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Prevalence, histological patterns and genetic variations of chronic kidney disease among HIV-infected patients in Kano, Nigeria

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Prevalence, histological patterns and genetic variations of chronic kidney disease among HIV-infected patients in Kano, Nigeria

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A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Internal Medicine

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Declaration

I, Aliyu Abdu, declare that this research is my original work. It is hereby submitted, in fulfilment for the award of degree of Doctor of Philosophy in the Faculty of Health Sciences at the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination at this or any other Institution.



Aliyu Abdu

October 2021

Dedication

This work is dedicated to all HIV-infected patients with kidney disease.

Research Outcomes

Publications

1. Abdu A, Akinfewa A, Bala SM, Ademola B, Nalado A, Obiagwu P, Duarte R, Naicker S. Histopathological pattern of kidney diseases among HIV-infected treatment-naïve patients in Kano, Nigeria. International Journal of Nephrology and Renovascular Disease 2021;14: 143–148.
2. Abdu A, Duarte R, Dickens C, Dix-Peek T, Bala SM, Ademola B, Naicker S. High risk *APOL1* genotypes and kidney disease among treatment naïve HIV positive patients at Kano, Nigeria. PlosOne. 2021. Under review.
3. Prevalence, risk factors and outcomes of CKD among treatment naïve HIV infected patients at Kano Nigeria. Draft manuscript.

Conference Presentations

1. Abdu A, Bala SM, Atanda A, Duarte R, Dickens C, Dix-Peek T, Naicker S. *APOL1* susceptibility gene variants among treatment naive HIV positive patients and its association with kidney disease at Kano Nigeria. Oral Presentation at the African Association of Nephrology Conference at Yaoundé, Cameroon. 14- 18 March 2017. African Journal of Nephrology 2017; 20(1):86
2. Abdu A, Bala SM, Atanda A, Ademola B, Mosiane P, Duarte R, Naicker S. Histopathological pattern of renal diseases among HIV-infected treatment naïve patients at Kano, Nigeria. Presented at The South African Renal Congress 2018, Johannesburg 11- 14 October 2018. African Journal of Nephrology 2018; 21(1): 86
3. Abdu A, Bala SM, Babatunde A, Dix-Peek T, Dickens C, Akinfenwa A, Duarte R, Naicker S. High risk *APOL1* genotypes and kidney disease among HIV positive patients at Kano, Nigeria. Presentation at the ISN World Congress of Nephrology 26 - 29 March 2020. Kidney International Reports March 2020; 5(3):S174

Abstract

The burden of Human Immunodeficiency Virus (HIV) infection is highest in sub-Saharan Africa. Increase in numbers of patients with HIV infection coupled with their improved long-term survival has led to increasing prevalence of HIV-associated organ dysfunction, including kidney diseases. Comorbidity of HIV with kidney disease has been associated with faster progression to AIDS and increased mortality. Early detection allows for targeted therapy to delay or prevent development of end stage kidney disease (ESKD). Proteinuria, reduced glomerular filtration rate (GFR), or both, have been utilized to define kidney disease in these patients. Definitive diagnosis by kidney biopsy, when indicated, is critical to optimal management. A wide variety of histopathological lesions have been reported among HIV-infected patients, with the variation depending on many factors such as geographical and ethnic diversity of the studied population. Kidney diseases such as HIV-associated nephropathy (HIVAN) are commonly known to occur among HIV-infected populations. This lesion has a higher prevalence among individuals of African descent compared to Caucasians and has been associated with rapid progression to ESKD. The possibility of genetic factors contributing to the development of kidney disease among people of African ancestry was supported by findings that variants in the gene located on chromosome 22 which encode for *Apolipoprotein L1* (*APOL1*) have strong association with increased risk of various forms of kidney diseases.Despite the strong association between certain histopathological lesions, such as HIVAN, and African ancestry as described among African-Americans, there is paucity of literature detailing the spectrum of histopathologic lesions among HIV-infected patients with kidney disease in West Africa. Not many studies were reported on the *APOL1* variants and kidney diseases among native Africans. The present study conducted in Northern Nigeria explored the prevalence and outcomes of chronic kidney disease (CKD) in a relatively large cohort of antiretroviral therapy (ART)-naive HIV-infected patients,

evaluated the histopathologic patterns among those that had biopsy and determined the frequencies of *APOL1* genotypes among them in comparison to those with no evidence of kidney disease.

Methods

This cross-sectional study was conducted at the dedicated HIV clinic of Aminu Kano Teaching Hospital (AKTH), which is one of the approved United State President's Emergency Plan for AIDS Relief (PEPFAR) centres in Nigeria. Following ethical approval, consecutive treatment naïve HIV patients were evaluated clinically and screened for the presence of CKD defined as persistent proteinuria and/ or reduced eGFR confirmed after three months, and were followed up every month for the first 3 months and subsequently every 3 months for 12 months. Those with CKD and no contraindication to kidney biopsy and had given consent to participate, had a kidney biopsy following established unit protocols. DNA samples obtained from the whole blood of 142 participants were genotyped for *APOL1* G1 and G2 variants after initial baseline investigations including assessment of kidney function. Participants comprised 50 HIV positive patients with no evidence of kidney disease, 52 HIV negative individuals with no kidney disease and 40 HIV positive patients with CKD.

Results

Nine hundred patients were studied; 63% were females with mean age of 34.17±10 years and mean CD4 count of 238 ±210 cells/ml. CKD prevalence was 22.8% using the CKD-EPI equation (without the race factor) with the majority 146 (71.21%) having stage 3 CKD. Multivariable analysis identified age, CD4 count <200 cells/mL, body mass index (BMI) and a history of use of traditional medications as being independently associated with CKD. Fourteen patients with stage 5 CKD were followed up at the Nephrology Clinic for the first year. Among the 14 patients

with stage 5 CKD, eight of them were dialysis-requiring within the first 6 months of follow up. Two were lost to follow up and the remaining 4 died of other complications within the follow up period. Among those with CKD, 53 had kidney biopsy and the commonest histological type was focal segmental glomerulosclerosis (FSGS) present in 20 patients (37.7%), followed by HIVAN in 17 (32.1 %) patients, and chronic interstitial nephritis in 7 patients (13.2%) among others. Compared to non-HIVAN patients, HIVAN patients tended to have higher serum creatinine levels (p= 0.005); lower eGFR (p= 0.030) and higher urine protein to creatinine ratio (uPCR; p= 0.020).

The prevalence of the individual risk alleles was 26.1% for G1 and 19.0% for G2. The frequency of the High Risk Genotype (HRG) was 12.5% among those with CKD, compared to 5.8% in the HIV negative group. Having the HRG was associated with a higher odds for developing HIVAN (2 vs 0 risk alleles: OR 10.83, 95% CI 1.38 - 84.52; p= 0.023; 2 vs 0 or 1 risk alleles: OR 5.5, 95% CI 0.83 - 36.29; p= 0.07). The HRG was also associated with higher odds for FSGS (2 vs 0 risk alleles: OR 13.0, 95% CI 2.06 - 81.91; p=0.006 and 2 vs 0 or 1 risk alleles: OR 9.0, 95%CI 1.62 - 50.12; p= 0.01) when compared to the control group.

Conclusions

There was a high prevalence of CKD and its risk factors in this cohort of HIV- infected patients, which has implications for overall organisation of HIV care in this environment. It calls for the incorporation of screening for CKD and its risk factors, and implementation of strategies targeted at early detection, and institution of therapies to reduce and slow progression of CKD to ESKD. Kidney involvement is a common form of presentation among HIV-infected treatment-naïve patients, with FSGS and HIVAN being the most common histologic types especially among

those carrying two copies of the HRG. There was high frequency of the high risk *APOL1* genes among HIV-positive CKD patients compared to those without CKD.

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List of Abbreviations

ACEI Angiotensin converting enzyme inhibitor

AIDS Acquired Immune Deficiency Syndrome

A1M Alpha-1 microglobulin

AKI Acute kidney injury

ARB Angiotensin receptor blocker

B2-M Beta-2 microglobulin

BMI body mass index

APOL1 Apolipoprotein L-1 gene

APOL1 Apolipoprotein L-1 protein

ART Antiretroviral therapy

CCR5 Chemokine co-receptor type 5

CD4 Cluster of differentiation 4

CD8 Cluster of differentiation 8

CG Cockcroft–Gault equation

CKD Chronic kidney disease

CKD-EPI Chronic Kidney Disease Epidemiology Collaboration

CRP C-reactive protein

CXCR4 Chemokine co-receptor type 4

DNA Deoxyribonucleic acid

EDTA Ethylenediaminetetraacetic acid

eGFR Estimated glomerular filtration rate

EM electron Microscopy

ESKD End stage kidney disease

FBS Fasting blood sugar

FSGS Focal segmental glomerulosclerosis

GBM Glomerular basement membrane

GFR Glomerular filtration rate

GN Glomerulonephritis

GWAS Genome wide association study

HAT Human African Trypanosomiasis

HDL3 High density lipoprotein class 3

H&E Hematoxylin and Eosin

HERS HIV Epidemiology Research Study

HGP Human Genome Project

HIV Human Immunodeficiency Virus

HIVAN HIV associated nephropathy

HIVICK HIV immune complex kidney disease

HRG High-risk genotype

HWE Hardy Weinburg equilibrium

IDMS Isotope dilution mass spectrometry

IF Immunofluorescence

INDELs Insertion and deletions

IDH Infectious Disease Hospital

IRIS Immune Reconstitution Inflammatory Syndrome

KIM-1 Kidney injury molecule-1

LDL Low density lipoprotein

L-FABP Liver-type fatty acid binding protein

MPGN Membranoproliferative glomerulonephritis

MDRD Modification of Diet in Renal Disease

MTA Material Transfer Agreement*MYH9* Non-muscle myosin heavy chain 9 gene

Myosin IIA Non-muscle myosin heavy chain IIA protein

NF-kβ Nuclear factor kappa-light-chain-enhancer of activated B cells

NGAL Neutrophil gelatinase-associated lipocalin

NNRTIs Non-nucleoside reverse transcriptase inhibitors

NRTIs Nucleoside reverse transcriptase inhibitors

NSAID Nonsteroidal anti-inflammatory drug

PAS Periodic Acid Schiff

PCR Polymerase chain reaction

PEPFAR United State President's Emergency Plan for AIDS Relief

PLA2R M-type phospholipase A2 receptor.

PIGN Post-infectious glomerulonephritis

RFLP Restriction Fragment Length Polymorphism

RNA Ribonucleic acid

RT Reverse transcriptase

RTECs Renal tubular epithelial cells

SIV Simian immunodeficiency virus

SNPs Single nucleotide polymorphisms

SSA Sub-Saharan Africa

Tat Trans-activator of transcription

TMA Thrombotic microangiopathy

uPCR Urine protein: creatinine ratio

USRDS US Renal Data System

WHO World Health Organization

WIHS The Women's Interagency HIV Study

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CHAPTER ONE. INTRODUCTION AND LITERATURE REVIEW

1.1 HIV Infection

The acquired immunodeficiency syndrome (AIDS) was first recognized among homosexual men in the United States in 1981.[1] Since then it has spread widely to become a major health problem with a diverse mode of transmission.

The infection is caused by a human retrovirus called the Human Immunodeficiency Virus (HIV). Molecular phylogenetic studies indicated that HIV evolved from a lentivirus, simian immunodeficiency virus (SIV), of the *Lentivirus* genus and *Retroviridae* family, which was reported in some subspecies of monkeys from Bioko (an island off the East African coast) and some subspecies of chimpanzees in the Cameroon.[2, 3]

Two types of HIV were described based on their zoonotic origin; the HIV-type 1 (HIV-1) strain, isolated from chimpanzees, is the most predominant type worldwide and HIV-type 2 (HIV-2) from the sooty Mangabey (Cercocebus atys), mostly reported in Western and Central Africa as well as Southern and Western India with some sporadic occurrences noted globally. HIV-2 results in a less virulent infection than HIV-1, with reported lower viral loads, lower rates of vertical transmission and slower disease progression.[4, 5]

HIV-1 consists of four distinct viral groups described as M, N, and O groups transmitted from chimpanzees and group P from a gorilla. Group M ("Main") HIV is considered the pandemic strain and is divided into subtypes also called clades, denoted with letters, and sub-subtypes, with numerals. The A1, A2, A3, A4, B, C, D, F1, F2, G, H, J, and K subtypes were the most widely reported.[6] This classification of HIV-1 into groups and subgroup types has clinical significance

in terms of the rate of transmission or disease progression and poses challenges for the development of an inclusive and effective vaccine.[7]

The retrovirus genome comprises two single-stranded RNA copies and is characterized by the structural genes *env*, *gag* and *pol*. The *gag* gene codes proteins of the core (p24, p7, p6) and matrix (p17) while *env* encodes the envelope glycoproteins, gp120 and gp41, which recognize cell surface receptors. The *pol* gene codes for reverse transcriptase, the enzyme that transcribes viral RNA into DNA, integrase that incorporates the viral DNA into host chromosomal DNA (the provirus) and the protease that cleaves the Gag and Pol precursors. These processes are vital for the replication of the virus.

There are other non-structural genes which encode for regulatory proteins that play important roles in virus replication process such as the viral protein r (*vpr*) negative factor (*nef*) and trans-activator of transcription (*tat*).[8] *Vpr* promotes nuclear import of the viral preintegration complex and apoptosis.[9] *Tat* increases HIV transcriptional efficiency and can induce expression of proinflammatory cytokines. *Nef* has effects such as enhancing viral transcriptional activity, activating T cells, reducing cell surface CD4 expression, and activation of cell signalling pathways. These regulatory proteins contribute to the pathogenesis of most organ involvement including kidney diseases seen in HIV-infected patients.[9]

The HIV attaches its viral envelope protein (gp 120) to a molecule expressed on CD4 cells and it also attaches to other target cells such as dendritic cells and macrophages mediated by different co-receptors, such as the chemokine receptor CCR5. [10, 11]

The viral RNA is transcribed into double-stranded DNA by reverse transcriptase following entry into the cell. This proviral double-stranded DNA moves into the nucleus of the target cell and

becomes integrated into the infected cell's chromosomal DNA. Viral replication may become activated and proviral DNA is transcribed back into genomic RNA and RNA that is translated into viral proteins, both of which are packaged and bud from the infected T cell as infectious virus.

There are various ways of transmitting HIV infection such as exposure to infected blood, sexual intercourse, or perinatal transmission though in a small proportion of infected individuals it could not be determined.

Risk factors for HIV transmission include certain sexual behaviours, high viral load, the lack of circumcision, the presence of ulcerative sexually transmitted infections, as well as host and genetic factors.[12]

1.2 Natural History of HIV infection

HIV infection can be divided into the following stages:[12]

●Viral transmission

●Acute HIV infection (primary HIV infection or acute sero-conversion syndrome)

● Chronic HIV Infection.

- Asymptomatic
- Early symptomatic HIV infection (previously known as AIDS-related complex [ARC])
- AIDS characterized by a CD4 cell count <200 cells/μL or the presence of any AIDS-defining condition
- Advanced HIV infection characterized by a CD4 cell count <50 cells/μL

The acute retroviral syndrome is recognised clinically by a "flu-like" illness characterized by fever, sore throat, lymphadenopathy, rash, headache, and myalgia/arthralgia, but could also be asymptomatic in a proportion of patients. It occurs a few weeks after exposure to the virus and its subsequent replication leading to high levels of plasma viremia. Seroconversion state ensues with detectable antibodies against HIV antigens, envelope (gp 160, gp 120, gp41) and core (p24). Chronic HIV infection is characterized by stability of viral levels and a progressive decline in CD4 cell count. The acquired immunodeficiency syndrome (AIDS) is defined as a CD4 cell count <200 cells/μL or the presence of any AIDS-defining condition regardless of the CD4 cell count. AIDS-defining conditions include mainly opportunistic infections, but also certain malignancies as well as conditions without clear alternative aetiology thought to be related to uncontrolled HIV infection itself, such as wasting or encephalopathy. These AIDS defining conditions are listed by the WHO as well as the CDC. [12]

1.3 Epidemiology of HIV infection

Worldwide there were approximately 37.9 million people living with HIV/AIDS at the end of 2018 and around 21% of these people do not know that were infected with the virus.[13] An estimated 32 million people have died from AIDS-related causes, with 770,000 deaths in 2018.[13]

Sub-Saharan Africa is the most affected region housing 68% of the world's HIV-infected persons in 2018.[13] In addition; the number of people with HIV receiving treatment in resource-poor countries has increased in 2018 with about 62% of all people living with HIV accessing treatment, out of which 53% were virally suppressed. [13]

The overall HIV prevalence rate in this region is 4.7%. The major burden of the HIV infection in sub-Saharan Africa is borne by South Africa with 25% and Nigeria with 13%.[7]

There are about 1,600,000 adults living with HIV in Nigeria in 2019, with a prevalence rate of 1.3%.[14] Many risk factors contribute to the spread of HIV, including prostitution, high-risk practices among itinerant workers, a high prevalence of sexually transmitted infections, concealed high-risk heterosexual and homosexual practices, irregular screening of blood and the international trafficking of women.[15]

There is still no cure for HIV infection but treatment with antiretroviral drugs (ART) helps in the control of the virus through improved healthy lives and reduces the risk of transmission to others among people living with the virus. Prevention has helped to reduce HIV prevalence rates in many countries and new HIV infections are believed to be on the decline.

1.4 Kidney Disease in HIV

1.4.1 Impact

Kidneys are among the internal organs commonly affected by the HIV infection. Rao *et al* described a rapidly progressive form of kidney disease affecting 12% of patients with AIDS who were mainly black drug abusers.[16] There were other reports subsequently from histopathological and autopsy studies describing similar kidney lesions among AIDS patients by Gardenswatch in New York City and Pardo *et al* from Miami.[17, 18]

Kidney disease among HIV-infected individuals is known to have implications for clinical outcomes, such as increased mortality and worse outcomes, regardless of the specific histological lesion or underlying cause.[19] The presence of kidney disease among HIV-infected patients was recognized by the World Health Organization (WHO) as stage 4 disease, eligible for ART initiation regardless of CD4 count.[20] The Women's Interagency HIV Study (WIHS) and the HIV Epidemiology Research Study (HERS), both from a cohort in the US, demonstrated a 2 to 2.5-

fold increased risk of death in HIV-infected women with serum creatinine ≥ 1.4 mg/dL. Proteinuria (defined by a dipstick reading of 2+ or greater on urinalysis) and an elevated creatinine level (≥1.4 mg/dL) were associated with an increased risk of death due to all causes (HR, 2.5; $p < 0.0001$) after controlling for predictors of AIDS-related death, including the CD4 count, the HIV RNA level, and the use of ART.[21, 22] However, all the participants in these studies were females and a possibility of gender bias may not be ruled out. Mulenga *et al* reported in a study involving 25,779 adults of both sexes in Lusaka, that presence of kidney disease at the time of ART initiation had 4-fold greater mortality than individuals with unimpaired kidney function.[23] George *et al* at the Johns Hopkins HIV Clinic noted that patients with an eGFR of less than 60 ml/min per 1.73 m^2 had a 15.9-fold increased odds of a cardiovascular event compared with an eGFR of at least 60 ml/min per 1.73 m^2 $p< 0.001$).[24] Similarly, Choi *et al* in a large cohort of US veterans with HIV reported that those with albuminuria and a GFR <30 mL/minute/1.73 m^2 had a 6-fold greater risk of cardiovascular events compared with those without albuminuria and a normal GFR.[25] Nadkarni *et al* reported from US hospital admissions data that dialysis-requiring AKI was associated with an increased risk of in-hospital mortality among HIV-positive adults (adjusted OR 2.64, 95% CI 2.04-3.42).[26]

1.5 Renal Syndromes in HIV infection

A number of renal syndromes have been described in the course of HIV infection, ranging from acute kidney injury (AKI) from a variety of causes, to chronic kidney disease (CKD) of varied aetiologies including HIV-associated nephropathy (HIVAN). In addition to these specific kidney lesions, a number of acid base and electrolyte abnormalities occur among HIV-infected patients during the course of the disease. Verma *et al* in a study of 510 HIV patients from India to elucidate the clinical spectrum of kidney diseases, reported electrolyte disorders in 71% of

patients, with the most frequent being hyponatremia (61%), AKI in 15.8% and CKD in 13% of these patients.[27]

1.6 Acute Kidney Injury

Acute kidney injury (AKI) is common and clinically significant among HIV patients with increased morbidity, mortality and associated life-threatening complications such as metabolic acidosis, electrolyte derangements and fluid overload. Naicker *et al* reported that in sub-Saharan Africa, AKI in ART naïve HIV patients carried a six-fold higher risk of in-hospital mortality related to severe immunosuppression, high viraemia, and late commencement of ART.[28] The increase in AKI among HIV patients was also reported by Wyatt *et al* from the US and Roe *et al* from a cohort study in the UK. A report from Cape Town, South Africa showed that dialysis for HIV infected patients with AKI was among the commonest indications for acute dialysis in the center.[29,30, 31]

1.7 Electrolytes and Acid Base Disorders in HIV infection

Electrolyte disorders are frequently found in patients infected with HIV occurring in various proportions and associated with an increased morbidity and mortality.[32] Impaired homeostatic responses may put them at increased risk of water, electrolyte and acid based disorders which may result from many factors such as infections and systemic inflammation, multiple drug regimens leading to electrolytes abnormalities. Hyponatraemia is the commonest electrolyte disorder seen in as many as 35 to 55% of hospitalized HIV-infected patients and can be seen even among ambulatory patients. Hypernatraemia was reported in up to 31% of patients in another report; hypokalaemia was reported in 17% to 24% of these patients. Hyperkalaemia was reported in 7% to 15% of patients.[33,34,35] Acidosis is also common among these patients and may result from various causes such as diarrhoea with loss of base, renal acidosis from adrenal

insufficiency, use of drugs such as Amphotericin and tenofovir which is known to cause proximal tubular damage or the syndrome of hyporeninemic hypoaldosteronism.[35]

1.8 Chronic Kidney Disease

1.8.1 Epidemiology of Kidney Disease in HIV-infected patients

There is worldwide variability in the reported prevalence of CKD among HIV-infected patients and this depends largely on the populations being studied. Naicker and Fabian summarised studies from different parts of the world including parts of Africa with varying prevalence ranging from 5.5- 48.5%.[36] The wide variation in the prevalence was further confirmed in a meta-analysis of 61 studies that reported on 209,078 HIV-infected adults from 60 countries using different equations to estimate eGFR.[37] The overall prevalence of CKD was 6.4% using the Modification of Diet in Renal Disease (MDRD) equation, 4.8% with CKD Epidemiology Collaboration (CKD-EPI) formula and 12.3% with the Cockcroft–Gault (CG) equation. The countries in the study were grouped according to WHO regional groupings, and using MDRD equation, Africa had the highest prevalence estimated at 7.9% while Europe had the lowest estimate at 3.7%.[37] This wide variation could be ascribed in part to differences in study design, the populations being studied and the criteria used for defining kidney disease.

