiserum against transforming growth factor β1. *Nature.* 1990;346(6282):371.
7. Border WA, Noble NA, Yamamoto T, et al. Natural inhibitor of transforming growth factor-beta protects against scarring in experimental kidney disease. *Nature.* 1992;360(6402):361-364.
8. Yamamoto T, Noble NA, Miller DE, et al. Increased levels of transforming growth factor-beta in HIV-associated nephropathy. *Kidney international.* 1999;55(2):579-592.
9. Yushchuk ND, Gadzhikulieva MM, Balmasova IP, et al. [The role of immune factors in the progression of chronic kidney diseases in HIV infection]. *Terapevticheskii arkhiv.* 2016;88(3):56-61.
10. Mitchell BI, Byron MM, Ng RC, et al. Elevation of Non-Classical (CD14+/lowCD16++) Monocytes Is Associated with Increased Albuminuria and Urine TGF-beta1 in HIV-Infected Individuals on Stable Antiretroviral Therapy. *PloS one.* 2016;11(4):e0153758.
11. Levey AS, Coresh J, Bolton K, et al. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *American Journal of Kidney Disease.* 2002;39(2 SUPPL. 1).
12. Levey AS, Bosch JP, Lewis JB, et al. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Annals of Internal Medicine.* 1999;130(6):461-470.
13. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Annals of Internal Medicine* . 2009;150(9):604-612.
14. *CORTESTI: Stata module to test equality of two correlation coefficients* [computer program]. 2000.
15. Meng X-m, Nikolic-Paterson DJ, Lan HY. TGF-β: the master regulator of fibrosis. *Nature Reviews Nephrology.* 2016;12(6):325.
16. Yoshioka K, Takemura T, Murakami K, et al. Transforming growth factor-beta protein and mRNA in glomeruli in normal and diseased human kidneys. *Laboratory Investigation.* 1993;68(2):154-163.
17. Bruijn JA, Roos AJ. Transforming growth factor-beta and the glomerular extracellular matrix in renal pathology. *The Journal of Laboratory Clinical Medicine.* 1994;123(1):34-47.
18. Tsakas S, Goumenos DS. Accurate measurement and clinical significance of urinary transforming growth factor-beta1. *American Journal of Nephrology.* 2006;26(2):186-193.
19. Honkanen E, Teppo A-M, Törnroth T, et al. Urinary transforming growth factor-beta 1 in membranous glomerulonephritis. *Nephrology, dialysis, transplantation.* 1997;12(12):2562-2568.

20. Goumenos DS, Tsakas S, El Nahas AM, et al. Transforming growth factor-β1 in the kidney and urine of patients with glomerular disease and proteinuria. *Nephrology Dialysis Transplantation.* 2002;17(12):2145-2152.
21. Nel M, Buys J-M, Botha F, et al. The functionality of African-specific variants in the TGFB1 regulatory region and their potential role in HIVAN. *Clinical Experimental Nephrology.* 2018;22(4):764-772.
22. Füth R, Herder C, Förster S, et al. Evaluation of diagnostic relevance of mRNA levels in peripheral blood: predictive value for mortality in hemodialysis patients. *Cytokine* 2004;27(6):166-172.
23. Knerr K, Füth R, Hemsen P, et al. Chronic inflammation and hemodialysis reduce immune competence of peripheral blood leukocytes in end-stage renal failure patients. *Cytokine.* 2005;30(3):132-138.
24. Kumar A, Gupta V, Chaudhary M, et al. Depleted TGF-β1 levels in end stage renal disease patients from North India. *Gene.* 2014;534(2):440-443.
25. Eikmans M, Baelde HJ, Hagen EC, et al. Renal mRNA levels as prognostic tools in kidney diseases. *Journal of the American Society of Nephrology.* 2003;14(4):899-907.
26. Iwano M, Akai Y, Fujii Y, et al. Intraglomerular expression of transforming growth factor-beta 1 (TGF-β1) mRNA in patients with glomerulonephritis: quantitative analysis by competitive polymerase chain reaction. *Clinical Experimental Immunology.* 1994;97(2):309-314.
27. Bódi I, Kimmel PL, Abraham AA, et al. Renal TGF-β in HIV-associated kidney diseases. *Kidney International.* 1997;51(5):1568-1577.
28. Park HC, Xu ZG, Choi S, et al. Effect of losartan and amlodipine on proteinuria and transforming growth factor-β1 in patients with IgA nephropathy. *Nephrology Dialysis Transplantation.* 2003;18(6):1115-1121.
29. Haramaki R, Tamaki K, Fujisawa M, et al. Steroid therapy and urinary transforming growth factor-β1 in IgA nephropathy. *American Journal of Kidney Diseases.* 2001;38(6):1191-1198.
30. Sheerin N, Sacks SJ. Leaked protein and interstitial damage in the kidney: is complement the missing link? *Clinical Experimental Immunology.* 2002;130(1):1.
31. Rangan GK, Pippin JW, Coombes JD, et al. C5b-9 does not mediate chronic tubulointerstitial disease in the absence of proteinuria. *Kidney International.* 2005;67(2):492-503.

FIGURE 1: Median urinary transforming growth factor levels across CKD stages

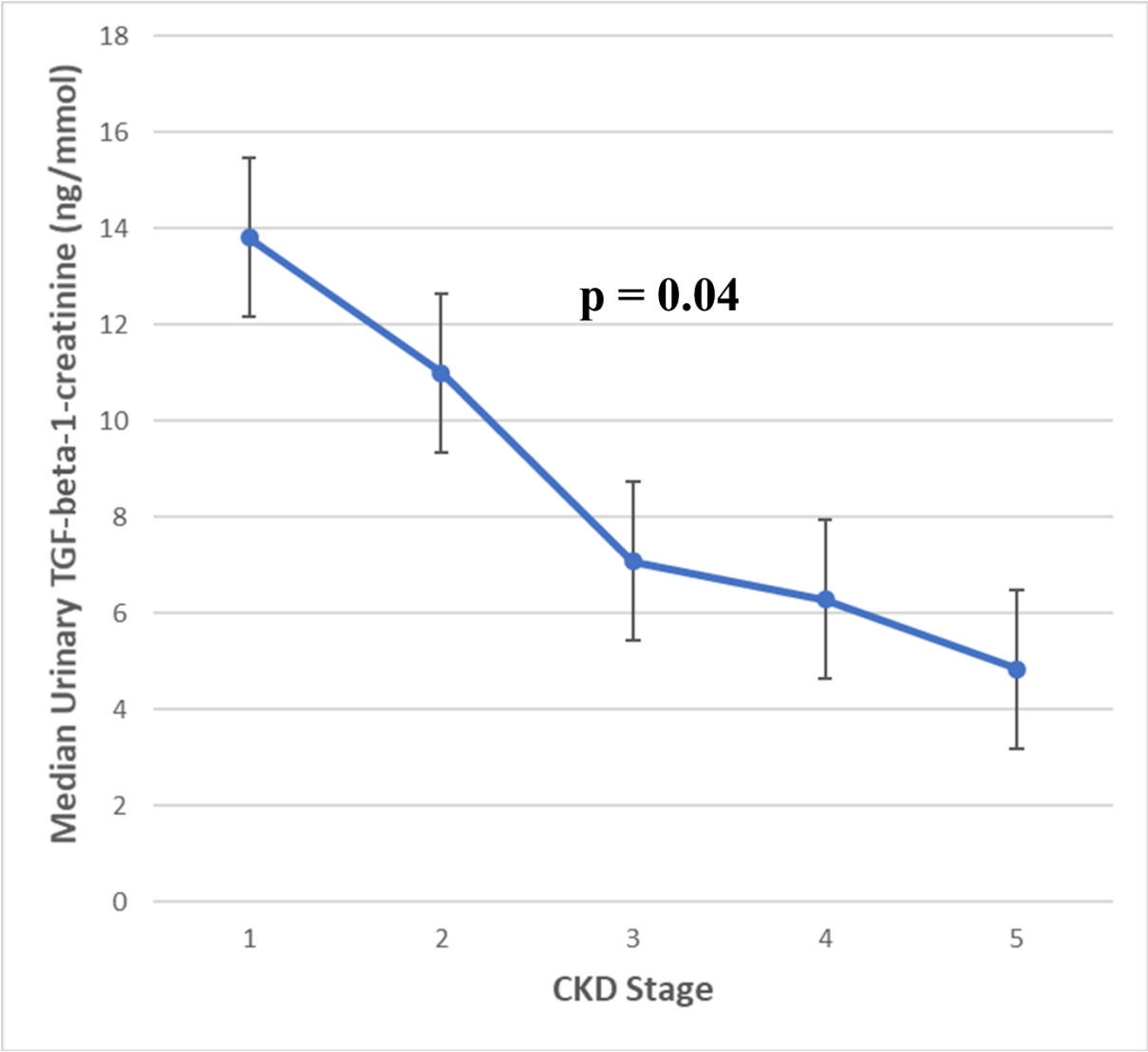

FIGURE 1: Median urinary transforming growth factor levels across CKD stages

CHAPTER 7: Association of Genetic Polymorphisms of *TGF-β1, HMOX1* and *APOL1* with chronic kidney disease in Nigerian Patients with HIV-related nephropathies

Chapter 7 investigates the association between polymorphisms of *TGF-β1, HMOX1* and *APOL1* polymorphisms with CKD in the adult HIV population in Nigeria, West Africa. Following the finding of higher levels of urinary transforming growth factor beta-1 in HIV-infected patients with CKD, it was pertinent to investigate the association of selected *TGF-β1* SNPs with CKD using the same population in Chapter 5. We went further to determine the frequency of APOL1 renal risk variants in a population not previously studied in West Africa and analyzed for any possible interaction with *TGF-β1* and HMOX1 (which has recently been found to be associated with Sickle cell nephropathy in a contiguous population).

Chapter 7 presents the findings in publication-ready format and is undergoing peer-review with the American Journal of Kidney disease (AJKD).

Association of Genetic Polymorphisms of *TGF-β1, HMOX1* and *APOL1* with chronic kidney disease in Nigerian Patients with HIV-related nephropathies

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ABSTRACT

Rationale & Objective: We investigated the association of genetic polymorphisms of Apolipoprotein-1 (*APOL1)*, transforming growth factor-β1 (*TGF-β1*)-an important renal pro-fibrotic cytokine, and Heme oxygenase-1 (*HMOX1)* with prevalent chronic kidney disease (CKD) among adult HIV-infected patients in West Africa.

Study design: Genetic case-control association study.

Setting & Participants: Two hundred and seventeen HIV-infected patients with CKD (Group A), 595 HIV-infected patients without CKD (Group B), 269 with CKD from other aetiologies (Group C) and 114 adults from the normal population (Group D).

Exposure: rs1800469 (*TGF-β1*), rs1800470 (*TGF-β1*), rs121918282 *(TGF-β1*); rs60910145 (*APOL1 G1*), rs73885319 (*APOL1 G1*), rs71785313 *(APOL1 G2)* and rs743811 *(HMOX1)* genetic polymorphisms, HIV.

Outcome: Chronic kidney disease

Analytical approach: Single Nucleotide Polymorphism (SNP) genotyping of rs1800469 (*TGF-β1*), rs1800470 (*TGF-β1*), rs121918282 *(TGF-β1*); rs60910145 (*APOL1*), rs73885319 (*APOL1*), rs71785313 *(APOL1)* and rs743811 *(HMOX1)* was undertaken. Hardy-Weinberg equilibrium was evaluated for all SNPs and minor allele frequencies (MAF) reported. A case-control analysis was performed, and multivariable logistic regression was used to control for potential confounders.

Results: The MAF for rs1800469, rs1800470, rs1800471, rs60910145, rs73885319, rs71785313 and rs743811 was 0.25, 0.46, 0.46, 0.44, 0.45, 0.17 and 0.14, respectively. For Group A versus B, in the multivariable model, there was reduced odds of CKD with rs1800470 [OR 0.44 (95% CI 0.20-0.97)]; increased odds of CKD with *APOL1* [2.54 (1.44-4.51)]; and no association with CKD with rs743811 (0.81 (0.21-3.08). Hypertension had increased odds [2.17 (1.35-3.48)]. For Group A versus D, *APOL1* was associated with prevalent CKD [OR 2.69 (1.23-5.86)].

Limitations: The lack of histopathological data for proper categorization of the type of HIV-related nephropathy.

Conclusion: *APOL1* polymorphisms was highly prevalent in this population and associated with increased CKD risk among the adult HIV patients, as did co-morbid hypertension. While *TGF-β1* (rs1800470) polymorphisms reduced the risk of CKD in the adult HIV-infected population.

Words count – 295

Keywords: *APOL1, TGF-β1, HMOX1, Chronic kidney disease, HIV*

Rationale & Objective

Chronic kidney disease (CKD) in the human immunodeficiency virus (HIV) population has become an increasingly important cause of morbidity and mortality especially in Sub-Saharan Africa (SSA), the epicenter of the HIV pandemic. Among the HIV population of SSA, estimated to be about 25 million individuals in 2013 (1), about 8% have CKD (2). Multiple factors contribute to the development of CKD in the HIV population. These include the effects of the virus itself on renal glomerular and tubular epithelial cells; the high prevalence and premature onset of cardiovascular risk factors like hypertension, diabetes mellitus and dyslipidemia (3); medications (e.g. Tenofovir disoproxil fumarate) used for the treatment of HIV and prevention of associated opportunistic infections; and recurrent acute kidney injury among HIV patients (4).

Recent studies have suggested a genetic predisposition to CKD in the HIV population, especially in SSA. A retrospective cohort study conducted in the United Kingdom that comprised HIV patients of different African ethnicities documented a 6-times increased risk of progression to end-stage renal disease (ESRD) in HIV-infected individuals of West African origin compared to those of East African origin (5). In the same study, black individuals of Caribbean origin (who have a shared ancestry with those of the West African sub-region) had a 5-time increased risk of progression to ESRD compared to those of East African origin. This finding corroborated a meta-analysis finding of the highest prevalence of CKD among HIV patients in the West African sub-region compared to other regions in SSA (2). Regardless of genetic polymorphisms of apolipoprotein L1 (*APOL1*) that have been clearly documented as an important genetic risk factor for CKD of various aetiologies including HIV (6-8), there is need to identify other genetic risk factors for CKD in the HIV population (9).

Transforming growth factor beta-1 (*TGF-β1*) is a 25KDa cytokine that plays a central role in extracellular matrix expansion and renal fibrosis (10).

Genetic regulation of its expression may be an important factor for CKD occurrence and progression in the HIV population (11, 12). Other genetic factors like heme oxygenase (*HMOX1*) polymorphisms implicated in sickle cell nephropathy (13) may also play a role in HIV-related nephropathy and the occurrence of microalbuminuria (14). In this study, we investigate the association of *TGF-β1*, *APOL1* and *HMOX1* genetic polymorphisms with prevalent CKD in the HIV population of West Africa.

METHODS

Ethical approval

The study was approved by the University of Cape Town, Faculty of Health Sciences Human Research Ethics Committee (*HREC REF: 733/2016*), Cape Town, South Africa and the University of Uyo Teaching Hospital Research Ethics Committee (*UUTH/AD/S/96/VOL.XIX/15*). All patients signed consent forms in accordance with the declaration of Helsinki. All data was anonymized to ensure confidentiality.

Study Participants

A total of 1195 adults [217 HIV-infected adults with CKD (Group A); 595 HIV-infected without CKD (Group B); 269 with CKD from other aetiologies (Group C); and 114 from the general population without CKD and HIV (Group D)] were recruited prospectively. Socio-demographic (age, gender, ethnicity), anthropometric (height, weight, body mass index [BMI], waist circumference, hip circumference, waist-hip ratio), family or personal history of diabetes mellitus and hypertension were collected. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using standard techniques. Mean arterial blood pressure (mABP) was calculated using the equation [DBP + (SBP-DBP)/3]. Other clinical variables collected include CD4 count at initiation of care and at the time of sample collection; viral load at initiation of care and at the time of sample collection; hepatitis B surface antigen and hepatitis C status; and the antiretroviral (ARV) regimen administered to the HIV-infected patient. All participants in the main subject group (Group A) were HIV-infected, confirmed using western blot assays and on treatment using defined antiretroviral (ARV) regimens for at least 6 months and had CKD.

Renal function measurement

Serum creatinine was measured using isotope dilution mass spectrophotometry, IDMS-traceable methods. The glomerular filtration rate was estimated using the 4-variable modification of diet in renal disease (MDRD) (15) and chronic kidney disease epidemiology collaboration (CKD-EPI) (16) equations. The CKD-EPI and MDRD equations without the race factor was utilized for estimating GFR in this study given that recent studies (17-19) have suggested that the inclusion of race may generate less precise estimates in the black population. Urinary protein was measured using turbidometric method employing trichloroacetic acid (Merck, USA) while urinary creatinine was measured using RANDOX creatinine kit (RANDOX, UK). Urine protein-creatinine ratio (uPCR) was calculated in g/mmol creatinine. Chronic Kidney Disease was defined as two consecutive values of estimated glomerular filtration rate (eGFR) <60mL/min/1.73m^2 and/or urine protein-creatinine ratio ≥ 0.05g/mmol recorded in a period at least 3 months apart (20).

