

**CLINICAL PRESENTATION AND  
HISTOPATHOLOGICAL DESCRIPTION OF THE  
SPECTRUM OF RENAL DISEASES IN HIV  
INFECTED ADULTS PRESENTING WITH RENAL  
INSUFFICIENCY AT THE UNIVERSITY  
TEACHING HOSPITAL, LUSAKA ZAMBIA**

**BY**

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**(MBCHB)**

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MEDICINE IN INTERNAL MEDICINE**

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**DECLARATION:**

I, Dr Chansa Abidan, declare that this dissertation represents my own work and is being submitted for the Master's degree in Internal Medicine at the University of Zambia, Lusaka. It has not been previously submitted for any degree or other qualifications at this or any other University.

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**CERTIFICATION OF APPROVAL**

I certify that this study, “Clinical presentation and histopathological description of spectrum of renal diseases in HIV infected adult patients presenting with renal insufficiency at the University Teaching Hospital, Lusaka” is the result of my own independent investigation.

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Examiner 1

Signature..... Date.....

Examiner 2

Signature..... Date.....

Examiner 3

Signature..... Date.....

## **ABSTRACT**

**Introduction:** The spectrum of renal diseases in HIV infected adults undergoing biopsy is vast. Previous studies have indicated that HIV-associated nephropathy (HIVAN) is the commonest diagnosis in HIV-infected patients presenting with renal insufficiency. More than 90% of patients with HIVAN are black. The natural history of the renal diseases associated with HIV infection has been radically changed by antiretroviral therapy. There are other diseases, however, that account for a good percentage to the spectrum of renal diseases other than HIVAN. A group of diseases known as HIV immune complex kidney diseases have gained prominence in this regard. Other diseases include acute tubular necrosis, acute and chronic interstitial nephritis, haemolytic uremic syndrome and many others.

**Objective:** To determine the clinical presentation and histopathological description of the spectrum of renal diseases in HIV infected adult patients presenting with renal insufficiency at the University Teaching Hospital, Lusaka

**Methods:** This was a descriptive cross-sectional study of HIV infected adults presenting with renal insufficiency who underwent kidney biopsy. It was conducted at the University Teaching Hospital from June 2014 up to November 2015. The primary outcome was proportion of major histological diagnosis of renal diseases in this patient population while secondary outcomes included proportion of other renal histological diagnoses and the patient's clinical characteristics. We compared the clinical characteristics of the commonest histological diagnoses.

**Results:** The commonest histological diagnoses in this study were HIV immune complex kidney disease (HIVICK) (32%) and focal segmental glomerulosclerosis (FSGS) (29%) of various histologic variants other than the collapsing type. We did not see the classic HIVAN on histopathology in our study population. All the patients presented with severe renal dysfunction with mean eGFR of 17 ml/min/1.73m<sup>2</sup> and massive proteinuria of 3+. Patients had advanced HIV infection with mean CD4 count of 197 cells/mm<sup>3</sup>. Majority of patients (64.5%) were not yet been initiated cART. 16% of the study patients were hypertensive.

**Conclusion:** HIVICK and FSGS were the commonest histological diagnoses. Classical HIVAN on histopathology was not found in this patient population at the UTH.

**Recommendation:** Kidney biopsy should be mandatory to make a definitive diagnosis in all patients presenting with renal dysfunction let alone those with HIV. In view high prevalence of HIVICK seroprevalence of Hepatitis C needs to be further explored in HIV positive population.

**Keywords:** Renal insufficiency, biopsy, HIV immune complex kidney disease, HIVAN, FSGS

## **DEDICATION**

This work is dedicated to my dear loving, ever supportive and understanding wife, Dr Bwendo Nduna-Chansa. My precious children, Chilupe, Jennifer Mweshi and Anna Kabwe. My parents Mr and Mrs O. C. Chansa and Mr and Mrs C.F. Nduna for always believing in me and holding my hand every step of the way from the time that I enrolled into the program of study and beyond. My understanding nieces and nephews that have always been inspired by me and in turn inspire me to strive for excellence. My brothers Pedaiah, Heman, Shemaiah, Ngosa, Sunday, Chileshe, Mweshi, Mwewa, Lombe and Danny. My sisters Merab, Shiphra, Dorcas, Lydia, Musonda, Louisa, Chenge and Carol.

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## **LIST OF ABBREVIATIONS**

**AIDS** - acquired immune deficiency syndrome

**ARF** - acute renal failure

**ART** - antiretroviral therapy

**ATN** - acute tubular necrosis

**cART** - combination antiretroviral therapy

**CRF** - chronic renal failure

**CKD** - chronic kidney disease

**eGFR** - estimated glomerular filtration rate

**ESKD** - end-stage kidney disease

**FSGS** - focal segmental glomerulosclerosis

**HAART** - highly active antiretroviral therapy

**HIV** - Human immunodeficiency syndrome

**HIVAN** - HIV associated nephropathy

**HIVICK** - HIV immune complex kidney disease

**IgA** - Immunoglobulin A

**KIDGO** - Kidney Disease Improving Global Outcomes

**MCD** - minimal change disease

**NOS** - not otherwise specified

**UTH** - University Teaching Hospital

**ZDHS** - Zambia Demographic Health Survey

## **OPERATIONAL DEFINITIONS**

**HIVAN** - collapsing focal segmental glomerulosclerosis, chronic interstitial nephritis with microcystic tubular dilatation, podocyte proliferation and effacement of the foot processes on histology