The true prevalence of kidney disease among HIV-infected patients in Nigeria is not known, as the majority of the studies were not population-based and had small sample sizes. . Reports from available studies show a prevalence rate of 38% in Ile-Ife, South Western Nigeria;[38] 53.3% in Benin City in South Southern part;[39] 31% in Zaria, North Western region [40] and 51.8% in Jos and 47.6% in Ilorin respectively, both in the central part of the country.[41.42]

Reports from African countries also revealed wide variations, with 36% prevalence in Tanzania.[43] In a cross-sectional study from western Kenya, a prevalence of 11.5% for reduced

eGFR and 6.2% of proteinuria was reported.[44] A community based investigation in Rakai, Uganda, noted that 8% of HIV-infected persons initiating HAART had reduced eGFR.[45] In Cameroonian ART naive patients, proteinuria was reported in 36% and 43% had reduced kidney function with eGFR of 60 or less.[46]

Wyatt *et al* reported an ESKD prevalence of 4%, and 11% among HIV patients in a cross-sectional study in New York City.[47] A study from the US using data from the US Renal Data System (USRDS) reported that the annual number of patients with incident ESKD secondary to HIVAN increased from 1989 to 1995 and thereafter remained stable until 2006. The substantial reduction in both the incidence of ESKD due to HIVAN and the mortality among these patients was attributed to increasing availability of ART.[48] In a cross-sectional study involving HIV-infected individuals from 31 European countries, Israel and Argentina the prevalence of kidney disease was reported as 3.5–4.7%.[49] Studies from other parts of the globe have reported rates of kidney disease among HIV-infected individuals to be 39% in France,[50] 18% in Hong Kong,[51] 1.1–5.6% in Brazil[52], and 27% in India.[53] Ramezani *et al* reported 12.3% prevalence of proteinuria in a screening study among HIV patients in Iran.[54] In a cross-sectional study involving 248 patients from Saudi Arabia, proteinuria was reported in 12% of them.[55]

1.8.2 Diagnosis of Chronic Kidney disease in HIV

To diagnose kidney disease among HIV- infected patients, proteinuria or reduced glomerular filtration rate (GFR), or both, are used and very few studies include a kidney biopsy. Proteinuria is used as a marker of kidney damage and is usually assessed by use of dipstick analysis of the urine. It provides a semi-quantitative measure of protein concentration in the urine as well as other pathologic indicators such as presence of glucose and blood. Among its limitations is that it

detects predominantly albumin and this can lead to an underestimation or overestimation of true protein excretion depending on the patient's condition such as the hydration status. Quantitative measures of urinary excretion of albumin or protein are preferred and 24 hour urine collections are the reference standard but are limited by common errors associated with urine collection. In clinical practice, other methods for quantifying albumin or protein excretion rates are preferred, such as ratios of albumin or protein concentrations to creatinine concentration in a spot urine sample respectively. Both are shown to correlate closely with a 24-hour urine collection and eliminate the collection errors associated with the latter.

Serum creatinine is frequently used in clinical practice and research to estimate the GFR using various equations such as the CG equation, the MDRD formula and the CKD-EPI formula. [,56,57] Despite the popular use of the CG and MDRD equations, there is evidence to suggest that the CKD-EPI estimate is more accurate than the MDRD equation in HIV-infected individuals.[58] Serum Cystatin C has advantages in patients that receive medications that alter tubular creatinine handling, though it has limitations in the setting of inflammation, and Cystatin C based GFR estimation was not shown to have superiority among HIV-infected patients.[58] Though this conclusion was deduced from a population with near-normal eGFR and therefore not surprising, the CKD-EPI equation is better at higher (near-normal) levels of GFR. There is limited data on the validation of these GFR estimating equations among HIV-infected populations in sub-Saharan Africa.

A kidney biopsy is important among these patients when indicated, as it allows for a definitive diagnosis which is critical to optimal management. Definitive diagnosis of certain kidney diseases associated with HIV infection such as HIVAN is best made histologically. However, in

resource limited settings there are lack of kidney biopsies, leading to limited information on the histological pattern of HIV-related kidney diseases in many countries in sub-Saharan Africa.

Several biomarkers have been evaluated in HIV associated kidney disease in the early detection, determination of the severity of the diseases and correlation with HIVAN histology, and in predicting long-term clinical outcomes. Among those well studied include tubular proteins, such as neutrophil gelatinase-associated lipocalin (NGAL), alpha-1 microglobulin (A1M), beta 2-microglobulin (B2-M), kidney injury molecule-1 (KIM-1), liver-type fatty acid binding protein (L-FABP), and retinol-binding protein (RBP), among others.[59,60,61,] These biomarkers were shown to be sensitive markers of tubular dysfunction, and their measurements in urine potentially facilitate the early identification of HIV-infected patients at risk of kidney injury. Temesgen *et al* concluded that more sensitive and specific markers in addition to the traditional markers are needed in order to identify HIV-infected patients with early kidney disease who may benefit from interventions known to delay disease progression and prevent complications.

It has been recommended that individuals with HIV infection be screened for proteinuria and eGFR determined at presentation and annually thereafter if CKD is absent *ab initio*. This recommendation is a practical means of detecting CKD at an early stage and enables early diagnosis and treatment, as with type 2 diabetes mellitus.[62]

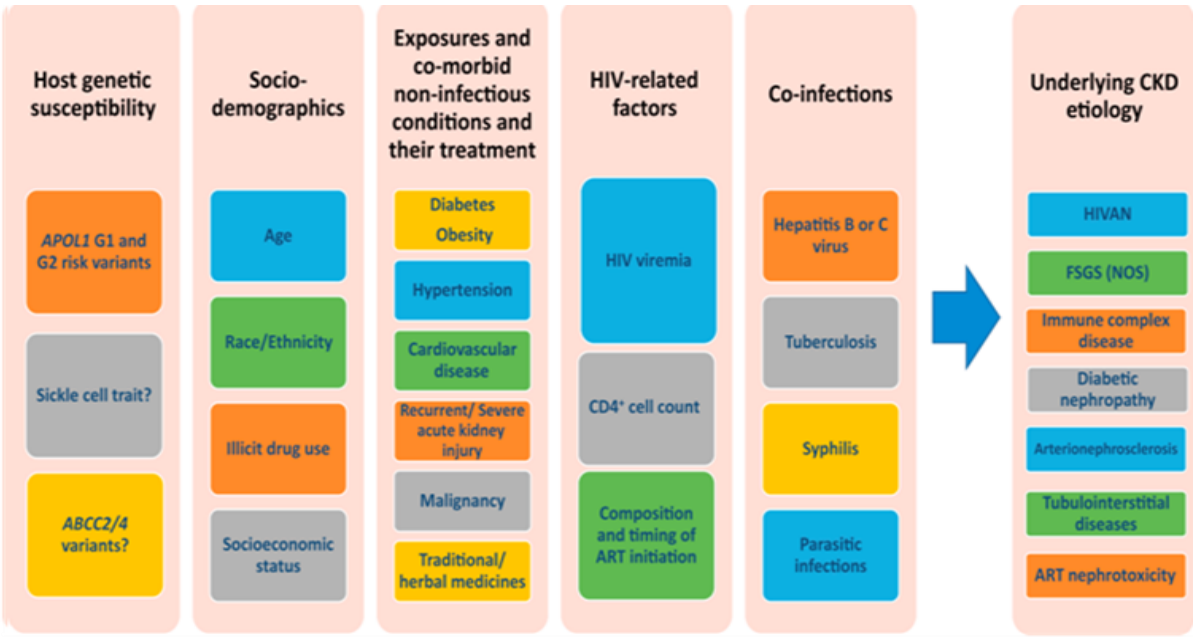
1.8.3 Risk factors for CKD among HIV-infected patients

Among HIV-infected patients, both traditional CKD and HIV-related risk factors contribute to the occurrence of kidney disease seen among HIV-infected patients. Hypertension, diabetes, increasing age, black race, male gender, hepatitis C virus co-infection, history of intravenous drug use, low CD4 count, high viral load and proteinuria were reported as risk factors for CKD

among these patients.[63] The prevalence of diabetes and hypertension were reported to be higher among HIV-infected patients. In a Multicenter AIDS Cohort Study, the prevalence of hypertension was 3-fold higher than age and sex matched controls whilst the prevalence of diabetes was 4-fold higher (34.2% vs. 11.9%; P<.001); and hypertensive persons with HIV infection are more likely to have insulin resistance and metabolic syndrome (64.3% vs. 16.9%; P<.001).[63] In a retrospective study involving HIV-infected patients in the Veterans Health Administration in the USA, Jotwani *et al* reported that ESKD risk can be attributed to a combination of traditional and HIV-related risk factors.[64] They reported factors such as diabetes, hypertension and cardiovascular disease, as well as others including HIV disease severity as measured by the CD4 lymphocyte counts < 200 cells/mm^3, HIV viral load, hepatitis C co-infection, and hypoalbuminaemia, lower baseline eGFR and higher baseline proteinuria to be associated with higher ESKD risk in various proportions.[64] Race is considered an important risk factor due to the fact that the burden of kidney disease in HIV-infected patients is disproportionately higher in Blacks. Over 90% of dialysis patients with HIV infection in the USA are blacks.[65] Eggers *et al* reported that HIV-infected African-American have at least 50 times higher odds of developing kidney disease than Caucasians. [66]

It has been hypothesised that genetic factors may play a vital role in explaining this racial disparity. This was further strengthened by the observation that most African-Americans trace their ancestral origin to the West African region and a meta-analysis has shown a high prevalence of CKD in HIV among West African populations.[37]

The interplay of the various risk factors in the development of CKD among HIV-infected individuals is summarised in a pictorial view as depicted in Figure 1 below from the paper by Swanepoel *et al*.[67]



Swanepoel et al *Kidney International* 2018 93545-559 *APOL1*, apolipoprotein L1; *ABCC*, ATP-binding cassette transporter proteins; ART, antiretrovial therapy; CKD, chronic kidney disease; FSGS (NOS), focal segmental glomerulosclerosis, not otherwise specified; GN, glomerulonephritis; HIVAN, HIV-associated nephropathy.
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Figure 1. Risk factor of CKD in HIV-positive individuals

1.8.4 Pathogenesis of Kidney Disease in HIV Patients

Various factors including viral protein expression in the kidney, host factors such as genetics, environmental and behavioural factors such as access to care and ART contribute to the pathogenesis of kidney disease in HIV-infected individuals.

HIV is known to infect the podocytes, parietal epithelial cells and renal tubular epithelial cells (RTECs); the mechanisms by which infection occurs is not fully elucidated especially as RTECs do not express CD4 or CCR5, CXCR6 receptors and co-receptors respectively. Direct transfer of HIV from infected lymphocytes was suggested, with the infected RTECs also transferring the virus to uninfected primary T lymphocytes. This, together with findings that isolated DNA from RTECs in kidney biopsies from patients with HIVAN are different from those in the blood of the

same patient, suggest a possibility of renal epithelium serving as reservoirs with evidence of on-going replication and evolution of the virus.[48] This viral replication and inflammation are essential in the pathogenesis of kidney disease among HIV-infected patients. The RTECs serving as a reservoir has implications for treatment and prevention of spread of the infection.[68] It also has implications for kidney transplantation. Canaud *et al* in a study involving 19 HIV-positive patients who received HIV-negative kidney allografts, found that 13 allografts had detectable HIV RNA in podocytes and/or RTECs, despite having undetectable plasma viral RNA with much more podocytes and RTECs injury and rapid loss of kidney function.[69]

Infection of the RTECs is associated with increased expression of many pro-inflammatory genes controlled by nuclear factor kappa B (NF-kB) leading to increased cytokine/ chemokine release and this may cause more infiltration by macrophages and lymphocytes with further cellular injury.[9] This role for NF-kB-induced inflammation in the pathogenesis of kidney disease in HIV has been corroborated in studies illustrating that pharmacologic inhibition of NF-kB prevents kidney disease in murine models of HIV-induced kidney disease[70] and clinical studies demonstrating improved kidney outcomes in HIVAN with glucocorticoids was associated with reduced tubulointerstitial inflammation.[71,72.]

HIVAN is associated with podocytes' cytoskeletal dysregulation induced by *nef* leading to injury and dedifferentiation which results in widespread foot process effacement and contributing to the pathogenesis of HIVAN. Several factors were implicated in this, including HIV gene expression and activation of other signalling pathways.[68]

Similarly formation of circulating immune complexes that deposit in the glomerular capillary tuft and the *in situ* deposition of antibodies that bind to glomerular antigens and activated

complement were suggested in the pathogenesis of HIV immune complex disease (HIVICD). These result from specific antibody responses to HIV epitopes and polyclonal gammopathy.[68]

Thrombotic microangiopathy via P24 antigen may follow endothelial injury precipitated by indirect cytopathic effects of HIV. Coexisting medical conditions, such as malignant conditions, opportunistic infections or antiretroviral therapy may also contribute to the pathogenesis of thrombotic microangiopathy.[68]

Rednor *et al* in a recent review highlighted these signalling pathways and other pathogenetic mechanisms and is depicted in Figure 2 below.

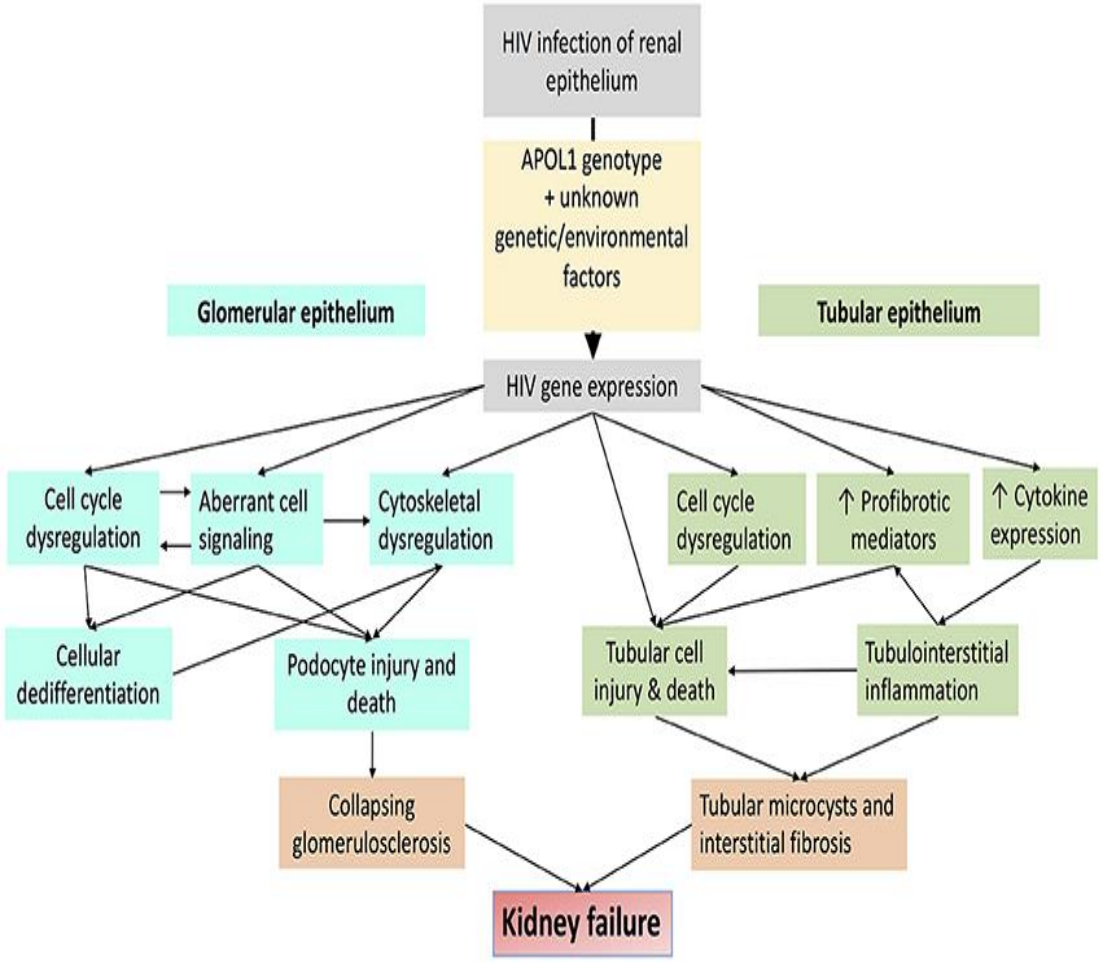

Figure 2. Major mechanisms by which HIV-1 infection promotes glomerular and tubular injury.

Rednor SJ Front. Med.2018; 5:177. An Open Access Source reproduced under Creative Commons Attribution License (CC BY)

1.8.5 Types of CKD in HIV-Infected Patients

There is a wide spectrum of CKD among HIV-infected patients although HIVAN was the first to be well described.[16] Various factors including geographical location, the ethnicity of the population and the nature of the studies being conducted contribute to this wide variability of kidney diseases seen in these patients. Some of the diseases may be due to lesions directly related to the HIV virus on the kidneys and lesions related to co morbidities, immune dysregulation, drug effects, and co-infections. Certain kidney diseases like HIVAN are limited to

populations infected with HIV and a diagnosis requires histology. Kidney biopsy is therefore significant for treatment and prognosis. In the setting of HIV a number of immune complex diseases have also been noted, namely IgA nephropathy, a lupus-like glomerulonephritis (GN), mesangiocapillary GN (MCGN) and post-infectious GN (PIGN).[73] HIV independent disorders such as diabetes and hypertension and non-recovered AKI are increasingly being reported especially with the widespread use of ART and the ageing of the HIV-infected individuals through improved long-term survival.

The lack of resources required to carry out kidney biopsies in most sub-Saharan African countries has contributed to the paucity of information specifying the spectrum of kidney morphology associated with disease in the region and with most reports emanating from South Africa.[74,75]

A comprehensive pathological classification have recently been proposed by the Pathology Working Group of the KDIGO Controversies Conference on HIV and Kidny Disease based on the major tissue compartment involved such as the glomerulus, tubules, and interstitium as depicted in Table 1 below.[67]

Table 1. Pathologic classification of HIV-related kidney diseases

I. Glomerular-dominant

A. **Podocytopathies** (all characterized by extensive foot process effacement)
i. Classic HIVAN
ii. FSGS (NOS) in the setting of HIV
iii. Minimal change disease in the setting of HIV
iv. Diffuse mesangial hypercellularity in the setting of HIV
v. Other podocytopathy in the setting of HIV

B. **Immune complex-mediated glomerular disease**
i. IgA nephropathy in the setting of HIV
ii. Lupus-like glomerulonephritis in the setting of HIV
iii. Lupus nephritis in the setting of HIV

iv. Membranous nephropathy in the setting of HIV
v. Membranoproliferative pattern glomerulonephritis in the setting of HIV
vi. Endocapillary proliferative and exudative glomerulonephritis in the setting of HIV
vii. Fibrillary or immunotactoid glomerulonephritis in the setting of HIV
viii. Other immune complex disease in the setting of HIV

II. Tubulointerstitial-dominant

A. **Tubulointerstitial injury in the setting of classic HIVAN**
i. Hyaline droplet tubulopathy
ii. Tubular microcysts
iii. Tubulointerstitial inflammation

B. **Acute tubular injury or acute tubular necrosis**
i. Ischemic
ii. Toxic (associated with ART vs. other)

C. **Drug-induced tubulointerstitial nephritis (other than ART)**
i. Antibiotics
ii. Proton pump inhibitors
iii. NSAIDs
iv. Other

D. Direct renal parenchymal infection by pathogens (bacterial, viral, fungal, protozoal, etc.

E. Immunologic dysfunction-related tubulointerstitial inflammation
i. Diffuse infiltrative lymphocytosis syndrome (DILS)
ii. Immune reconstitution inflammatory syndrome (IRIS)

F. Other tubulointerstitial inflammation in the setting of HIV

III. Vascular-dominant
a. Thrombotic microangiopathy in the setting of HIV
b. Arteriosclerosis

IV**. Other, in the setting of HIV infection**
a. Diabetic nephropathy
b. Age-related nephrosclerosis

Modified from Swanepoel et al *Kidney International* 2018 93545-559 ART= antiretroviral therapy; HBV, hepatitis B virus; HCV, hepatitis C virus; FSGS, focal segmental glomerulosclerosis; HIVAN, HIV-associated nephropathy; NOS, not otherwise specified; NSAID, nonsteroidal anti-inflammatory drug; PLA2R, M-type phospholipase A2 receptor.

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Patients with HIVAN present with body swelling and to a lesser extent hypertension with proteinuria (sometimes nephrotic range), with associated decline in kidney function that rapidly progresses to ESKD within a few weeks to months without treatment. It is usually seen in the late stages of HIV infection although a few cases were reported to occur early in the disease.[76] Kidneys may appear enlarged and echogenic on kidney ultrasound. Histologically HIVAN is characterised by collapsing FSGS, microcystic tubular dilatation and interstitial inflammation; there may be podocyte swelling, intra-cytoplasmic protein droplets. Electron microscopy changes may show retraction, wrinkling, pleating, and thickening of the glomerular basement membrane and effacement of the foot processes.[77]

1.8.5. HIV-associated immune complex disease (HIVICD)

HIV-associated immune complex disease **(**HIVICD) is characterized by immune complex deposition that results in a range of kidney histopathologies. Gerntholtz *et al*[78] described a unique immune complex glomerulonephritis with a characteristic 'ball in cup' basement membrane reaction; however, a variety of immune complex diseases were described including IgA nephropathy, lupus-like glomerulonephritis, PIGN, membranoproliferative glomerulonephritis (MPGN), and cryoglobulinaemic glomerulonephritis.[79]

HIVICD can present with raised blood pressure, though with a lower prevalence, haematuria, proteinuria, kidney failure and low levels of serum complement. It is less commonly seen in patients of black ancestry and is more common among those co-infected with hepatitis B or C virus. Higher levels of HIV RNA, hypertension and diabetes are considered independent risk factors for the development of HIVICD. Histologic features include the presence of crescents, endocapillary and/or mesangial proliferation including an exudative pattern, fibrinoid necrosis, double contours of the GBM, 'spikes' or a 'moth-eaten' appearance on silver stain, and an

orange precipitate following Masson trichrome stain. It has been recommended that due to the heterogeneous nature of these conditions, a specific description of the pattern of immune complex deposition in the setting of HIV is preferred.[67, 79]

1.1.8.2 HIV-Associated Thrombotic Microangiopathy

HIV-associated thrombotic microangiopathy is a spectrum characterized by variable expression of the following findings: fever, thrombocytopenia, neurologic dysfunction, microangiopathic haemolytic anaemia, and impaired kidney function with haematuria. Nephrotic range proteinuria is uncommon and assists in differentiating these syndromes from HIV-associated nephropathy and the immune-mediated classes of disease.