Molecular methods

DNA was extracted from peripheral blood using manufacturer's instructions (Quick-DNA^{TM} Miniprep plus kit, Zymo Research, California, USA). Targeted SNPs in *TGF-β1, APOL1* and *HMOX1* genes—SNP genotyping of rs1800469 (*TGF-β1*), rs1800470 (*TGF-β1*), rs1800471 (*TGF-β1*), rs121918282 *(TGF-β1*); rs60910145 (*APOL1 G1*), rs73885319 *(APOL1 G1*), rs71785313 (*APOL1 G2*) and rs743811 (*HMOX1*) was undertaken. The PCR protocols were performed on the Bio-Rad CFX96 real time PCR system (Bio-Rad Laboratories, Hercules, CA, USA) by using TaqMan assay, fragment analyses using ABI prism and iPLEX GoldSequenom Mass Genotyping (Inqaba biotech, Pretoria South Africa). Thereafter, Direct Cycle Sequencing using the ABI Prism 3130 xl was performed on a subset (10%) of the samples for validation.

Statistical Analysis

Data analysis was performed using Stata 15.1 (StataCorp Inc., College Station, TX, USA). Descriptive analyses were performed, and data presented as frequencies (and percentages), means (± standard deviation), or medians [25th-75th percentiles] as appropriate.

Pearson's Chi-square was used to compare proportions across the four study groups while ANOVA was used to compare means for quantitative variables and the Kruskal-Wallis test for skewed variables. The Bonferroni correction was used with the ANOVA for pairwise comparison of the means. A $\chi 2$ test with one degree of freedom was used to test SNPs data for any departure from Hardy-Weinberg equilibrium (HWE). A case-control analysis was performed using recessive, dominant and additive models to assess for association between polymorphism and prevalent CKD by fitting univariable (unadjusted) logistic regression models with the SNPs being the independent variables. The Cochran-Armitage trend test was employed to further analyze for genotypic association in the additive model. Analysis involving group C was performed with or without individuals with diabetes mellitus. Multivariable logistic regression models were used to identify independent association of genetic variables with prevalent CKD after adjusting for the effect of known possible clinical confounders. For the *APOL1* SNPs, the joint effects of the G1 and G2 SNPs were combined for the multivariable models. Those with G1 and G2 homozygotes and G1/G2 compound heterozygotes were deemed to have 2 risk alleles; the G1/G0 and G2/G0 heterozygotes had 1 risk allele while those with G0/G0 were coded to have zero risk allele. The multivariable logistic regression models were fitted with *APOL1 G1/G2* in recessive model. *p*-values less than 0.05 were deemed statistically significant.

RESULTS

Study Population

Most (84.7%) of the participants were of similar geographic (bordering the Atlantic Ocean along the west coast of Africa) and linguistic origin. This includes the Ibibio (60.7%), Annang (17.8%), Efik/Ekoi (3.0%), and Oron (3.2%). Other ethnic groups were Ibo (7.3%) and Yoruba (4.8%). Table 1 summarizes the sociodemographic and clinical characteristics of the study participants. Individuals in group C were older, had fewer females and higher mABP compared with the other groups. Group C individuals also had higher waist circumference and greater proportion of obese individuals.

The aetiology of CKD among group C individuals included hypertension (35.2%), diabetes mellitus (27.8%), chronic glomerulonephritis (15.9%), obstructive uropathy (4.4%), polycystic kidney disease (1.5%), sickle cell nephropathy (0.4%) and indeterminate (12.2%). There was no significant difference in CD4 count, viral load, proportion using tenofovir disoproxil fumarate (TDF)-based regimen, hepatitis B and C co-infection and duration on antiretrovirals (ARVs) between group A and B. The HIV positive participants had higher female preponderance than the other groups. Within group A, the proportion of individuals with the various CKD stages, 1 – 5 respectively was 21.5%, 21.1%, 35.3%, 5.9% and 16.2% respectively. For group C, it was 0.4%, 2.6%, 10.7%, 16.3% and 70.0% for stages 1-5 respectively.

Comparison of MAF with the 1000 genomes project

Table 2 summarizes the minor allele frequencies (MAF) for the studied SNPs and compares these with that obtained for the African, African American and European populations from the 1000 genomes project. The MAF for the *APOL1* SNPs were twice the proportion found in other African populations and significantly higher compared to the African American and European populations. The MAF for rs71785313 (*APOL1* G2 SNP) of our cohort was similar to the African and African American values but significantly different from the European values. The MAF for the *TGF-β1* SNPs (rs1800470 and rs1800471) and *HMOX1* were also comparable to that found in other African and African American populations but different from the European population. rs121918282 was monomorphic and not used for subsequent analyses.

Genetic associations with CKD between cases and controls

The p-values for the genotypic trend test comparing groups A and B for rs1800469 (***TGF-β1)***, rs1800470 (***TGF-β1)***, rs1800471 (***TGF-β1)***, rs60910145 (***APOL1)***, rs73885319 (***APOL1)***, rs71785313 (***APOL1)***, rs743811 (***HMOX1)*** were 0.64, 0.04, 0.17, <0.001, <0.001, 0.87 and 0.96, respectively.

The p-values for the genotypic trend test comparing groups A to D for rs1800469, rs1800470, rs1800471, rs60910145, rs73885319, rs71785313, rs743811 were 0.22, 0.45, 0.18, 0.001, 0.001, 0.70, and 0.66, respectively.

A summary of the odds ratios (and 95% confidence intervals) comparing groups A to B; A to D and C to D using the dominant, recessive and additive models is given on Table 3. rs1800470 (*TGF-β1),* in the dominant model, was significantly associated with prevalent CKD when groups A and B were compared. There was increasing risk of CKD as the number of renal risk alleles increased for the *APOL1 G1* SNPs but not for the *APOL1 G2* SNP. In the recessive model, the *APOL1 G1* SNPs consistently showed increased risk for CKD across comparison categories.

Multivariable models

Multivariable logistic regression models for A versus B, A versus D and C versus D (Table 4) showed independent association between *APOL1* and CKD in the HIV population. This relationship persisted when the HIV-CKD group was compared to the general population. There was a 2.5 times increased odds of CKD among HIV patients with the *APOL1* genetic risk even after adjustment for the effect of other potential confounders. There was statistically significant reduction in the odds of having CKD with the *TGF-β1* SNP, rs1800470, suggesting a possible protective effect. Traditional CKD risk factors like hypertension also significantly predicted CKD in the HIV population (Table 4).

DISCUSSION

This analysis is one of the few large studies investigating genetic risk factors for CKD in West Africa. Overall, there was a high prevalence of hypertension and other cardiovascular risk factors among the study participants. All the SNPs analyzed had MAF significantly different from the available European data.

For *TGF-β1*, the MAF of rs1800469 and rs1800471 were lower while rs1800470 was higher in our population compared to the European population. The MAF for *HMOX1* was also higher in the European data compared to that found in our population. The MAF for all the SNPs were similar in our data compared to the African American data except for the *APOL1 G1* SNPs which were about double the frequency in the African American population. The MAF for the *APOL1 G2* SNP was like that found among the African Americans. Both *APOL1 G1* SNPs were associated with significantly higher odds of CKD in the HIV population and when compared to non-HIV controls. The *APOL1 G2* SNPs did not show significant difference between the HIV patients with CKD compared with the other control groups. In multivariable models, rs1800470 (*TGF-β1)* was associated with a 56% reduction in CKD odds among the HIV population while the combined *APOL1* gene, in the recessive model, had about 2.5 times increased odds of CKD.

Since the discovery of *APOL1* genetic odds among the African American population, there has been keen interest in determining the frequency and contribution of the *APOL1* to the increasing CKD burden in SSA. The findings have been quite variable, ranging from the complete absence of *APOL1* odds variants in Ethiopian populations (21) to intermediate *APOL1* prevalence in other Eastern African communities (22) and then to the very high prevalence in West Africa (23, 24). In the Ethiopian study, none of the HIV patients had the *APOL1 G1* SNP while the *APOL1 G2* SNP was found in only 2 patients in the heterozygous state out of 338 patients. The frequency of the *APOL1* renal odds variants was 9-11% in a study from East Africa (22) and this value corresponds closely to many other studies in the region. In West Africa, some studies have documented one of the highest *APOL1* renal odds variant frequencies and this corresponds to the highest CKD prevalence in Sub-Saharan Africa (25). Among the Yoruba ethnic group (24), the MAF for rs60910145 (G1), rs73885319 (G1) and rs71785313 (G2) was 40.6%, 35.8% and 10.5% respectively compared to the current study which was 44.0%, 45.3% and 16.8% (p=0.39, 0.02 and 0.04 respectively) indicating a higher frequency of the APOL1 odds alleles in this population. This study was undertaken among the Niger-Congo tribes living next to the Atlantic Ocean along the west coast of Africa.

This region still bears the relics of the trans-Atlantic slave trade and ports and may well be responsible for a good proportion of the APOL1 bearing individuals in the Caribbean Islands and North America. These regions also have some of the highest proportions of CKD patients among the HIV population (2, 5).

We found a 2.5 times increased odds of CKD among our HIV patients with *APOL1* genetic odds which is a much smaller effect size compared with that found in studies in South Africa (7) (89 times increased odds) or that found among African Americans (26) (29 times increased odds). This large disparity in effect size may be due to the lack of histopathologic data and therefore the heterogenous nature of the HIV-CKD group in this study. Chronic kidney disease in the HIV population may be due to HIV-associated nephropathy (HIVAN), HIV immune complex kidney disease (HIVICK), HIV thrombotic microangiopathy (HIVTMA), CKD from hypertension or diabetes mellitus with co-existent HIV infection or CKD arising from tenofovir-induced renal injury. Renal histopathologic data would have made it possible to compare *APOL1*-driven odds for CKD in biopsy proven HIVAN in our population. HIVTMA and HIVICK do not appear to be associated with APOL1 genetic odds (7, 27) and therefore their presence in our sample may have attenuated the *APOL1* effect size. Considering the high proportion of HIV patients with hypertension in our sample, there is a possibility that a good proportion of these patients have hypertension-attributable CKD with co-existent HIV disease. This may also dilute the effect of *APOL1*. Renal histopathologic data in the HIV population in Africa is largely unavailable.

Even in patients with biopsy proven HIVAN, 20-30% do not carry the high odds variants of *APOL1* and as much as 8% carry no *APOL1* odds variant (7, 28) suggesting other possible genetic or environmental factors. Another notable finding in this study, was the protective effect of rs1800470 on CKD. However, previous studies have reported conflicting results relating to the association between TGF-β SNPs and CKD. An increased odds was noted with rs1800471 in a Caucasian population (29) while a recent meta-analysis (30) has shown increased CKD odds (in the non-HIV population) among Asians and a CKD-protective effect of another *TGF-β1*.

Therefore, the effect of *TGF-β1* polymorphisms on CKD remains inconclusive.

HMOX1 polymorphisms, on the other hand, was not associated with CKD odds regardless of genetic model used, and it appears its relationship with CKD may be limited to sickle cell nephropathy (13, 31). Therefore, the search for other potential genetic factors predisposing to CKD in the HIV sub-population should continue while not taking attention from the multiple environmental factors that promote the development of CKD among individuals with *APOL1* genetic odds variants.

While genetic odds factors for CKD in the HIV population are complex and generally non-modifiable, environmental odds factors are modifiable and may serve as an avenue for the reduction of CKD odds in the HIV population of Sub-Saharan Africa. Combating the high prevalence of hypertension, diabetes mellitus, obesity and dyslipidemia (3, 32, 33) in the relatively young population of patients with HIV in Africa remains the most viable option for controlling CKD odds apart from the use of combined antiretroviral medications.

The major limitation of this study is the lack of histopathological data. This limitation is further enhanced by the lack of local data on the distribution and frequency of different glomerular and tubular lesions among the HIV patients with CKD. This has highlighted the need for large renal clinicopathologic studies among the HIV-CKD population in this region.

CONCLUSION

APOL1 is associated with increased odds of CKD in the adult HIV population of West Africa while at least one SNP of *TGF-β1* may be protective against CKD. Although the role of genetics in the initiation and progression of CKD in the HIV population of West Africa is increasingly becoming evident, it is important to also pay close attention to the threat of non-communicable disease in the HIV population where interventions may serve as means of reducing the proportion who progress to end-stage kidney disease.

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Table 1: Sociodemographic and clinical characteristics of study participants

	Overall (N=1195)	Group A [HIV-CKD] (n=217)	Group B [HIV-no CKD] (n=595)	Group C CKD (other aetiologies) (n=269)	Group D [Population controls] (n=114)	*p*
Age (years)	42.3±12.9	42.2±10.9	39.2±10.8	50.6±14.6	39.7±13.5	<0.001
Female gender n (%)	750 (62.7)	146 (67.3)	442 (74.3)	96 (35.7)	66 (57.9)	<0.001
SBP (mmHg)	132.8±27.0	136.4±29.0	126.6±23.7	147.3±29.8	126.3±18.7	<0.001
DBP (mmHg)	80.9±15.8	83.4±17.5	77.6±13.9	87.3±17.5	79.2±12.5	<0.001
mABP (mmHg)	98.2±18.4	101.1±20.3	93.9±16.3	107.3±19.9	95.0±13.1	<0.001
Hypertension n (%)	559 (46.8)	108 (49.8)	176 (29.6)	237 (88.1)	38 (33.3)	<0.001
Diabetes Mellitus n (%)	112 (9.4)	15 (6.9)	13 (2.2)	79 (29.4)	5 (4.5)	<0.001
BMI (kg/m^2)	24.2±5.2	23.6±5.5	23.6±4.8	25.8±5.0	25.3±6.0	<0.001
Obesity n (%)	143 (12.0)	22 (10.1)	56 (9.4)	40 (14.9)	25 (21.9)	0.001
Waist circumference (cm)	84.3±21.9	82.4±11.4	83.1±28.4	88.3±13.2	84.6±12.2	0.01
Hip circumference (cm)	93.8±10.8	91.8±9.7	93.7±9.5	94.7±13.5	96.5±12.1	0.002
Waist-hip ratio	0.90±0.23	0.89±0.08	0.89±0.31	0.95±0.10	0.88±0.07	0.003
Family history of HTN n (%)	346 (28.9)	45 (20.7)	123 (20.7)	133 (49.4)	45 (39.5)	<0.001
Family history of DM n (%)	166 (13.9)	24 (11.1)	59 (9.9)	63 (23.4)	20 (17.5)	<0.001
uPCR (g/mmol)	0.04 (0.02-0.07)	0.14 (0.05-0.27)	0.03 (0.02-0.05)	0.12 (0.06-0.53)	0.03 (0.02-0.04)	0.002
Serum creatinine (umol/L)	91 (73-138)	126 (83-183)	81 (67-95)	512 (243-981)	85 (71-97)	<0.001

$eGFR_{MDRD}$ (ml/min/1.73m^2)	64.8 (63.6-83.9)	46.9 (30.3-70.8)	74.5 (61.7-93.2)	9.7 (4.8-21.3)	76.2 (63.6-89.0)	<0.001
$eGFR_{CKD-EPI}$ (ml/min)	71.1 (34.0-96.2)	49.6 (31.1-78.7)	84.4 (70.3-105.4)	8.3 (5.4-18.2)	88.7 (73.3-106.0)	<0.001
CD4 count (cells/uL)	283 (157-473)	273 (148-425)	291 (160-492)	-	-	0.54
Viral load (log_{10})	2.2 (1.3-3.8)	2.4 (1.4-4.3)	2.2 (1.3-3.7)	-	-	0.53
TDF-regimen n (%)	223 (18.7)	41 (18.9)	182 (30.6)	-	-	0.001
HBV co-infection n (%)	16 (1.9)	4 (1.8)	12 (2.0)	-	-	0.88
HCV co-infection n (%)	2 (0.2)	1 (0.5)	1 (0.2)	-	-	0.46
Duration on ARVs (years)	5.0 (2.0-9.0)	5.0 (2.0-9.0)	5.0 (2.0-9.0)	-	-	0.63

HIV=Human immunodeficiency virus; CKD=Chronic kidney disease; SBP=Systolic blood pressure; DBP=Diastolic blood pressure; mABP=mean arterial blood pressure; BMI=Body mass index; HTN=hypertension; DM=diabetes mellitus; uPCR=urine protein-creatinine ratio; $eGFR_{MDRD}$=estimated glomerular filtration rate using 4-variable modification of diet in renal disease equation; $eGFR_{CKD-EPI}$= estimated glomerular filtration rate using chronic kidney disease epidemiology equation; TDF=tenofovir disoproxil fumarate; HBV=Hepatitis B virus; HCV=Hepatitis C virus; ARVs=antiretrovirals.