**HIVICK** - parenchymal renal diseases with immune complexes with a typical ball in cup appearance of glomeruli on histology

**Hypertension** - use of antihypertensive medication at the time of renal biopsy

**Microalbuminuria** - presence of albumin less than 30mg/L in urine

**Renal dysfunction** - raised serum creatinine above 146  $\mu\text{mol/L}$  and/or persistent proteinuria

- **Proteinuria** - urine dipstick reading of >1+ protein
- **Persistent proteinuria** - persistent positive urine protein lasting more than two weeks

## CHAPTER ONE

### 1.0 INTRODUCTION

HIV prevalence in sub-Saharan Africa accounts for a significant proportion of the world's burden of the disease (1). Zambia, a country in this region of the world, is not spared; with the HIV prevalence being 14.3% according to the 2010 Zambia Demographic Health Survey. HIV seropositive patients have an increased risk for the development of a variety of acute and chronic renal diseases (2). Thus the spectrum of renal diseases in HIV infection is wide. Renal failure causes so much morbidity and mortality and the cost of managing these disease entities is huge.

Kidney dysfunction in HIV infection is a cause of concern for practising clinicians in the care of patients. It can be caused by opportunistic infections like sepsis, which is more prevalent in untreated HIV patients than the general public. It can also arise due the drugs that are used in the treatment of HIV and opportunistic infections. Drugs that have been implicated in renal dysfunction or worsening of renal dysfunction are the antiretroviral agents like *tenofovir*, *indinavir* and *lamuvidine*. Drugs used in the treatment of opportunistic infection like amphotericin B and septrin can also cause renal dysfunction. The HIV has been implicated in the pathogenesis of parenchymal renal diseases like HIVAN and HIVICK. Other concurrent diseases like diabetes mellitus, hypertension and autoimmune diseases have tendency to increase the burden of renal dysfunction in HIV patients.

Previously HIV-associated nephropathy (HIVAN) has been shown to be the commonest diagnosis in HIV-infected patients presenting with renal insufficiency with more than 90% of patients with HIVAN being of African descent (3-5). The natural history of the renal diseases associated with HIV infection has, however, been radically changed by antiretroviral therapy. Most investigators believe that combination antiretroviral therapy (cART) needs to be initiated in patients with renal disease in the presence of HIV infection more so in those patients found to have HIVAN and immune mediated kidney diseases.

There are other renal parenchymal diseases seen in HIV infection that account for a good percentage to the spectrum of renal diseases other than HIVAN. A group of diseases known as HIV immune complex kidney diseases have gained prominence in this regard (6). Other

diseases include acute tubular necrosis, acute and chronic interstitial nephritis, haemolytic uremic syndrome and many others. The latter disease entities usually account for the majority of patients with acute renal failure in HIV infection (7). The clinical presentation of the majority of acute renal failure patients does not differ significantly from one another hence necessitating kidney biopsy. The treatment modalities and clinical outcomes also differ depending on the histological diagnosis made.

In this study, renal insufficiency was defined as raised serum creatinine level above 146  $\mu\text{mol/L}$  and/or persistent proteinuria of 1+ and above. This definition was adopted from the Kidney Disease Improving Global Outcomes (KDIGO) technical working group of 2012. Persistent proteinuria in the study was defined as proteinuria that remained positive for two weeks after the initial urine dipstick test.

Literature concerning histologically proven renal diseases in HIV positive patients in most countries in Sub-Saharan Africa remains scant (8). Most of the histological studies conducted have been retrospective in nature. HIVAN is thought to be the commonest cause of end stage renal disease in black individuals. However most of the earlier studies that found HIVAN to be the most common diagnosis were based on clinical and radiological data rather than histopathological findings.

The commonest diagnosis among the spectrum of kidney diseases in HIV infection, HIVAN, is most often a complication of advanced HIV infection, associated with low CD4 cell counts and high HIV viral load (9). However, it was found that it can develop even in patients with undetectable viral loads and high CD4 cell counts (8). CD4 cell count  $<200$  cells/ $\text{mm}^3$  is a risk factor for HIVAN, and there is a short period of time to commence renal replacement therapy in untreated patients with lower CD4 cell counts. In their study, Szczech LA et al. found that the average time to commence renal replacement therapy in HIVAN patients was 254 days (10). HIVAN is characterized by collapsing focal segmental glomerulosclerosis with marked podocyte proliferation, microcystic dilatation of the tubules and interstitial nephritis. Patients generally present with advanced HIV-1 infection, renal insufficiency and marked proteinuria (11).

The other prominent renal diseases in HIV infection are HIVICK, focal segmental glomerulosclerosis of varying variants, minimal change disease. Patients who are diagnosed



with these diseases usually present as chronic kidney disease. Those patients that present with acute kidney injury and undergo biopsy are found to have acute tubular necrosis, immune thrombocytopenic purpura, interstitial nephritis and many others. Patients could also have multiple diagnoses. Drug toxicity is also an important cause of renal dysfunction in HIV infection. The drugs range from antiretroviral drugs used to treat HIV to drugs used in the management of opportunistic infection in HIV.