1.9 Epidemiology of various forms of CKD in HIV kidney disease

There are noticeable changes in the reported histologic lesions among HIV-infected patients due to factors such as prolonged survival, improved access to ARTs and better management of complications such as opportunistic infections among others with a reported decline in HIVAN prevalence and increase in the prevalence of non-HIV-associated diseases such as diabetes and hypertension.[80, 75]

In Nigeria there have been limited studies with HIV patients where a kidney biopsy had been performed. Emem-Chioma *et al*[38] reported that 7 of 10 patients (70%) that had been biopsied had exhibited features of HIVAN histologically. A previous study from Kano in the Northern part of Nigeria had shown that among 25 patients that had undergone a kidney biopsy, 55% exhibited features of HIVAN although this may not be a true representative of the Nigerian population.[81]

There were reports of variable histological findings among studies from South Africa similar to the global pattern. For example, HIVAN was present in more than 80% of biopsies in the study by Han *et al*[82] in Durban and in 27% of biopsies in the study by Gerntholtz *et al* [78] in

Johannesburg, with immune complex disease and other glomerular diseases present in 21%. A single center in Cape Town reported the diagnosis of HIVAN in 25% of all kidney biopsies,[83] while in a retrospective study of all biopsies in another centre in the western cape, HIVAN was the most commonly identified pathology and was the second most common secondary glomerular disease reported in the study [84]. Similarly, there were varied reports on the prevalence of these kidney diseases from other part of the globe. In a study involving HIV positive patients that were mostly whites from Italy, Casanova *et al*[85] reported that none of the patients had histological features suggestive of HIVAN. A study in US reported a 35% prevalence for HIVAN, 22% for FSGS, and 7.9% for acute interstitial nephritis in addition to other low prevalent forms, [86] In another study from US the commonest histologic finding was immune complex disease seen in 17% followed by diabetic nephropathy (DN) in 16%, HIVAN in 14% and FSGS in 12%.[87]

In India, a meta-analysis of 10 studies showed a prevalence of 16.5% of HIVAN, 3.9% mesangioproliferative glomerulonephritis, 7.7% of FSGS and 22.7% of tubulointerstitial lesions.[88]

Worldwide HIVICD is seen in varying proportions among HIV-infected individuals. An analysis of kidney biopsy samples from a clinical cohort at Johns Hopkins found HIVICD in 32.2% of patients.[89] A study in SA reported only 1% of 30 HIV-infected black patients having HIVICD, [82] while in another biopsy series report from SA HIVICD was reported in 21.2%. [83] Another SA study from Cape Town reported HIVICD in 8.3%, and 30.2% had features of both HIVAN and HIVICD.[73] A multicenter study in France found that 23.3% (14/60) had HIVICD.[90] There is thus a need for a multicentre study with adequate power to elucidate the true prevalence of these various types of kidney disease among populations in sub-Saharan Africa.

1.10 Genetic factors in Kidney Diseases among HIV-infected patients

The contribution of genetic factors to the pathogenesis of HIVAN was suggested by the racial/ ethnic disparity in its occurrence with an excess risk among people of African descent in the US. Studies have shown an increase in the risk of various forms of kidney diseases among people of African ancestry, with three times greater risk of developing lupus nephritis, seven times greater risk of developing hypertensive kidney disease, seventeen times greater risk of developing primary FSGS, 29 times greater risk of HIVAN.[91,92]

Other studies also corroborated the higher odds of developing kidney disease observed among people of African ancestry which further supported the possibility of genetic factors in the racial disparity. [93,94] Furthermore, this excess risk could not entirely be explained by factors such as access to care and other socioeconomic factors among African Americans.[95] This racial/ ethnic disparity in the occurrence of kidney disease is also noted among HIV-infected patients. Eggers *et al* reported that the possibility of developing CKD among HIV-infected African-Americans is at least 50 times higher than that of Caucasians [66]

The genetic basis of this disparity was initially thought to be associated with the *MYH9* gene located on chromosome 22, encoding non-muscle myosin heavy chain IIa as reported in studies based on mapping by admixture linkage disequilibrium.[96] Further studies using fine-mapping did not reveal variants to denote the association of *MYH9* single nucleotide polymorphisms with kidney disease.[97,98]

Subsequent studies interrogating the '1000 Genomes Project,' data by Genovese *et al*[99] suggested that it was due largely to variants in the *APOL1* gene, on chromosome 22 which encodes for apolipoprotein L1 (APOL1). Kopp *et al* and Limou *et al* reported that *APOL1* coding variants are present only on African-ancestry haplotypes.[92,100] The *APOL1* genotype was strongly

associated with FSGS (OR = 16.9, 95%CI: 11.0 to 26.5, p = 1.3 x 10-48) and HIVAN (OR = 29.2, 95% CI: 13.1-68.5, P = 6 x 10-22) among African-Americans and with HIVAN among black South Africans (OR, 89.1; 95% CI, 17.68 to 911.72; p=1.24×10^{-14}).[92,101] Behar *et al* reported the lack of HIVAN and the *APOL1* risk variants among HIV-infected individuals of Ethiopian ancestry.[102] Table 2 below summarises some studies of the association of *APOL1* risk variants with kidney disease among HIV infected and non HIV infected individuals.

It has been reported that no similar association with *APOL 1* among HIVICD was observed as the association was noted in only 3% of patients with biopsy-proven immune complex disease in a US study, and in 25% of those with HIV and immune complex kidney disease in an African study.[103] Despite the high prevalence of the risk alleles and the strong association with HIV kidney disease among African Americans, in a subset of individuals approximately 20–30% of HIVAN patients have zero or one *APOL1* risk allele, suggesting that other susceptibility genetic, viral, or environmental factors that are triggered or unmasked in the presence of HIV infection contribute to this genetic association seen among these patients.[104] Many potential 'second hits' have been suggested including infections such as viral hepatitis and others, interferon, other CKD risk factors, gene-gene interactions and illicit drug use.[104]

Table 2. Prevalence of *APOL*1 risk alleles among HIV Kidney Disease

Disease	**Country**	**N cases**	***APOL1* 2 RA prevalence in reference population (HIV status)**	***APOL1* 2 RA prevalence in disease**	**OR (CI) for kidney disease, *APOL1* HRG present**
HIVAN	USA[92]	54	8% (HIV+)	72%	29 (14, 68)
	USA[104]	60	ND	62%	ND
	South Africa[101]	38	3.7% (HIV+)	79%	89 (18, 912)

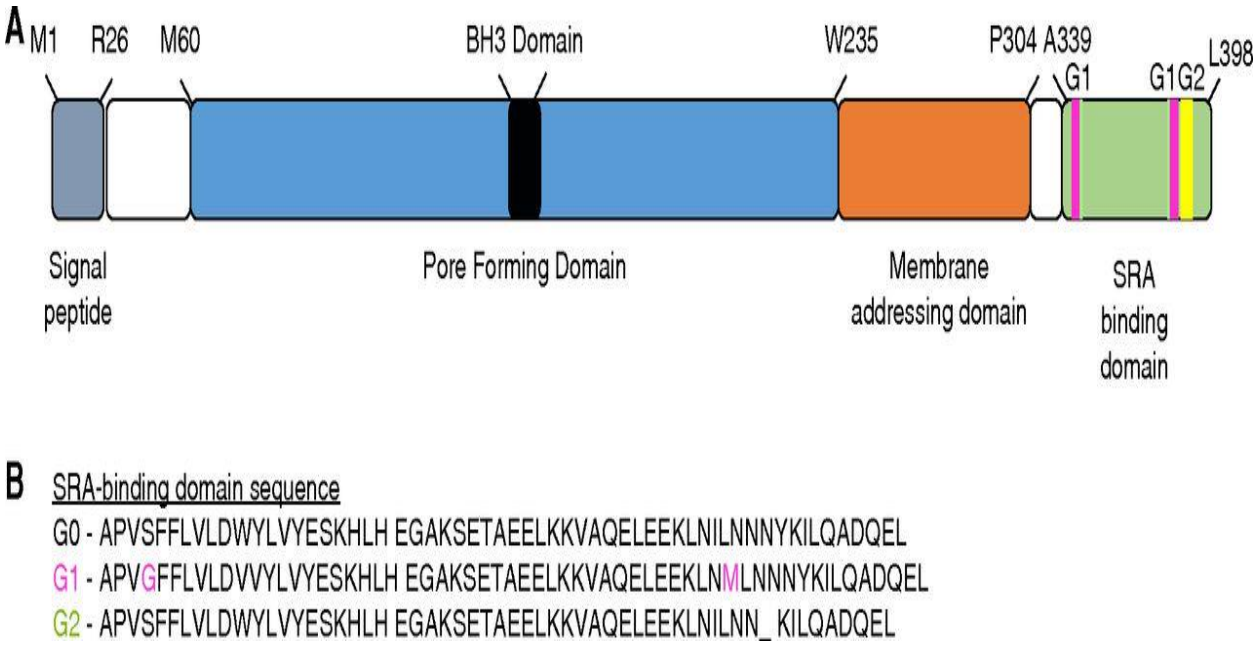
HIVICD	USA[104]	31	ND	3%	ND
	South Africa[101]	49	3.7% (HIV+)	9%	2.6 (NS)
HIV+ FSGS	USA[105]	35	8% (HIV+)	63%	20 (8, 45)
	USA[106]	203-	ND		3 (1.5, 6)
	South Africa[101]	22	3.7% (HIV+)	8%	2.1 (NS)
HIV-negative FSGS	USA[92]	217	13% (HIV-)	72%	17 (11,26)

Modified from Heymann J et al. Semin Nephrol. 2017 Nov;37(6):514-519. Reproduced with permission under Licence no 5077711198399 of 28/05/2021 HIVAN= HIV nephropathy. HIVICD= HIV-associated immune complex disease. ND= not determined. NS= not significant, HRG= High risk genotype, OR= Odds Ratio

1.15 *APOL 1* Gene and Genotype

The *APOL1* gene is one of the six *APOL* gene clusters on human chromosome 22 and codes for a high density lipoprotein (HDL) which binds to Apolipoprotein A-I. It is abundant in the plasma and complexes in HDL3 particles. It is involved in the formation of most cholesteryl esters in plasma and also promotes efflux of cholesterol from cells. Immunohistochemically, *APOL1* is expressed in the podocytes, proximal tubules and medium-sized arteries and arteriolar endothelial cells in kidney biopsies in patients with HIVAN and FSGS,[107] although the relevance of this expression in relation to the pathogenesis is not fully elucidated. Many intracellular functions of *APOL1* have been proposed though not yet fully understood, such as the role in the regulation of autophagy, intracellular vesicle trafficking, ion-channel activity, and providing a protective effect from HIV infection.[107] Figure 3 depicts a schematic representation of APOL1 protein domains and the amino acid variants.

Two kidney disease risk variants G1 {a haplotype comprising of two missense mutations, rs73885319 and rs60910I45 (-/TTATAA)} and G2 (comprising of two amino acid deletion at rs71785313) have been described. *APOL1* high-risk status (HRG) for kidney disease occurs in an autosomal recessive pattern defined by carriage of 2 risk alleles (G1/G1, G1/G2, and G2/G2) and low-risk (LRG) status is defined by carriage of 1 or 0 kidney risk alleles.[99]



(A) Schematic representation of APOL1 protein domains. (B) The amino acid sequence of the C-terminal SRA-interacting domain showing the locations of G1 and G2 amino-acid changes compared with reference protein G0. SRA, serum resistance–associated protein.

From Sethu M. Madhavan and Matthias Buck Kidney 360 January 2021, 2 (1) 134-140; An Open Access

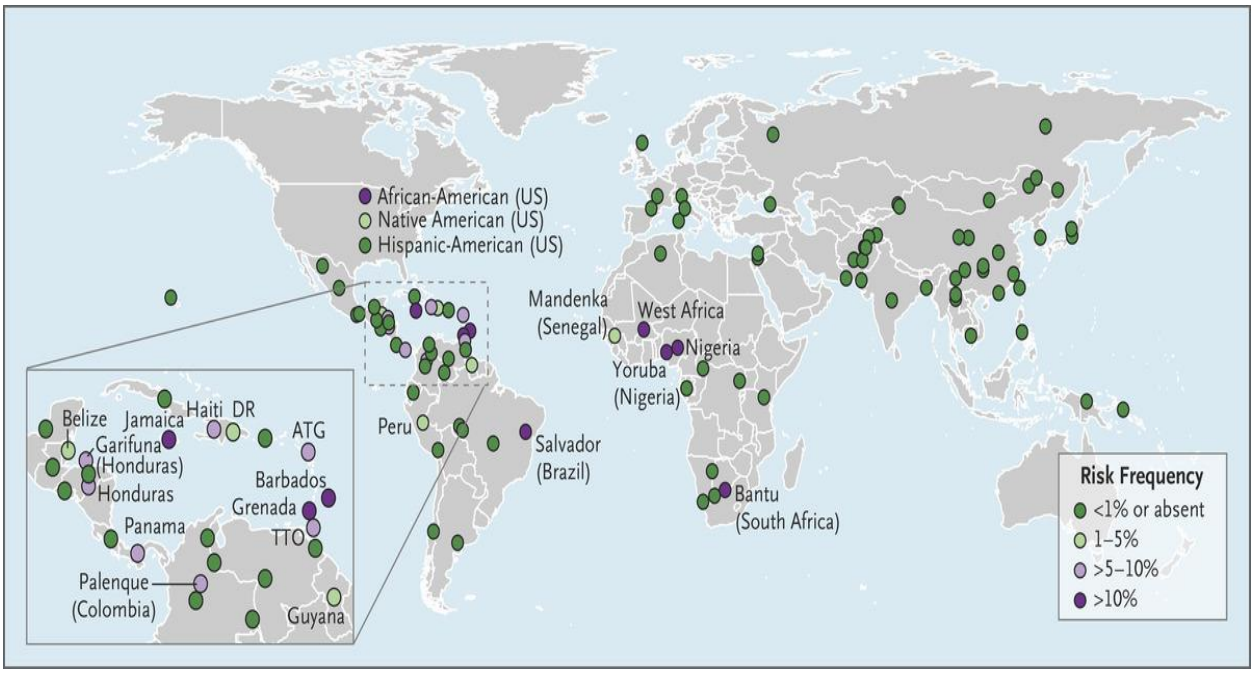
Figure 3. APOL1 protein domain organization and location of kidney risk variants

1.11 Population Frequencies of *APOL1* genotypes

APOL1 risk variants exist at varied frequencies among the diverse African ethnic populations. This variation in frequencies was noted in a multicenter and multi ethnic study from 12 African

populations including Ethiopia (4 ethnic groups), Congo, Cameroon (2 ethnic groups), Ghana (2 ethnic groups), Mozambique Malawi and Sudan.[108]

Nadhkarni *et al* using data from 111 populations in two large studies also found variations in frequencies of the *APOL1* haplotype in African-American, sub-Saharan African, and Western African populations ranging from 11 to 32% and their findings are summarised in Figure 4.[109]



Nadkarni et al. N Engl J Med 2018; 379:2571-2572. Reproduced with permission from (scientific reference citation), Copyright Massachusetts Medical Society.

Figure 4. *APOL*1 G1 and G2 Risk Alleles in 111 Global Reference Populations

There is the need to study the population frequencies of these alleles and associations with kidney disease among HIV-infected native Africans, especially as the HRG association with kidney disease was mostly described among African-Americans with limited information from sub-Saharan Africa. The processes linking the HRG need to be studied as well so that targeted preventive measures could be encouraged.

1.12 Trypanolytic activity of *APOL1*

The high frequency of *APOL1* kidney disease risk alleles in populations with African ancestry is believed to have evolutionary origins resulting from natural selection of a trait that is protective against infection with certain forms of protozoan infection, *Trypanosoma brucei rhodesiense* that causes sleeping sickness, which is endemic in sub-Saharan Africa. However, this positive selection advantage against sleeping sickness has an associated increased risk of developing CKD among the carriers of the HRG.

Two models for *APOL1* trypanolytic activity were suggested, lysosome permeabilization or colloid-osmotic swelling of the cell body.[110] *APOL1* lyses trypanosomes by causing osmotic swelling of parasite lysosomes through a pore-mediated mechanism and makes humans resistant to infection. *Trypanosoma brucei rhodesiense* however, produces a virulence factor, serum resistance associated-factor (SRA),that neutralizes the wild type *APOL1* through the binding of its C-terminus. *APOL1* risk variants (G1, G2) alter amino acid residues directly within the C-terminal SRA binding site thus preserving lytic activity and conferring *Trypanosoma brucei rhodesiense* resistance to heterozygous carriers.[103]

1.13 Role of *APOL1* in the pathogenesis of kidney disease

The exact mechanism of *APOL1* injury to the kidneys is not fully understood though a number of potential mechanisms have been suggested with resultant damage to the podocytes and other kidney cells. However, the majority of the studies were *in vitro* and animal model studies that lack *APOL1* in their genomes and not many of these studies were focused on the *APOL1* mediated injury in the context of HIV infection. Several factors including host factors such as hypoxia, hydrogen peroxide, TNF-α, and puromycin aminonucleoside, augmented *APOL1* and

APOL1-induced podocyte injury, as well as the effect of HIV on podocytes contributing to glomerular damage.[111] *APOL1* risk variants also cause lysosomal disruption mediated by increased chloride permeability, similar to the lysosomal disruption in trypanosomes.[111] The podocyte injury induced is associated with impaired endosomal trafficking and autophagic flux leading to pyroptosis as depicted in transgenic mice expressing G2.[68] *APOL 1* risk variants also impair mitochondrial function and mitochondrial membrane potential, reduce maximum respiratory rate, and reduce respiratory reserve.[112]

The innate immune response to HIV results in generation of interferons (IFN); this is known to cause upregulation of *APOL1* production. This is supported by a finding that intravenous interferon infusion was associated with increased *APOL1* levels and was associated with the development of FSGS in patients with *APOL1* HRG.[113] However, there is need for further studies to establish the role of type 1 IFN expression in the up regulation of *APOL1* in HIV infection and possibly the mechanisms involved. Additionally, down regulation of miRNA 193a (a repressor of *APOL1*) results in the up regulation of *APOL1*[114] and it has been postulated that glomerular damage affected by *APOL1* risk variants is a result of gain- and loss of function changes in the protein. [107]

1.14 Management of HIV Kidney Disease

There are benefits of using ART in the recovery of kidney function in patients with HIVAN. Epidemiologic data from USRDS also suggested a 60% reduction in the risk of ESKD attributed to HIV with the use of ART.[115] Atta *et al*, in a retrospective cohort study of 36 biopsy-proven HIVAN patients, showed superior kidney survival among the 26 who were treated with ART compared with 10 who received no ART.[116] Several antiretroviral regimens necessitate dose adjustment in individuals with decreased eGFR and some of these drugs may induce or inhibit

absorption of co-administered medications, hence the need for attention to detail in prescribing medications to these patients.

Renin angiotensin aldosterone system blockade through the use Angiotensin Converting Enzyme Inhibitors (ACEi) and Angiotensin receptor Blockers (ARB) reduces intraglomerular pressure, decreases mediators of inflammation and proteinuria in mechanisms independent of the control of blood pressure. Wei *et al* studied 44 patients with biopsy-proven HIVAN in which 28 patients received fosinopril 10 mg/day while another 16 patients served as controls and both groups were followed up for a study period of 5.1 years with end point of death or ESKD. Median kidney survival of treated patients was significantly better with only 1 patient developing ESKD (relative risk [RR] =0.003, $P<0.0001$) and patient survival was also better ($p<0.001$).(109).[117] Kimmel *et al* studied 18 patients with biopsy-proven HIVAN, nine of whom were treated with Captopril. They reported a significantly better kidney survival in patients treated with Captopril vs controls (mean kidney survival 156 ±71 vs 37 ±5 days, respectively; $p<0.002$)[118] Major clinical practice guidelines have now recommended the use of ACEi and ARBs in the treatment of patients with confirmed or suspected HIVAN or those with significant proteinuria.[59]

There are benefits of using ART in the recovery of kidney function in patients with HIVAN. Epidemiologic data from USRDS also suggested a 60% reduction in the risk of ESKD attributed to HIV with the use of ART.[117] Atta *et al,* in a retrospective cohort study of 36 biopsy-proven HIVAN patients, showed superior kidney survival among the 26 who were treated with ART compared with 10 who received no ART.[118]

Studies in some parts of sub Saharan Africa suggested similar improvement of kidney function with ART initiation.[119,120,121] Initiation of ART is part of the recommendations of management of HIV infected patients with Kidney disease. Several antiretrovirals require dose adjustment in

individuals with decreased eGFR and some of these drugs may induce or inhibit absorption of co-administered medications, hence the need for attention to detail in prescribing medications to these patients.

1.15 Justification

There is a need to know the prevalence of kidney disease and its associated factors among HIV patients in Nigeria and especially in the Northern region t, as this will facilitate the initiation of adequate preventive measures in those at risk, as well as delay or prevent progression to ESKD in those with established kidney disease. There is limited information from this part of Nigeria on the histological pattern of kidney disease among HIV-infected patients beyond light microscopy with all its associated limitations. Hence this study will provide data on the histological pattern of kidney diseases using immunoflourescence, which will help in identifying specific kidney diseases and instituting appropriate treatment. It will also provide baseline data for further research in this field. Genetic predilection to HIVAN has been described among African-Americans, but has not been widely ascertained among native Africans making this aspect of the study relevant. Some drugs such as tenofovir used in the current regimens of ART are known to be nephrotoxic, thus there is a need for long term follow up of patients on these drugs to ensure kidney safety.