Table 2: Comparison of minor and major allele frequencies of *TGF-β1, APOL1, HMOX1* between the 1000 Genomes Project

Gene	*SNP*	*Chr*	*Minor/Major*	*Alleles*	*Allele frequency (allele count)*				*P-val*		
					Current study	**African**	**A. American**	**European**	**Current study vs African**	**Current vs African American**	**Current study vs European**
TGF-β1	rs1800469	19:41354391	Major	G	0.748 (1730)	0.782 (1034)	0.795 (97)	0.688 (692)	0.04	0.30	0.003
			Minor	A	0.252 (582)	0.218 (288)	0.205 (25)	0.312 (314)			
	rs1800470	19:41353016	Major	A	0.542 (1147)	0.586 (775)	0.623 (76)	0.618 (622)	0.06	0.17	0.002
			Minor	G	0.458 (969)	0.414 (547)	0.377 (46)	0.382 (384)			
	rs1800471	19:41352971	Major	C	0.954 (2153)	0.943 (1247)	0.934 (114)	0.929 (935)	0.16	0.33	0.005
			Minor	G	0.046 (103)	0.057 (75)	0.066 (8)	0.071 (71)			
	rs121918282	11:123653773	Major	A	1.00 (1223)	1.00 (15304)	-	-	-	-	-
			Minor	-	-	-	-	-			
APOL1	rs60910145	22:36265988	Major	T	0.560 (1193)	0.741 (980)	0.779 (95)	1.000 (1006)	<0.001	<0.001	<0.001
			Minor	G	0.440 (939)	0.259 (342)	0.221 (27)	-			
	rs73885319	22:36265860	Major	A	0.547 (1151)	0.741 (979)	0.779 (95)	1.000 (1006)	<0.001	<0.001	<0.001
			Minor	G	0.453 (955)	0.259 (343)	0.221 (27)	-			
	rs71785313	22:36266000-36266005	Major	TTATAA	0.832 (1320)	0.855 (1004)	0.891 (98)	1.000 (1006)	0.13	0.13	<0.001
			Minor		0.168 (266)	0.155 (171)	0.119 (12)	-			
HMOX1	rs743811	22:35396981	Major	T	0.856 (1879)	0.863 (1141)	0.844 (103)	0.747 (751)	0.59	0.74	<0.001
			Minor	C	0.144 (317)	0.137 (181)	0.156 (19)	0.253 (255)			

TGF-β1=transforming growth factor beta-1 gene; APOL1=apolipoprotein-1 gene; HMOX1=heme oxygenase-1 gene; Chr=chromosome.

Table 3: SNP associations with CKD among HIV+/CKD- (control) and HIV+/CKD+ (cases)

Gene	SNP	HWE	Association mode*	Unadjusted OR (95% CI), p (A versus B)	Unadjusted OR (95% CI), p (A versus D)	Unadjusted OR (95% CI), p (C versus D)
TGF-β1	rs1800469	0.73	Dominant	0.94 (0.69-1.30) 0.73	1.33 (0.81-2.19) 0.26	1.18 (0.72-1.91) 0.51
			Recessive	0.81 (0.45-1.48) 0.50	1.79 (0.58-5.54) 0.31	2.08 (0.70-6.23) 0.19
			Additive	0.93 (0.73-1.19) 0.58	1.31 (0.87-1.98) 0.19	1.23 (0.84-1.82) 0.29
TGF-β1	rs1800470	0.61	Dominant	**0.69 (0.48-0.98) 0.04**	1.20 (0.71-2.01) 0.50	1.33 (0.80-2.21) 0.58
			Recessive	0.77 (0.51-1.16) 0.22	1.20 (0.62-2.32) 0.58	1.20 (0.63-2.28) 0.27
			Additive	**0.78 (0.62-0.99) 0.04**	1.14 (0.80-1.63) 0.45	1.20 (0.84-1.71) 0.30
TGF-β1	rs1800471	0.81	Dominant	1.45 (0.85-2.45) 0.17	1.81 (0.75=4.36) 0.19	1.50 (0.63-3.58) 0.36
			Recessive[μ]	-	-	-
			Additive	1.40 (0.83-2.34) 0.20	1.81 (0.75-4.36) 0.19	1.52 (0.65-3.56) 0.33
APOL1	rs60910145	0.08	Dominant	**1.95 (1.33-2.85) 0.001**	**2.09 (1.23-3.57) 0.007**	1.25 (0.77-2.05) 0.37
			Recessive	**1.72 (1.17-2.51) 0.006**	**2.71 (1.38-5.38) 0.004**	**2.17 (1.11-4.25) 0.02**
			Additive	**1.56 (1.24-1.97) <0.001**	**1.89 (1.31-2.73) 0.001**	1.37 (0.98-1.92) 0.06
APOL1	rs73885319	0.61	Dominant	**2.01 (1.36-2.97) <0.001**	2.08 (1.20-3.58) 0.009	1.16 (0.70-1.92) 0.55
			Recessive	**1.70 (1.16-2.49) 0.006**	**2.74 (1.39-5.39) 0.004**	**2.19 (1.12-4.30) 0.02**
			Additive	**1.58 (1.25-2.00) <0.001**	**1.91 (1.32-2.77) 0.001**	1.34 (0.95-1.87) 0.09
APOL1	rs71785313	0.09	Dominant	0.96 (0.65-1.43) 0.86	1.11 (0.61-2.01) 0.74	1.33 (0.74-2.40) 0.34
			Recessive	1.03 (0.46-2.28) 0.95	1.41 (0.37=5.37) 0.61	2.10 (0.59-7.53) 0.26
			Additive	0.98 (0.71-1.34) 0.91	1.12 (0.69-1.82) 0.65	1.33 (0.84-2.13) 0.22
HMOX1	rs743811	0.08	Dominant	1.03 (0.71-1.48) 0.89	1.14 (0.66-1.97) 0.65	0.75 (0.43-1.31) 0.32
			Recessive	0.63 (0.21-1.90) 0.41	0.97 (0.18-5.44) 0.98	0.80 (0.14-4.45) 0.80
			Additive	0.98 (0.71-1.34) 0.89	1.11 (0.68-1.81) 0.69	0.48 (0.43-1.28) 0.34

For the additive model, the first row represents the heterozygous state while the second row represents the homozygous state for the minor allele. The homozygous state of the major allele is the reference group.

[μ]***Only 2 individuals were homozygous for the minor allele. The numbers were too few for computations.***

TGF-β1=Transforming growth factor beta-1 gene; APOL1=Apolipoprotein-1 gene; HMOX1=Heme oxygenase 1 gene; SNP=Single nucleotide polymorphism; OR=Odds ratio; CI=confidence interval; HWE=Hardy-Weinberg equilibrium

Table 4: Multivariable logistic regression models controlling for environmental factors

	Group A versus B Odds ratio (95% CI) *p*-value	Group A versus D Odds ratio (95% CI) *p*-value	Group C versus D Odds ratio (95% CI) *p*-value
rs1800469			

GG	1	1	1
GA	1.35 (0.79-2.29) 0.27	1.20 (0.53-2.68) 0.66	0.59 (0.20-1.70) 0.33
AA	1.32 (0.43-4.01) 0.63	1.64 (0.28-963) 0.58	1.67 (0.23-11.87) 0.61
rs1800470			
AA	1	1	1
GA	0.59 (0.34-1.03) 0.06	1.08 (0.49-2.41) 0.84	2.06 (0.74-5.71) 0.17
GG	**0.44 (0.20-0.97) 0.04**	0.89 (0.27-2.90) 0.35	2.14 (0.46-9.89) 0.33
rs1800471			
CC	1	1	1
CG	1.45 (0.68-3.08) 0.34	1.80 (0.52-6.24) 0.35	0.54 (0.11-2.61) 0.45
GG	-	-	-
rs743811			
TT	1	1	1
TC	1.30 (0.81-2.08) 0.28	1.80 (0.84-3.87) 0.13	0.89 (0.35-2.27) 0.80
CC	0.81 (0.21-3.08) 0.76	2.29 (0.21-25.07) 0.50	2.56 (0.09-75.71) 0.59
Age (years)	1.02 (0.99-1.04) 0.17	1.01 (0.98-1.05) 0.38	**1.04 (1.01-1.07) 0.01**
APOL1 (recessive)	**2.54 (1.44-4.51) 0.001**	**2.69 (1.23-5.86) 0.01**	1.13 (0.46-2.72) 0.79
Sex			
Female	1	1	1
Male	1.24 (0.78-1.99) 0.36	**0.48 (0.23-0.96) 0.04**	1.93 (0.86-4.36) 0.11
Hypertension	**2.17 (1.35-3.48) 0.001**	1.79 (0.83-3.86) 0.14	**12.63 (5.41-29.53) <0.001**
Diabetes Mellitus	2.48 (0.90-6.78) 0.08	1.19 (0.22-6.25) 0.83	**5.68 (1.14-28.26) 0.03**
Waist circumference (cm)	0.99 (0.97-1.01) 0.61	1.02 (0.97-1.07) 0.34	0.98 (0.94-1.03) 0.45
BMI (Kg/m^2)	0.98 (0.92-1.05) 0.56	0.91 (0.82-1.01) 0.07	1.03 (0.93-1.14) 0.51

BMI=Body mass index

Supplementary Table 3: *APOL1* associations with CKD between Groups C versus D (without patients with diabetes)

Gene	SNP	Association mode	Unadjusted OR (95% CI), p (C versus D)
APOL1	rs60910145	Dominant	1.17 (0.69-1.97) 0.56
		Recessive	**2.65 (1.33-5.30) 0.006**
		Additive	1.40 (0.99-1.98) 0.05
APOL1	rs73885319	Dominant	1.08 (0.64-1.83) 0.78
		Recessive	**2.71 (1.36-5.40) 0.005**
		Additive	1.37 (0.97-1.94) 0.07
APOL1	rs71785313	Dominant	1.26 (0.68-2.34) 0.46
		Recessive	2.03 (0.54-7.64) 0.29
		Additive	1.28 (0.79-2.09) 0.32

REFERENCES

1. Kharsany ABM, Karim QA. HIV Infection and AIDS in Sub-Saharan Africa: Current Status, Challenges and Opportunities. The Open AIDS Journal. 2016;10:34-48.
2. Ekrikpo UE, Kengne AP, Bello AK, Effa EE, Noubiap JJ, Salako BL, et al. Chronic kidney disease in the global adult HIV-infected population: A systematic review and meta-analysis. PloS one. 2018;13(4):e0195443.
3. Ekrikpo UE, Akpan EE, Ekott JU, Bello AK, Okpechi IG, Kengne AP. Prevalence and correlates of traditional risk factors for cardiovascular disease in a Nigerian ART-naive HIV population: a cross-sectional study. BMJ open. 2018;8(7):e019664.
4. Rosenberg AZ, Naicker S, Winkler CA, Kopp JB. HIV-associated nephropathies: epidemiology, pathology, mechanisms and treatment. Nature Reviews Nephrology. 2015;11(3):150.
5. Jose S, Hamzah L, Jones R, Williams D, Winston A, Burns F, et al. Chronic Kidney Disease Risk in African and Caribbean Populations With HIV. The Journal of Infectious Diseases. 2018:jiy397-jiy.
6. 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. Journal of the American Society of Nephrology. 2011:ASN. 2011040388.
7. 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. Journal of the American Society of Nephrology. 2015;26(11):2882-90.
8. 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. Journal of the American Society of Nephrology. 2011:ASN. 2011040434.
9. Cohen SD, Kopp JB, Kimmel PL. Kidney diseases associated with human immunodeficiency virus infection. New England Journal of Medicine. 2017;377(24):2363-74.
10. Meng X-M, Tang PM-K, Li J, Lan HY. TGF-β/Smad signaling in renal fibrosis. Frontiers in physiology. 2015;6:82.
11. Rao M, Wong C, Kanetsky P, Girndt M, Stenvinkel P, Reilly M, et al. Cytokine gene polymorphism and progression of renal and cardiovascular diseases. Kidney International. 2007;72(5):549-56.
12. Rao M, Guo D, Jaber BL, Tighiouart H, Pereira BJG, Balakrishnan VS. Transforming growth factor-beta 1 gene polymorphisms and cardiovascular disease in hemodialysis patients. Kidney International. 2004;66(1):419-27.
13. Saraf SL, Zhang X, Shah B, Kanias T, Gudehithlu KP, Kittles R, et al. Genetic Variants and Cell-Free Hemoglobin Processing in Sickle Cell Nephropathy. Haematologica. 2015.
14. Geard A, Pule GD, Chetcha Chemegni B, Ngo Bitoungui VJ, Kengne AP, Chimusa ER, et al. Clinical and genetic predictors of renal dysfunctions in sickle cell anaemia in Cameroon. 2017;178(4):629-39.
15. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Annals of internal medicine. 1999;130(6):461-70.
16. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Annals of internal medicine. 2009;150(9):604-12.
17. Anker N, Scherzer R, Peralta C, Powe N, Banjeree T, Shlipak M. Racial disparities in creatinine-based kidney function estimates among HIV-infected adults. Ethnicity & disease. 2016;26(2):213.
18. Yayo E, Ayé M, Yao C, Gnionsahé A, Attoungbré M-L, Cavalier E, et al. Measured (and estimated) glomerular filtration rate: reference values in West Africa. Nephrology Dialysis Transplantation. 2017.

19. Madala ND, Nkwanyana N, Dubula T, Naiker IP. Predictive performance of eGFR equations in South Africans of African and Indian ancestry compared with 99m Tc-DTPA imaging. International urology and nephrology. 2012;44(3):847-55.
20. Levey AS, Eckardt K-U, Tsukamoto Y, Levin A, Coresh J, Rossert J, et al. Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney international. 2005;67(6):2089-100.
21. Behar DM, Kedem E, Rosset S, Haileselassie Y, Tzur S, Kra-Oz Z, et al. Absence of APOL1 risk variants protects against HIV-associated nephropathy in the Ethiopian population. American journal of nephrology. 2011;34(5):452-9.
22. Stanifer JW, Karia F, Maro V, Kilonzo K, Qin X, Patel UD, et al. APOL1 risk alleles among individuals with CKD in Northern Tanzania: A pilot study. PloS one. 2017;12(7):e0181811.
23. Ulasi II, Tzur S, Wasser WG, Shemer R, Kruzel E, Feigin E, et al. High population frequencies of APOL1 risk variants are associated with increased prevalence of non-diabetic chronic kidney disease in the Igbo people from south-eastern Nigeria. Nephron Clinical Practice. 2013;123(1-2):123-8.
24. Tayo BO, Kramer H, Salako BL, Gottesman O, McKenzie CA, Ogunniyi A, et al. Genetic variation in APOL1 and MYH9 genes is associated with chronic kidney disease among Nigerians. International urology and nephrology. 2013;45(2):485-94.
25. Stanifer JW, Jing B, Tolan S, Helmke N, Mukerjee R, Naicker S, et al. The epidemiology of chronic kidney disease in sub-Saharan Africa: a systematic review and meta-analysis. The Lancet Global Health. 2014;2(3):e174-e81.
26. 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. Journal of the American Society of Nephrology. 2011;22(11):2129-37.
27. Fine DM, Wasser WG, Estrella MM, Atta MG, Kuperman M, Shemer R, et al. APOL1 risk variants predict histopathology and progression to ESRD in HIV-related kidney disease. Journal of the American Society of Nephrology. 2012;23(2):343-50.
28. Atta MG, Estrella MM, Kuperman M, Foy MC, Fine DM, Racusen LC, et al. HIV-associated nephropathy patients with and without apolipoprotein L1 gene variants have similar clinical and pathological characteristics. Kidney Int. 2012;82(3):338-43.
29. Nabrdalik K, Gumprecht J, Adamczyk P, Górczyńska-Kosiorz S, Żywiec J, Grzeszczak W. Association of rs1800471 polymorphism of TGFB1 gene with chronic kidney disease occurrence and progression and hypertension appearance. Archives of medical science: AMS. 2013;9(2):230.
30. Mao S, Yan B, Zhang J. Association of transforming growth factor-β 1 polymorphisms with the risk of chronic kidney diseases. Renal failure. 2015;37(10):304-11.
31. Geard A, Pule GD, Chetcha Chemegni B, Ngo Bitoungui VJ, Kengne AP, Chimusa ER, et al. Clinical and genetic predictors of renal dysfunctions in sickle cell anaemia in Cameroon. British Journal of Haematology. 2017;178(4):629-39.
32. Dimala CA, Atashili J, Mbuagbaw JC, Wilfred A, Monekosso GL. Prevalence of hypertension in HIV/AIDS patients on highly active antiretroviral therapy (HAART) compared with HAART-naïve patients at the Limbe Regional Hospital, Cameroon. PloS one. 2016;11(2):e0148100.
33. Nguyen KA, Peer N, Mills EJ, Kengne AP. A meta-analysis of the metabolic syndrome prevalence in the global HIV-infected population. PloS one. 2016;11(3):e0150970.