At present there are no serologic markers that exist to make a diagnosis on the various types of the spectrum of renal diseases in HIV, and the differential diagnosis for renal failure in HIV patients renal is broad. This therefore necessitates the need for renal biopsy (12). Viral infection of renal cells, and genetic factors are assumed to play a central role in the pathogenesis of some of the diseases seen in HIV (13). In HIVAN for example there is MYH9 gene that has been implicated. Also the Apo 1 gene has been found to have an association with the development of HIVAN.

The University Teaching Hospital in Lusaka, has a high burden of patients with HIV who present with renal insufficiency (9%) (14). Thus, the need for a study to identify the various types of histopathological confirmed HIV related renal diseases and the clinical characteristics that these patients present with as this imparts greatly on the choice of therapy offered to these patients was necessary.

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

Many studies that have been done in patients with HIV infection have shown a high susceptibility to the development of renal disease in patients of African descent (15, 16). There has been varying prevalence of renal diseases across the global. HIVAN has been shown to be the commonest histological diagnosis in patients of African descent (17, 18) while other diseases like membranous nephropathy were found in increasing proportions in Indian patients with HIV, and immune complex diseases being common in Caucasians. (11) The spectrum of renal diseases differs from populations and race. The pathogenesis and clinical manifestations in the various patient populations also differs. There has also been a dramatic change in data concerning the spectrum of renal diseases with the advent of antiretroviral diseases.

Studies conducted in Europe, United States of America and recently in South Africa show varying prevalence and presentation of patients with HIVAN and other HIV related renal diseases. It is a known fact that the classical HIVAN is very common among patients of African descent with genetics playing an important role (13, 17, 19). Proteinuria, acute renal failure and chronic kidney disease are the commonest presentation of HIVAN in most studies that have been conducted (20). These patients tend to have severe immunosuppression and severe renal dysfunction. They were often found to be younger patients with little exposure to antiretroviral diseases. In contrast, in the general population patients at risk of renal insufficiency are older, present with hypovolemia, sepsis and have underlying non-communicable diseases like diabetes mellitus and hypertension.

The U.S. Renal Data System (USRDS) report of 2004 indicated that, of the incident patients initiating chronic dialysis between January 1992 and June 1997, 1% had HIVAN (19) and 87% of these were African-American (17). The other patient populations like Hispanics and Caucasians had immune complex kidney diseases and autoimmune diseases contributing to the causes of renal insufficiency. Four thousand new cases of end stage renal disease (ESRD) were attributed to HIV in the U.S.A in 2005, and HIVAN was found to be the third leading cause of ESRD in African-American between the ages of 20–64 years (19). The incidence of

HIVAN peaked in the U.S. during the mid-1990s and declined by 50% in the 1998–2001 time period, relative to 1995–1997, in association with the widespread use of HAART (2, 21).

In their paper, Philippe Flandre, Pascal Pugliese, Lise Cuzin et al, indicated that CKD and decline in renal function have been reported in association with older age, female gender, hepatitis B and C infections, diabetes, hypertension, and ART exposure. They concluded that, AKI, hypertension and low CD4 cell count is also a risk factor to developing CKD (22). AKI and a decline in renal function have been reported in association with indinavir, atazanavir, and ritonavir. Proximal tubular dysfunction and acute tubular necrosis have been reported in patients starting tenofovir or didanosine, often precipitated by drug interactions. The Swiss cohort has reported a reduction of the estimated GFR (eGFR) with prolonged ART exposure, and among ART, tenofovir and indinavir exposures have been associated with increased risk of CKD in the EuroSIDA study.

The incidence of HIVAN has continued to decrease during the cART era, though its prevalence is now increasing due to the aging of patients as a result of improved survival among those with HIV infection (21). The true prevalence of HIVAN remains unknown since the diagnosis requires viral testing and renal histologic analysis. It is estimated that more cases likely exist than those that are reported since renal biopsy is not routinely done in most cases (12). In addition, there are other disease entities that cause kidney disease in HIV-infected patients, some of which result from HAART (10, 17).

The prevalence of HIVAN in HIV-infected African Americans ranges from 3.5% for proteinuria to 12% in postmortem studies (5, 19). A report in South Africans revealed higher frequencies of HIV immune complex kidney disease (HIVICK), as well as membranous, mesangial hyperplasia, and IgA nephropathy, relative to African-Americans (6). In a retrospective chart review conducted by Williams et al. at a London Hospital on patients with HIV and renal disease who had biopsy or necropsy done in the period 1992-1996; the commonest abnormality was HIVAN. The commonest presentation in these patients was acute renal failure (ARF) in 10 patients (59%), chronic renal failure (CRF) in five (29%), and proteinuria alone in two (12%) (20).

In a small cohort of ten black patients that underwent kidney biopsy after 2004, at the Royal London Hospital in the UK, the prevalence of HIVAN and immune complex mediated-

glomerulonephritis was similar at 30% (23). Berliner AR, Fine DM et al. in a cohort study between 1995 and 2004 of HIV infected patients undergoing biopsy, the leading biopsy diagnosis was HIVAN at 35%. There was a decreasing trend in yearly incidence of HIVAN diagnosis, which coincided with the use of HAART (18).