1.16 Hypothesis

There is a high prevalence of kidney disease among HIV-infected individuals in Kano, and not much is known about the histological pattern or prevalence of HIVAN in this group. High risk alleles of *APOL1* may contribute to susceptibility for CKD in this population. The ART regimen used may be associated with kidney dysfunction on a long term basis.

1.17 Objectives

1. To study the prevalence of CKD among ART-naive HIV-infected patients in Kano.
2. To evaluate the risk factors associated with CKD among these patients.
3. To study the histological pattern of kidney disease among HIV-infected patients.
4. To study the prevalence of kidney risk variants of *APOL1* among HIV-infected patients with CKD.
5. To study long term kidney function in HIV-infected adults initiating ART.

CHAPTER TWO. MATERIALS AND METHODS

2.1 Study area

This study was conducted at the Aminu Kano Teaching Hospital, Kano, Nigeria a facility with a 540 bed capacity. The hospital, established in 1988, is situated in the North Western Nigeria. The 100 bed medical wards have an average admission of 15 daily. The hospital is a referral center for Kano and its surrounding states such as Yobe, Gombe, Bauchi, Zamfara, Katsina and Jigawa. The hospital is an approved United State President's Emergency Plan for AIDS Relief (PEPFAR) center. Over 12,000 registered HIV positive patients attend the center and are followed up in a dedicated HIV clinic. The clinic, established in 2002, offers first and second line anti-retroviral treatments and has a well-equipped laboratory for determining CD4 counts and carrying out other routine investigations.

2.2 Study Design: This is a cross-sectional study with a longitudinal component.

2.3 Sample Size

The minimum sample size to detect CKD in this study to allow for meaningful statistical analyses of result was obtained using the Fisher's formula and prevalence of CKD among HIV positive patients in Nigeria from a previous study.[38]

$\mathbf{N=Z^2PQ/d^2}$ where

N=Minimum sample size

Z= the standard normal deviate corresponding to a 95% confidence level. The value is 1.96 obtained from a normal distribution table.

P= prevalence of HIV related CKD in a Nigerian study (38%) i.e 0.38[38]

Q= complementary probability i.e 1-P=1-0.38=0.62

d=absolute precision limit desired (5%) 0.05 Thus

N= $(1.96)^2$ (0.38)0.62/ $(0.05)^2$ =362.

However, 900 patients were continuously recruited aiming at a sizable number of histologically confirmed HIVAN patients for the genetic component of the study.

A minimum sample size for the genetic component of this study was determined by statistical analysis using frequency estimates and effect size for the risk alleles in the Yoruba and Igbo population from Nigeria and African Americans.[110, 122,123] The general population risk allele frequency for the two *APOL1* risk allele frequency combined is at least 15%; 2.3% are expected to be homozygous or compound heterozygotes for the two *APOL1*risk alleles. Based on the assumption that among HIVAN 20% would be homozygous for the risk allele then a minimum sample of 20 cases in the study group and 50 population controls was considered adequate to obtain an alpha =5% with 97% power in a two tailed test. However to factor in possible problems that could occur during the extraction and calling protocol of the genotyping, a total of 155 participants were studied in the genetic component. They comprise of 50 treatment naive HIV positive patients with no evidence of kidney disease (HIV positive no CKD), 52 HIV negative participants with no kidney disease (HIV negative no CKD) and 53 HIV positive patients with kidney disease evidenced by persistent proteinuria and/or reduced eGFR, who had also had a kidney biopsy (HIV positive CKD). The HIV positive CKD patients had no obvious other known causes of kidney disease, such as diabetes and hypertension, and were not undergoing any other treatment for other kidney disease prior to enrolment and had no contraindication to kidney biopsy

2.4 Sampling method

All consecutive patients who fulfilled the inclusion criteria were enrolled into the study until the required sample was obtained.

2.5 Study Population.

Patients referred to the HIV clinics who were 18 years and older. Controls for the genetics study were recruited from the HIV voluntary test counselling unit of the hospital as well as from informed and willing healthy volunteers from the hospital community who are ready to undergo pre-test counselling and testing for HIV at the unit and were not previously known to have kidney disease.

2.5.1 Inclusion criteria

1. Diagnosed HIV positive
2. Not previously diagnosed with diabetes mellitus, hypertension, obstructive uropathy, and clinical features of glomerulonephritis preceding HIV seropositivity.

2.5.2 Exclusion criteria

1. Pregnancy
2. Congestive cardiac failure
3. Unable or unwilling to give consent
4. Evidence of active infections such a tuberculosis, urinary tract infection(UTI) and other opportunistic infections until treated

2.5.3 Control Group for genetic studies:

1. 50 HIV positive patients without evidence of CKD
2. 52HIV negative individuals without HIV and evidence of CKD **2.6 Methods**

Patients were recruited from the HIV clinic at the SS Wali Center of the hospital after signing an informed consent form. Their demographic variables including age, sex, ethnic origins, address, religion, marital status etc. Each study participant was assigned a unique identification number and underwent a focused medical examination by the researcher and findings including systolic and diastolic blood pressures, height, weight and body mass index, among others, were recorded. Urinalysis was performed by the researcher on a mid-stream urine samples using a Combi-screen 9 urine Dipstick (Biotechnologies AG, Lichtenfels, Germany). Patients with a protein positive urine test underwent a repeat urinalysis after two weeks.

Spot urine protein/creatinine ratios (uPCR) were carried out for those individuals with persistent proteinuria. Those patients who had persistent proteinuria (uPCR ≥ 150 mg/g on 2 occasions) were referred for further Nephrology review and possible kidney biopsy.

Approximately 10mls of venous blood was collected for routine baseline assessments that included complete blood count, urea, creatinine, CD_4 count, lipid profiles, Hepatitis C antibody and Hepatitis B surface antigen which were all part of the routine workup of the patients prior to commencement of ARTs in the clinic. Another 5 mls of blood was collected at 12 weeks interval during their routine follow up for repeat creatinine in those who had elevated serum creatinine to establish the diagnosis of CKD.

Percutaneous kidney biopsy under ultrasound guidance was performed by the researcher on patients who have kidney disease based on reduced eGFR and or persistent proteinuria that were confirmed after a minimum of three months interval.

For the purpose of this study, persistent proteinuria was defined as the "presence of dipstick protein ≥ + 1 or a urine protein: creatinine ratio (uPCR) ≥ 150 mg/g (on 2 occasions at least 2 weeks apart)" and considered a reduced eGFR to be less than 60 mls/ min/ 1.73m^2. Only those

that had a repeat after 3 months were considered as having CKD. The patients were followed up every month for the first 3 months and subsequently every 3 months for 12 months.

Figures 5 and 6 below depict a flow chart of the methods for both the CKD prevalence and the genetic study components respectively.

Figure 5. Flow chart showing the proposed methodology for the CKD prevalence study

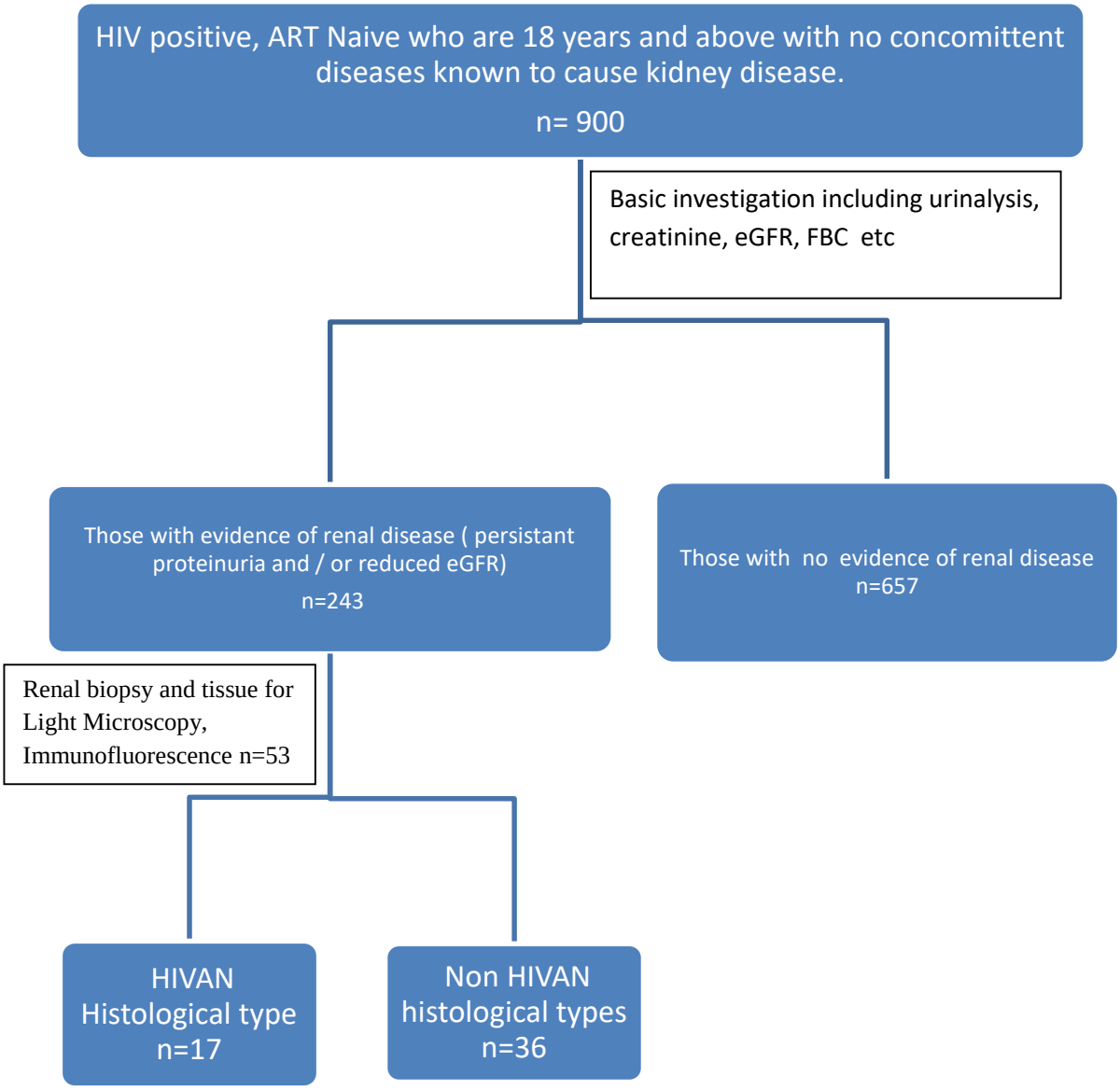
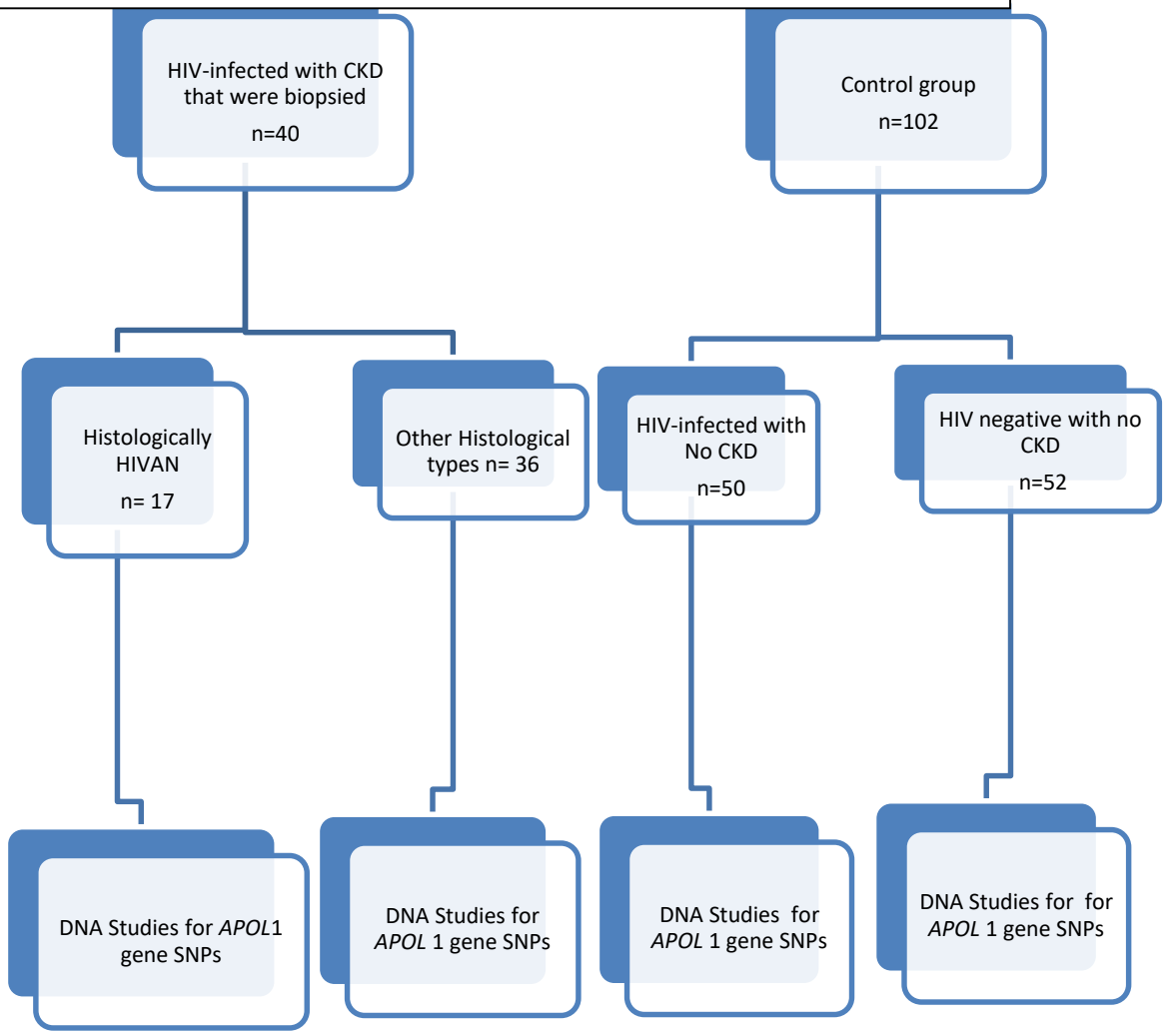


Figure 6 : Flow diagram for the genetics aspect of the study



2.7 Procedures

2.7.1 Kidney Biopsy

Patients for kidney biopsy were all counselled on the procedure and requested to sign a consent form before the procedure. They also had some basic laboratory evaluation including complete blood counts, platelets and clotting profiles to rule out any possibility of bleeding.

Patients were positioned in a prone position on a pillow that was rolled up firmly to compress the upper abdomen and lower ribs in order to fix the kidneys in place. Pre- biopsy vital signs were recorded after setting up intravenous saline. After scrubbing and draping the patient, a 5-10ml of 2% Xylocaine injection were used to infiltrate the site of needle insertion. The biopsy was carried out under ultrasound to guide the insertion of the spring-loaded biopsy gun (BARD 16/18 G, 22 mm cutting edge) and the tissue from the biopsy was placed in a labelled container holding 10% neutral buffered formalin solution.

Following the biopsy procedure the vital signs of the patients were recorded and they were confined to a prone position for a minimum of 30 minutes, to ensure pressure haemostasis. The procedure was carried out on an outpatient basis except for inpatients that might be admitted for other reasons and have no contraindications to kidney biopsy. Following observation for a minimum of eight hours post biopsy, the patients were discharged with instructions to monitor and report any complications.

2.7.2 Processing of Tissue Samples

Tissues obtained from the biopsies were fixed in 10% neutral buffered formalin solution. Histological sections were stained with Haematoxylin and Eosin (H&E) and Periodic Acid Schiff (PAS), Jones methenamine silver as well as Congo red and Masson's trichrome stains. An experienced and well trained Consultant Histopathologist with a bias in kidney pathology at

AKTH reviewed and reported on the histological slides sections. All tissue samples underwent light microscopy. At the time the study commenced no facilities for immunofluorescence (IF) were available. However, towards the end of the study an IF service became available and 10 of the samples underwent IF analysis. As electron microscopy was not available in our center it did not form part of the tissue evaluation.

2.7.3 Genomic DNA extraction:

Venous blood samples of about 7.5mls were collected in an EDTA bottle and stored in a special fridge at -20^{0}C in the laboratory section of the SS Wali Center and subsequently transported to the University of Witwatersrand, Johannesburg (Department of Internal Medicine Research Laboratory) where the DNA extractions were carried out. The blood samples were stored until a batch of 50 were collected and these were subsequently transported.

The Maxwell DNA purification kit (Promega AS1010, USA) was used to extract DNA from the whole blood, as per the manufacturer's protocol. DNA concentrations were determined by the NanoDropTM 2000 spectrophotometer (Thermo Scientific, USA) and the quality was assessed by means of the A260/280 ratios. A 260/280 ratio of 1.8 to 2.0 was considered as indicative of a pure DNA preparation free of contamination.

DNA samples were genotyped for G1 (SNPs rs73885319, rs60910145) and G2 (rs71785313), the known kidney disease risk variants using a PCR–Restriction Fragment Length Polymorphism (PCRRFLP) as described in Nqebelele *et al.*[124] Briefly, a 420–base pair product was amplified using forward primer (5′-ACC AAC TCA CAC GAG GCA TT-3′) and reverse primer (5′-CTG CCA GGC ATA TCT CTC CT-3′) and KAPA2G Robust HotStart ReadyMix PCR Kit (Kapabiosystems, Boston, MA). Cycling conditions for PCR: 95^0 C for 3 minutes, 40 cycles of 95^0 C for 15 seconds, 60^0 C for 15 seconds, and 72^0 C for 20 seconds. The 3 *Apo*L1 single

nucleotide polymorphisms rs73885319, rs60910145, and rs71785313, were genotyped by RFLP analysis using restriction enzymes *Hind*III, *Nsp*I, and *Mlu*CI, respectively, followed by resolution on 1.5% agarose gels.[124]

2.7.4 Other Laboratory Measurements

Creatinine in both the serum and Urine was measured using modified Jaffe method using the Clinical Chemistry Auto Analyser (Cobas C 311, Roche Diagnostics, Rotkreuz, Switzerland) which is based on the principle that creatinine in an alkaline solution will form a yellow-orange complex with picrate such that the colour intensity is proportional to creatinine concentration. The creatinine assay is calibrated to isotope dilution mass spectrometry (IDMS)-traceable reference material. The CKD-EPI equation,[57] with no ethnicity factor, was used to calculate the eGFR.

2.8 Data Analysis

Data was analysed using a computer based software STATA v14.2 (Stata Corp, College Station, TX). Demographic and clinical characteristics were expressed as means and standard deviation (SD) Student T test was used to compare means of quantitative variables in between groups such as between those with CKD and with no CKD, while the ANOVA test was used for comparison in more than 2 groups eg in the analysis of clinical parameters between 3 groups (those with FSGS, those with HIVAN and those with other histologic types). Categorical variables were expressed as frequencies and compared using a chi-squared test such as in the comparison of the prevalence of the various risk factors of CKD between those with CKD and those with no CKD. Associations between the risk of kidney disease and *APOL1* risk alleles were explored using logistic regression models. A two-tailed p-value <0.05 was considered significant.

2.9 Ethical Approval

Ethical approval to conduct this study was obtained from the Research Ethics Committee of AKTH (NHREC/21/08/AKTH/1090) and the Human Research Ethics Committee of the University of the Witwatersrand (M140116). Prospective study participants were counseled about the study prior to receiving their consent and the study was conducted in accordance with the declaration of Helsinki.

No added costs were incurred by patients because of this study and there was no coercion. Patients continued to receive the same standard of care even if they did not consent to participate in this study.

An MTA agreement was drawn up between the two institutions for the transfer of the biological material.

CHAPTER THREE. RESULTS

Demographic characteristics of the study population

Nine hundred patients were studied; the mean age of our study population was 34.17 ± 10.0 years with a range of 20-50 years. Most of the study participants were females [588 (63%)]. Majority of the participants were married [595(55%)], followed by singles (185) while 15.7% were widows. Eighty percent of the participants had western education with the majority (40%) having secondary level, 25% having tertiary level education. Many of the participants were currently not employed (39%) and 31% were businessmen, 11.8% were civil servants, 2.4% were long distance drivers, 2.1% were factory workers. The remaining had various types of employment. Most of the participants self-reported Hausa as their ethnic group (63.5%) with 6.2% as Fulani, 3.7% Igbo, and 3.2% Yoruba while other minor ethnic groups constituted the rest.

Clinical and laboratory characteristics of the study population

Table 3 depicts the clinical and laboratory parameters of the study population.The mean systolic blood pressure (SBP) of the study population was 122.5 ± 24.8 mm Hg and diastolic blood pressure (DBP) was 79.9 ± 18.2 mm Hg. Hypertension was diagnosed in only 25 (2.7%) of the study population. There was also no significant difference in either SBP or DBP between those with and those without CKD.

Obesity was present in 128 (28.2%) of the participants and there was no significant difference observed in the prevalence of obesity between the CKD group and the no CKD group.

The mean uPCR of the study population was 236.23 ± 130.7 1 mg/g, it was however it significantly lower among those without CKD compared to those with CKD. Only 4 patients had

proteinuria of nephrotic range, one of whom had kidney biopsy with no significant pathological findings on light microscopy, suggesting a probable minimal change disease.