CHAPTER 8: SYNTHESIS-CONCLUSION AND RECOMMENDATION

Approximately 36.7 million people live with HIV infection worldwide with about two-third of them living in sub-Saharan Africa (118, 119). Access to ARVs in sub-Saharan Africa is still below desired levels with about 64% of those in needs, receiving such treatment 120. However, there has been significant improvement in the life expectancy of HIV-positive individuals for those who have access to treatment increasing the likelihood of the development of NCDs. These NCDs (including hypertension, diabetes mellitus, obesity and dyslipidaemia) make the occurrence of CKD more likely, apart from the direct effect of HIV on renal tissues. If the ambitious 90-90-90 target (121) of diagnosing 90% of HIV infections, getting 90% of HIV patients on treatment and achieving viral suppression in 90% of those on treatment is successful or nearly so, then we should envisage an upsurge in the prevalence of NCDs in the HIV population. Increasingly, it appears that the overall success and long-term sustainability of the various HIV care programs soon will be dependent on the systems created now for dealing with NCDs in the HIV population. Akin to this will be the need to set up systems to identify and mitigate the effects of genetic predisposition to NCDs (including CKD) in the HIV population.

The spectrum of kidney disease in the HIV population ranges from AKI, through CKD to opportunistic infections of the kidney and infiltrative lesions of the kidney like lymphoma and Kaposi sarcoma (122). AKI could result from prerenal azotemia, acute tubular necrosis, ARV-associated acute kidney injury and acute tubulointerstitial nephropathy from immune reconstitution syndrome.

Other causes of AKI among HIV-positive patients include the HIV-immune complex kidney disease, thrombotic microangiopathies and bacterial urinary tract infection. Opportunistic infections of the kidneys including viral, fungal and mycobacterial infections also may cause significant morbidity in this patient group. CKD could occur from HIVAN, HIVICK, ARV-associated CKD, chronic tubulointerstitial nephropathy and crystal nephropathy. Ultimately, if not diagnosed early and appropriate treatment instituted, all forms of kidney disease in this population will result in CKD and possible progression to ESRD if the HIV-positive patient lives long enough.

The overarching aim of this work was to document the prevalence of CKD in the global adult HIV population and compare the prevalence across different WHO regions; determine the prevalence of CKD and other NCDs in the extreme southern Nigeria which has a high HIV prevalence; investigate the association between urinary TGF-β1 and prevalent CKD in the adult HIV population and investigate any association between *TGF-β1, APOL1 and HMOX1* polymorphisms with CKD in the HIV population. Using meta-analytic methods, the prevalence of CKD among HIV patients across the WHO regions were synthesized and comparisons made. In sub-Saharan Africa, the epicenter of the HIV pandemic, comparison was made across the African Union sub-regions in order to establish a geographical differentia in CKD-HIV prevalence. It was then necessary to determine the prevalence of CKD in the adult HIV population from which the genetic and urinary TGF-β1 samples will be obtained.

The overall prevalence of CKD in the global HIV population was 4.8-12.3% depending on the GFR estimating equation used [6.4% (95%CI 5.2–7.7%) with MDRD, 4.8% (95%CI 2.9–7.1%) with CKD-EPI and 12.3% (95%CI 8.4–16.7%) with Cockcroft–Gault]. Of all the WHO regions, the highest prevalence was found in sub-Saharan Africa while within Africa, the sub-region with the highest prevalence was West Africa. This finding may not be unconnected with the genetic epidemiology of *APOL1* which has the highest worldwide prevalence of *APOL1* genetic risk in West Africa and high occurrence in the Caribbean islands and among African Americans, both being of African ancestry with forefathers having been part of the transatlantic slave route (112, 123). This assertion is corroborated by a recent study (124) which followed up HIV-positive individuals of West African, East African and Caribbean origin living in the United Kingdom which showed significantly increased rate of progression to CKD in individuals of West African and Caribbean origin compared to those of East African origin. This finding has far-reaching consequences for ESRD patients and their relatives living in West Africa because the predominant source of kidneys in Nigeria for transplantation is from living-related donors. There may be a high risk of development of *APOL1*-associated FSGS in the related donor.

The wide disparity in CKD prevalence noted across the three GFR estimating equations suggests significant lack of concordance in the eGFR derived from existing serum creatinine-based equations in the black HIV population. This disparity has been noted by other studies among HIV-positive patients in Africa (125) and among African Americans (126) and is made more complicated by the choice of including (or not) the race factor in the equation.

An effort to determine the most accurate equation in this population was undertaken in Kenya using measured GFR from iohexol clearance (127) indicated the CKD-EPI equation (without the race factor) as the most accurate creatinine-based equation. This study however had certain limitations including a small sample size (99 individuals) and lack of generalizability because the participants were mostly in early CKD with a very small proportion with GFR less than 60ml/min/1.73m^2 and all patients were ARV-naïve. Therefore, this signals a need to develop new equations or modify existing serum creatinine-based equations for the black HIV population which will be accurate enough for the purposes of dosing of ARV (and other medications) and monitoring of renal function in the long-term.

In West Africa, the prevalence of CKD in the HIV population using the most conservative of the three studied equations, the CKD-EPI equation, was 9.2% (4.8-14.8%) from our summary estimates. We recorded a prevalence of 13.4% using the same equation in the population of Nigeria's Niger-Delta where the samples for this work was collected which is within 95% confidence interval of our summary estimate. This is a high prevalence, considering that prevalence was based on eGFR<60ml/min/1.73m^2 without taking into cognizance those with persistent proteinuria. This means that at least 455,600 of the 3.4 million HIV-positive patients in Nigeria (128) are in at least stage 3 CKD. This is a grim picture in a country with a dysfunctional health system where most patients pay out-of-pocket for treatment. It is therefore necessary to incorporate CKD screening and treatment programs into HIV/AIDS program for the purpose of early identification and appropriate management of CKD and its traditional risk factors (which have high prevalence in this patient group as documented in this work).

We documented a high prevalence of hypertension [26.7% (95% CI 25.5% to 28.0%)], diabetes mellitus [5.6% (95% CI 4.5% to 6.7%)], and dyslipidemia [29.1% (95% CI 26.1% to 32.1%)], among our HIV-positive patients especially considering the relatively low average age (34.3 ± 9.9 years).

The need for early identification of CKD in the HIV population led to the investigation of the utility of urinary TGF-β1 as a possible biomarker for CKD progression. We considered that for a biomarker to be useful in this clime it should have the following characteristics:

1. Have great discriminatory ability to detect CKD using one sample and not repeated samples as currently obtained with urine albumin-creatinine ratio. There is usually great tendency to have loss to follow up in NCDs clinics in resource-poor settings.
2. Be affordable for people living in resource-poor communities or at least cheap enough to be integrated into screening programs in HIV clinics.
3. Should not require expensive equipment for assay.
4. Assay should be reproducible.
5. Sample used for assay should be easy to collect, preferably by the patient.

Using urine samples will ensure easy collection of samples, but this has already been achieved with the widespread use of urine albumin-creatinine ratio for early detection of CKD. TGF-β1 ELISA assay is reproducible using standard techniques but the kits are not cheaper than urine albumin-creatinine ratio and ELISA equipment may be beyond the reach

of many laboratories in low income countries of Sub-Saharan Africa therefore potentially limiting its widespread use. Also, there is need to do proper cohort studies with follow up periods long enough to determine if high urinary TGF-β1 levels at initiation of care is a good predictor of progression to CKD in the HIV cohort. Our finding from this cross-sectional study showed higher urinary TGF-β1 levels in patients with early CKD with waning levels as CKD stage worsened. It is possible that urinary TGF-β1 will be at least, a component of a battery of biomarkers used for the non-invasive prediction of CKD progression in the HIV population in the near future. We also had theorized that polymorphisms of this cytokine gene may be involved in the pathogenesis of CKD in the HIV population similar to that documented in ESRD caused by diabetes and chronic glomerulonephritis (129).

This work is, to the best of our knowledge, the first investigation of *TGF-β1* SNPs and their association with CKD in Sub-Saharan Africa. There may be a protective association between rs1800470 polymorphisms and CKD in the HIV population even at the multivariable regression level. There is a possibility that certain *TGF-β1* SNPs may be protective against ESRD as suggested in this study. A study from India (130) had noted that polymorphisms of the "low TGF producing" SNPs was associated with ESRD in patients with a wide range of CKD aetiologies including glomerulonephritis, diabetes, reflux nephropathy, FSGS and Alport syndrome. It is worthy of note that TGF-beta, though believed to be profibrotic also has anti-inflammatory properties which may be associated with its CKD preventive effect. More detailed transcriptomic and proteomic studies may help decipher the processes involved in the CKD-protective effect of *TGF-β1.* On the other hand, *HMOX1* polymorphism did not

show any relationship with CKD in the HIV population compared to the borderline association seen in sickle cell patients with nephropathy. The minor allele frequency of the APOL1 SNPs continue to differ across regions of Africa, even in contiguous communities. While the *APOL1* SNPs do not occur in Ethiopia (101), some communities along the west coast of Africa may have almost half of the population carrying the *APOL1* genetic risk. In our study, we documented a high [44.0% for rs60910145 (*G1*) and 45.3% for rs73885319 *(G1)*] minor allele frequency. This was higher than that obtained among the Yoruba of Ibadan (131), Nigeria (27.7-40.6%). Our values were also higher than the recorded frequency among the Igbo in Enugu (132). The region where this study was undertaken is more southerly and nearer the Atlantic coast where the slave trade ports were located. This may be responsible for the equally high *APOL1* genetic risk among the population of the Caribbean and African Americans. We documented a 2.5-fold increased CKD odds among the HIV patients due to the presence of the *APOL1* risk but this was not as high as that recorded in South Africa (113) where an 89-fold increased odds was noted. It is worthy of note, however, that the South African study was done among biopsy-proven HIVAN patients unlike this study. Albeit, APOL1 remains an important factor in the pathogenesis of CKD in sub-Saharan Africa.

This work was not without limitations. The meta-analysis and cross-sectional studies used to document the prevalence of CKD in the global HIV population and the local population employed a definition of CKD that did not take into cognizance individuals with eGFR greater than 60ml/min/1.73m^2 who have persistent proteinuria. The inclusion of individuals with persistent proteinuria would have greatly increased the documented CKD prevalence in the HIV population and probably drawn more attention to the need for regular use of urine

albumin-creatinine ratio for detecting CKD in the HIV population, even before elevation of serum creatinine (reduction of creatinine-based eGFR). It is obvious that early detection of CKD is key to prevention of progression to CKD especially in SSA. Another limitation was the lack of properly validated serum creatinine-based eGFR equation for the HIV population in SSA. It is possible that there may have been some misclassification of CKD status in the component study that addressed the question of CKD prevalence in the local HIV population. We attempted to circumvent this problem by using three serum creatinine-based equations for the estimation of GFR. However, this gap in knowledge presents an opportunity to investigate the best GFR estimation equation for HIV patients in SSA which will help, not just for screening of CKD purposes but for medication dosing in the HIV population.

A major limitation of this work is the lack of histopathological data which would have helped decipher the type of HIV-related nephropathy present in the CKD-HIV group. This limitation arose because of the lack of facilities for proper histopathologic characterization of renal lesions in the country where this work was undertaken. The delineation of individuals with HIVAN would have made for more precise statistical analysis regarding the potential of urinary TGF-β1 as a biomarker for CKD diagnosis in HIVAN and HIVICK individuals. Again, while *APOL1* genetic risk lies at the heart of the pathogenesis of HIVAN, it is not known to play a role in the pathogenesis of HIVICK (100). If the proportion of our patients with HIVICK is relatively high, it may reduce the magnitude of the computed association between CKD occurrence and the *APOL1* genetic risk.

Recommendations & future work

Considering the foregoing, we wish to make the following recommendations:

1. It has become imperative to incorporate into HIV care programs, systems that are able to integrate HIV treatment with continuous screening and treatment of NCDs in an integrated care model that is sustainable in SSA.
2. Early and regular screening for CKD in the HIV population as suggested by available guidelines.
3. APOL1 screening in the HIV population may be instituted to help predict CKD risk if proven by well conducted studies to be cost effective.
4. We need to evolve the most dependable equation for estimating GFR in the HIV population of SSA.

Conclusion

There is a high CKD prevalence among HIV patients globally with the highest prevalence occurring in West Africa. The prevalence of NCDs is high in the relatively young HIV population of the Niger-Delta and may be the most important factor predicting CKD among HIV-infected adults in this region. The levels of urinary TGF-β1-creatinine ratio reduces as CKD becomes more advanced. Polymorphism of rs1800470 (*TGF-β1*) may be protective against CKD occurrence in the HIV population while there is no association between *HMOX1* and CKD in the HIV population. One of the highest minor allele frequencies for *APOL1* G1 and G2 occurs among the people of Nigeria's Niger-Delta.

There is an increased risk of CKD among HIV-positive adults with *APOL1* renal risk variants in Nigeria's Niger-Delta region which may be potentiated by the high prevalence of hypertension and other NCDs within the HIV population.

REFERENCES

1. Rao TK, Filippone EJ, Nicastri AD, Landesman SH, Frank E, Chen CK, et al. Associated focal and segmental glomerulosclerosis in the acquired immunodeficiency syndrome. N Engl J Med. 1984;310(11):669-73.
2. Gardenswartz MH, Lerner CW, Seligson GR, Zabetakis PM, Rotterdam H, Tapper ML, et al. Renal disease in patients with AIDS: a clinicopathologic study. Clin Nephrol. 1984;21(4):197-204.
3. Carbone L, D'Agati V, Cheng JT, Appel GB. Course and prognosis of human immunodeficiency virus-associated nephropathy. Am J Med. 1989;87(4):389-95.
4. Cantor KP, Weiss SH, Goedert JJ, Battjes RJ. HTLV-I/II seroprevalence and HIV/HTLV coinfection among U.S. intravenous drug users. J Acquir Immune Defic Syndr. 1991;4(5):460-7.
5. Bruggeman LA, Ross MD, Tanji N, Cara A, Dikman S, Gordon RE, et al. Renal epithelium is a previously unrecognized site of HIV-1 infection. J Am Soc Nephrol. 2000;11(11):2079-87.
6. Katz IT, Maughan-Brown B. Improved life expectancy of people living with HIV: who is left behind?. The lancet HIV. 2017 Aug 1;4(8):e324-6.
7. Han TM, Naicker S, Ramdial PK, Assounga AG. A cross-sectional study of HIV-seropositive patients with varying degrees of proteinuria in South Africa. Kidney Int. 2006;69(12):2243-50.
8. Szczech LA, Menezes P, Quinlivan EB, van der Horst C, Bartlett JA, Svetkey LP. Microalbuminuria predicts overt proteinuria among patients with HIV-infection: HIV Med. 2010 Aug;11(7):419-26. Epub 2010 Jan 4 doi:10.1111/j.1468-1293.2009.00805.x.
9. Musso CG, Belloso WH, Glassock RJ. Water, electrolytes, and acid-base alterations in human immunodeficiency virus infected patients: World J Nephrol. 2016 Jan 6;5(1):33-42. Epub 2016 Jan 6 doi:10.5527/wjn.v5.i1.33.
10. Meng X-M, Tang PM-K, Li J, Lan HY. TGF-β/Smad signaling in renal fibrosis. Frontiers in physiology. 2015;6:82.
11. Szczech LA, Gupta SK, Habash R, Guasch A, Kalayjian R, Appel R, et al. The clinical epidemiology and course of the spectrum of renal diseases associated with HIV infection. Kidney Int. 2004;66(3):1145-52.
12. Bruggeman LA, Bark C, Kalayjian RC. HIV and the Kidney: Curr Infect Dis Rep. 2009 Nov;11(6):479-85.
13. Berliner AR, Fine DM, Lucas GM, Rahman MH, Racusen LC, Scheel PJ, et al. Observations on a cohort of HIV-infected patients undergoing native renal biopsy. Am J Nephrol. 2008;28(3):478-86.
14. Mallipattu SK, Wyatt CM, He JC. The New Epidemiology of HIV-Related Kidney Disease: J AIDS Clin Res. 2012;Suppl 4:001-. Epub 2012 Jan 17 doi:10.4172/2155-6113.S4-001.
15. Rachakonda AK, Kimmel PL. CKD in HIV-infected patients other than HIV-associated nephropathy. Adv Chronic Kidney Dis. 2010;17(1):83-93.
16. Wyatt CM, Morgello S, Katz-Malamed R, Wei C, Klotman ME, Klotman PE, et al. The spectrum of kidney disease in patients with AIDS in the era of antiretroviral therapy. Kidney Int. 2009;75(4):428-34.
17. Mocroft A, Lundgren JD, Ross M, Fux CA, Reiss P, Moranne O, et al. Cumulative and current exposure to potentially nephrotoxic antiretrovirals and development of chronic kidney disease in HIV-positive individuals with a normal baseline estimated glomerular filtration rate: a prospective international cohort study. Lancet HIV. 2016;3(1):00211-8.
18. Poignet JL, Desassis JF, Chanton N, Litchinko MB, Zins B, Kolko A, et al. [Prevalence of HIV infection in dialysis patients: results of a national multicenter study]. Nephrologie. 1999;20(3):159-63.
19. Vigneau C, Guiard-Schmid JB, Tourret J, Flahault A, Rozenbaum W, Pialoux G, et al. The clinical characteristics of HIV-infected patients receiving dialysis in France between 1997 and 2002. Kidney Int. 2005;67(4):1509-14.