In a retrospective chart review by Gernholtz et al. of 104 renal biopsies obtained between 2003 and 2004 in a South African hospital, HIVAN was found in approximately 30% of the renal biopsies reviewed and 20% were classified as HIV immune-complex kidney disease (6). They concluded that biopsy in HIV-infected patients presenting with proteinuria identified the typical features of HIVAN in about 60% of cases. Han et al conducted a study among HIV infected patients in Durban, South Africa in which they screened HIV patients for proteinuria and microalbuminuria. They found persistent proteinuria in 6%; of these 72.4% had HIVAN and the prevalence of HIVAN in those with persistent microalbuminuria was 85.7% (14).

In their retrospective study (24), Francois-Xavier et al, where they reviewed 88 renal biopsies which included 66% Black patients, HIV-associated nephropathy (HIVAN) was observed in 26 cases, classic focal segmental glomerulosclerosis (FSGS) in 23 cases, immune complex glomerulonephritis in 20 cases and other glomerulopathies in 19 patients. They noted that the decrease in HIVAN cases coincided with the use of HAART in the period 2004-07, while FSGS emerged as the most common cause of glomerular diseases. In this study similar clinical characteristic like lower CD4 cell counts (<200/mL) and glomerular filtration rate <30 mL/min/1.73m<sup>2</sup> and black race were risk factors for HIVAN just like in other studies. Compared to HIVAN, patients with classic FSGS were less often Black, had HIV infection for longer periods, were more often co-infected with hepatitis C virus, showed more often cardiovascular (CV) risk factors, had less often CD4 <200/mL, lower HIV viral load and were older.

Literature indicates decrease in renal function with prolonged use of certain antiretroviral drugs. Tenofovir and certain protease inhibitors (PI) have been implicated in this regard. In their study (25) Goicoechea et al found that patients receiving TDF+PI/r had a greater rate of decline in creatinine clearance than did the TDF+NNRTI group. They concluded that treatment with TDF and PI/r was associated with greater declines in renal function over the 48 weeks study period compared with TDF+NNRTI-based regimens. Tenofovir is associated

with hypophosphatemia and tubular dysfunction. Stephanie B et al (26) found no worsening effect on phosphatemia and tubular phosphate reabsorption in patients receiving tenofovir over a 6 months period after introduction of tenofovir in treatment experienced patients.

A crystal nephropathy, characterized by serum creatinine elevation, loss of concentrating ability of the kidney, leukocyturia, and renal parenchymal image abnormalities, is a frequent complication of indinavir therapy (27). Simon S et al (28) performed a retrospective analysis on kidney biopsies of 30 HIV-positive patients. Of these Twenty-two of them received highly active antiretroviral therapy (HAART). Tenofovir containing HAART together with atazanavir, was administered to three patients. All the three patients developed acute renal failure. The kidney biopsies of these patients showed an acute interstitial nephritis or a chronic interstitial nephritis with an acute component. Withdrawal of atazanavir and tenofovir resulted in recovery of renal function in all three patients. Acute interstitial nephritis was observed only in 1 of 19 patients without atazanavir or tenofovir treatment. They concluded that acute interstitial nephritis and consecutive acute renal failure is a relevant side effect of atazanavir and tenofovir therapy in HIV-positive patients.

In Zambia there is no published data on renal biopsy proven diseases except some studies on clinical parameters describing renal diseases.

## **CHAPTER THREE**

### **3.0 STATEMENT OF THE PROBLEM**

Since the emergence of acquired immune deficiency syndrome (AIDS) more than 30 years ago, renal disease has been found to be a common complication of HIV infection and/or treatment. It is also now known that there are several renal syndromes and diseases associated with HIV infection. In a country like Zambia where there is a high prevalence of HIV the above is true. However studies to determine the histopathological pattern and diagnosis of HIV associated renal diseases have not been conducted and hence the need for this study

### **3.1 STUDY JUSTIFICATION**

The UTH being a tertiary hospital attends to a lot of HIV infected patients with severe disease and complicated cases like renal insufficiency and the burden of care and cost of treatment for these patients is huge. A study of HIV patients with renal insufficiency, undergoing biopsy, is necessary to identify the spectrum of renal diseases in HIV infection proven by histopathology and their clinical characteristics as this will assist to guide practice and improve patient outcomes and the overall care of these patients.

### **3.2 RESEARCH QUESTION**

What is the clinical presentation and histopathological description of the spectrum of renal diseases in HIV infected adult patients with renal failure at the UTH?

### **3.4 STUDY OBJECTIVES**

#### **3.4.1 General Objective**

To determine the clinical presentation and histopathological description of the spectrum of renal diseases in adult HIV infected patients presenting with renal insufficiency at the UTH

#### **3.4.2 Specific Objectives**

- To determine the proportion of HIVAN patients that present with renal insufficiency
- To determine the proportion of other renal diseases in patients that present with renal insufficiency
- To determine the clinical characteristics of patients presenting with the various histological diagnoses
- To compare the clinical characteristics of patients presenting with commonest histological diagnoses

## **CHAPTER FOUR**

### **4.0 RESEARCH METHODS**

#### **4.1 Study Design**

Descriptive cross sectional study

#### **4.2 Study site**

UTH

#### **4.3 Study Population**

The study population was HIV infected patients presenting with renal insufficiency defined as persistent proteinuria of 1+ and above, and/or serum creatinine of >146umol/L and consenting to undergo renal biopsy

#### **4.4 Sampling**

Consecutive sampling of consenting patients fulfilling the inclusion and exclusion criteria into the study was done.