Table 3. Clinical characteristics and laboratory parameters of the study population [n=900]

Age, years	34.2 ±10.0
Female sex	n=588 (63.5%)
Mean Systolic BP, mm Hg	122.5 ± 24.8
Mean Diastolic BP, mm Hg	79.9 ±18.2
Hypertension	n=25 (2.7%)
Mean fasting plasma glucose, mmol/L	5.28 ±2.1
Diabetes mellitus	n=72 (8.0%)
Mean BMI, (kg/m2)	21.4 ± 6.1
Underweight	n=162 (18%)
Normal BMI	n=540 (60%)
Overweight	n= 135 (15%)
Obese	n=63 (7%)
Mean Haemoglobin, (g/dL)	10.3 ± 2.0
Mean WBC count (10^9/L)	4.8 ± 1.7
Mean Platelets counts (10^9/L)	238.0 ± 117.8
Hepatitis C Antibody	n=11 (1.2%)
Hepatitis B Surface antigen	n=95 (10.58%)
Mean CD4 count, (cells/mm3)	238.36 ± 120.8
Mean uPCR (mg/g)	236.23 ± 130.7

Mean eGFR (ml/min/1.74m^2)	86.18 ±38.10

uPCR= urine protein creatinine ratio, eGFR = estimated glomerular filtration rate, WBC= white blood cells, CD4= Cluster of differentiation 4, BMI= Body Mass Index

Risk factors for CKD

Among the study participants, the majority (67.8%) had a positive history of use of traditional herbal medications, 8.8% admitted to the use of analgesic drugs, alcohol ingestion was noted in 10.8%, cigarette smoking in 19% and use of medicated soap was reported in 16%. There was a family history of hypertension in 35% and family history of kidney disease in 5.7%, and 16.1% had positive family history of diabetes, as shown in Table 4. Significant differences in the occurrences of most of the traditional CKD risks factors were noted, with higher prevalence among those with CKD compared to those with no CKD.

Table 4. Comparison of the risk factors for CKD among the study population

Variables	**All [n=900]**	**With CKD [n=205]**	**No CKD [n=695]**	χ^2/student t	**p value**
Systolic BP (mmHg)	122.5± 24.8	112.06±10.58	112.87±9.10	t = 1.02	0.720
Diastolic BP (mmHg)	79.9±18.2	69.34±9.16	69.65±9.40	t=0.39	0.920
Hypertension	24	11	13	t= 0.89	0.620
Traditional medicine use	572	126	446	$\chi^{2\ =}$62.5	0.002
Skin lightening cream	136	20	116	$\chi^{2=}$62.8	0.001
Analgesic use	75	14	62	$\chi^{2=}$62.8	0.001
Alcohol ingestion	92	17	75	$\chi^{2=}$61.4	0.001
Cigarette smoking	163	36	127	$\chi^{2=}$ 56.4	0.001
FH of hypertension	297	61	236	$\chi^{2=}$59.4	0.001

FH of diabetes	137	27	110	$\chi^{2=}$56.2	0.004
FH of kidney disease	48	8	40	$\chi^{2=}$59.1	0.001
eGFR (ml/min/1.74m^2)	86.18 ±38.10	39.34±13.94	101.20±30.42	t= 28.99	p<0.001
BMI (kg/m2)	21.4 ± 6.1	19.20±5.3	22.10±6.3	t =6.10	0.001

BP= Blood Pressure, BMI- Body Mass Index, eGFR estimated glomerular filtration rate, FH= Family History, CKD= Chronic Kidney Disease

Prevalence of CKD

The prevalence of CKD using the single eGFR measurement at recruitment was 27% when the CKD-EPI equation, without race factor, was adopted.

Among those individuals that had creatinine measurements, one at initiation of care and another at least 3 months later, the prevalence of CKD using the CKD-EPI equation without race was 22.8%. The majority of those with CKD 146 (71.21%) had stage 3 disease while 45 (21.95%) and 14 (6.82%) had stages 4 and 5 using the KDIGO classification.[125]

Multivariate logistic regression analysis identified age, female sex, low BMI, low CD4 cell count and positive history of cigarette smoking as factors with high odds associated with CKD that reached statistical significance as shown in table 5 .

Table 5. Multivariate logistic regression modelling factors associated with the presence of CKD

Parameter	OR, 95%(CI)	P
Low BMI	3.73 (1.56-4.69)	<0.010

Cigarette smoking	1.65 (1.47-4.8)	<0.010
CD4 cell count (<200 cells/mm3)	2.44 (2.20-5.39)	<0.010
Age	1.61 (1.12-2.54)	<0.010
Female gender	1.93 (1.28-2.92)	<0.010

OR: Odds ratio, CI: Confidence interval, BMI: Body mass index, CD4: Cluster of differentiation

Outcomes

All the patients in the study were commenced on ART based on the national treatment guidelines at the SS Wali Center of the hospital. Similarly, those that were diagnosed with CKD had further follow up at the nephrology unit with further evaluations including kidney biopsy and specific treatments such as ACEi or ARTs as appropriate.

The 14 patients with stage 5 CKD were followed up at the Nephrology Clinic for a year. Eight of them became dialysis-requiring within the first 6 months of follow up. Two were lost to follow up and the remaining 4 died of other complications such as sepsis within the follow-up period.

Among the 200 patients with CKD that we followed up, 69 (34.5%) showed improvement in kidney function after starting ART within the first 6 months of follow up. The mean CD4 improved from 238.36 ±120.8 cells/mm^3 to 368.36 ± 114 cells/mm^3 (p=0.0001), while the mean eGFR improved from 86.18±38.10 ml/min/1.73m^2 to 94.40 ml/min/1.74m^2 (p=0.0002) and the mean uPCR decreased from 236.23 ±130.7mg/g to 134.35 ± 92.6mg/g (p=0.0001)

Histological Characteristics

Histology showed that the most commonly reported lesion (in 37.7% of patients) was FSGS while HIVAN, characterised by lesions including cystic tubular dilatation, collapsing FSGS,

interstitial infiltrates, and dilated tubules was noted in 32% (17) of the patients. Six patients (11.3%) were reported to have no pathological finding. Among these 6, one patient had proteinuria of nephrotic range and is most probably minimal change disease. Table 6 depicts the histopathological lesions on kidney biopsy while table 7 depicts the frequencies of the various histological diagnoses among the patients that had kidney biopsy,.

Compared to non-HIVAN patients, patients with HIVAN tended to have higher serum creatinine levels (p= 0.005); lower eGFR (0.030) and higher uPCR; (p= 0.020), while there were no significant differences in parameters such as age, haematological measurements, diastolic BP, BMI, and CD4 count. When comparing the clinical and laboratory parameters, no differences between HIVAN and FSGS were observed. Those with HIVAN had lower eGFR compared to those with other histologic types of CKD and this difference reached statistical significance (p=0.030).

Table7. Clinical and laboratory parameters of the patients who had kidney biopsies

Parameter	All patients; n= 53	Non-HIVAN; n=36 (68 %)	HIVAN; n=17(32 %)	p-value
Age (years)	38.3± 9.3	40.5± 11.3	37.2± 8.1	0.510
Gender (M/F)	36/17	25/11	11/6	
Body Mass Index (kg/m^2)	22.26± 26	22.5± 7.7	21.9± 6.6	0.770
Systolic BP (mm Hg)	124± 67	121.3± 26.3	122.7± 16.3	0.050
Diastolic BP (mm Hg)	79.00± 8.5	73.31± 10.8	76.4± 10.8	0.160
Urea (mmol/L)	28.7± 18.3	17.67± 12.0	29.6± 18.1	0.040
Creatinine (µmol/L)	249.62± 164.6	237± 89.9	256.7± 195.2	0.050
eGFR (ml/min/1.74m^2)	40.0± 19.2	41.0± 14.3	37.1± 17.4	0.030
uPCR (mg/g)	846.23± 130.7	233.8± 65.0	402.0± 16	0.020

CD4 (cells/mm3)	238.36± 210.8	238.6±191.0	184.9± 82.7	0.090
Haemoglobin g/dL	10.3± 2.0	10.4± 1.8	10.1± 2.2	0.770
White Blood Cells (10^9/L)	4.8± 1.7	4.8± 1.8	4.7± 1.5	0.810
Platelets (10^9/L)	238.0± 117.8	244.6± 122.1	225.1± 111.4	0.340
Albumin g/L	30.2± 1.6	31.8± 1.5	26.9± 1.5	0.330

uPCR= urine protein creatinine ratio, eGFR = estimated glomerular filtration rate.

Table 6. Histopathological findings of the study population

Histologic Diagnosis	**Frequency (%)**
Focal Segmental Glomerulosclerosis (FSGS)	20 (37.7)
HIV Associated Nephropathy (HIVAN)	17 (32.1)
Chronic Interstitial Nephritis	7 (13.2)
No significant pathological finding	6 (11.3)
Acute Tubular Necrosis	1 (1.9)
Hypertensive Nephrosclerosis	1 (1.9)
Membranoproliferative glomerulonephritis	1 (1.9)

***APOL1* genotype**

A total of 155 participants were studied with a mean age of 36.0 ±9.32 years and 55% were males. Complete data on genotyping was available for 142 participants (40 HIV positive CKD

patients, 50 HIV positive controls and 52 HIV negative controls). The SNP data was tested for any departure from Hardy-Weinberg equilibrium.

Table 8 shows the characteristics of the participants. The HIV positive CKD patients had higher blood pressure (systolic: 120 vs 110 mmHg, p=0.001) when compared with the no-CKD controls, both HIV positive and negative.

Table 8. Comparison of the clinical characteristics of *APOL1* gene study participants

Characteristic	**HIV CKD (n=53)**	**HIV positive no CKD controls (n=50)**	**HIV negative controls (n=52)**	**p-value**
Male, n (%)	34 (64.2%)	20 (40.0%)	32 (61.5%)	0.027
Creatinine (µmol/L)	210 (143 - 302)	82 (70 - 90)	82 (73 - 87.5)	<0.001
eGFR (ml/min/1.74m^2)	34.4 (26.4 - 48.9)	107 (87 - 128)	110.5 (100.5 - 120.5)	<0.001
Systolic BP (mm Hg)	120 (110 - 132)	110 (110 - 120)	110 (110 - 120)	0.001
Diastolic BP (mm Hg)	77 (65 - 84)	60 (60 - 70)	69.5 (60 - 70)	<0.001
BMI (kg/m^2)	22.26 ± 2.6	22.5 ± 7.7	21.9 ± 6.6	0.770
CD4 (cells/mm3)	115 (74 - 304)	265 (179 - 402)	-	0.005

BMI= Body Mass Index, eGFR , estimated Glomerular Filtration Rate

Table 9 shows the frequencies of the various haplotypes across different groups in the study participants.The individual risk allele frequency among all study participants was 26.1% (37/142) for G1 and 19.0% (27/142) for G2. The haplotype G-G-I (G1^{GM}), comprising the derived allele at both rs73885319 (p.S342G) and rs60910145 (p.I384M) and the ancestral insertion (I) allele at rs71785313, was the most frequent G1 configuration seen in 20.4% of all the participants for the genotype study.

Table 9. *APOL1* risk allele frequencies

***APOL 1* Variants**	**HIV positive CKD (n=40)**	**HIV Positive no-CKD (n=50)**	**HIV Negative no-CKD controls (n=52)**	**p-value**	**HIV+ CKD vs HIV+ no-CKD p-value**	**HIV+ CKD vs HIV- no-CKD p-value**
rs73885319						
AA, n (%)	32 (80.0%)	43 (89.0%)	36 (69.2%)	0.008	0.143	0.023
AG, n (%)	5 (12.5%)	7 (14.0%)	16 (30.8%)			
GG, n (%)	3 (7.5%)	0	0			
rs60910145						
TT, n (%)	30 (75.0%)	43 (86.0%)	36 (69.2%)	0.153	0.289	0.373
TG, n (%)	9 (22.5%)	7 (14.0%)	16 (30.8%)			
GG, n (%)	1 (2.5%)	0	0			
rs71785313						
Insertion/Insertion	28 (70.0%)	46 (92.0%)	41 (78.8%)	0.027	0.007	0.331
Insertion/Deletion	12 (30.0%)	4 (8.0%)	11 (21.2%)			
Deletion/Deletion	0	0	0			
Haplotypes						
G0	35	50	49			

$G1^{GM}$	8	6	15			
$G1^{G+}$	2	1	1			
$G1^{+M}$	2	1	1			
G2	12	4	11			

HIV= Human Immune deficiency Virus, CKD= Chronic Kidney Disease,

The frequency of the HRG (a haplotype comprising of two missense mutations, rs73885319 and rs60910I45 (-/TTATAA)) and G2 (comprising of two amino acid deletion at rs71785313) was 5.6% (8/142) among all the study participants. Three participants had the homozygous G1/G1 genotype and five had the compound heterozygote G1/G2 form; none of the study participants had the homozygous G2/G2 form. However the frequency of the HRG was 12.5% among those with HIV CKD compared to 5.8% in the HIV negative controls and none in HIV positive no-CKD group; this difference was statistically significant (p=0.025). Table 10 compares the frequencies of the risk alleles among the various study groups.

Table 10. *APOL1* risk alleles among the study participants

Characteristic	**HIV positive CKD (n=40)**	**HIV positive no-CKD controls (n=50)**	**HIV-negative no-CKD controls (n=52)**	**p-value**	**p-value HIV+ CKD vs HIV+ no-CKD**	**p-value HIV+ CKD vs HIV- no-CKD**
0 risk alleles	20 (50.0%)	38 (76.0%)	27 (51.9%)	0.016	0.007	0.516
1 risk alleles	15 (37.5%)	12 (24.0%)	22 (42.3%)			
2 risk alleles	5 (12.5%)	0	3 (5.8%)			
0 risk alleles						

G0/G0	20 (50.0%)	38 (76.0%)	27 (51.9%)	0.013	0.015	1.000
1 risk allele						
G0/G1	5 (12.5%)	8 (16.0%)	14 (26.9%)	0.13	0.175	0.673
G0/G2	10 (25.0%)	4 (8.0%)	8 (15.4%)			
2 risk alleles						
G1/G1	3 (7.5%)	0	0	0.025	0.015	0.288
G1/G2	2 (5.0%)	0	3 (5.8%)			
G2/G2	0	0	0			

The study showed a 10.8 fold higher odds for HIVAN among those with 2 risk alleles vs no risk alleles (OR 10.83, 95%CI 1.38 - 84.52; p=0.023) and a 5.5 fold higher odds for those with 2 vs 1or 0 risk alleles (OR 5.50, 95%CI 0.83 - 36.29; p= 0.070) compared to controls. These associations however do not reach significance after adjusting for age and gender. Associations were stronger among those with FSGS (2 vs 0 risk alleles: OR 13.0, 95%CI 2.06 - 81.91; p=0.006 and 2 vs 0 or 1 risk alleles: OR 9.0, 95% CI 1.62 - 50.12; p= 0.012) and remained significant after adjusting for age and gender. Table 11 depicts the OR in the various groups, both unadjusted and after adjustments for age and gender.

Table 11. Odds ratios for associations of *APOL1* risk alleles with HIVAN and FSGS

	Unadjusted OR (95% CI)	p-value	Adjusted OR* (95% CI)	p-value
HIVAN				
No of Risk Alleles				

1 vs 0	3.82 (1.07 - 13.61)	0.038	2.51 (0.63 - 9.92)	0.189
2 vs 0	10.83 (1.38 - 84.52)	0.023	4.81 (0.52 - 44.90)	0.168
2 vs 1	2.83 (0.40 - 19.87)	0.295	2.58 (0.36 - 18.63)	0.347
2 vs 1 or 0	5.50 (0.83 - 36.29)	0.077	3.76 (0.54 - 26.35)	0.182
FSGS				
No of Risk Alleles				
1 vs 0	2.29 (0.65 - 8.07)	0.196	1.55 (0.40 - 6.00)	0.526
2 vs 0	13.0 (2.06 - 81.91)	0.006	14.00 (1.77 - 110.67)	0.012
2 vs 1	5.67 (0.92 - 34.99)	0.062	5.30 (0.83 - 33.95)	0.079
2 vs 1 or 0	9.0 (1.62 - 50.12)	0.012	8.85 (1.55 - 50.74)	0.014

*for age, gender

CHAPTER FOUR. DISCUSSION

This study reported the prevalence of CKD and its risk factors, histopathologic pattern of kidney diseases and prevalence of *APOL1* kidney risk variants among a cohort of treatment naïve HIV-infected patients in the northern part of Nigeria. The majority of the study population were females, similar to reports by many others.[37, 38.39] Also reflecting findings from other studies, the mean age of the study population was in the young productive age range between 30 and 50 years.[37, 38]

The prevalence of risk factors for CKD was noted to be high among the study population, especially the positive history of use of traditional medicines noted in the majority of them (68.7%). This is similar to our earlier findings among civil servants in the Kano Metropolis during a screening exercise.[126] This is important as most of these herbal preparations were not accurately characterized, thus the active ingredient is not known and studies from the southern part of Nigeria reported the nephrotoxic nature of some of them.[127] This high prevalence of CKD risk factors may also have additive effects on CKD among these patients as they already have HIV specific kidney risk factors, such as low CD4 cell count and HIV viremia. It is of note that the prevalence of some of the risk factors are relatively low such as cigarette smoking and alcohol consumption, and may be due to the socio cultural background of the study population. The prevalence of hypertension and diabetes in the study population were also very low, 2.7% and 8.0 % respectively. This may be as a result of our exclusion criteria that did not allow those previously known to be hypertensive or diabetic to be enrolled into the study.

This study found a high prevalence of CKD among treatment naive HIV patients similar to previous reports from different parts of the country as well as in a systematic review of studies from different parts of Africa.[124] Although CKD prevalence in this study was lower than earlier

reports from other regions of the country such as the rate of 38% in Ile-Ife, South Western Nigeria;[38] 53.3% in Benin City in South Southern part;[39] 31% in Zaria, North Western region [40] and 51.8% in Jos and 47.6% in Ilorin respectively, both in the central part of the country. The finding is also lower than our previous report from a study done in this center.[82] However the finding in this study is similar to the 23.5% and 22.9% prevalence reported from Lagos in South West and Oweri in South East respectively. [129,130] These previous studies were however limited by the use of single eGFR measurement which cannot discriminate between CKD and AKI among these patients. The finding in this study is higher than the CKD prevalence of 13.4% reported recently by Ekripo *et al*[131] in the South Southern part of the country, that used 2 measurements of GFR and also used the CKD-EPI equation, similar to this study

Majority of the patients with CKD in this study had stage 3 disease. This has implications for management, as initiation of measures such as early initiation of ART and other kidney-specific interventions like the use of ACE-inhibitors (or angiotensin receptor blockers) to reduce proteinuria and control of blood pressure as well as avoidance of nephrotoxic agents among others**,** will help in slowing down or stopping progression to ESKD. This provides a cost effective means of reducing CKD associated mortality, given that access to treatment of ESKD in this environment is limited and expensive.

Histopathological pattern of kidney diseases

The results from this study are in keeping with the reported wide spectrum of kidney changes that accompany HIV infection and are very similar to what has been shown in other studies; with reported pathologies of the tubules, glomerulus, tubules, and interstitium.[73, 132] It has been postulated that the many pathogenetic mechanisms of HIV underlie these observations and are a result of HIV gene expression in the kidney compartments and lesions related to co-infections, comorbidities, immune dysregulation and administration of various drugs. These observations highlight the critical importance of the utilisation of a kidney biopsy in HIV patients.

The predominant lesion in our study was FSGS (37.7%), followed by HIVAN (32.1%) and then interstitial nephritis together with various others. This broad variation in the observed histological pattern has also been observed globally and is believed to be due to a combination of factors such as geographical location, the ethnicities of the populations involved and the sample size of the studies.[86,87]

In Nigeria, only very few studies have been reported that show sufficient numbers of HIV patients that have undergone a kidney biopsy. In a study by Emem-Chioma *et al*[38] it was shown that 7 of 10 biopsied patients revealed HIVAN diagnosis on histology. Earlier reports from our center reported biopsy-proven HIVAN in 55% of a 25 patient series.[82] Although these earlier reported frequencies of HIVAN are higher than that reported in this current study, at 32.1%, the sample size of this current study is somewhat larger, and therefore provides a better indication of true prevalence. Another possibility may be the hypothesized change in kidney disease epidemiology in the HIV-infected population that has seen a decline over time in the frequency of HIVAN.[73]

Most of the histopathological studies from sub-Saharan Africa emanate from South Africa, with a 104 patient biopsy review in Johannesburg, reporting classic HIVAN in 27%, with immune complex disease and other glomerular diseases present in 21%. A single center in Cape Town reported HIVAN as the most frequent (57.2%) histopathologic lesion, followed by "HIVAN with immune complex glomerulonephritis (ICGN)" in 21.8%; 12.5% with no HIVAN or ICGN; and 8.3% with ICGN.[73] In Kenya, Koech *et al*[133] reported that of the 27 patients that underwent a biopsy, none had classic HIVAN and the majority of patients (41%) had interstitial nephritis. The rarity of HIVAN as a histologic diagnosis was reported in a study of a white HIV-positive Italian cohort as well as another study from Thailand with varying degrees of proteinuria.[82,134] These observations are at odds with the reports from our center that HIVAN is the second most frequent diagnosis. Genetic variation could explain some of the variability, especially the observed lower prevalence of high-risk *APOL1* genotypes reported in the current study population, especially when compared to the population prevalence observed in West Africa. Studies from the USA also reported variable findings; whilst a study from Baltimore showed that HIVAN and FSGS were the major diagnoses, another study from Columbia University reported DN and ICGN as the most common forms of disease.[85,86]

Globally kidney biopsy reviews commonly report on the presence of HIV-related tubulointerstitial disease. In our study cohort 15% had tubulointerstitial disease; while this was higher than the 7.9% reported by Berliner *et al*[85], it is lower than that reported from India and Kenya at 27.7 % and 41%, respectively.[133] The use of concurrent medications, use of traditional herbal medicines and other infections, may account for the higher frequency of tubulointerstitial disease in this patient cohort.

In the HIV setting, immune complex diseases are also prevalent and include "IgA nephropathy, lupus-like glomerulonephritis (GN), membranous glomerulopathy, post-infectious GN, mesangiocapillary GN and the 'ball-in-cup' phenomenon".[13] In this study, of those samples that underwent IF none had immune complex lesions. In contrast, a study of 20 cases from Johannesburg showed HIV immune-complex kidney to be present in 8 (40%) of their patients.[135] This variation may be explained by the smaller number of samples that underwent IF (10) in this study or it may actually reflect a lower immune-complex disease prevalence in Kano. This highlights the need for additional studies to be conducted since to date there has been no other study utilizing IF in disease characterization.