20. Barril G, Trullas JC, Gonzalez-Parra E, Moreno A, Bergada E, Jofre R, et al. [Prevalence of HIV-1-infection in dialysis units in Spain and potential candidates for renal transplantation: results of a Spanish survey]. Enferm Infecc Microbiol Clin. 2005;23(6):335-9.
21. Trullas JC, Barril G, Cofan F, Moreno A, Cases A, Fernandez-Lucas M, et al. Prevalence and clinical characteristics of HIV type 1-infected patients receiving dialysis in Spain: results of a Spanish survey in 2006: GESIDA 48/05 study. AIDS Res Hum Retroviruses. 2008;24(10):1229-35.
22. Halle MP, Takongue C, Kengne AP, Kaze FF, Ngu KB. Epidemiological profile of patients with end stage renal disease in a referral hospital in Cameroon. BMC Nephrol. 2015;16(59):015-0044.
23. Madala ND, Thusi GP, Assounga AG, Naicker S. Characteristics of South African patients presenting with kidney disease in rural KwaZulu-Natal: a cross sectional study. BMC Nephrol. 2014;15(61):1471-2369.
24. Gardner LI, Holmberg SD, Williamson JM, Szczech LA, Carpenter CC, Rompalo AM, et al. Development of proteinuria or elevated serum creatinine and mortality in HIV-infected women. J Acquir Immune Defic Syndr. 2003;32(2):203-9.
25. Choi AI, Rodriguez RA, Bacchetti P, Volberding PA, Havlir D, Bertenthal D, et al. Low rates of antiretroviral therapy among HIV-infected patients with chronic kidney disease. Clin Infect Dis. 2007;45(12):1633-9.
26. Wyatt CM, Winston JA, Malvestutto CD, Fishbein DA, Barash I, Cohen AJ, et al. Chronic kidney disease in HIV infection: an urban epidemic. Aids. 2007;21(15):2101-3.
27. Santiago P, Grinsztejn B, Friedman RK, Cunha CB, Coelho LE, Luz PM, et al. Screening for decreased glomerular filtration rate and associated risk factors in a cohort of HIV-infected patients in a middle-income country. PLoS One. 2014;9(4).
28. Mocroft A, Kirk O, Reiss P, De Wit S, Sedlacek D, Beniowski M, et al. Estimated glomerular filtration rate, chronic kidney disease and antiretroviral drug use in HIV-positive patients. Aids. 2010;24(11):1667-78.
29. Cheung CY, Wong KM, Lee MP, Liu YL, Kwok H, Chung R, et al. Prevalence of chronic kidney disease in Chinese HIV-infected patients. Nephrol Dial Transplant. 2007;22(11):3186-90.
30. Wools-Kaloustian K, Gupta SK, Muloma E, Owino-Ong'or W, Sidle J, Aubrey RW, et al. Renal disease in an antiretroviral-naive HIV-infected outpatient population in Western Kenya. Nephrol Dial Transplant. 2007;22(8):2208-12.
31. Adedeji TA, Adedeji NO, Adebisi SA, Idowu AA, Fawale MB, Jimoh KA. Prevalence and Pattern of Chronic Kidney Disease in Antiretroviral-Naive Patients with HIV/AIDS. J Int Assoc Provid AIDS Care. 2015;14(5):434-40.
32. Emem CP, Arogundade F, Sanusi A, Adelusola K, Wokoma F, Akinsola A. Renal disease in HIV-seropositive patients in Nigeria: an assessment of prevalence, clinical features and risk factors. Nephrology Dialysis Transplantation. 2008;23(2):741-6.
33. Okani CO, Ekrikpo UE, Okolo CA, Asinobi AO, Salako BL, Akang EEU. IS THE ART OF RENAL BIOPSY ON THE DECLINE IN NIGERIA? Annals of Ibadan Postgraduate Medicine. 2014;12(1):38-41.
34. Ayokunle DS, Olusegun OT, Ademola A, Adindu C, Olaitan RM, Oladimeji AA. Prevalence of chronic kidney disease in newly diagnosed patients with Human immunodeficiency virus in Ilorin, Nigeria. J Bras Nefrol. 2015;37(2):177-84.
35. Anyabolu EN, Chukwuonye, II, Arodiwe E, Ijoma CK, Ulasi I. Prevalence and predictors of chronic kidney disease in newly diagnosed human immunodeficiency virus patients in Owerri, Nigeria. Indian J Nephrol. 2016;26(1):10-5.
36. Medapalli RK, He JC, Klotman PE. HIV- Associated Nephropathy: Pathogenesis. Current opinion in nephrology and hypertension. 2011;20(3):306-11.
37. Bruggeman LA, Dikman S, Meng C, Quaggin SE, Coffman TM, Klotman PE. Nephropathy in human immunodeficiency virus-1 transgenic mice is due to renal transgene expression. Journal of Clinical Investigation. 1997;100(1):84-92.

38. Eitner F, Cui Y, Hudkins KL, Stokes MB, Segerer S, Mack M, et al. Chemokine receptor CCR5 and CXCR4 expression in HIV-associated kidney disease. J Am Soc Nephrol. 2000;11(5):856-67.
39. Ray PE, Liu XH, Henry D, Dye L, 3rd, Xu L, Orenstein JM, et al. Infection of human primary renal epithelial cells with HIV-1 from children with HIV-associated nephropathy. Kidney Int. 1998;53(5):1217-29.
40. Chen P, Chen BK, Mosoian A, Hays T, Ross MJ, Klotman PE, et al. Virological Synapses Allow HIV-1 Uptake and Gene Expression in Renal Tubular Epithelial Cells. Journal of the American Society of Nephrology : JASN. 2011;22(3):496-507.
41. Ross MJ, Fan C, Ross MD, Chu TH, Shi Y, Kaufman L, et al. HIV-1 infection initiates an inflammatory cascade in human renal tubular epithelial cells. J Acquir Immune Defic Syndr. 2006;42(1):1-11.
42. Bruggeman LA, Nelson PJ. Controversies in the pathogenesis of HIV-associated renal diseases. Nature reviews Nephrology. 2009;5(10):574-81.
43. Cohen SD, Kimmel PL. Immune complex renal disease and human immunodeficiency virus infection. Semin Nephrol. 2008;28(6):535-44.
44. Foy MC, Estrella MM, Lucas GM, Tahir F, Fine DM, Moore RD, et al. Comparison of risk factors and outcomes in HIV immune complex kidney disease and HIV-associated nephropathy. Clin J Am Soc Nephrol. 2013;8(9):1524-32.
45. Becker S, Fusco G, Fusco J, Balu R, Gangjee S, Brennan C, et al. HIV-associated thrombotic microangiopathy in the era of highly active antiretroviral therapy: an observational study. Clin Infect Dis. 2004;1(39):S267-75.
46. Ahmed S, Siddiqui R, Siddiqui A, Zaidi S, Cervia J. HIV associated thrombotic microangiopathy. Postgraduate Medical Journal. 2002;78(923):520-4.
47. Wikman P, Safont P, Del Palacio M, Moreno A, Moreno S, Casado JL. The significance of antiretroviral-associated acute kidney injury in a cohort of ambulatory human immunodeficiency virus-infected patients. Nephrol Dial Transplant. 2013;28(8):2073-81.
48. Wyatt CM. Antiretroviral therapy and the kidney. Top Antivir Med. 2014;22(3):655-8.
49. Roe J, Campbell LJ, Ibrahim F, Hendry BM, Post FA. HIV care and the incidence of acute renal failure. Clin Infect Dis. 2008;47(2):242-9.
50. Reid A, Stohr W, Walker AS, Williams IG, Kityo C, Hughes P, et al. Severe renal dysfunction and risk factors associated with renal impairment in HIV-infected adults in Africa initiating antiretroviral therapy. Clin Infect Dis. 2008;46(8):1271-81.
51. Le MP, Landman R, Koulla-Shiro S, Charpentier C, Sow PS, Diallo MB, et al. Tenofovir plasma concentrations related to estimated glomerular filtration rate changes in first-line regimens in African HIV-infected patients: ANRS 12115 DAYANA substudy. J Antimicrob Chemother. 2015;70(5):1517-21.
52. Scherzer R, Estrella M, Li Y, Choi AI, Deeks SG, Grunfeld C, et al. Association of tenofovir exposure with kidney disease risk in HIV infection. Aids. 2012;26(7):867-75.
53. Reilly RF, Tray K, Perazella MA. Indinavir nephropathy revisited: a pattern of insidious renal failure with identifiable risk factors. Am J Kidney Dis. 2001; 38(4): E23
54. Marinescu CI, Leyes M, Ribas MA, Peñaranda M, Murillas J, Campins AA, et al. Relationships between serum levels of atazanavir and renal toxicity or lithiasis. AIDS research and treatment. 2015;2015.
55. Zimmermann AE, Pizzoferrato T, Bedford J, Morris A, Hoffman R, Braden G. Tenofovir-associated acute and chronic kidney disease: a case of multiple drug interactions. Clin Infect Dis. 2006;42(2):283-90.
56. Jones R, Stebbing J, Nelson M, Moyle G, Bower M, Mandalia S, et al. Renal dysfunction with tenofovir disoproxil fumarate-containing highly active antiretroviral therapy regimens is not observed more frequently: a cohort and case-control study. J Acquir Immune Defic Syndr. 2004;37(4):1489-95.

57. Olyaei AJ, DeMattos AM, Bennett WM. Renal toxicity of protease inhibitors. Current opinion in nephrology and hypertension. 2000 Sep 1;9(5):473-6.
58. Achhra AC, Nugent M, Mocroft A, Ryom L, Wyatt CM. Chronic Kidney Disease and Antiretroviral Therapy in HIV-Positive Individuals: Recent Developments. Curr HIV/AIDS Rep. 2016;30:30.
59. Steiniche D, Jespersen S, Erikstrup C, Krarup H, Handberg A, Ostergaard L, et al. Diabetes mellitus and impaired fasting glucose in ART-naive patients with HIV-1, HIV-2 and HIV-1/2 dual infection in Guinea-Bissau: a cross-sectional study. Trans R Soc Trop Med Hyg. 2016;110(4):219-27.
60. Tzur F, Chowers M, Agmon-Levin N, Mekori YA, Hershko AY. Increased Prevalence of Diabetes Mellitus in a Non-Obese Adult Population: HIV-Infected Ethiopians. Isr Med Assoc J. 2015;17(10):620-3.
61. Hernandez-Vazquez LR, Martinez JH, Rivera-Anaya C, Laboy J, Miranda S, Mancilla P, et al. Prevalence of Diabetes Mellitus in Human Immunodeficency Virus positive Patients in Puerto Rico--San Juan City Hospital Experience. Bol Asoc Med P R. 2015;107(3):5-8.
62. Karamchand S, Leisegang R, Schomaker M, Maartens G, Walters L, Hislop M, et al. Risk Factors for Incident Diabetes in a Cohort Taking First-Line Nonnucleoside Reverse Transcriptase Inhibitor-Based Antiretroviral Therapy. Medicine. 2016;95(9):0000000000002844.
63. Nsagha DS, Assob JC, Njunda AL, Tanue EA, Kibu OD, Ayima CW, et al. Risk Factors of Cardiovascular Diseases in HIV/AIDS Patients on HAART. Open AIDS J. 2015;9:51-9.
64. Dimala CA, Atashili J, Mbuagbaw JC, Wilfred A, Monekosso GL. Prevalence of Hypertension in HIV/AIDS Patients on Highly Active Antiretroviral Therapy (HAART) Compared with HAART-Naive Patients at the Limbe Regional Hospital, Cameroon. PLoS One. 2016;11(2).
65. Muhammad S, Sani MU, Okeahialam BN. Cardiovascular disease risk factors among HIV-infected Nigerians receiving highly active antiretroviral therapy. Niger Med J. 2013;54(3):185-90.
66. Gazzaruso C, Bruno R, Garzaniti A, Giordanetti S, Fratino P, Sacchi P, et al. Hypertension among HIV patients: prevalence and relationships to insulin resistance and metabolic syndrome. J Hypertens. 2003;21(7):1377-82.
67. Mocroft A, Lundgren JD, Ross M, Law M, Reiss P, Kirk O, et al. Development and validation of a risk score for chronic kidney disease in HIV infection using prospective cohort data from the D:A:D study. PLoS Med. 2015;12(3).
68. van Deventer HE, George JA, Paiker JE, Becker PJ, Katz IJ. Estimating glomerular filtration rate in black South Africans by use of the modification of diet in renal disease and Cockcroft-Gault equations. Clin Chem. 2008;54(7):1197-202.
69. Mauss S, Berger F, Kuschak D, Henke J, Wolf E, Schauseil S. et al. Cystatin C as a marker of renal function is affected by HIV replication leading to an underestimation of kidney function in HIV patients/S. Mauss [at al.]. Antivir. Ther. 2008;13:1091-5
70. Wasung ME, Chawla LS, Madero M. Biomarkers of renal function, which and when? Clin Chim Acta. 2015;438:350-7.
71. Jotwani V, Scherzer R, Abraham A, Estrella MM, Bennett M, Cohen MH, et al. Association of urine alpha1-microglobulin with kidney function decline and mortality in HIV-infected women. Clin J Am Soc Nephrol. 2015;10(1):63-73.
72. Kang J, Liu J, Ding H, Li X, Wang Q, Guo X, et al. Urine alpha1-microglobulin is a better marker for early tubular dysfunction than beta2-microglobulin among tenofovir-exposed human immunodeficiency virus-infected men who have sex with men. Braz J Infect Dis. 2015;19(4):410-6.
73. Peralta C, Scherzer R, Grunfeld C, Abraham A, Tien P, Devarajan P, et al. Urinary biomarkers of kidney injury are associated with all-cause mortality in the Women's Interagency HIV Study (WIHS). HIV Med. 2014;15(5):291-300.