#### **4.5 Sample size calculation**

The sample size was calculated using the Epi Infor version 7 population survey formula.

The following assumptions were made:

Study population of HIV positive patients being followed up at UTH at 11000

Estimated renal dysfunction in study population at 9%

Worldwide prevalence of commonest HIV related renal diseases (HIVAN) at 3.5%

Confidence interval 95% and power at 80%

Total sample size 51



#### **4.6 Inclusion criteria**

1. HIV positive
2. Age 18-65
3. Renal insufficiency defined as creatinine >146umol/L and/or persistent proteinuria or microalbuminuria of 1+ and above
4. Willingness to undergo study procedures
5. Signed informed consent

#### **4.7 Exclusion criteria**

1. Shrunken kidneys or single kidney on abdominal US scan
2. Patient with overt heart failure
3. Moribund patients
4. Patients with uncontrolled hypertension
5. Patients with bleeding diathesis
6. Pregnancy
7. Patients on aspirin, clopidogrel, heparin and warfarin

#### **4.8 Clinical procedures**

Patients were screened and recruited on a daily basis in Adult Medical Emergency Unit, Adult Infectious Disease Centre and Medical Admission Ward based on the inclusion/exclusion criteria.

A detailed history was obtained at the screening points and included patient demographics, presenting complaints, past medical and drug history.

Physical examination was done to elicit physical signs and vital signs such as body temperature, pulse and respiratory rates and blood pressure. Urinalysis was conducted by a single research assistant and findings recorded.

Investigations that were conducted included serum creatinine, urea and electrolytes, full blood count, hepatitis B surface antigen, clotting profile, random blood sugar, liver function tests, CD4 cell counts.

Abdominal ultrasound scans were done by an experienced radiographer and the researcher and the findings were documented.

After satisfying the criteria for entry in the study and that there was no contraindication to biopsy, a renal biopsy was done. Samples were sent to the pathologist for reading and reports obtained.

### **Renal biopsy**

Renal biopsy is a necessary procedure as experts in the field of nephrology believe that renal biopsy is gold standard of diagnosis of parenchymal renal diseases (29, 30, 31). Furthermore, renal biopsy has been shown to guide therapy and thus a necessary procedure. Kidney biopsy in critically ill patients in Intensive care units in France guided therapy (32, 33).

In this study, percutaneous kidney biopsy was performed under local anaesthesia in the radiology department, of the UTH, under ultrasound guidance. The local anaesthetic agent used was lignocaine 1%. The biopsy was done with the patient lying in the prone position and breath held. This was done after careful selection of patients and necessary precautions.

Blood tests were done before the biopsy to ensure that there was no evidence of infection or a blood clotting abnormality. Further, an ultrasound scan of the kidney was performed before biopsy to exclude structural defects of the kidney which include hydronephrosis, cystic kidney disease and small, shrunken kidneys.

To decrease the risk of bleeding, patients taking medications like aspirin, clopidogrel, heparin were excluded from the study as indicated in the section on exclusion criteria. Patients with kidney failure at danger of uremic coagulopathy were put on hemodialysis prior to biopsy. Strict control of blood pressure was done to reduce bleeding risk in patients that had hypertension. In this study only 5/31 patients had hypertension which was well controlled on antihypertensive drugs. Prior to the procedure, informed consent was obtained and consent forms signed.

Post-biopsy care and supervision was done by monitoring any signs of bleeding. Post-procedure monitoring included monitoring for blood in urine, monitoring patients' vital signs which included blood pressure, pulse rate and quality of pulse. In our study no patient experienced worsening renal function or post biopsy complications. This was in agreement with studies which have shown that renal biopsy is a safe procedure with very few adverse events (32, 33). Paracetamol and tramadol were given for pain relief after the procedure.

#### **4.9 Data Entry/Management**

Patients' information was anonymized with study identification numbers rather than names.

Specially designed data collection forms (Appendix 3) were used to collect data which was later entered into the excel spread sheet and later on the Epidata software database.

The forms were then checked for inconsistencies and completeness.

Double entry was used to enter data on the Epidata software database.

#### **4.10 Outcomes**

##### **4.10.1 Primary outcome**

Major histological diagnoses of HIV related renal diseases

##### **4.10.2 Secondary outcomes**

Other histological diagnoses

Clinical characteristics of patients with major renal histological diagnoses

Characteristics of patients with other renal diagnoses other than the major diagnoses (these include HIVICK, acute tubular necrosis, IgA nephropathy and other HIV associated microangiopathy)

#### **4.11 Variables**

##### **4.11.1 Independent variables**

These included age, sex, proteinuria, serum creatinine, haemoglobin and CD4 cell counts

##### **4.11.2 Dependent variables**

Proportions of histological diagnosis on renal biopsy reports

#### **4.11.3 Categorical variables**

These included sex, histological diagnoses and level of proteinuria

#### **4.11.4 Continuous variables**

These include age, level of proteinuria, serum creatinine, haemoglobin and CD4 cell counts

### **4.12 Statistical analysis**

#### **4.12.1 Descriptive statistics**

Continuous variables which are age, CD4 cell counts, level of haemoglobin, creatinine levels were expressed as means and/or medians, and percentages.