In the HIV-infected patient, the involvement of the kidney can present at any stage of the illness although HIVAN is thought to occur later in the disease. For this study, newly diagnosed and treatment naïve HIV patients were recruited, and were therefore presumed to be at an earlier stage of their illness. However, the low level of observed CD4 cell counts is suggestive of advanced disease.

There were no complications noted from the kidney biopsy procedure in this study. However, it was not expected that HIV-infected patients should have a greater risk of complications than the general population.

***APOL1* genotype**

The prevalence of the individual risk alleles of G1 (26.1%) and G2 (19.0%) in this population was lower than that reported in previous studies involving Nigerian populations; 45 to 50% for G1 among Yoruba people in the South Western and the 30% among the Igbo speaking people in the South Eastern parts of the country.[122,123] It is also lower than the 40% reported for the Ashanti group and higher than the 11.4% reported among the Bulsa group, both from Ghana.[10o]

The G1 prevalence in our study participants was similar to the 20-22% reported from 3 surveys among African-Americans.[92,100] The prevalence was however higher than the 7.3% for G1 and 11.1% for G2 reported in a South African black population.[101] The G2 prevalence of 19% in this study was higher than the 7.5 % and 16.6% reported in the Yoruba speaking people.[100,123] The G2 frequency in this study was lower than the 24.4% in the Igbo and 21.4 % in the Bulsa in Ghana.[100,122] It was however similar to the 13-15% among African-Americans in the US and the 12.9% among the Asante group in Ghana.[92,100] Variations in the frequencies of these alleles among diverse African ethnic populations was also reported by Limou[100] when they studied 676 samples from 12 African populations that included Cameroon (2 ethnic groups), Congo, Ethiopia (4 ethnic groups), Ghana (2 ethnic groups), Malawi, Mozambique and Sudan. The risk allele frequencies differ between groups from the same country and also across the continent; for example in the Bulsa and Asante populations of Ghana, the frequencies are 11% and 41% respectively.[100] The findings in this study also differ from that of an Ethiopian population where they reported zero frequencies of these *APOL*-1 risk alleles among HIV-infected patients with associated absence of HIVAN.[102]

From the lower frequencies of these individual alleles in our study, it may be possible to speculate on a possible relaxation of the natural selection push exerted by the Trypanosome infection in these regions by ancestors of the ethnic populations in this Northern part of Nigeria. The study area is some distance from the trypanosome infection belt and the Atlantic coastal areas of West Africa, the hub for the trans-Atlantic slave trade from which most African Americans trace their ancestral journeys to the Caribbean and the Americas. [134] There is a need for a multicenter comparative study with a large sample size to explain the differences in the frequencies of these alleles in different geographic locations within the same country. The

frequencies of the HRG defined by the presence of 2 risk alleles (G1/G1, G2/G2, or G1/G2) among all participants in this study was 5.6% and it was 12.5% among the HIV positive CKD group. The overall frequency was thus lower than the 11% reported for African-Americans and 7% for DRC children.[136] The differences between the groups was also noted by Kasembeli *et al* in which the frequency of the HRG was 17% among all participants but was as high as 79% among those with HIVAN.[101]

Our study found that having the 2 high risk allele genotype was a predictor of FSGS compared to zero risk alleles and this association was maintained even in the recessive form.The association was also strong with HIVAN with a less strong association in the recessive form. Kopp *et al* also[92] reported this association in a cohort of FSGS and HIVAN; a greater association was observed among HIVAN compared to FSGS. Kasembeli *et al* reported an even greater association with HIVAN among a black South African cohort but no significant association with FSGS.[101] This lower strength of the association among our patients may be due to a smaller sample size compared to both the Kopp and Kasembeli studies.[92,102] This calls for further study with a larger sample size on the effect of this HRG and other *APOL*1 nephropathies, not only among HIV-infected patients but also in the general population.

A strength of this study is the relatively large number of patients that were recruited and the ability to diagnose CKD with two measurements of eGFR at least 3 months apart, thereby fulfilling the diagnostic criteria for CKD by KDIGO, as most other previous studies based their CKD diagnosis on a single serum creatinine and eGFR measurement, with all its limitations. A further strength is the number of patients with kidney disease that underwent a kidney biopsy from a broader geographical area compared to other studies from Nigeria that had smaller numbers of biopsied patients. Due to advantages in relation to resources and the capacity to

perform kidney histology, our institution is the referral center for kidney biopsies from other centers in the region. Despite the small number, ten of the biopsy specimens underwent IF and this is the first report of IF from our region. Most of the other studies emanating from this region only report light microscopic evaluations.

This study provides the first information on the high frequencies of the *APOL1* genotypes among treatment naïve HIV-infected patients from this environment, though the frequencies were not as high as those reported among ethnic groups in the western and eastern parts of Nigeria. The study also confirmed the association between the occurrence of these HRGs and kidney disease, and specifically FSGS and HIVAN histological types among HIV-infected patients despite the relatively lower frequencies; this was hitherto not reported from this environment. This calls for the inclusion of evaluation of kidney function among newly diagnosed HIV patients as recommended, [131] to allow for early detection and subsequent initiation of ART.

This study is limited by the fact that we were not able to continue with the longitudinal aspect of the study because of funding issues that resulted in inadequate follow up and withdrawal of support for routine investigations in the HIV clinic. In spite of this limitation, the study found improvement of kidney function with the initiation of HAART, similar to findings by others.

Szczech reviewed the relevant literature supporting early initiation of ART among persons with HIV and kidney disease and concluded that early initiation of ART probably improved outcomes among persons with HIVAN. Studies in some parts of sub Saharan Africa such as Fabian et al, Mulenga et al and the DART study suggested similar improvement of kidney function with ART initiation.[117,118,119] Although Szczech et al. found no benefit for non-HIVAN kidney disease, the South African studies by Wearne et al. and Fabian et al. reported benefit irrespective of the histological lesion.

The small sample size and cross sectional design of the genetic component of the study did not allow for the assessment of the impact of the HRG on the progression of the kidney disease.

Recommendations

1. The high prevalence of CKD and its risk factors among this cohort of treatment naïve HIV-infected patients from this environment calls for HIV programs to include CKD screening in the initial evaluation as well as regularly at follow-up visits of these patients. This will allow for early detection and management of those with established CKD to prevent or slow progression to ESKD.
2. Early referrals to nephrologists for advanced care should be encouraged in those that have persistent proteinuria and advanced kidney disease.
3. Multicenter studies with larger sample size need to be carried out to evaluate the histological pattern of kidney disease with advanced tissue evaluation including IF studies and possibly EM.
4. Multicenter comparative study with a large sample size to explain the differences in the frequencies of the *APOL1* risk alleles in different geographic locations within the same country. Future genetic studies of *APOL1* in Africans should attempt to look at the effects of different genotypes on outcomes and treatment response.
5. Long term follow of patients with HIV infection with monitoring of kidney function should be part of routine care. Longer term studies are also needed to evaluate the impact of ART on renal outcomes in patients with different histological lesions.

CHAPTER FIVE. REFERENCES

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APPENDIX 1 AKTH ETHICS APPROVAL

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25th Nov 2013

AKTH/MAC/SUB/12A/P-3/VI/1190

Dr Aliyu Abdu
Department of Internal Medicine
AKTH

Ufs:

Head of Department
Internal Medicine
AKTH,Kano

ETHICAL APPROVAL

Further to your application in respect of your research proposal titled "prevalence, histologic pattern and genetic associations of renal disease among HIV infected patients in Kano" The committee has reviewed your proposal and noted same as a prospective study.

In view of the above, Ethical approval is hereby granted to conduct the research.

However, the approval is subject to periodic reporting of the progress of the study and its completion to the ethical committee.

Regards

Barrister BaraatuAliyu-Datti
Secretary Research Ethical Committee
For:Chairman

M140116

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140116

NAME: (Principal Investigator) Dr Aliyu Abdu

DEPARTMENT: Dept of Internal Medicine/Pulmonology
CM Johannesburg Academic Hospital

PROJECT TITLE: Prevalence, Histological Patterns and Genetic Variations of Chronic Kidney Disease among HIV Infected Patients in Kano

DATE CONSIDERED: 31/01/2014

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR: Prof S Naicker

APPROVED BY: _____
Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 08/08/2014

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

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

_____ _____
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INTRODUCTION

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APPENDIX 4 Article published in International Journal of Nephrology and Renaovascular Disease

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ORIGINAL RESEARCH

Histopathological Pattern of Kidney Diseases Among HIV-Infected Treatment-Naïve Patients in Kano, Nigeria

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Introduction: Kidney biopsy in patients with HIV-associated kidney diseases allows for histopathologic diagnosis and institution of appropriate treatment as well as proper prognostication. There is a paucity of data on the histopathological pattern of HIV-associated kidney diseases in most sub-Saharan African countries. This study was aimed at evaluating the histopathologic patterns of kidney diseases seen among HIV-infected treatment-naive patients in our center as this will allow for proper diagnosis and institution of appropriate treatment.

Methods: In this cross-sectional study, consecutive patients who satisfied inclusion criteria and consented to participate were recruited. Percutaneous kidney biopsies were carried out as day procedures under real-time ultrasound guidance using an automatic spring-loaded biopsy gun as per our unit protocols. Baseline investigations including urea, creatinine, electrolytes, CD4 count, complete blood count, and glomerular filtration rate (eGFR) calculations, urinalysis and urine protein creatinine ratios were done on all the participants.

Results: Fifty-five patients who satisfied the inclusion criteria were studied. The mean age of the study population was 38.34± 9.26 years, with 32% females. Mean serum creatinine was 249.6±164.6 µmol/L, and mean CD4 count was 238 ±210 cells/mL. The commonest histological type was FSGS seen in 20 patients (37.7%), followed by HIVAN seen in 17 (32.1%) patients; chronic interstitial nephritis in 7 patients (13.2%) and 6 (11%) had no significant pathological finding. Compared to non-HIVAN, HIVAN patients tended to have higher systolic BP ($p= 0.05$); higher serum creatinine levels ($p= 0.05$); lower eGFR (0.03) and higher urine protein to creatinine ratio [uPCR; $p= 0.02$].

Conclusion: Kidney involvement is still a form of presentation among HIV-infected treatment-naïve patients and though a wide range of glomerular and tubulointerstitial lesions may be seen, FSGS and HIVAN are still the most common. We recommend assessment of kidney function, including urinalysis, as part of the routine evaluation of newly diagnosed HIV patients and biopsy where indicated to prognosticate and institute appropriate early treatment.

Keywords: HIV, kidney disease, histology

Introduction

Human Immunodeficiency Virus (HIV) infection remains a huge burden in sub-Saharan Africa; including Nigeria.[1] Increase in numbers of HIV infection coupled with improved long-term survival has led to increasing prevalence of HIV-associated organ dysfunction, including kidney diseases.

Comorbidity of HIV with kidney disease has been associated with faster progression to AIDS and increased mortality. Mulenga et al[2] in Lusaka, Zambia reported that

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anti-retroviral therapy (ART)-naïve adults living with both HIV and kidney disease had a 4-fold higher mortality rate than those without kidney disease. Reports on the prevalence of kidney disease among HIV-infected patients have varied across the globe. In a recent meta-analysis of studies on the prevalence of kidney disease among HIV patients the greatest burden was reported to be in the West African subregion.[3] Proteinuria, reduced glomerular filtration rate (GFR), or both, have been utilized to define kidney disease in most studies; however, definitive diagnosis by kidney biopsy, when indicated, is important among these patients and is critical to optimal management.[4] A wide variety of histopathological lesions have been reported among HIV-infected patients, with the variation depending on many factors such as geographical and ethnic diversity of the population and type of study involved. Certain kidney diseases such as HIV-associated nephropathy (HIVAN) are exclusive to HIV-infected populations. This lesion has a higher prevalence among individuals of African descent and has been associated with an 18-fold higher relative risk of developing HIV-related end stage renal disease (ESRD) among African-Americans compared to Caucasians.[3,5] Diagnosis of HIVAN is best made histologically and thus, kidney biopsy is important for instituting appropriate treatment and for prognostication.

Despite the strong association between certain histopathological lesions, such as HIVAN, and African ancestry there is paucity of literature detailing the spectrum of kidney morphologic changes of the disease in West Africa. This has been attributed to a scarcity of resources needed to perform or interpret a kidney biopsy on which the diagnosis of HIVAN or other HIV-related renal disease is based.[7,8] This study aimed at having a histological diagnosis of HIV patients with kidney disease to help in instituting appropriate diagnosis and treatment.

Materials and Methods

This cross-sectional study was conducted at the dedicated HIV clinic of Amino Kano Teaching Hospital (AKTH), one of the approved United States President's Emergency Plan for AIDS Relief (PEPFAR) centers in Northern Nigeria. All the participants are Nigerians receiving treatment at this center. Ethical approval to conduct the study was granted by the Research Ethics Committee of AKTH (NHREC/21/08/AKTH/1090) and university of Witwatersrand Human Research Ethics Committee (M140116). All participants were aware of the purpose before giving their consent and the research was conducted in accordance with Helsinki Declaration. Consecutive patients who satisfied the inclusion criteria were recruited into the study and the criteria included:

- Treatment-naïve HIV positivity
- Persistent proteinuria and/or reduced eGFR
- Non-diabetic
- Not being treated for any kidney disease
- No contraindication to kidney biopsy and
- Consent to participate

Demographic variables including age, sex, ethnicity, history of kidney disease in the family and current use of medications were recorded using a questionnaire with specific identification numbers assigned to each participant.

A focused medical examination was conducted on all study participants and findings recorded included weight, height, body mass index (BMI) and systolic and diastolic blood pressures. Urinalysis was performed on mid-stream urine samples using Combi-9 urine Dipstick (Biotechnologies AG, Lichtenfels, Germany). All patients whose urine tested positive for protein were seen again after two weeks for a repeat of urinalysis to ascertain persistent proteinuria.

Venous blood samples were collected for baseline investigations including urea, creatinine, electrolytes, CD4 count, complete blood count, Hepatitis B surface antigen and Hepatitis C antibody. Glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation with no ethnicity factor used. Traceable Jaffe's method was used to determine creatinine levels in both the blood and urine samples using Cobas C 311 Clinical Chemistry Analyser (Roche Diagnostics)

Percutaneous kidney biopsies were carried out as day procedures under real-time ultrasound guidance using an automatic spring-loaded biopsy gun (BARD 16/18 G, 22 mm cutting edge). Before the biopsy, patients were counseled on the procedure and had complete blood counts including platelets measured. Clotting profiles were also checked to exclude any bleeding tendencies and they were requested to sign a consent form. Patients were monitored both pre- and post-biopsy according to established unit protocols and were discharged after a minimum of 8 hours observation with no obvious complication.

Each biopsy specimen was fixed and transported in 10% neutral buffered formalin solution for analysis in the pathology laboratory of the hospital, where they were embedded in paraffin wax and stained with Haematoxylin and Eosin, Jones methenamine silver, Periodic Acid

Schiff, Congo red and Masson's trichrome stains. Light microscopy was performed on all the tissue samples; there were no facilities for immunofluorescence (IF) at the time when the study commenced. However, IF service became available before the end of the study and 10 of the tissue specimens were subjected to IF analysis. Electron microscopy is not available in our center hence it was not included in the evaluation of the renal tissues obtained in this study.

For the purpose of this study, we considered eGFR to be reduced at values less than 60 mls/min/1.73m^2 and defined persistent proteinuria, as the presence of dipstick protein ≥ + 1 or a urine protein: creatinine ratio (uPCR) ≥ 150 mg/g on 2 occasions at least 2 weeks apart.

Data was analysed using SPSS version 24 and statistical significance was set at p-value >0.05 at 95% confidence interval.

Results

Fifty-five patients who satisfied the inclusion criteria were studied; however, complete data was available for only 53 patients. The mean age of the study population was 38.3± 9.3 years, with 32% females and 68% males. None of the patients had symptoms suggestive of kidney failure such as body swelling, nausea or vomiting. Mean serum creatinine was 249.6± 164.6 µmol/L; mean eGFR 40.0± 19.2, mean CD4 count was 238 ±210 cells/mL details as depicted in Table 1. Although all the patients had persistent proteinuria, only two had nephrotic range proteinuria at the time of review. All the tissue samples obtained had renal cortex with a number of glomeruli ranging between 9 and 26. None of the patients had a major bleeding complication that required blood transfusion or any invasive intervention following the kidney biopsy procedure. One patient had haematuria that lasted for more than 24 hours necessitating an overnight hospital stay, he was later discharged in a stable condition without requiring blood transfusion.

The histopathological lesions on kidney biopsy are shown in Table 2. The characteristic lesions consistent with HIVAN including collapsing Focal Segmental Glomerulosclerosis (FSGS), cystic tubular dilatation, interstitial infiltrates, and dilated tubules were reported in 17 (32%) of the patients; however the majority (37.7%) had FSGS as the histological diagnosis and 6 (11.3%) had no significant pathological finding. Majority of the FSGS seen in this study were of the NOS sub type with only a few being of the tipped sub type. Among the 10 patients that had IF evaluation of their tissues, none showed features of immune complex disease.

Patients with HIVAN (compared to non-HIVAN patients) tended to have higher systolic BP (p= 0.05); higher serum creatinine levels (p= 0.05); lower eGFR (0.03) and higher uPCR; (p= 0.02). Other clinical and laboratory parameters including age, BMI, diastolic BP, CD4 count and hematological indices did not show any statistically significant differences between the two groups. Similarly, there were no significant differences between those with HIVAN and those with FSGS when the clinical and laboratory parameters were compared.

Similarly, only uPCR showed a statistically significant difference (p=0.00) on analysis of variance between the 3

Table 1 Clinical and Laboratory Parameters of the Study Patients

Parameter	All Patients; n= 53	Non-HIVAN; n=36	HIVAN; n=17	p-value
Age (years)	38.3± 9.3	40.5± 11.3	37.2± 8.1	0.51
Gender (M/F)	36/17	25/11	11/6	
Body Mass Index	22.28± 26	22.5± 7.7	21.9± 6.6	0.77
Systolic BP (mmHg)	134± 67	121.3± 26.3	122.7± 16.3	0.05
Diastolic BP (mmHg)	79.00± 8.5	73.8± 18.8	78.4± 18.8	0.16
Urea (mmol/L)	28.7± 18.1	17.67± 12.0	29.6± 18.1	0.04
Creatinine (µmol/l)	249.62± 164.6	257± 89.9	256.7± 195.2	0.05
eGFR (mL/min/1.73m^2)	40.0± 19.2	41.0± 14.3	37.1± 17.4	0.03
uPCR (mg/g)	846.28± 132.7	253.8± 65.0	482.0± 16	0.02
CD4 count	238.36± 210.6	258.6±191.8	184.9± 82.7	0.09
Haemoglobin	10.3± 2.0	10.4± 1.8	10.1± 2.2	0.77
White Blood Cells	4.8± 1.7	4.8± 1.8	4.7± 1.5	0.81
Platelets	238.0± 117.8	244.6± 122.1	225.1± 111.4	0.34
Albumin	30.3± 1.6	31.8± 1.5	28.9± 1.5	0.33

Abbreviations: uPCR, urine protein creatinine ratio; eGFR, estimated glomerular filtration rate.

Table 3 Summary of the Histopathological Findings of the Study Population

Histologic Diagnosis	Frequency (%)
Focal Segmental Glomerulosclerosis	20 (37.7)
HIVAN	17 (32.1)
Chronic Interstitial Nephritis	7 (13.2)
No significant pathological finding	6 (11.3%)
Acute Tubular Necrosis	1 (1.9)
Hypertensive Nephrosclerosis	1 (1.9)
Membranoproliferative glomerulonephritis	1 (1.9)

Abbreviations: HIVAN, human immune deficiency virus associated nephropathy.

groups those with FSGS, those with HIVAN and those with other histologic types.

Discussions

We report on the histopathologic pattern of kidney lesions seen among newly diagnosed, treatment-naive HIV patients with either persistent proteinuria and/or reduced eGFR in a tertiary health facility in Nigeria.

The majority of patients in our study were males. Though this is similar to the findings by Vali et al[9] who reported a greater male preponderance, it differs from that reported by Emem-chioma et al[10] who reported a female preponderance in their study. The mean age of the study population is also similar to findings by others who have documented that most patients are young and in the most productive age group (30 to 50 years of age).[10] The youngest patient in our study was 20 years while the oldest was 54 years and mean age was 38.3±9.3 years.

The results from this study corroborate the wide spectrum of renal changes that have been observed in HIV infection; with pathologies involving the glomerulus, tubules, and interstitium, and similar to what has been reported in other studies.[11,12] This may be due to multiple pathogenetic mechanisms underlying renal affectation by the virus including a direct intrarenal HIV gene expression and lesions related to comorbidities, effects of drugs, immune dysregulation, and co-infections. This underscores the relevance of kidney biopsy among these patients.[13]

The predominant lesion was FSGS (37.7%), followed by HIVAN (32.1%) and then interstitial nephritis together with various others. This wide variation in observed histopathologic lesions has also been reported globally and has been attributed to factors such as the geographical and ethnic diversity as well as sample sizes of the reported studies.[6]

There are only a few studies with sizable numbers of HIV patients that had kidney biopsy done in Nigeria. Emem-Chioma et al[10] reported that 7 (70%) of their 10 patients that were biopsied had histopathologic features of HIVAN. We have also earlier reported HIVAN in 55% of 25 patients that had kidney biopsy in our center.[14] Though these frequencies of HIVAN are higher than the 32.1% found in this study, our current sample size is larger and thus may give a better representation of the true prevalence. This may also be due to the changing epidemiology of kidney disease among HIV-infected patients, characterized by reports of decrease in frequency of HIVAN over time.[9,15,16] Although common genetic susceptibility to CKD and limited resources for the diagnosis and management of chronic diseases may allow HIVAN to still be relevant in this region.[8]

There are limited numbers of histopathological studies from other sub-Saharan African countries as well, with most of the reports from South Africa (SA). Reports from various studies in SA revealed that HIVAN in more than 80% of biopsies in Durban as reported by Han et al,[17] in 27% in Johannesburg by Gerntholtz et al[18] and in 57.2% in report from Cape Town by Wearne et al.[11] A study from the Congo by Assounga et al[19] reported a lower rate of HIVAN at 19% among black Africans. Koech et al[20] reported that, out of the 27 patients with persistent proteinuria that underwent renal biopsy in Kenya, none had the classic HIVAN with the majority (41%) only having interstitial Nephritis.