74. Shlipak MG, Scherzer R, Abraham A, Tien PC, Grunfeld C, Peralta CA, et al. Urinary markers of kidney injury and kidney function decline in HIV-infected women. J Acquir Immune Defic Syndr. 2012;61(5):565-73.
75. Scherzer R, Lin H, Abraham A, Thiessen-Philbrook H, Parikh CR, Bennett M, et al. Use of urine biomarker-derived clusters to predict the risk of chronic kidney disease and all-cause mortality in HIV-infected women. Nephrol Dial Transplant. 2016;10.
76. Lan HY. Diverse Roles of TGF-β/Smads in Renal Fibrosis and Inflammation. International Journal of Biological Sciences. 2011;7(7):1056-67.
77. Hirschberg R. Introduction An Oscar for TGF-β and Its Performance in the Kidney. Mineral and Electrolyte Metabolism. 1998;24(2-3):107-10.
78. Assoian RK, Komoriya A, Meyers CA, Miller DM, Sporn MB. Transforming growth factor-beta in human platelets. Identification of a major storage site, purification, and characterization. J Biol Chem. 1983;258(11):7155-60.
79. Blobe GC, Schiemann WP, Lodish HF. Role of Transforming Growth Factor β in Human Disease. New England Journal of Medicine. 2000;342(18):1350-8.
80. Wrana JL, Attisano L, Cárcamo J, Zentella A, Doody J, Laiho M, et al. TGFβ signals through a heteromeric protein kinase receptor complex. Cell. 1992;71(6):1003-14.
81. Meng XM, Tang PM, Li J, Lan HY. TGF-beta/Smad signaling in renal fibrosis. Front Physiol. 2015;6(82).
82. Tsapenko MV, Nwoko RE, Borland TM, Voskoboev NV, Pflueger A, Rule AD, et al. Measurement of urinary TGF-β1 in patients with diabetes mellitus and normal controls. Clinical Biochemistry. 2013;46(15):1430-5.
83. De Muro P, Faedda R, Fresu P, Masala A, Cigni A, Concas G, et al. Urinary transforming growth factor-β1 in various types of nephropathy. Pharmacological Research. 2004;49(3):293-8.
84. Bobkova IN, Chebotareva NV, Kozlovskaia LV, Varshavskii VA, Golitsyna EP. [Urine excretion of a monocytic chemotaxic protein-1 and a transforming growth factor beta1 as an indicator of chronic glomerulonephritis progression]. Ter Arkh. 2006;78(5):9-14.
85. Shankland SJ, Johnson RJ. TGF-β in Glomerular Disease. Mineral and Electrolyte Metabolism. 1998;24(2-3):168-73.
86. Haramaki R, Tamaki K, Fujisawa M, Ikedo H, Haramaki N, Okuda S. Steroid therapy and urinary transforming growth factor-beta1 in IgA nephropathy. Am J Kidney Dis. 2001;38(6):1191-8.
87. Goumenos DS, Tsakas S, El Nahas AM, Alexandri S, Oldroyd S, Kalliakmani P, et al. Transforming growth factor-beta(1) in the kidney and urine of patients with glomerular disease and proteinuria. Nephrol Dial Transplant. 2002;17(12):2145-52.
88. Park HC, Xu ZG, Choi S, Goo YS, Kang SW, Choi KH, et al. Effect of losartan and amlodipine on proteinuria and transforming growth factor-beta1 in patients with IgA nephropathy. Nephrol Dial Transplant. 2003;18(6):1115-21.
89. Mitchell BI, Byron MM, Ng RC, Chow DC, Ndhlovu LC, Shikuma CM. Elevation of Non-Classical (CD14+/lowCD16++) Monocytes Is Associated with Increased Albuminuria and Urine TGF-beta1 in HIV-Infected Individuals on Stable Antiretroviral Therapy. PLoS One. 2016;11(4).
90. August P, Sharma V, Ding R, Schwartz JE, Suthanthiran M. Transforming growth factor beta and excess burden of renal disease. Trans Am Clin Climatol Assoc. 2009;120:61-72.
91. Suthanthiran M, Khanna A, Cukran D, Adhikarla R, Sharma VK, Singh T, et al. Transforming growth factor-beta 1 hyperexpression in African American end-stage renal disease patients. Kidney Int. 1998;53(3):639-44.
92. Suthanthiran M, Li B, Song JO, Ding R, Sharma VK, Schwartz JE, et al. Transforming growth factor-beta 1 hyperexpression in African-American hypertensives: A novel mediator of hypertension and/or target organ damage. Proc Natl Acad Sci U S A. 2000;97(7):3479-84.
93. Tsakas S, Goumenos DS. Accurate measurement and clinical significance of urinary transforming growth factor-beta1. American journal of nephrology. 2006;26(2):186-93.

94. Khalil MS, El Nahas AM, Blakemore AI. Transforming growth factor-β1 SNPs: genetic and phenotypic correlations in progressive kidney insufficiency. Nephron Experimental nephrology. 2005;101(2):e31-41.
95. Akai Y, Sato H, Ozaki H, Iwano M, Dohi Y, Kanauchi M. Association of transforming growth factor-β1 T29C polymorphism with the progression of diabetic nephropathy. American Journal of Kidney Diseases. 2001;38(4, Supplement):S182-S5.
96. Buraczynska M, Baranowicz-Gaszczyk I, Borowicz E, Ksiazek A. TGF-β1 and TSC-22 Gene Polymorphisms and Susceptibility to Microvascular Complications in Type 2 Diabetes. Nephron Physiology. 2007;106(4):p69-p75.
97. Babel N, Gabdrakhmanova L, Hammer MH, Schoenemann C, Skrypnikov V, Poliak N, et al. Predictive value of cytokine gene polymorphisms for the development of end-stage renal disease. J Nephrol. 2006;19(6):802-7.
98. Valladares-Salgado A, Angeles-MartÍNez J, Rosas M, GarcÍA-Mena J, Utrera-Barillas D, GÓMez-DÍAz R, et al. Association of polymorphisms within the transforming growth factor-β1 gene with diabetic nephropathy and serum cholesterol and triglyceride concentrations. Nephrology. 2010;15(6):644-8.
99. Prasad P, Tiwari AK, Kumar KP, Ammini A, Gupta A, Gupta R, et al. Association of TGFβ1, TNFα, CCR2 and CCR5 gene polymorphisms in type-2 diabetes and renal insufficiency among Asian Indians. BMC Medical Genetics. 2007;8(1):1-6.
100. Limou S, Nelson GW, Kopp JB, Winkler CA. APOL1 kidney risk alleles: population genetics and disease associations. Adv Chronic Kidney Dis. 2014;21(5):426-33.
101. Behar DM, Kedem E, Rosset S, Haileselassie Y, Tzur S, Kra-Oz Z, et al. Absence of APOL1 risk variants protects against HIV-associated nephropathy in the Ethiopian population. Am J Nephrol. 2011;34(5):452-9.
102. Atta MG, Estrella MM, Kuperman M, Foy MC, Fine DM, Racusen LC, et al. HIV-associated nephropathy patients with and without apolipoprotein L1 gene variants have similar clinical and pathological characteristics. Kidney Int. 2012;82(3):338-43.
103. Tenhunen R, Marver HS, Schmid R. The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase. Proceedings of the National Academy of Sciences. 1968;61(2):748-55.
104. Saraf SL, Zhang X, Shah B, Kanias T, Gudehithlu KP, Kittles R, et al. Genetic variants and cell-free hemoglobin processing in sickle cell nephropathy. Haematologica. 2015;100(10):1275-84.
105. Bean CJ, Boulet SL, Ellingsen D, Pyle ME, Barron-Casella EA, Casella JF, et al. Heme oxygenase-1 gene promoter polymorphism is associated with reduced incidence of acute chest syndrome among children with sickle cell disease. Blood. 2012;120(18):3822-8.
106. Ashley-Koch AE, Okocha EC, Garrett ME, Soldano K, De Castro LM, Jonassaint JC, et al. MYH9 and APOL1 are both associated with sickle cell disease nephropathy. British journal of haematology. 2011;155(3):386-94.
107. Schaefer BA, Flanagan JM, Alvarez OA, Nelson SC, Aygun B, Nottage KA, et al. Genetic Modifiers of White Blood Cell Count, Albuminuria and Glomerular Filtration Rate in Children with Sickle Cell Anemia. PLoS One. 2016;11(10):e0164364.
108. Geard A, Pule GD, Chetcha Chemegni B, Ngo Bitoungui VJ, Kengne AP, Chimusa ER, et al. Clinical and genetic predictors of renal dysfunctions in sickle cell anaemia in Cameroon. 2017;178(4):629-39.
109. Divers J, Palmer ND, Lu L, Langefeld CD, Rocco MV, Hicks PJ, et al. Gene-gene interactions in APOL1-associated nephropathy. Nephrol Dial Transplant. 2014;29(3):587-94.
110. Bostrom MA, Kao WH, Li M, Abboud HE, Adler SG, Iyengar SK, et al. Genetic association and gene-gene interaction analyses in African American dialysis patients with nondiabetic nephropathy. Am J Kidney Dis. 2012;59(2):210-21.

111. Langefeld CD, Comeau ME, Ng MCY, Guan M, Dimitrov L, Mudgal P, et al. Genome-wide association studies suggest that APOL1-environment interactions more likely trigger kidney disease in African Americans with nondiabetic nephropathy than strong APOL1-second gene interactions. Kidney Int. 2018;94(3):599-607.
112. 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. 2011:ASN. 2011040388.
113. 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. 2015;26(11):2882-90.
114. Freedman BI, Skorecki K. Gene–gene and gene–environment interactions in apolipoprotein L1 gene-associated nephropathy. Clinical Journal of the American Society of Nephrology. 2014;9(11):2006-13.
115. Thomas B. Akwa Ibom 2016 [cited 2016 10th May]. Available from: http://www.citypopulation.de/php/nigeria-admin.php?adm1id=NGA003.
116. Akpan EE, Ekrikpo UE. Changing aetiologies of End Stage Kidney Disease in a Resource Poor Environment: What is the way forward? World Journal of Biomedical Research. 2014;1(2):1 - 5.
117. Saraf SL, Zhang X, Shah B, Kanias T, Gudehithlu KP, Kittles R, et al. Genetic variants and cell-free hemoglobin processing in sickle cell nephropathy. Haematologica. 2015;100(10):1275-84.
118. Wang H, Wolock TM, Carter A, Nguyen G, Kyu HH, Gakidou E, et al. Estimates of global, regional, and national incidence, prevalence, and mortality of HIV, 1980–2015: the Global Burden of Disease Study 2015. 2016;3(8):e361-e87.
119. Bärnighausen TJCOiH, AIDS. The HIV treatment cascade and antiretroviral impact in different populations. 2015;10(6):391-4.
120. WORLD HEALTH ORGANISATION. Antiretroviral coverage among all age groups [online]. Geneva. Available from https://www.who.int/gho/hiv/epidemic_response/ART_text/en/
116. Bain LE, Nkoke C, Noubiap JJ. UNAIDS 90–90–90 targets to end the AIDS epidemic by 2020 are not realistic: comment on “Can the UNAIDS 90–90–90 target be achieved? A systematic analysis of national HIV treatment cascades”. BMJ global health. 2017 Mar 1;2(2):e000227.
117. Cohen SD, Kopp JB, Kimmel PL. Kidney diseases associated with human immunodeficiency virus infection. New England Journal of Medicine. 2017;377(24):2363-74.
118. Rotimi CN, Tekola-Ayele F, Baker JL, Shriner D. The African diaspora: history, adaptation and health. Current Opinion in Genetics & Development. 2016;41:77-84.
119. Jose S, Hamzah L, Jones R, Williams D, Winston A, Burns F, et al. Chronic Kidney Disease Risk in African and Caribbean Populations With HIV. 2018;218(11):1767-72.
120. Stohr W, Walker AS, Munderi P, Tugume S, Gilks CF, Darbyshire JH, et al. Estimating glomerular filtration rate in HIV-infected adults in Africa: comparison of Cockcroft-Gault and Modification of Diet in Renal Disease formulae. 2008;13(6):761-70.
121. Anker N, Scherzer R, Peralta C, Powe N, Banjeree T, Shlipak MJE, et al. Racial disparities in creatinine-based kidney function estimates among HIV-infected adults. 2016;26(2):213.
122. Wyatt CM, Schwartz GJ, Ong'or WO, Abuya J, Abraham AG, Mboku C, et al. Estimating kidney function in HIV-infected adults in Kenya: comparison to a direct measure of glomerular filtration rate by iohexol clearance. 2013;8(8):e69601.
123. UNAIDS. Country fact sheet: Nigeria: UNAIDS; 2018 [Available from: http://www.unaids.org/en/regionscountries/countries/nigeria.
124. Babel N, Gabdrakhmanova L, Hammer MH, Schoenemann C, Skrypnikov V, Poliak N, et al. Predictive value of cytokine gene polymorphisms for the development of end-stage renal disease. 2006;19(6):802-7.
125. Mittal RD, Manchanda PKJD, biology c. Is Low-Frequency Distribution of TGF-β Genotype Associated with Increased Risk for End-Stage Renal Disease? 2007;26(3):172-7.

126. Tayo BO, Kramer H, Salako BL, Gottesman O, McKenzie CA, Ogunniyi A, et al. Genetic variation in APOL1 and MYH9 genes is associated with chronic kidney disease among Nigerians. 2013;45(2):485-94.
127. Ulasi II, Tzur S, Wasser WG, Shemer R, Kruzel E, Feigin E, et al. High population frequencies of APOL1 risk variants are associated with increased prevalence of non-diabetic chronic kidney disease in the Igbo people from south-eastern Nigeria. 2013;123(1-2):123-8.

APPENDIX 1: ETHICAL APPROVAL CERTIFICATE FROM THE UNIVERSITY OF UYO TEACHING HOSPITAL INSTITUTIONAL HEALTH RESEARCH ETHICS COMMITTEE

UNIVERSITY OF UYO TEACHING HOSPITAL
P.M.B. 1136, UYO, AKWA IBOM STATE
e-mail: info@uuthuyo.net

Prof. Etete J. Peters, FWACP, FCCP, Cert Pulm (Tel Aviv)
CHIEF MEDICAL DIRECTOR

Dr. Patience A. Udo, MBBS, FWACP, FPNP
CHAIRMAN, MEDICAL ADVISORY COMMITTEE

Mr. Thompson T. Ikpe, B.Sc., M.Sc., MNIM, ACIPM, AHAN, Fcna
DIRECTOR OF ADMINISTRATION

UUTH/AD/S/96/VOL.XIX/15
Our Ref:

Your Ref:

AUGUST 19, 2016
Date:

UNIVERSITY OF UYO TEACHING HOSPITAL, UYO INSTITUTIONAL HEALTH RESEARCH ETHICAL COMMITTEE (IHREC)

APPROVAL CERTIFICATE LETTER

Principal Investigator: DR. UDEME EKPENYONG EKRIKPO

Protocol Title: **"CHRONIC KIDNEY DISEASE IN HIV POPULATIONS IN SOUTH-SOUTH NIGERIA: PREVALENCE, RISK FACTORS AND ROLE OF TRANSFORMING GROWTH FACTOR BETA (TGF-B) POLYMORPHISMS".**

STATUS

The University of Uyo Teaching Hospital, Uyo Institutional Review Committee has reviewed your protocol title: **"Chronic Kidney Disease in HIV Populations in South-South Nigeria: Prevalence, Risk Factors and Role of Transforming Growth Factor Beta (TGF-B) Polymorphisms"**

The research protocol described above has been approved by the University of Uyo Teaching Hospital, Uyo Institutional Health Research Ethical Committee (IHREC) as indicated.

Signature Removed

J. E. Inyang
Secretary, IHREC
UUTH, Uyo.

APPENDIX 2: ETHICAL APPROVAL CERTIFICATE FROM THE UNIVERSITY OF CAPE TOWN

HUMAN RESEARCH ETHICS COMMITTEE

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

04 October 2016

HREC REF: 733/2016

A/Prof I Okpechi
Division of Nephrology
C-17
NGSH

Dear A/Prof Okpechi

PROJECT TITLE: CHRONIC KIDNEY DISEASE IN HIV POPULATIONS IN SOUTH-SOUTH NIGERIA: PREVALENCE, RISK FACTORS AND ROLE OF TRANSFORMING GROWTH FACTOR BETA (TGF-B) POLYMORPHISMS (PhD-candidate-Dr U Ekrikpo)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 October 2017.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

We acknowledge that the student, Dr U Ekrikpo will also be involved in this study.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval before the research may occur.

Yours sincerely

Signature Removed

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

HREC 733/2016

Appendix 3: Letter of award of the postgraduate publication Incentive (PPI award) of the Department of Medicine, UCT

Deputy Dean: Research
Faculty of Health Sciences

Professor Ambroise Wonkam

Private Bag X3, Rondebosch, 7701, South Africa
Barnard Fuller Building, Anzio Road, Observatory, Cape Town
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10 December 2018

Prof Ikechi Okpechi
Division of Medicine

Dear Prof Okpechi

Faculty Research Committee (FRC) Awards: Postgraduate Publication Incentive

I have pleasure in informing you that the Faculty Research Committee has awarded your student Udeme Ekrikpo (student number: EKRUDE001) an amount of **R10 000** to complete research papers resulting from their postgraduate work.

This amount will be transferred to your internal research fund and will be released once the Faculty Research Office has received proof that your student has submitted their thesis. Please submit such proof (obtainable from the Postgraduate Office) to Jacqui.steadman@uct.ac.za

You are reminded of the following:

- Six months after the funds have been released; the supervisor should submit a report with proof that the paper/s have been submitted an *ISI-accredited* journal.
- Please submit to only *ISI-accredited* journals (please see a list of accredited journals at http://www.researchsupport.uct.ac.za/publication-count-resources
- The student should acknowledge the source of funding in any conference presentation or publication emanating from this research.
- In addition, please be sure to state your affiliation with the University on any publication (journal articles, book(s)/chapters and conference proceedings. This will assist us to claim the corresponding research output component of the University's state subsidy.

Best wishes for your students' continued growth as a researcher.

Yours sincerely

Signature Removed

Ambroise Wonkam
Deputy Dean: Research
Faculty of Health Sciences
University of Cape Town

Cc: Mr Salie Nassiep
Research Management Accountant

Appendix 4: Published manuscript from Chapter 6

KI REPORTS
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CLINICAL RESEARCH

Urinary Transforming Growth Factor-Beta 1 (uTGF-β1) and Prevalent CKD Risk in HIV-Positive Patients in West Africa

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Introduction: This study investigated the association of urinary transforming growth factor-β1 (uTGF-β1) with prevalent chronic kidney disease (CKD) in the HIV-infected population.