Categorical variables like sex, histological diagnoses and level of proteinuria were expressed as percentages or proportions.

#### **4.12.2 Analytical statistics**

Fisher's exact test was used to quantify correlations between dichotomous variables (e.g the main histological diagnosis and patient characteristics).

Multivariate analysis was used for independent variables.

A p-value  $\leq 0.05$  was considered statistically significant.

### **4.13 Ethical considerations**

The study was approved by the ERES Converge IRB. Permission to conduct the study at the University Teaching Hospital was also sought from the office of the Senior Medical Superintendent of the UTH.

The study was conducted in accordance with internationally recognized standards like the Helsinki declaration

Only patients with written informed consent were enrolled in the study. No patient was coerced to enter the study by monetary, preferential care or otherwise. Some patients opted to leave the study at any point without compromising their clinical care. Patients' safety was the priority in the study by adhering to safety issues in the study procedures.

Investigations and procedures were performed by the researcher in collaboration with the supervisor and qualified personnel to ensure patient safety. All enrolled patients who were not on antiretroviral drugs were commenced on government Antiretroviral Treatment program.

Confidentiality was maintained at all levels of the study. Study participants were given an identification code rather than their name for identity purposes.

Data collected was kept under lock and key in the Renal Unit of the UTH and was only accessible to the investigator and supervisors.

## CHAPTER FIVE

### 5.0 RESULTS

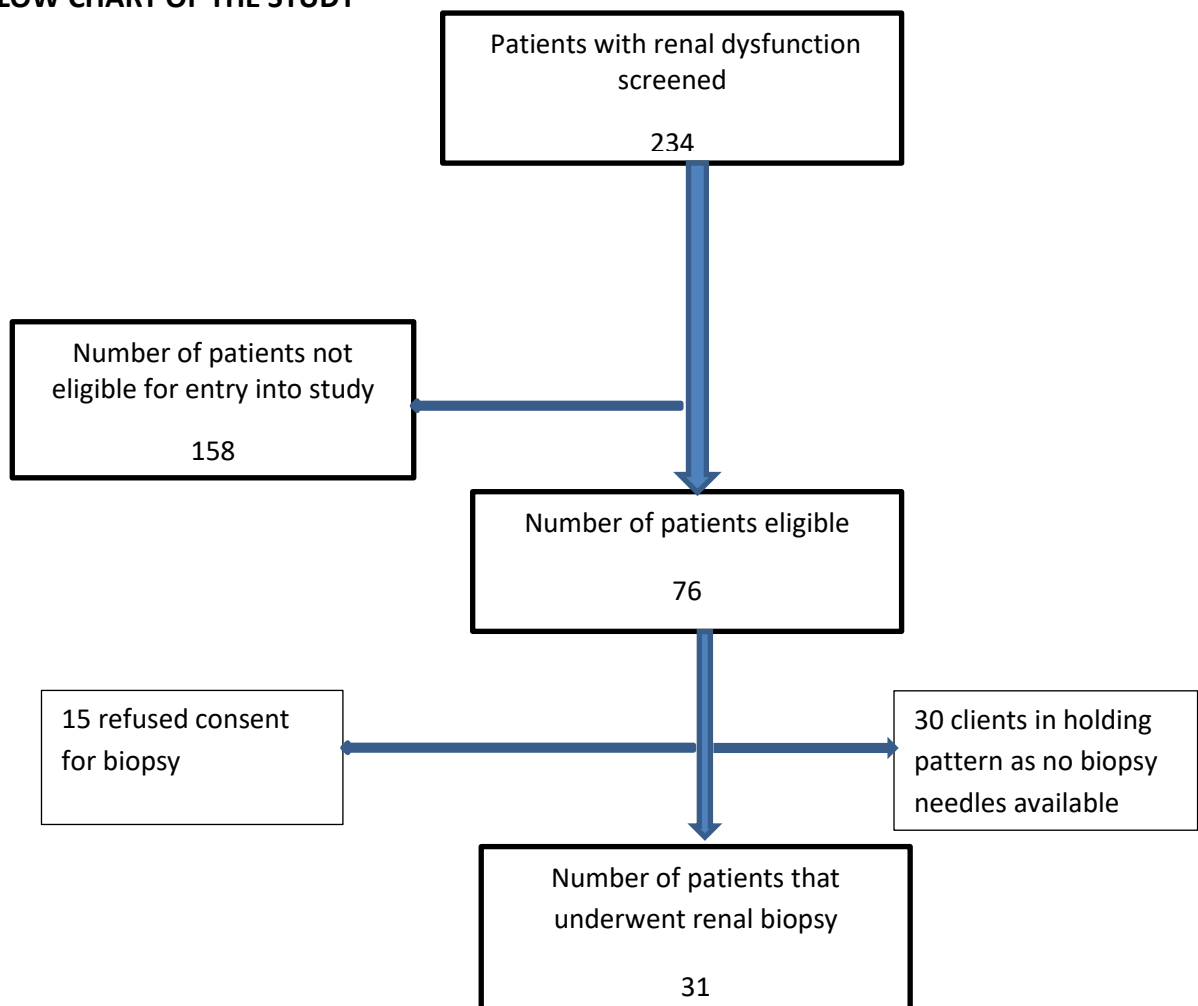
#### 5.1 Recruitment procedure

Two hundred and thirty-four patients were screened during the period of the study, July 2014-December 2015. The extended period of the study from the Ethic Board was due to non-availability of kidney biopsy needles. Of these patients, we performed 31 percutaneous kidney biopsies. Patients were mainly recruited from the Adult Infectious Disease Centre of the University Teaching Hospital. The patients were approached for entry into the study if kidney dysfunction was noted from the patients' files. After accepting entry into the study, urinalysis, kidney and liver function tests, full blood count, CD4 cell counts and abdominal ultrasound were performed.