Casanova et al,[21] in a study among white Italian HIV-positive patients, reported that none had the classic HIVAN. Similarly, Praditpornsilpa et al[22] in their study among 26 HIV-positive patients presenting with varying degrees of proteinuria in Thailand did not demonstrate a single case of HIVAN. These are at variance with the findings of this study, where HIVAN was the second most frequent histopathologic diagnosis. This may be related to genetic variations, especially the low prevalence of high-risk genotypes of APOL1 reported in these populations, compared to those reported among the West African population.

Reports from the Indian sub-continent also demonstrated wide variation in the prevalence of HIVAN ranging from 0 to 83%. However, a pooled analysis of data from 10 studies in India showed that HIVAN constituted 16.5%, followed by mesangioproliferative glomerulonephritis (MPGN) 13.9%, FSGS 7.7% and tubulointerstitial lesions 22.7% among other histologic diagnoses.[23] Though this

prevalence of HIVAN is lower than what we found in this study, the wide variation in the histopathologic findings is similar to that of our study.

Berliner et al[24] from Baltimore in USA reported a frequency of 35% for HIVAN, 22% cases of FSGS, and 7.9% cases of acute interstitial nephritis along with other less frequent lesions. Kudose et al[25] in a retrospective analysis of a cohort of 437 HIV-positive patients who had kidney biopsy at Columbia University also reported diverse pathologic diagnoses with the commonest being ICGN (17%), diabetic nephropathy (DN) (16%), classic HIVAN (14%) and FSGS (12%). The figures from Baltimore were similar to our finding as HIVAN and FSGS were the majority of the histologic diagnoses, the finding of ICGN and DN as the commonest diagnosis in the study from Baltimore was however at variance with our findings.

Presence of HIV-related tubulointerstitial lesions is commonly reported in all kidney biopsy series across the world, and may be present in up to 70% of cases.[12] In this study, tubulointerstitial disease was seen in only 15% of our patients; and although higher than the 7.9% reported by Berliner et al, it is lower than the 27.7% and the 41% reported from India and Kenya respectively.[23,24] Concurrent medications, use of herbal concoctions and infections, among other causes, may underlie the reasons for the high frequency of tubulointerstitial disease among these patients. A number of immune complex diseases have also been described in the setting of HIV, including IgA nephropathy, lupus-like glomerulonephritis (GN), membranous glomerulopathy, post-infectious GN, mesangiocapillary GN and the "ball-in-cup" phenomenon.[15] However, none of those who had IF showed features of immune complex disease in our study. In contrast, Fabian et al[26] in Johannesburg reported HIV immune-complex kidney disease as the predominant diagnosis in 8 (40%) of their 20 cases. This variation may be due to relatively small number (10) that had IF in our study or may actually be a result of low prevalence of immune-complex disease in this part of the world. This calls for further studies as there was no study from Nigeria that had IF evaluation to our knowledge.

Kidney involvement in HIV-infected patients can present at any stage of the illness although HIVAN commonly occurs late in the course of the illness.[27] In this study we recruited newly diagnosed and treatment-naïve patients, presumably at an early stage of their illness. However, low CD4 cell counts may suggest advanced disease.

There was no major complication noted from the kidney biopsy procedure in this study, although HIV-infected patients that underwent renal biopsy are not expected to be at greater risk of major complications than the general population. Fine et al reported that 6 (3.2%) out of 187 HIV-infected patients who underwent ultrasound-guided biopsies in their center experienced a major complication and all were stabilized and discharged without further complications.[28]

Management of kidney disease in HIV-infected individuals poses some challenges, however suppression of HIV viral load, use of ARBs and ACEi in those with with confirmed or suspected HIVAN or those with significant proteinuria as well as special attention to therapeutic issues such as drug-drug interactions and drug-toxicity are important.[29]

Among the strengths of our study is the relatively high number of HIV-positive patients with kidney disease that had renal biopsy from a broad geographic area compared to other previous Nigerian studies that were limited by the small numbers of biopsied patients. Our institution receives referrals for kidney biopsy from many centers in our region because of our advantage in terms of resources and capacity for renal histology. Ten of the biopsy tissues had IF evaluation which is the first report from this locality even though limited by the small number; other studies from this region were limited by the fact that only light microscopic evaluation was done.

In conclusion, this study has demonstrated the safety and relevance of a kidney biopsy in the assessment of HIV-infected patients with renal involvement. It has demonstrated that although a wide range of histopathologic glomerular and tubulointerstitial lesions may be seen, FSGS and HIVAN are still the most common, with the frequency of HIVAN dropping from an earlier 55% to 32.1%.

Disclosure

The authors reported no conflicts of interest for this work.

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HIGH RISK APOL1 GENOTYPES AND KIDNEY DISEASE AMONG TREATMENT NAÏVE HIV PATIENTS AT KANO, NIGERIA

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Keywords:	Apol1 Genotype, Kidney Disease, HIV, Native Africans
Abstract:	Introduction Racial disparities are known in the occurrence of kidney disease with excess risks found among black populations. Apolipoprotein L1 (APOL1) gene variants G1 and G2 are associated with kidney disease among HIV infected individuals of African descent in the USA as well as among black population in South Africa. We set out to investigate the prevalence of these high-risk variants and their effects on kidney disease among HIV infected patients in Northern Nigeria with hitherto limited information despite earlier reports of high population frequencies of these alleles from the Southern part of the country. Methods DNA samples obtained from the whole blood of 142 participants were genotyped for APOL1 G1 and G2 variants after initial baseline investigations including assessment of kidney function. Participants comprised 50 HIV positive patients with no evidence of kidney disease, 52 HIV negative individuals with no kidney disease and 40 HIV positive patients with chronic kidney disease (CKD) evidenced by persistent proteinuria and/or reduced eGFR, who had also had a kidney biopsy. All the HIV positive patients were newly diagnosed and treatment naïve. Results The prevalence of the individual risk alleles among all the study participants was 26.1% for G1 and 19.0% for G2. The frequency of the High Risk Genotype (HRG) was 12.5% among those with CKD compared to 5.8% in the HIV negative group and none in HIV positive no CKD group. Having the HRG was associated with a higher odds for developing HIVAN (2 vs 0 risk alleles: OR 10.83, 95% CI 1.38 - 84.52; P= 0.023; 2 vs 0 or 1 risk alleles: OR 5.5, 95% CI 0.83 - 36.29; P= 0.07). The HRG was also associated with higher odds for FSGS (2 vs 0 risk alleles: OR 13.0, 95% CI 2.06 - 81.91; p=0.006 and 2 vs 0 or 1 risk alleles: OR 9.0, 95%CI 1.62 - 50.12; P= 0.01) when compared to the control group. Conclusion This study showed a high population frequency of the individual risk alleles of the APOL1 gene with higher frequencies noted among HIV positive patients with kidney disease. There is high association with the presence of kidney disease and especially FSGS and HIVAN among treatment naive HIV patients carrying two copies of the HRG.
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HIGH RISK *APOL1* GENOTYPES AND KIDNEY DISEASE AMONG TREATMENT NAÏVE HIV POSITIVE PATIENTS AT KANO, NIGERIA

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Abstract

Introduction

Racial disparities are known in the occurrence of kidney disease with excess risks found among black populations. *Apolipoprotein L1* (*APOL1*) gene variants G1 and G2 are associated with kidney disease among HIV infected individuals of African descent in the USA as well as among black population in South Africa. We set out to investigate the prevalence of these high-risk variants and their effects on kidney disease among HIV infected patients in Northern Nigeria with hitherto limited information despite earlier reports of high population frequencies of these alleles from the Southern part of the country.

Methods

DNA samples obtained from the whole blood of 142 participants were genotyped for *APOL1* G1 and G2 variants after initial baseline investigations including assessment of kidney function. Participants comprised 50 HIV positive patients with no evidence of kidney disease, 52 HIV negative individuals with no kidney disease and 40 HIV positive patients with chronic kidney

disease (CKD) evidenced by persistent proteinuria and/or reduced eGFR, who had also had a kidney biopsy. All the HIV positive patients were newly diagnosed and treatment naïve.

Results

The prevalence of the individual risk alleles among all the study participants was 26.1% for G1 and 19.0% for G2. The frequency of the High Risk Genotype (HRG) was 12.5% among those with CKD compared to 5.8% in the HIV negative group and none in HIV positive no CKD group. Having the HRG was associated with a higher odds for developing HIVAN (2 vs 0 risk alleles: OR 10.83, 95% CI 1.38 - 84.52; P= 0.023; 2 vs 0 or 1 risk alleles: OR 5.5, 95% CI 0.83 - 36.29; P= 0.07). The HRG was also associated with higher odds for FSGS (2 vs 0 risk alleles: OR 13.0, 95% CI 2.06 - 81.91; p=0.006 and 2 vs 0 or 1 risk alleles: OR 9.0, 95%CI 1.62 - 50.12; P= 0.01) when compared to the control group.

Conclusion

This study showed a high population frequency of the individual risk alleles of the *APOL1* gene with higher frequencies noted among HIV positive patients with kidney disease. There is high association with the presence of kidney disease and especially FSGS and HIVAN among treatment naive HIV patients carrying two copies of the HRG.

Key words; HIV Infection, Kidney Disease, *APOL 1* genotypes, Northern Nigeria.

Introduction

There is global disparity in the occurrence of kidney disease with an excess risk for persons of African Ancestry as they were shown to have four times higher incidence of chronic kidney disease (CKD) compared to Caucasians, [1] together with a more progressive course of the disease, as reported in the Multi-Ethnic Study of Atherosclerosis (MESA) during 5 years of follow-up.[2] Data analysis from the US revealed that African-Americans showed a consistent yearly increase in the incidence of end stage renal disease (ESRD) that is 3.5 to 5-fold higher than Caucasians.[3] This racial/ ethnic disparity in the occurrence of kidney disease is also noted among HIV infected patients. Eggers et al reported that the possibility of developing kidney disease among HIV infected African-Americans is at least 50 times higher than that of Caucasians. [4] Nearly 90% of ESRD attributed to HIVAN was likely to occur in African-Americans.[5]

The excess risk of kidney disease among African-Americans could not entirely be explained by factors such as access to care and other socioeconomic factors. [6] The possibility of genetic factors being significant in the development of kidney disease among this population group was supported by findings from many studies. [7,8] Variants in the gene located on chromosome 22 that encode for *Apolipoprotein L1* (*APOL1*) have been found to be strongly associated with increased risk of various forms of kidney diseases among people of African Ancestry with three times greater risk of developing lupus nephritis, seven times greater risk of developing hypertensive nephrosclerosis, seventeen times greater risk of developing primary FSGS and twenty nine times greater risk to develop HIV-associated nephropathy (HIVAN).[9,10] There were reports of even stronger associations with the development of HIVAN. [11,12] Kasembeli et al, in a study among black people in South Africa, showed that the presence of these high risk variants was associated with an 89-time odds for developing HIVAN.[13]

These variants include the *APOL1* G1 allele comprising two non-synonymous coding variants rs73885319 (S342G) and rs60910145 (I384M) and the G2 allele with a 6 base pair deletion that removes two amino acids (rs71785313).

APOL1 high-risk genotypes (HRGs) are defined as the occurrence of two risk alleles in any of the combination (homozygous G1/G1, homozygous G2/G2 and compound heterozygous G1/G2). Patients with the HRG carry an increased risk of kidney disease. The heterozygous forms (G1/G0 or G2/G0), with only one risk allele, are known as low risk genotypes and are associated with a lesser risk of kidney disease. These kidney disease risk variants occur in higher frequency among individuals of West African descent. [14] This was later found to have evolutionary origins by way of natural selection for a trait that offers protection against infection with *Trypanosoma brucei*, a protozoan that causes sleeping sickness, which is endemic in parts of Sub-Saharan Africa. These traits encode for circulating *APOL1* that functions as a trypanolytic factor leading to death of the trypanosome parasites in human serum. [15] However this positive selective advantage against sleeping sickness has associated increased risk of developing CKD among the carriers of the high-risk genotype.

Genetic predilection to HIVAN has been described among African-Americans but has not been widely studied among native Africans except for a few studies such as the study among the South African black population by Kasembeli et al and another study among children in the DRC.[13,16] We therefore set out to investigate the frequencies of these variants and their association with kidney disease among HIV infected natives of Northern Nigeria, a region with scarce information regarding these risk alleles despite previous reports of high population frequencies of these alleles from the South Western and South Eastern parts of the country which were predominantly inhabited by the Yoruba and Igbo ethnic groups respectively.[14,17]

Methods

Participants in this cross-sectional study were recruited consecutively from the dedicated HIV clinic of Aminu Kano Teaching Hospital (AKTH), one of the approved United States President's Emergency Plan for AIDS Relief (PEPFAR) centers in Northern Nigeria. One hundred and fifty five participants comprising 50 treatment naive HIV positive patients with no evidence of kidney disease (HIV positive no CKD), 52 HIV negative participants with no kidney disease (HIV negative no CKD) and 53 HIV positive patients with kidney disease evidenced by persistent proteinuria and/or reduced eGFR, who had also had a kidney biopsy (HIV positive CKD). The HIV positive CKD patients had no obvious other known causes of kidney disease, such as diabetes and hypertension, and were not being treated for any kidney disease prior to enrolment and had no contraindication to kidney biopsy.

All participants gave consent to participate and underwent an initial assessment with a focused clinical examination and baseline investigations including serum creatinine, CD4 count, complete blood count, Hepatitis B surface antigen and Hepatitis C antibody tests. The IDMS traceable Jaffe method was used to determine creatinine levels in both the blood and urine samples using the Cobas C 311 Clinical Chemistry Analyser (Roche Diagnostics) at Aminu Kano Teaching Hospital (AKTH). Glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation with no ethnicity factor.

Genomic DNA was extracted from whole blood using the Maxwell DNA purification kit (Promega AS1010, USA) as per the manufacturer's protocol. DNA concentrations were determined by the NanoDropTM 2000 spectrophotometer (Thermo Scientific, USA) and the

quality was assessed by means of the A260/280 ratios. A 260/280 ratio of 1.8 to 2.0 was considered as indicative of a pure DNA preparation free of contamination. DNA samples were genotyped for SNPs rs73885319, rs60910145 and rs71785313 using a PCR-restriction fragment length polymorphism assay as described by Nqebelele *et al*[18]. Genomic studies were carried out in the Department of Internal Medicine Research Laboratory at the University of Witwatersrand, Johannesburg.

Ethical approval to conduct the study was granted by the Research Ethics Committee of AKTH (NHREC/21/08/AKTH/1090) and University of Witwatersrand Human Research Ethics Committee (M140116).

Statistics

Data was analysed using STATA v14.2 (Stata Corp, College Station, TX). Demographic and clinical characteristics were expressed as medians and interquartile ranges, except for age and BMI which were expressed as mean ± SD. Comparisons of continuous variables were made using a Kruskal-Wallis test or ANOVA test, as appropriate. Categorical variables were expressed as frequencies and compared using a chi-squared test. Associations between the risk of kidney disease and *APOL1* risk alleles were explored using logistic regression models. A two-tailed p-value <0.05 was considered significant.

Results

A total of 155 participants were studied with a mean age of 36.08 ± 9.32 and 55% were males. Complete data on genotyping was available for 142 participants (40 HIV positive CKD patients, 50 HIV positive controls and 52 HIV negative controls). Table 1 shows the characteristics of the participants. The HIV positive CKD patients were older than the HIV positive no-CKD patients

(38.8 ± 9.3 vs. 31.2 ± 7.4 years, p<0.001); however the age was similar to the HIV negative no-CKD controls (38.8 ± 9.3 vs 38.0 ± 9.3, p=0.648). The HIV positive CKD patients also had higher blood pressure (systolic: 120 vs 110 mm Hg, p=0.001) when compared with the no-CKD controls, both HIV positive and negative. All the HIV positive CKD patients had a kidney biopsy and the commonest histological diagnosis was FSGS seen in 20 (37.7%) patients followed by HIVAN seen in 19 (35.9 %) patients, chronic interstitial nephritis in 6 (11.3%) patients, and 6 (11.3%) with no significant pathological finding.

Table 1 Characteristics of the study participants

Characteristic	**HIV CKD (n=53)**	**HIV positive controls(n=50)**	**HIV negative controls (n=52)**	**p-value**
Male, n (%)	34 (64.2%)	20 (40.0%)	32 (61.5%)	0.027
Age (years)	38.8 ± 9.3	31.2 ± 7.4	38.0 ± 9.3	0.083
Serum Creatinine (µmol/L)	210 (143 - 302)	82 (70 - 90)	82 (73 - 87.5)	<0.001
eGFR (ml/min/1.74m^2)	34.4 (26.4 - 48.9)	107 (87 - 128)	110.5 (100.5 - 120.5)	<0.001
Systolic BP (mmHg)	120 (110 - 132)	110 (110 - 120)	110 (110 - 120)	0.001
Diastolic BP (mmHg)	77 (65 - 84)	60 (60 - 70)	69.5 (60 - 70)	<0.001
Body mass index (kg/m^2)	22.26 ± 2.6	22.5 ± 7.7	21.9 ± 6.6	0.770
CD4	115 (74 - 304)	265 (179 - 402)	-	0.005

The individual risk allele frequency among all study participants was 26.1% (37/142) for G1 and 19.0% (27/142) for G2. The haplotype G-G-I (G1^{GM}), comprising the derived allele at both rs73885319 (p.S342G) and rs60910145 (p.I384M) and the ancestral insertion (I) allele at rs71785313, was the most frequent G1 configuration seen in 20.4% of all the study participants.

Table 2 depicts the frequencies of the various haplotypes across different groups in the study participants.

Table 2 *APOL1* risk allele frequencies

***APOL 1* variants**	**HIV positive CKD (n=40)**	**HIV Positive no-CKD (n=50)**	**HIV Negative no-CKD controls (n=52)**	**p-value**	**p-value HIV+ CKD vs HIV+ no-CKD**	**p-value HIV+ CKD vs HIV- no-CKD**
rs73885319						
AA, n (%)	32 (80.0%)	43 (89.0%)	36 (69.2%)	0.008	0.143	0.023
AG, n (%)	5 (12.5%)	7 (14.0%)	16 (30.8%)			
GG, n (%)	3 (7.5%)	0	0			
rs60910145						
TT, n (%)	30 (75.0%)	43 (86.0%)	36 (69.2%)	0.153	0.289	0.373
TG, n (%)	9 (22.5%)	7 (14.0%)	16 (30.8%)			
GG, n (%)	1 (2.5%)	0	0			
rs71785313						
Insertion/Insertion	28 (70.0%)	46 (92.0%)	41 (78.8%)	0.027	0.007	0.331
Insertion/Deletion	12 (30.0%)	4 (8.0%)	11 (21.2%)			
Deletion/Deletion	0	0	0			
Haplotypes						
G0	35	50	49			
$G1^{GM}$	8	6	15			
$G1^{G+}$	2	1	1			
$G1^{+M}$	2	1	1			
G2	12	4	11			

The frequency of the HRG was 5.6% (8/142) among all the study participants. Three participants had the homozygous G1/G1 genotype and five had the compound heterozygote G1/G2 form; none of the study participants had the homozygous G2/G2 form. However the frequency of the HRG was 12.5% among those with HIV CKD compared to 5.8% in the HIV negative controls and none in HIV positive no-CKD group; this difference was statistically significant (p=0.025). Table 3 compares the frequencies of the risk alleles among the various study groups.

Table 3 *APOL1* risk alleles among the study participants

Characteristic	**HIV positive CKD (n=40)**	**HIV positive no-CKD controls (n=50)**	**HIV-negative no-CKD controls (n=52)**	**p-value**	**p-value HIV+ CKD vs HIV+ no-CKD**	**p-value HIV+ CKD vs HIV- no-CKD**
0 risk alleles	20 (50.0%)	38 (76.0%)	27 (51.9%0	0.016	0.007	0.516
1 risk alleles	15 (37.5%)	12 (24.0%)	22 (42.3%)			
2 risk alleles	5 (12.5%)	0	3 (5.8%)			
0 risk alleles						
G0/G0	20 (50.0%)	38 (76.0%)	27 (51.9%)	0.013	0.015	1.000
1 risk allele						
G0/G1	5 (12.5%)	8 (16.0%)	14 (26.9%)	0.13	0.175	0.673
G0/G2	10 (25.0%)	4 (8.0%)	8 (15.4%)			
2 risk alleles						
G1/G1	3 (7.5%)	0	0	0.025	0.015	0.288
G1/G2	2 (5.0%)	0	3 (5.8%)			
G2/G2	0	0	0			

We found a 10.8 fold higher odds for HIVAN among those with 2 risk alleles vs no risk alleles (OR 10.83, 95%CI 1.38 - 84.52; p=0.023) and a 5.5 fold higher odds for those with 2 vs 1or 0 risk alleles (OR 5.50, 95%CI 0.83 - 36.29; p= 0.07) compared to controls. These associations however were not significant after adjusting for age and gender. Associations were stronger

among those with FSGS (2 vs 0 risk alleles: OR 13.0, 95%CI 2.06 - 81.91; p=0.006 and 2 vs 0 or 1 risk alleles: OR 9.0, 95% CI 1.62 - 50.12; p= 0.012) and remained significant after adjusting for age and gender. Table 4 depicts the OR in the various groups, both unadjusted and after adjustments for age and gender.

Table 3. **Odds ratios for associations of *APOL1* risk alleles with HIVAN and FSGS versus controls**

	Unadjusted OR (95% CI)	p-value	Adjusted OR* (95% CI)	p-value
HIVAN				
No of Risk Alleles				
1 vs 0	3.82 (1.07 - 13.61)	0.038	2.51 (0.63 - 9.92)	0.189
2 vs 0	10.83 (1.38 - 84.52)	0.023	4.81 (0.52 - 44.90)	0.168
2 vs 1	2.83 (0.40 - 19.87)	0.295	2.58 (0.36 - 18.63)	0.347
2 vs 1 or 0	5.50 (0.83 - 36.29)	0.077	3.76 (0.54 - 26.35)	0.182
FSGS				
No of Risk Alleles				
1 vs 0	2.29 (0.65 - 8.07)	0.196	1.55 (0.40 - 6.00)	0.526
2 vs 0	13.0 (2.06 - 81.91)	0.006	14.00 (1.77 - 110.67)	0.012
2 vs 1	5.67 (0.92 - 34.99)	0.062	5.30 (0.83 - 33.95)	0.079
2 vs 1 or 0	9.0 (1.62 - 50.12)	0.012	8.85 (1.55 - 50.74)	0.014

*for age, gender

Discussion

This study conducted in Northern Nigeria determined the frequencies of *APOL1* genotypes among treatment naïve HIV infected patients with biopsy-proven kidney disease and those with no evidence of kidney disease.