Methods: HIV-positive patients without CKD (HIV^+CKD^-, $n = 194$) and 114 with CKD (HIV^+CKD^+) who did not have hypertension, diabetes mellitus, or hepatitis B or C, had their urinary protein-creatinine ratio (uPCR), serum transforming growth factor (TGF)–β1, and uTGF-β1 measured. uTGF-β1-creatinine ratios (uTGF-β1Cr) were calculated. Spearman correlation was used to determine the association between uTGF-β1Cr and various attributes, and the Cuzick trend test was used to assess the presence of a linear trend in median uTGF-β1Cr levels across the stages of CKD. Multivariable robust linear regression models were used to assess independent association with variability in uTGF-β1Cr and estimated glomerular filtration rate (eGFR) levels.

Results: The age of the participants was 38.3 ± 10.3 years with 73.4% women. The median uTGF-β1Cr was higher among HIV^+CKD^+ (4.85 ng/mmol [25th–75th percentile 1.96–12.35] vs. 2.95 [1.02–5.84]; $P = 0.001$]). There was significant correlation between uTGF-β1Cr and age ($P = 0.02$), eGFR ($P = 0.001$), and uPCR ($P < 0.001$) in the HIV^+CKD^+ group. Among the HIV^+CKD^+ patients, there was gradual reduction in the median level of uTGF-β1Cr with CKD severity ($P = 0.04$). HIV^+CKD^+ patients had significantly higher levels of uTGF-β1Cr after controlling for potential confounders. Using eGFR as dependent variable, proteinuria explained the changes associated with uTGF-β1Cr levels.

Conclusion: HIV^+CKD^+ patients express higher levels of uTGF-β1 especially in the early stages of CKD apparently related to proteinuria levels.

Kidney Int Rep (2019) **4**, 1698–1704; https://doi.org/10.1016/j.ekir.2019.07.011
KEYWORDS: CKD; HIV; Nigeria; urinary TGF-β1

The early diagnosis and treatment of CKD presents a definite opportunity for reducing the incidence of end-stage renal disease. This is especially important in regions of high HIV prevalence, which, unfortunately, tend to have dysfunctional health care systems. TGF-β1 is a 112-amino acid, 25-KDa ubiquitous protein believed to play a central role in renal fibrosis,[1] both as a proliferative, and in some conditions, anti-proliferative factor.[2]

In diabetic nephropathy, overexpression of TGF-β1 has been found to occur in the kidneys, and *in vivo* studies have documented increased expression of TGF-β1 in proximal tubular cells and mesangial cells cultured in high glucose concentrations.[3] Also, in glomerulosclerosis, TGF-β1 has been observed to be centrally involved in extracellular matrix expansion.[4] Indeed, renal disease associated with expansion of extracellular matrix (diabetic nephropathy, lupus nephritis, focal and segmental glomerulonephritis, and IgA nephropathy)

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have all been associated with increased expression of TGF-β1, whereas renal conditions that do not have extracellular matrix accumulation (thin basement membrane disease, minimal change disease) seem not to have increased expression of TGF-β1.[5] The former may be the case with HIV-related nephropathy in which expansion of the extracellular matrix occurs. Studies have shown that the administration of anti-TGF-β1 substances attenuate extracellular matrix production[6,7] and may lead to retardation of CKD progression in renal diseases associated with increased extracellular matrix production.

Some studies in patients with CKD who are HIV-positive have demonstrated elevated levels of TGF-β1 in renal tissue,[8] serum,[9] and in the urine[10] of patients with HIV-associated nephropathy. Our study aim was to investigate the association of uTGF-β1 with prevalent CKD in HIV-positive patients in Nigeria.

METHODS

Study Participants

This was a cross-sectional study involving 194 HIV-positive patients without CKD (HIV^+CKD^-) and 114 HIV-positive patients with CKD (HIV^+CKD^+) seen at the HIV clinic and the renal unit of the University of Uyo Teaching Hospital, Nigeria (Ethical review number: UUTH/AD/S/96/VOL.XIX/15). The facility's HIV clinic offers care to approximately 50 ambulant HIV-positive patients daily with approximately 3500 patients on active enrollment. Screening for renal disease is not routinely done because patients pay out-of-pocket. Ambulant HIV-positive patients on antiretroviral (ARV) treatment at the HIV clinic who gave informed consent were recruited into the study. CKD was defined as eGFR of <60 ml/min per 1.73 m^2 persisting in 2 measurements at least 3 months apart and/or urine protein-creatinine ratio (uPCR) of $\geq$ 0.05 g/mmol creatinine.[11] The patients' sociodemographic (age, sex, ethnicity) and clinical characteristics (e.g., body mass index and blood pressures) were collected using standard techniques. Data regarding the patients' CD4 count at initiation of care and current CD4 count levels, viral load at initiation of care, and current levels and ARV regimen also were collected. All patients were on ARVs. The ARV regimen used in this program included zidovudine-based regimens (AZT-3TC-EFV or AZT/3TC/NVP), tenofovir-based regimen (TDF-3TC-EFV or TDF-FTC-EFV), abacavir-based regimen (ABC-3TC-EFV), and less frequently, protease-inhibitor regimen. Patients with hypertension, diabetes mellitus, or hepatitis B or C coinfection were excluded from the study.

Spot urine samples for uTGF-β1 assay were collected, processed, and stored using the manufacturer's instructions. All samples were centrifuged with the supernatant stored in 2 aliquots at $-20\,^{\circ}C$ within 2 hours of collection. Blood samples (4 ml) were collected, serum separated, and stored in 2 aliquots (for serum creatinine and TGF-β1 assay). Serum and urine creatinine were measured with RANDOX creatinine kits (RANDOX, Crumlin, UK) using the modified Jaffe reaction. Serum and urinary TGF-β1 were measured using Biovision (San Francisco, CA) TGF-β1 (human) enzyme-linked immunosorbent assay kit (catalog #K4342–100) in duplicates. The intra-assay and inter-assay coefficient of variation was 8.6% and 7.0%, respectively. The sensitivity of the assay was <1 pg/ml. To ameliorate the effect of varying urine concentrations, uTGF-β1Cr ratio (pg/mmol) was derived by dividing urinary TGF-β1 (in pg/l) by urinary creatinine (in mmol/l) and later converted to ng/mmol. We estimated the glomerular filtration rate using the 4-variable Modification of Diet in Renal Disease[12] and the CKD-Epidemiology Collaboration[13] equations. Participants' kidney function was staged using the Kidney Disease Outcome Quality Initiative classification.[11]

All data analysis was performed with Stata 15.1 (StataCorp, College Station, TX). Numerical variables were reported as means ($\pm$SD) or median (25th–75th percentile). Categorical variables are reported as frequencies (percentages). Comparison of quantitative variables was performed using the Student t test (or its nonparametric equivalent, the Mann-Whitney U test). Comparison of categorical variables was performed using the χ^2 test. The Spearman rank correlation test was used to investigate the continuous association between uTGF-β1Cr and certain demographic and clinical variables (age, serum creatinine, serum TGF-β1, uPCR, and CD4 count) and a formal comparison of the correlation coefficients undertaken using the Stata module for comparison of correlation coefficients based on the Steiger test.[14] The Cuzick trend test was used to assess the presence of a linear trend in median uTGF-β1Cr levels across the stages (1–5) of CKD. Univariable and multivariable robust linear regression models were built to determine factors independently associated with variability in uTGF-β1Cr and eGFR levels. The Akaike information criteria was used to determine the best multivariable model that explains the variability in uTGF-β1Cr levels. P value less than 0.05 was deemed statistically significant.

RESULTS

Demographic and Clinical Characteristics

A total of 194 HIV^+CKD^- and 114 HIV^+CKD^+ were recruited into the study. The mean age of the participants was 38.3 $\pm$ 10.3 years with statistically significant difference between the groups ($P = 0.02$). The sex

distribution showed a female preponderance (73% vs. 27%). Table 1 summarizes the demographic and clinical characteristics of the study participants by CKD status. The level of serum TGF-β1 and urinary TGF-β1 was higher among the HIV^+CKD^+ patients than in HIV^+CKD^- patients, although this difference did not achieve statistical significance. However, when urinary TGF-β1 level was standardized by using the uTGF-β1Cr ratio, those with HIV^+CKD^+ had significantly higher levels (Table 1).

Factors Associated With uTGF-β1Cr

Age and uPCR had significant correlation with uTGF-β1Cr, whereas no significant correlation was found with mean arterial blood pressure, body mass index, CD4 count, and serum TGF-β1 for the total study population (Table 2). However, in the HIV^+CKD^+ group there was significant correlation with age, serum creatinine, uPCR, and eGFR. There was significant positive correlation between uPCR and uTGF-β1Cr (rho = 0.32; $P < 0.001$). A significantly higher correlation was found between uTGF-β1Cr and eGFR in the HIV^+CKD^+ group than the HIV^+CKD^- group. For the HIV^+CKD^+ group, median uTGF-β1Cr significantly decreased with severity of CKD (Figure 1). There was no correlation between serum TGF-β1 and eGFR (rho = −0.04; $P = 0.52$) or between serum TGF-β1 and serum creatinine (rho = 0.09; $P = 0.19$).

Univariable and Multivariable Linear Regression Models

After adjusting for the effect of age at the time of data collection, sex differences, mean arterial blood pressure, waist circumference, eGFR, ARV regimen, and CD4 count at enrollment, patients with HIV^+CKD^+ persistently had higher levels of uTGF-β1Cr compared with HIV^+CKD^- (Table 3). The magnitude of the effect of uPCR on uTGF-β1Cr levels was noted to be much higher than that of eGFR. The type of ARV regimen administered, differences in sex, body mass index, CD4 count, and serum TGF-β1 were not significantly associated with uTGF-β1Cr at the multivariable level

Table 1. Demographic and clinical characteristics

Variables	Total ($N = 308$)	HIV^+CKD^+ ($n = 114$)	HIV^+CKD^- ($n = 194$)	P
Age (yr)	38.3 ± 10.3	39.9 ± 10.4	37.1 ± 10.1	0.02
Female sex, *n* (%)	226 (73.4)	85 (74.6)	141 (72.7)	0.72
Duration on ARV (25th–75th percentile)	5 (2–8)	5 (2–8)	5 (2–8)	0.27
Systolic blood pressure (mm Hg)	129.0 ± 26.3	137.0 ± 26.9	124.5 ± 24.8	<0.001
Diastolic blood pressure (mm Hg)	77.7 ± 14.5	80.8 ± 14.5	75.2 ± 14.1	0.001
Mean arterial pressure (mm Hg)	91.2 ± 13.9	93.0 ± 13.5	90.1 ± 14.1	0.07
Weight (kg)	61.4 ± 11.9	59.1 ± 11.5	62.7 ± 12.0	0.01
Height (m)	1.63 ± 0.09	1.63 ± 0.08	1.63 ± 1.0	0.38
Body mass index (kg/m^2)	23.2 ± 4.4	22.5 ± 4.4	23.6 ± 4.4	0.03
Waist circumference (cm)	80.1 ± 9.5	79.3 ± 9.2	81.3 ± 9.6	0.07
Serum creatinine (μmol/l)	85.3 (67.6–108.8)	110.8 (90–144)	74 (61–88)	<0.001
$eGFR_{CKD\text{-}EPI}$ (ml/min per 1.73 m^2)	80.3 (58.6–105.1)	56.1 (41.9–75.1)	97.6 (78.3–113.3)	<0.001
$eGFR_{MDRD}$ (ml/min per 1.73 m^2)	74.7 (55.8–97.9)	53.9 (39.9–69.5)	90.2 (72.5–111.2)	<0.001
uPCR (g/mmol creatinine)	0.04 (0.02–0.09)	0.11 (0.03–0.20)	0.03 (0.02–0.04)	<0.001
CD4 count at enrollment (cells/μl)	207 (80–380)	226 (69–384.5)	197 (89–372)	0.84
Current CD4 count (cells/μl)	499 (308–693)	472.5 (274–675)	500 (329–714)	0.32
Current Log viral load (copies/ml)	2.9 ± 1.5	2.8 ± 1.4	3.0 ± 1.6	0.49
CKD stage, *n* (%)				
1		18 (15.8)		
2		30 (26.3)		
3		48 (42.1)		
4		8 (7.0)		
5		10 (8.8)		
Serum TGF-β1 (ng/l)	13.7 (3.7–42.5)	20.1 (3.7–47.8)	13.0 (3.7–37.1)	0.22
uTGF-β1 (ng/l)	32.5 (19.2–46.6)	33.2 (21.4–45.5)	32.5 (18.2–47.9)	0.64
uTGF-β1Cr (ng/mmol)	3.7 (1.7–7.5)	4.8 (2.0–11.5)	2.9 (1.2–5.7)	<0.001
Antiretroviral regimens, *n* (%)				
• Zidovudine-based	205 (66.5)	83 (72.8)	122 (62.9)	
• Tenofovir-based	88 (28.6)	26 (22.8)	62 (31.9)	0.09
• Abacavir-based	11 (3.6)	4 (3.5)	7 (3.6)	
• Protease-inhibitor-based	4 (1.3)	1 (0.9)	3 (1.6)	

CKD, chronic kidney disease; $eGFR_{CKD\text{-}EPI}$, estimated glomerular filtration rate using CKD-EPI equation; $eGFR_{MDRD}$, estimated glomerular filtration rate using the 4-variable Modification of Diet in Renal Disease formula; HIV^+CKD^+, HIV-positive patients with CKD; HIV^+CKD^-, HIV-positive patients without CKD; TDF, tenofovir disoproxil fumarate; TGF- β1, transforming growth factor-beta1; uPCR, urine protein-creatinine ratio; uTGF-β1, urinary transforming growth factor-beta 1; uTGF-β1Cr, urinary transforming growth factor-beta-1–Creatinine ratio.

Table 2. Correlations of uTGF-β1Cr with demographic and biochemical parameters

	HIV^+CKD^+ group (n = 114)		HIV^+CKD^- group (n = 194)			Total (N = 308)	
	Rho	P	Rho	P	P-for difference	Rho	P
Age (yr)	0.20	0.02	0.09	0.22	0.35	0.19	0.001
Serum creatinine (μmol/l)	−0.35	<0.001	−0.01	0.85	0.003	0.01	0.98
Serum TGF-β1 (pg/ml)	−0.17	0.11	0.03	0.75	0.09	−0.03	0.66
mABP (mm Hg)	0.04	0.62	0.04	0.64	0.99	0.07	0.22
BMI (kg/m^2)	0.02	0.85	−0.14	0.07	0.18	−0.11	0.06
Waist circumference (cm)	−0.03	0.73	−0.07	0.32	0.74	−0.06	0.26
$eGFR_{MDRD}$ (ml/min per 1.73 m^2)	0.35	<0.001	0.03	0.70	0.01	0.003	0.95
$eGFR_{CKD\text{-}EPI}$ (ml/min per 1.73 m^2)	0.35	<0.001	−0.005	0.99	0.003	−0.02	0.81
CD4 count (cells/μl)	−0.05	0.68	−0.15	0.13	0.40	−0.09	0.20
uPCR (g/mmol)	0.31	<0.001	0.14	0.05	0.13	0.32	<0.001

BMI, body mass index; eGFR, estimated glomerular filtration rate; HIV^+CKD^+, HIV-positive patients with CKD; HIV^+CKD^-, HIV-positive patients without CKD; mABP, mean arterial blood pressure; TGF-β1, transforming growth factor-beta-1; uPCR, urine protein-creatinine ratio.

(Table 3). Older age was significantly associated with higher uTGF-β1Cr levels. A little more than a quarter (88 [28.6%]) of the sample population were on tenofovir disoproxil fumarate. Sensitivity analysis done (without patients using tenofovir disoproxil fumarate) did not show any significant qualitative difference. All independent associations seen in the initial model were maintained.

Table 4 shows univariable and multivariable robust linear regression models with $eGFR_{CKD\text{-}EPI}$ as dependent variable. The relationship between uTGF-β1Cr and renal function seen in previous analysis appears to be dependent on proteinuria levels. In both multivariable models, serum TGF-β was not independently associated with uTGF-β1Cr or $eGFR_{CKD\text{-}EPI}$ (Tables 3 and 4).

DISCUSSION

This study found significantly higher levels of uTGF-β1Cr in HIV^+CKD^+ patients compared with HIV^+CKD^- patients even after controlling for other potential confounders. This difference also occurred despite both groups having similar levels of serum TGF-β1. There was significant positive correlation between uTGF-β1Cr and uPCR. Older age and eGFR were also independently associated with differences in uTGF-β1Cr levels. Among the HIV^+CKD^+ individuals, the levels of uTGF-β1Cr progressively decreased across CKD stages 1 to 5. Ultimately, uTGF-β1Cr levels were fully dependent on uPCR levels.