Figure 1 shows the recruitment and enrolment flow chart of the study.

**Figure 1**

#### FLOW CHART OF THE STUDY



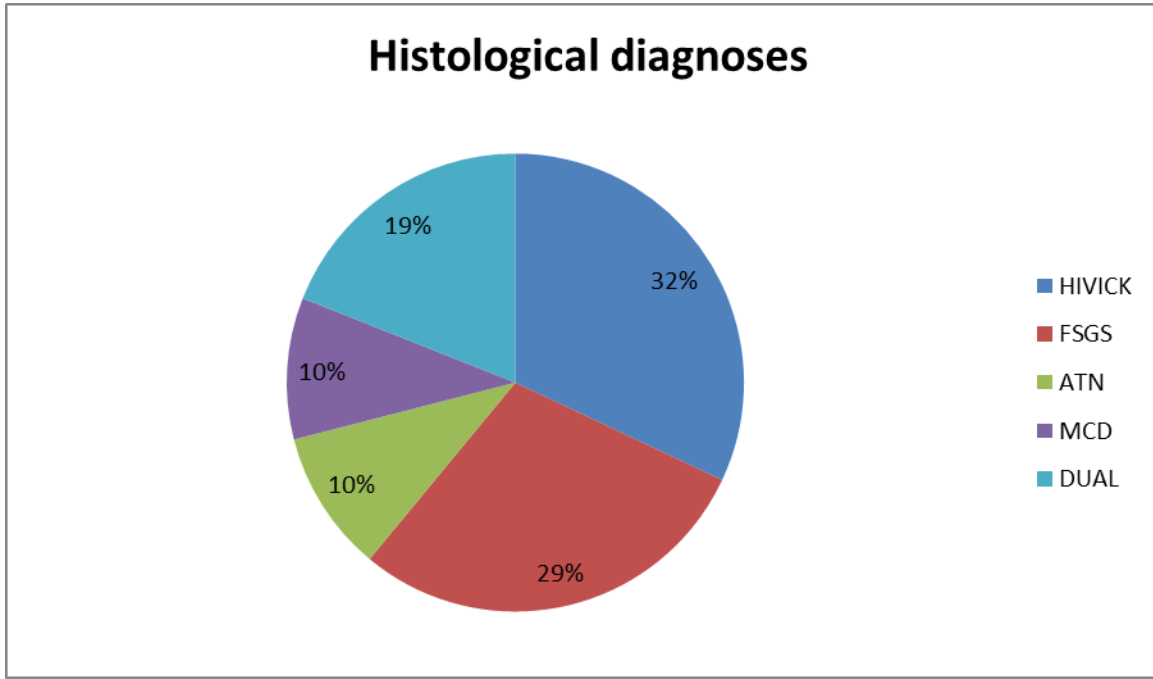
The reasons for non-eligibility into the study included the following:

1. Moribund and severe co-morbid conditions (54 patients)
2. Refused consent to enter into the study (45 patients)
3. Resolved renal dysfunction during hospital stay (these were mainly patients who presented with severe sepsis and kidney dysfunction) (37 patients)
4. Small kidneys on abdominal ultrasound scan (22 patients)
5. Refusal to sign consent to the study procedure (kidney biopsy) (15patients)

In this study, we had sought to describe the spectrum of renal diseases on histopathology with the assumption that HIVAN is the commonest diagnosis in the study population. However, of the 31 patients that underwent renal biopsy, none had the classical HIVAN diagnosis on histopathological description. The most common histological diagnoses were HIV immune complex kidney disease (HIVICK) and focal segmental glomerulosclerosis (FSGS). This is shown in Figure 2 below.

**Figure 2**

**Histological diagnosis distribution (N=31)**



***HIVICK**- HIV immune complex kidney disease, **FSGS**- Focal segmental glomerulosclerosis, **Dual** – dual diagnosis of HIVICK and FSGS, **MCD**- Minimal change disease and **ATN**- Acute tubular necrosis*

### **5.2 Clinical characteristics of the patients**

Most of the study participants, twenty of the 31 (64.5%), were pre cART exposure (not on combination antiretroviral therapy). The male to female ratio in the study participants was almost 1:1. All the patients had both severe renal dysfunction with average eGFR of 24 ml/min/m<sup>2</sup> and nephrotic range proteinuria (3+ on urine dipstick). The mean age of all study participants was 41.3 years with the range of 21 to 58 years. The clinical characteristics of all the study participants are shown in Table 1 below.