The prevalence of the individual risk alleles of G1 (26.1%) and G2 (19.0%) in this population was lower than that reported in previous studies involving Nigerian populations; 45 to 50% for G1 among Yoruba group in the South Western parts and the 30% among the Igbo speaking group in the South Eastern parts of the country. [12,14,17,19] It is also lower than the 40% reported for the Ashanti group and higher than the 11.4% reported among the Bulsa group, both from Ghana. [18] The G1 prevalence in our study participants was similar to the 20-22% reported from 3 surveys among African-Americans. [10,20] The prevalence was however higher than the 7.3% for G1 and 11.1% for G2 reported in a South African black population. [13] The G2 prevalence of 19% in this study was higher than the 7.5 % and 16.6% reported in the Yoruba group.[14,20] The G2 frequency in this study was lower than the 24.4% in the Igbo and 21.4 % in the Bulsa in Ghana. It was however similar to the 13-15% among African-Americans in the US and the 12.9% among the Asante group in Ghana.[10,19,] Variations in the frequencies of these alleles among diverse African ethnic populations was also reported by Tzur et al when they studied 676 samples from 12 African populations that included Cameroon (2 ethnic groups), Congo, Ethiopia (4 ethnic groups), Ghana (2 ethnic groups), Malawi, Mozambique and Sudan. The risk allele frequencies differ between groups from the same country and also across the continent; for example in the Bulsa and Asante populations of Ghana, the frequencies are 0.11 and 0.41 respectively.[19] The findings in this study also differ from that of an Ethiopian population where they reported zero

frequencies of these *APOL 1* risk alleles among HIV infected patients with associated absence of HIVAN. [21]

From the lower frequencies of these individual alleles in our study, it may be possible to speculate on a possible relaxation of the natural selection push exerted by the Trypanosome infection in these regions by ancestors of the ethnic populations in this Northern part of Nigeria. The study area is some distance from the trypanosome infection belt and the Atlantic coastal areas of West Africa, the hub for the trans-Atlantic slave trade from which most African Americans trace their ancestral journeys to the Caribbean and the Americas. [22] There is a need for a multicenter comparative study with a large sample size to explain the differences in the frequencies of these alleles in different geographic locations within the same country. The frequencies of the HRG defined by the presence of 2 risk alleles (G1/G1, G2/G2, or G1/G2) among all participants in this study was 5.6% and it was 12.5% among the HIV positive CKD group. The overall frequency was thus lower than the 11% reported for African Americans[7] and 7 % for DRC children.[17] The differences between the groups was also noted by Kasembeli et al in which the frequency of the HRG was 17% among all participants but was as high as 89% among those with HIVAN. [13]

This study found that having the 2 risk allele genotype was a predictor of FSGS compared to no risk alleles (OR= 13.0, 95% CI: 2.06 - 81.9; p= 0.006). The association was maintained in the recessive form (2 vs 1 or 0 risk alleles: OR= 9.0, 95% CI: 1.6 – 50.1; p= 0.01). The association was also strong with HIVAN (2 vs 0 risk alleles: OR= 10.83, 95%CI: 1.4 - 84.5; p= 0.023) with a less strong association in the recessive form (2 vs 1 or 0 risk alleles: OR=5.50 95% CI: 0.83 - 36.29; p= 0.07). Kopp et al also reported this association in a cohort of FSGS and HIVAN; a greater association was observed among HIVAN (OR = 29.2, 95% CI: 13.1-68.5, P = 6 x 10-22)

compared to FSGS (OR = 16.9, 95% CI: 11.0 to 26.5, P = 1.3 x 10-48)[10] Kasembeli et al reported an even greater association with HIVAN among a black South African cohort (OR 89.1; 95% CI, 17.68 to 911.72; P=1.24×10^{-14}) but no significant association with FSGS (OR 2.13; 95% CI, 0.03 to 44.30; P=0.48).[13] This lower strength of the association among our patients may be due to a smaller sample size compared to both the Kopp and Kasembeli studies. This calls for further study with a larger sample size on the effect of this HRG and other *APOL1* nephropathies, not only among HIV infected patients but also in the general population.

This study provides the first information on the high frequencies of the *APOL1* genotypes among treatment naïve HIV infected patients from this environment, though the frequencies were not as high as those reported among ethnic groups in the western and eastern parts of Nigeria. The study also confirmed the association between the occurrence of these HRGs and kidney disease, and specifically FSGS and HIVAN histological types among HIV infected patients despite the relatively lower frequencies; this was hitherto not reported from this environment. This calls for the inclusion of evaluation of kidney function among newly diagnosed HIV patients as recommended, [23] to allow for early detection and subsequent initiation of ART as extrapolation from a previous study showed that the life time risk of developing HIVAN among HIV patients carrying 2 alleles may reach as high as 50% .[10] This study is limited by a small sample size and cross sectional design that will not allow for the assessment of the impact of the HRG on the progression of the kidney disease.

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Appendix 6 Letter from PLOS ONE

Submission Confirmation for PONE-D-21-10394 - [EMID:a8cdf968e6560fbc]

From: PLOS ONE (em@editorialmanager.com)

To: aliyuabdu2000@yahoo.co.uk

Date: Tuesday, 30 March 2021, 08:59 GMT+1

PONE-D-21-10394
HIGH RISK APOL1 GENOTYPES AND KIDNEY DISEASE AMONG TREATMENT NAÏVE HIV PATIENTS AT KANO, NIGERIA
PLOS ONE

Dear Dr. Abdu,

Thank you for submitting your manuscript entitled 'HIGH RISK APOL1 GENOTYPES AND KIDNEY DISEASE AMONG TREATMENT NAÏVE HIV PATIENTS AT KANO, NIGERIA' to PLOS ONE. Your assigned manuscript number is PONE-D-21-10394.

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Appendix 7. Draft Manuscript

Prevalence, Risk Factors and Outcomes of CKD among treatment naïve HIV infected patients at Kano Nigeria

Abstract

The rise in the population of HIV infected patients and associated improvement in their long term survival has resulted in an increase in the prevalence of HIV-associated organ dysfunction, including kidney diseases. Comorbidity of HIV with kidney disease is associated with faster progression to AIDS and increased mortality. Wide variability exists in the reported prevalence of kidney disease among HIV infected patients globally. Most of the studies were limited by factors such as small sample size, the definition of the Chronic Kidney Disease (CKD) and the population studied. In this study we looked at the prevalence of CKD and its risk factors among a relatively large cohort of ART-naive HIV infected patients.

Methods: Consecutive treatment naïve HIV patients were studied using a structured questionnaire, after an initial clinical evaluation and screening for the presence of kidney disease defined as persistent proteinuria and/ or reduced eGFR confirmed at least three months after the initial assessment.

Results: Nine hundred patients were studied; 63% were females with mean age of 34.17±10 years and mean CD4 count of 238 ±210 cells/mm^3. prevalence of CKD was 22.8% using the CKD-EPI equation (without the race factor). Multivariable analysis identified age, CD4 count <200cells/mL, BMI and history of use of traditional medications as being independently associated with CKD.

Conclusion: We found high prevalence of CKD and its risk factors in this cohort of HIV infected patients and this has implications for overall organisation of HIV care in this environment. It calls for the incorporation of screening for CKD and its risk factors and implementation of strategies targeted at early detection, and institution of therapies to slow progression to End Stage Kidney Disease (ESKD).

Key words Chronic Kidney disease, HIV infection, Nigeria.

Introduction

There were approximately 37.9 million people worldwide living with HIV/AIDS at the end of 2018 with Sub-Saharan Africa being the most affected region housing 68% of the world's HIV-infected persons and an overall HIV prevalence rate of 4.7%.[1]The major burden of the HIV infection in sub-Saharan Africa is borne by South Africa with 25% and Nigeria with 13%.[2] There are about 1.6 million adults living with HIV in Nigeria in 2019, with a prevalence rate of 1.3%.[3]

Kidney disease among HIV-infected individuals is known to have implications for clinical outcomes, such as increased mortality and worse outcomes, regardless of the specific histological lesion or underlying cause.[4] Presence of kidney disease among HIV-infected patients was recognized by the World Health Organization (WHO) as stage 4 disease, eligible for Highly Active Anti-Retroviral Therapy (HAART) initiation regardless of the CD4 cell count.[5]

There is worldwide variability in the reported prevalence of Chronic Kidney Disease (CKD) among HIV-infected patients, depending on the population studied. Naicker and Fabian summarised studies from different parts of the globe including parts of Africa with varying prevalence ranging from 5.5- 48.5%.[6] The wide variation in the prevalence was also reported in a

meta-analysis of 61 studies that reported on 209,078 HIV-infected adults from 60 countries using different equations to estimate eGFR[7]

The prevalence of kidney disease among HIV-infected patients in Nigeria also varied widely with reports from available studies showing a prevalence rate of 38% in Ile-Ife, South Western geopolitical zone,[8] 53.3% in Benin City in South Southern zone ,[9] 31% in Zaria North zone [10] and 51.8% in Jos and 47.6% in Ilorin respectively, both in the North central zone of the country.[11.12] Majority of these studies were cross-sectional with small numbers. Reports from African countries also revealed wide variations, with 36% prevalence in Tanzania, 11.5% in Kenya, 8 % in Uganda and 43% in Cameroon [13],[14,15,16] In the USA the annual number of patients with incident End Stage Kidney Disease (ESKD) secondary to HIVAN increased steadily from 1989 to 1995 and then remained stable until 2006. The substantial reduction in both the incidence of ESKD due to HIVAN and the mortality among these patients was attributed to increasing availability of HAART .[17] This wide variation could be ascribed in part to differences in study design, the populations studied and the criteria used for defining kidney disease in these studies.

Serum creatinine is frequently used in clinical practice and research to estimate the GFR using various equations such as the Cockcroft–Gault (CG) equation, the Modification of Diet in Renal Disease (MDRD) formula and the CKD Epidemiology Collaboration (CKD-EPI) formula. [18,19,20] Despite the popular use of the CG and MDRD equations, there is evidence to suggest that the CKD-EPI estimate is more accurate than the MDRD equation in HIV-infected individuals.[21]

Kidney biopsy when indicated is important among these patients as it allows for a definitive diagnosis which is critical to optimal management. Definitive diagnosis of certain kidney diseases associated with HIV infection such as HIVAN is best made histologically.

Traditional CKD risk factors and HIV-related risk factors both contribute to the occurrence of kidney disease seen among HIV-infected patients. Diabetes, hypertension, black race, increasing age, male gender, history of intravenous drug use, hepatitis C virus co-infection, presence of proteinuria, low CD4 count, and high viral load were reported as risk factors for CKD among these patients.[22] There is a need to know the prevalence of kidney disease and its associated factors among HIV patients from this environment, as this will facilitate the initiation of adequate preventive measures in those at risk, as well as delay or prevent progression to ESKD in those with established kidney disease. This study was aimed at studying the prevalence of CKD and its risk factors among ART-naive HIV-infected patients in Kano.

Methods

The study was conducted at Aminu Kano Teaching Hospital, Kano, Nigeria one of the approved United State President's Emergency Plan for AIDS Relief (PEPFAR) centers in Nigeria. In this cross sectional study, consecutive patients who fulfilled the inclusion criteria were enrolled during the study period. Patients diagnosed with HIV who were 18 years and older and not previously diagnosed with diabetes mellitus, hypertension, obstructive uropathy, and clinical features of glomerulonephritis were recruited after signing an informed consent form. Their demographic variables including age, sex, ethnic origins, address, religion, marital status etc. were recorded using specific identification numbers assigned to each participant. A focused

medical examination was conducted on each study participant and findings recorded including weight, height, body mass index, systolic and diastolic blood pressures among others.

Urinalysis was performed on a mid-stream urine samples using Combi-9 urine Dipstick (Biotechnologies AG, Lichtenfels, Germany). All patients whose urine tested positive for protein were asked to come back after two weeks for a repeat urinalysis.

Spot urine protein/creatinine ratios (uPCR) were done for those with persistent proteinuria. Those patients who had persistent proteinuria (uPCR $\geq$ 150 mg/g on 2 occasions) were referred for further Nephrology review and possible kidney biopsy.

Venous blood samples of about 10mls were collected for baseline investigations including complete blood count, urea, creatinine, electrolytes, CD_4 count, lipid profiles, Hepatitis B surface antigen and Hepatitis C antibody which were all part of the routine workup of the patients prior to commencement of ARTs in the clinic. Another 5 mls of blood was collected at 12 weeks interval during their routine follow up for repeat creatinine in those who had elevated serum creatinine to establish the diagnosis of CKD.

Creatinine in both the serum and Urine was measured using modified Jaffe method using Cobas C 311 Clinical Chemistry Analyser (Roche Diagnostics) The eGFR was calculated using the CKD-EPI equation[20] with no ethnicity factor.

Data was analysed using a computer based software STATA v14.2 (Stata Corp, College Station, TX). Demographic and clinical characteristics were expressed as means and standard deviation

Ethical approval to conduct the study was granted by the Research Ethics Committee of AKTH (NHREC/21/08/AKTH/1090) and University of Witwatersrand Human Research Ethics

Committee (M140116). All participants were informed about the purpose of the study before giving their consent and the research was conducted in accordance with the declaration of Helsinki.

Results

The mean age of the study participants was34.17±10.00 years with a range of 20-50 years. Most of the study participants were females {588 (63%)}.

The mean systolic blood pressure (SBP) of the study population was 113 ±9.0 mm Hg while that of diastolic DBP was 69.0±9.4 mm Hg. Hypertension was diagnosed in only 25 (2.7%) of the study population. There was also no significant difference in both systolic and diastolic blood pressures between those with and those without CKD.

Obesity was found in 128 (28.2%) of the participants. There was no significant difference in the prevalence of obesity between those with and those without CKD.

The mean uPCR of the study population was 236.23± 130.7 150 mg/g it was however higher among those with CKD (846.23± 130) compared to those with no CKD (126.21± 91.11). Only 4 patients had proteinuria of nephrotic range and one of which had kidney biopsy with no significant pathological finding on light microscopy, suggesting a probable minimal change disease.

The mean eGFR of the study population was (86.18 ±38.10) it was significantly lower among those with CKD (39.34±13.94) compared to those with no CKD (101.20±30.42). Table 1 summarises the clinical and laboratory features of the study participants.

Table 1. Clinical characteristics and laboratory parameters of the study population-

Age, years	34.2±10.0
Female sex	n=588 (63.5%)
Mean Systolic BP, mm Hg	122.5± 24.8
Mean Diastolic BP, mm Hg	79.9±18.2
Hypertension	n=25 (2.7%)
Mean fasting plasma glucose, mmol/L	5.28±2.1
Diabetes mellitus	n=72 (8.0%)
Mean BMI, kg/m2	21.4 ± 6.1
Underweight	n=162 (18%)
Normal BMI	n=540 (60%)
Overweight	n= 135 (15%)
Obese	n=63 (7%)
Mean Haemoglobin, (g/dL)	10.3± 2.0
Mean WBC count (10^9/L)	4.8± 1.7
Mean Platelets counts (10^9/L)	238.0± 117.8
Hepatitis C Antibody	n=11 (1.2%)
Hepatitis B Surface antigen	n=95 (10.58%)
Mean CD4 count, (cells/mm^3)	238.36± 120.8
Mean uPCR (mg/g)	236.23± 130.7
Mean eGFR (ml/min/1.74m^2)	86.18 ±38.10

uPCR= urine protein creatinine ratio, eGFR = estimated glomerular filtration rate, WBC= white blood cells, CD4= Cluster of differentiation 4, BMI= Body Mass Index

Risk factors for CKD

Majority of the study participants (67.8%) had a positive history of use of traditional medications, 8.8% admitted to the use of analgesic drugs, alcohol ingestion was noted in 10.8%, and cigarette smoking in 19% and use of medicated soap was reported in 16%. There were significant differences in the occurrences of most of the traditional CKD risks for CKD, with higher prevalence among those with CKD compared to those with no CKD as shown in table 2.

Table 2. Comparison of the risk factors for CKD among the study population

Variables	**All**	**With CKD**	**No CKD**	χ^2/student t	**p value**
Traditional medicine use	572	126	446	$\chi^{2=}$62.5	0.002
FH of hypertension	297	61	236	$\chi^{2=}$59.4	0.001
FH of diabetes	137	27	110	$\chi^{2=}$56.2	0.004
FH of kidney disease	48	8	40	$\chi^{2=}$59.1	0.001
Skin lightening cream	136	20	116	$\chi^{2=}$62.8	0.001
Analgesic use	75	14	62	$\chi^{2=}$62.8	0.001
Alcohol ingestion	92	17	75	$\chi^{2=}$61.4	0.001
Cigarette smoking	163	36	127	$\chi^{2=}$ 56.4	0.001
eGFR (ml/min/1.74m^2)	86.18 ±38.10	39.34±13.94	101.20±30.42	t= 28.99	p<0.001
BMI (kg/m2)	21.4 ± 6.1	19.20±5.3	22.10±6.3	t =6.10	0.001
Systolic BP (mmHg)	122.5± 24.8	112.06±10.58	112.87±9.10	t = 1.02	0.72
Diastolic BP (mmHg)	79.9±18.2	69.34±9.16	69.65±9.40	t=0.39	0.92

BP= Blood Pressure, BMI- Body Mass Index, eGFR estimated glomerular filtration rate, FH= Family History, CKD= Chronic Kidney Disease

Prevalence of CKD

The prevalence of CKD using the single eGFR at recruitment was 27% using the CKD-EPI equation without the race factor.

Among those who had a repeat creatinine measured using the CKD-EPI equation without race after 3 months from the initial diagnoses, CKD was diagnosed in 205 participants giving a prevalence of 22.8%. Majority 146 (71.21%) of the CKD patients were in stage 3 using the KDIGO classification, while 45 (21.95%), and 14 (6.82%) had stages 4 and 5 respectively.

Multivariate logistic regression analysis identified age, female sex, low BMI, low CD4 cell count and positive history of cigarette smoking as factors with high odds associated with CKD that reached statistical significance.

Outcomes

The 14 patients with stage 5 CKD were followed up at the Nephrology clinic for the first one year. Eight of them were dialysis requiring within the first 6 months of follow up. Two were lost to follow up and the remaining 4 died of other complications within the follow up period.

Among the 400 patients with early CKD that we were able to follow, 69 (17.5%) showed improvement in the Kidney function after starting ART within the first 6 month of follow up period.

Table 5. Multivariate logistic regression model factors associated with the presence of CKD

Parameter	OR, 95%(CI)	P
BMI kg/m2	3.73 (1.56-4.69)	0.00
Cigarette smoking	1.65 (1.47-4.8)	0.00
CD4 cell count ((<200 cells/mm3)	2.44 (2.20-5.39)	0.00
Age years	1.61 (1.12-2.54)	0.00
Female gender	1.93 (1.28-2.92)	0.00

OR: Odds ratio, CI: Confidence interval, BMI: Body mass index, CD4: Cluster of differentiation

Discussion

This study reported the prevalence of CKD and its risk factors among newly diagnosed treatment naïve HIV infected patients at Kano, Northern Nigeria.

Majority of the study population were females similar to report by many others.[7, 8.9] The mean age of the study population is also similar to findings by others who have documented that most patients are young and in the most productive age group (30 to 50 years of age).[7,8]

The prevalence of traditional risk factors for CKD was noted to be high among the study population, most especially the positive history of use of traditional medicines was noted in the majority of them (68.7%). This is similar to our earlier findings among civil servants in Kano Metropolis during a screening exercise. [23] This is important as most of these herbal preparations were not accurately characterized, thus the active ingredient is not known and studies from other part of Nigeria reported the nephrotoxic nature of some of them.[24] This high prevalence may also have additive effects on CKD among these patients as they already have HIV associated renal risk factors, such as low CD4 cells count and HIV viraemia and use of Nephrotoxic medications

in some instances. It is of note that the prevalence of some of the risk factors are relatively low such as cigarette smoking and alcohol consumption, and may be due to the socio cultural background of the study population. The prevalence of hypertension and diabetes in the study population were also very low, 2.7% and 8.0 % respectively. This may be as a result of our exclusion criteria that did not allow those previously known to be hypertensive or diabetic to be enrolled into the study.

This study found high prevalence of CKD among treatment naive HIV patients similar to previous reports from different parts of the country as well as in a systematic review of studies from different parts of Africa.[25] Although the prevalence of CKD found in this study was lower than the prevalence rate reported earlier from other parts of the country such as the rate of 38% in Ile-Ife, South Western Nigeria;[8] 53.3% in Benin City in South Southern part;[9] 31% in Zaria, North Western region [10] and 51.8% in Jos and 47.6% in Ilorin respectively, both in the central part of the country[11.12]. The finding is also lower than our previous report from this center.[26] However the finding in this study is similar to the 23.5% and 22.9% prevalence reported from Lagos in South West and Owerri in South East respectively. [27,28] These previous studies were however limited by the use of single eGFR measurement which cannot discriminate between CKD and AKI among these patients. The finding in this study is higher than CKD prevalence of 13.4% reported recently by Ekripo *et al*[29] in the South Southern part of the Country that uses 2 measurements of GFR also using the CKD-EPI equation, similar to this study. There is evidence to suggest that the CKD-EPI estimate is more accurate than the MDRD equation in HIV-infected individuals as used in this study.[29]

Majority of the patients with CKD in this study had stage 3 disease. This has implications for management as initiation of measures such as early initiation of ART and other renal-specific

interventions like the use of ACE-inhibitors (or angiotensin receptor blockers) to reduce proteinuria and control of blood pressure as well as avoidance of nephrotoxic agents among others**,** will help in slowing down or stopping progression to ESKD. This provides a cost effective means of reducing CKD associated mortality, given that access to treatment of ESKD in this environment is limited and expensive.

This study is limited by the fact that we were not able continue with the longitudinal aspect of the study because of funding issues that resulted in inadequate follow up and withdrawal of support for routine investigations in the HIV clinic.. Despite this limitation the study found improvement of kidney with the initiation of HAART. This is similar to findings by others.[30]

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