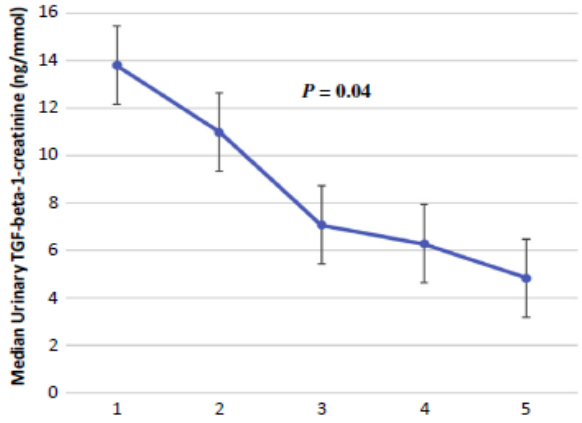

Figure 1. Median urinary transforming growth factor (TGF) levels across chronic kidney disease stages.

TGF-β1 is known to induce renal fibrosis through multiple pathways, including direct action on fibroblasts and other cells that cause extracellular matrix synthesis; inhibition of antifibrotic pathways, and induction of cell loss through apoptosis.[15] In the "canonical" pathway of TGF-β1–induced renal fibrosis, the binding of TGF-β1 to its twin transmembrane receptors leads to the activation (phosphorylation) of smad2 and smad3, which is then translocated to the nucleus with the help of smad4 protein. The activation of smad2 and smad3 is usually associated with the inhibition of smad7, which is known to have antifibrotic activity. The binding of smad3 to gene promoters leads to transcription of profibrotic molecules. This leads to increased laying down of extracellular matrix in the kidneys and subsequent fibrosis.[15] In the kidneys, TGF-β1 is expressed on the renal tubular epithelial cells and glomerular basement membrane[16] and also in the myofibroblasts occurring in the interstitium during chronic kidney injury.[5] The increased expression of TGF-β1 in persistent renal injury[17] manifests as increased urinary excretion of TGF-β1.[18] This has been documented in renal diseases associated with increase in extracellular matrix production,[6,7] including HIV-related nephropathy.[10] The finding of increased urinary excretion of TGF-β1 in patients with HIV and CKD was corroborated by our study. Others also have found a positive correlation between urinary albumin excretion and uTGF-β1,[19] which was also corroborated by our study. Indeed, we observed a stronger effect of

Table 3. Linear regression models for uTGF-β1Cr prediction

	Univariable β (95% CI) *P* value	Multivariable β (95% CI) *P* value
Study groups		
HIV^+CKD^-	1	1
HIV^+CKD^+	2.66 (1.85–3.47) <0.001	3.97 (2.49–5.45) <0.001
Age (yr)	0.04 (0.004–0.08) 0.03	0.07 (0.02–0.12) 0.01
Sex		
Female	1	1
Male	−0.98 (−1.13 to 0.94) 0.85	−0.51 (−1.78 to 0.76) 0.43
mABP (mm Hg)	0.04 (0.01–0.06) 0.01	0.01 (−0.02 to 0.05) 0.44
BMI (kg/m^2)	−0.13 (−0.23 to −0.03) 0.02	0.03 (−0.19 to 0.25) 0.83
Waist circumference (cm)	−0.03 (−0.74 to 0.02) 0.32	−0.05 (−0.15 to 0.06) 0.39
ARV regimen		
Non–TDF-based	1	1
TDF-based	−0.76 (−1.81 to 0.29) 0.15	−2.57 (−6.07 to 0.93) 0.15
CD4 count (cells/μl)	−0.01 (−0.01 to 0.006) 0.81	−0.0006 (−0.007 to 0.006) 0.91
eGFR (CKD-EPI), ml/min per 1.73 m^2	−0.10 (−0.02 to 0.01) 0.24	0.03 (0.01–0.05) 0.01
uPCR (g/mmol)	12.10 (10.58–13.62) <0.001	6.62 (2.04–11.20) 0.01
Serum TGF-β1 (pg/ml)	0.002 (−0.001 to 0.002) 0.99	0.0001 (−0.001 to 0.003) 0.93

ARV, antiretrovirals; BMI, body mass index; CI, confidence interval; CKD, chronic kidney disease; eGFR (CKD-EPI), estimated glomerular filtration rate using the chronic kidney disease epidemiology collaboration equation; HIV^+CKD^+, HIV-positive patients with CKD; HIV^+CKD^-, HIV-positive patients without CKD; mABP, mean arterial blood pressure; TDF, tenofovir disoproxil fumarate; TGF-β1, transforming growth factor-beta-1; uPCR, urine protein-creatinine ratio.

uPCR (compared with eGFR) on uTGF-β1Cr levels in this study. This is probably due to the activation of proximal tubular cells by persistent proteinuria leading to increased production of TGF-β1.[20] In the multivariable model with eGFR as the dependent variable, uPCR was shown to clearly explain the changes associated with uTGF-β1Cr.

We also noted a stepwise reduction in urinary TGF-β1 levels in individuals with more severe CKD (stages 4 and 5). In advanced CKD, where tubular atrophy and severe tubulointerstitial fibrosis has set in, there are reduced numbers of myofibroblasts and functional proximal tubular epithelial cells (and thus TGF-β expression) in the tubulointerstitium, which may lead to reduced excretion of urinary TGF-β1. This explanation suggests a type of "burn-out" of TGF-β1 activity as CKD progresses. Some investigators have reported lower interstitial density measurements for TGF-β1 in advanced CKD from patients with HIV-associated nephropathy and diabetic nephropathy compared with HIV-positive and HIV-negative controls.[21] Similarly, some studies have shown a reduction in circulating TGF-β1 and TGF-β1 mRNA in patients with end-stage kidney disease.[22–24] A 12-month follow-up study[25] among patients with CKD with a wide range of etiology (not including HIV), found significantly higher levels of tubulointerstitial TGF-β1 mRNA in patients with renal disease who did not have progressive CKD compared with those who had progressive CKD. This suggested a protective effect of TGF-β1, but a 12-month follow-up period may not be adequate to assess long-term renal outcomes, and serial TGF-β mRNA measures were not available in this study. Studies with contrary findings[26,27] (higher TGF-β1 immunohistochemical and mRNA expression in the renal tubulo-interstitium with increased fibrosis) were

Table 4. Linear regression models for eGFR prediction

	Univariate β (95% CI) *P* value	Multivariate β (95% CI) *P* value
Age (yr)	−0.87 (−1.16 to −0.57) <0.001	−0.86 (−1.15 to −0.56) <0.001
Sex		
Female	1	1
Male	−0.58 (−8.28 to 7.13) 0.88	4.00 (−3.41 to 11.41) 0.29
uPCR (g/mmol creatinine)	−24.27 (−36.18 to −12.36) <0.001	−29.57 (−42.68 to −16.46) <0.001
uTGF-β1Cr (ng/mmol)	−0.12 (−0.36 to 0.11) 0.31	0.18 (−0.09 to 0.44) 0.19
Serum TGF-β1 (pg/ml)	−0.001 (−0.01 to 0.01) 0.89	−0.001 (−0.01 to 0.01) 0.91
BMI (kg/m^2)	0.24 (−0.54 to 1.02) 0.55	0.09 (−0.64 to 0.82) 0.81
CD4 count (cells/μl)	−0.01 (−0.03 to 0.01) 0.24	−0.01 (−0.03 to 0.01) 0.28
ARV regimen		
Non-TDF regimen	1	1
TDF regimen	8.88 (1.57–16.19) 0.02	6.10 (−1.22 to 13.42) 0.10

ARV, antiretrovirals; BMI, body mass index; CI, confidence interval; eGFR, estimated glomerular filtration rate; TDF, tenofovir disoproxil fumarate; TGF-β1, transforming growth factor-beta1; uPCR, urine protein-creatinine ratio; uTGF-β1Cr, transforming growth factor-beta-1–creatinine.

noted to have been done in patients with stages 1 to 3 CKD and not very advanced disease. Indeed, one study[26] used only patients with serum creatinine less than 2.0 mg/dl (approximately 177 μmol/l), understandably because of the risk involved in the biopsy of fibrotic kidneys. It appears that serial measurements of urinary TGF-β1 in progressive $HIV^{+}CKD^{+}$ patients, being a noninvasive procedure, may more succinctly document the temporal profile of uTGF-β1 in progressive CKD.

The markedly elevated uTGF-β1Cr found in patients in stages 1 and 2 CKD present a window of opportunity to reverse or slow down progression if early diagnosis is made and available intervention, like angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, are used. Studies have shown reduction in uTGFβ1 levels attributable to administration of angiotensin-converting enzyme inhibitors[28] or prednisolone[29] in IgA nephropathy. The actions of these medications may not be unconnected with the fact that persistent proteinuria is known to activate complements in the renal tubular epithelial cells leading to a cascade of events that eventuate in renal fibrosis.[30,31] Reducing proteinuria using angiotensin receptor blockers or angiotensin-converting enzyme inhibitors may indirectly reduce TGF-β1 profibrotic activity in the kidneys and slow down CKD progression; however, it is yet to be shown, in a properly conducted clinical trial, that this will be the case among patients with HIV with early CKD. The type of ARV regimen used did not appear to have any effect on variability in uTGF-β1Cr levels in this study. This includes tenofovir use, which has been known to be associated with proximal tubular dysfunction. We are not aware of any direct link between tenofovir-related renal fibrosis and TGF-β1 levels. It may also have been more informative to compare uTGF-β1Cr levels between ARV-naïve and ARV-exposed patients. Unfortunately, all patients in this study had been commenced on ARV.

A major limitation of this study is the lack of renal histopathology among the patients with CKD. This could have determined if the degree of fibrosis (glomerular, tubular, and interstitial) correlated with uTGFB1 in the CKD population. Another limitation of our study is the cross-sectional design as this design does not allow for serial assessment of this marker as a measure of progressive deterioration of kidney function. Therefore, it is difficult to ascertain from this work if uTGF-β1Cr increase predates the occurrence of persistent proteinuria. Again, because the levels of uTGF-β1Cr appear to wane with advanced CKD, it is possible to miss cases of advanced CKD if this test is used as a stand-alone. Also, the lack of HIV-negative controls limited comparison of uTGF-β1Cr levels in the patients with HIV and the general population. Despite the limitations, the result of our study is an important addition to the current body of literature and suggests that uTGF-β1Cr is an important marker of CKD in patients who are HIV-positive. Whether this biomarker can be useful for monitoring kidney response to treatment still needs to be studied.

CONCLUSION

Patients with HIV and CKD express higher levels of TGF-β1 activity in urine, especially in the early stages of CKD, explained by proteinuria levels. Persistent proteinuria remains a veritable tool for early CKD detection in the HIV population.

DISCLOSURE

All the authors declared no competing interests.

REFERENCES

1. Sporn MB, Roberts AB, Wakefield LM, de Crombrugghe B. Some recent advances in the chemistry and biology of transforming growth factor-beta. *J Cell Biol.* 1987;105:1039–1045.
2. Lan HY. Diverse roles of TGF-beta/Smads in renal fibrosis and inflammation. *Int J Biol Sci.* 2011;7:1056–1067.
3. Sharma K, Ziyadeh FN. Hyperglycemia and diabetic kidney disease. The case for transforming growth factor-beta as a key mediator. *Diabetes.* 1995;44:1139–1146.
4. Isaka Y, Fujiwara Y, Ueda N, et al. Glomerulosclerosis induced by in vivo transfection of transforming growth factor-beta or platelet-derived growth factor gene into the rat kidney. *J Clin Invest.* 1993;92:2597–2601.
5. Yamamoto T, Noble NA, Cohen AH, et al. Expression of transforming growth factor-beta isoforms in human glomerular diseases. *Kidney Int.* 1996;49:461–469.
6. Border WA, Okuda S, Languino LR, et al. Suppression of experimental glomerulonephritis by antiserum against transforming growth factor β1. *Nature.* 1990;346:371.
7. Border WA, Noble NA, Yamamoto T, et al. Natural inhibitor of transforming growth factor-beta protects against scarring in experimental kidney disease. *Nature.* 1992;360:361–364.
8. Yamamoto T, Noble NA, Miller DE, et al. Increased levels of transforming growth factor-beta in HIV-associated nephropathy. *Kidney Int.* 1999;55:579–592.
9. Yushchuk ND, Gadzhikulieva MM, Balmasova IP, et al. [The role of immune factors in the progression of chronic kidney diseases in HIV infection]. *Terapevticheskii arkhiv.* 2016;88:56–61.
10. Mitchell BI, Byron MM, Ng RC, et al. Elevation of non-classical (CD14+/lowCD16++) monocytes is associated with increased albuminuria and urine TGF-beta1 in HIV-infected individuals on stable antiretroviral therapy. *PLoS One.* 2016;11:e0153758.
11. Levey AS, Coresh J, Bolton K, et al. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis.* 2002;39(2 Suppl. 1):S1–S266.

12. Levey AS, Bosch JP, Lewis JB, et al. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Ann Intern Med*. 1999;130:461–470.
13. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150:604–612.
14. Caci H. *CORTESTI: Stata Module to Test Equality of Two Correlation Coefficients. Statistical Software Components S407302*. Boston, MA: Boston College Department of Economics; 2000.
15. Meng X-M, Nikolic-Paterson DJ, Lan HY. TGF-β: the master regulator of fibrosis. *Nat Rev Nephrol*. 2016;12:325.
16. Yoshioka K, Takemura T, Murakami K, et al. Transforming growth factor-beta protein and mRNA in glomeruli in normal and diseased human kidneys. *Lab Invest*. 1993;68:154–163.
17. Bruijn JA, Roos AJ. Transforming growth factor-beta and the glomerular extracellular matrix in renal pathology. *J Lab Clin Med*. 1994;123:34–47.
18. Tsakas S, Goumenos DS. Accurate measurement and clinical significance of urinary transforming growth factor-beta1. *Am J Nephrol*. 2006;26:186–193.
19. Honkanen E, Teppo A-M, Törnroth T, et al. Urinary transforming growth factor-beta 1 in membranous glomerulonephritis. *Nephrol Dial Transplant*. 1997;12:2562–2568.
20. Goumenos DS, Tsakas S, El Nahas AM, et al. Transforming growth factor-β1 in the kidney and urine of patients with glomerular disease and proteinuria. *Nephrol Dial Transplant*. 2002;17:2145–2152.
21. Nel M, Buys J-M, Botha F, et al. The functionality of African-specific variants in the TGFB1 regulatory region and their potential role in HIVAN. *Clin Exp Nephrol*. 2018;22:764–772.
22. Füth R, Herder C, Förster S, et al. Evaluation of diagnostic relevance of mRNA levels in peripheral blood: predictive value for mortality in hemodialysis patients. *Cytokine*. 2004;27:166–172.
23. Knerr K, Füth R, Hemsen P, et al. Chronic inflammation and hemodialysis reduce immune competence of peripheral blood leukocytes in end-stage renal failure patients. *Cytokine*. 2005;30:132–138.
24. Kumar A, Gupta V, Chaudhary M, et al. Depleted TGF-β1 levels in end stage renal disease patients from North India. *Gene*. 2014;534:440–443.
25. Eikmans M, Baelde HJ, Hagen EC, et al. Renal mRNA levels as prognostic tools in kidney diseases. *J Am Soc Nephrol*. 2003;14:899–907.
26. Iwano M, Akai Y, Fujii Y, et al. Intraglomerular expression of transforming growth factor-beta 1 (TGF-β1) mRNA in patients with glomerulonephritis: quantitative analysis by competitive polymerase chain reaction. *Clin Exp Immunol*. 1994;97:309–314.
27. Bódi I, Kimmel PL, Abraham AA, et al. Renal TGF-β in HIV-associated kidney diseases. *Kidney Int*. 1997;51:1568–1577.
28. Park HC, Xu ZG, Choi S, et al. Effect of losartan and amlodipine on proteinuria and transforming growth factor-β1 in patients with IgA nephropathy. *Nephrol Dial Transplant*. 2003;18:1115–1121.
29. Haramaki R, Tamaki K, Fujisawa M, et al. Steroid therapy and urinary transforming growth factor-β1 in IgA nephropathy. *Am J Kidney Dis*. 2001;38:1191–1198.
30. Sheerin N, Sacks SJ. Leaked protein and interstitial damage in the kidney: is complement the missing link? *Clin Exp Immunol*. 2002;130:1.
31. Rangan GK, Pippin JW, Coombes JD, et al. C5b-9 does not mediate chronic tubulointerstitial disease in the absence of proteinuria. *Kidney Int*. 2005;67:492–503.

Appendix 5: Letter of acceptance of manuscript from Chapter 7

AJKD Editorial Office <em@editorialmanager.com> Sat, Jan 25, 1:02 PM (3 days ago)

to me

CC: "Ikechi Okpechi" ikechi.okpechi@uct.ac.za

Jan 25, 2020

Dear Dr Ekrikpo,

Thank you for providing a revised version of "Association of Genetic Polymorphisms of TGF-B1, HMOX1 and APOL1 with chronic kidney disease in Nigerian Patients with and without HIV" to *AJKD*. We are pleased to inform you that we are satisfied that the concerns raised in the letter requesting revision have been appropriately addressed, and we have accepted this Original Investigation for publication.

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