**Table 1****Patients' demographics and clinical characteristics**

<b>Variable</b>	<b>Total number N=31</b>	<b>On cART N=11</b>	<b>PreHAART N=20</b>	<b>HIVICK N=10</b>	<b>FSGS N=9</b>
Mean age in years ( $\pm$ SD)	41.3 $\pm$ 10 (21-58)	47.9 $\pm$ 9.7 (28-58)	37.6 $\pm$ 8.3 (21-52)	43.1 $\pm$ 7.1 (31-52)	42.4 $\pm$ 13.1 (21-58)
Males N [%]	16 [51.6]	3 [27.3]	13 [65]	4 [40]	3 [33.3]
Females N [%]	15 [49.4]	8 [72.7]	7 [35]	6 [60]	6 [66.7]
Mean CD4 cell counts in cells/UL ( $\pm$ SD)	197 $\pm$ 120 (21-425)	236.4 $\pm$ 110 (98-425)	175.5 $\pm$ 22.6 (21-423)	211.6 $\pm$ 137.9 (21-387)	229.5 $\pm$ 114.5 (103-425)
Mean creatinine in umol/L ( $\pm$ SD)	321 $\pm$ 112.9 (154-569)	359 $\pm$ 102.3 (243-569)	301 $\pm$ 115.6 (154-569)	318 $\pm$ 133.6 (154-569)	332 $\pm$ 108.8 (225-569)
Mean eGFR in ml/min/1.73m <sup>2</sup> ( $\pm$ SD)	24 $\pm$ 12 (8-57)	21 $\pm$ 13 (10-58)	23 $\pm$ 13 (8-57)	24 $\pm$ 12 (8-57)	23 $\pm$ 11 (12-44)
Hypertension N [%]	5 [16.2]	3 [9.7]	2 [6.5]	3 [9.7]	0 [0]
Mean Hb in g/dL ( $\pm$ SD)	9.7 $\pm$ 1.8 (6.8-13.7)	10.2 $\pm$ 1.7 (7.9-12.8)	9.5 $\pm$ 1.9 (6.8-13.7)	9.5 $\pm$ 1.8 (6.8-12.4)	8.8 $\pm$ 1.3 (6.8-11.2)

*N= Number, eGFR= estimated glomerular filtration rate, Hb= haemoglobin, HIVICK= HIV immune complex kidney disease, FSGS- Focal segmental glomerulosclerosis, c ART combined antiretroviral therapy*

There were more patients who were not on ART in this study (64.5%). The mean CD4 cell count for those that were pre cART was 175 cells/mm<sup>3</sup> while those on cART had a mean of 211 cells/mm<sup>3</sup>. There were more female patients on combination ART, 72.7%, compared to men at 27.3%. The patients who were on combination ART were older, with mean age at 47.9 years, than those patients who were not on ART whose mean age was 37.6 years. Patients that were on combination ART had a mean estimated GFR of 23 ml/min/m<sup>2</sup>

compared to 24 ml/min/m<sup>2</sup> those patients who were not on ART. The commonest ART regimens in those who were on combination ART was protease inhibitor based with lopinavir/ritonavir and tenofovir based as can be seen in Table 2.

**Table 2**

**Summary of patients on cART with histological diagnosis of renal failure cause**

Patient No.	Sex	Age	ART regimen	Duration	Histological diagnosis
1	F	58	ABC, 3TC, EFV	2 years	HIVICK with FSGS ( with chronic interstitial nephritis)
2	M	53	TDF, FTC, EFV	1 year	HIVICK with FSGS
3	M	45	TDF, FTC, LPV/r	4 years	HIVICK
4	M	45	ABC,3TC, LPV/r	6 years	HIVICK with FSGS (with immune complex mediated process)
5	F	58	ABC, 3TC, LPV/r	6 years	Primary FSGS with hyalinosis
6	F	58	TDF, FTC, LPV/r	9 years	FSGS with chronic interstitial nephritis
7	F	43	ABC,3TC, LPV/r	5 years	HIVICK
8	F	45	ABC, 3TC,EFV	3 years	FSGS
9	F	38	TDF, FTC, EFV	6 months	Minimal change disease
10	F	28	TDF, FTC, EFV	6 weeks	ATN
11	F	56	ABC,3TC, LPV/r	1 year	HIVICK and FSGS

### 5.3 Description of Histological diagnoses in study patients

The comparison of the clinical characteristics in the two commonest histological diagnoses is as shown in Table 3 below.

**Table 3**  
**Comparison of the main histological diagnoses variables**

Variable	HIVICK	FSGS	P value
Mean age in years (±SD)	43.1±7.1 (31-52)	42.4±13.1 (21-58)	1.00
Males N [%]	4 [44.4]	3 [30]	1.00
Females N [%]	5 [55.6]	7 [70]	1.00
Mean CD4 cell counts in cells/mm <sup>3</sup> (±SD)	211.6±137.9 (21-387)	229.5±114.5 (103-425)	1.00
Mean creatinine in umol/L (±SD)	318.1±133.6 (154-569)	332±108.8 (225-569)	1.00
Mean eGFR in ml/min/1.73m <sup>2</sup> (±SD)	24±12 (8-57)	23±11 (12-44)	1.00
Mean Hb in g/dL (±SD)	9.5±1.8 (6.8-12.4)	8.8±1.3 (6.8-11.2)	1.00

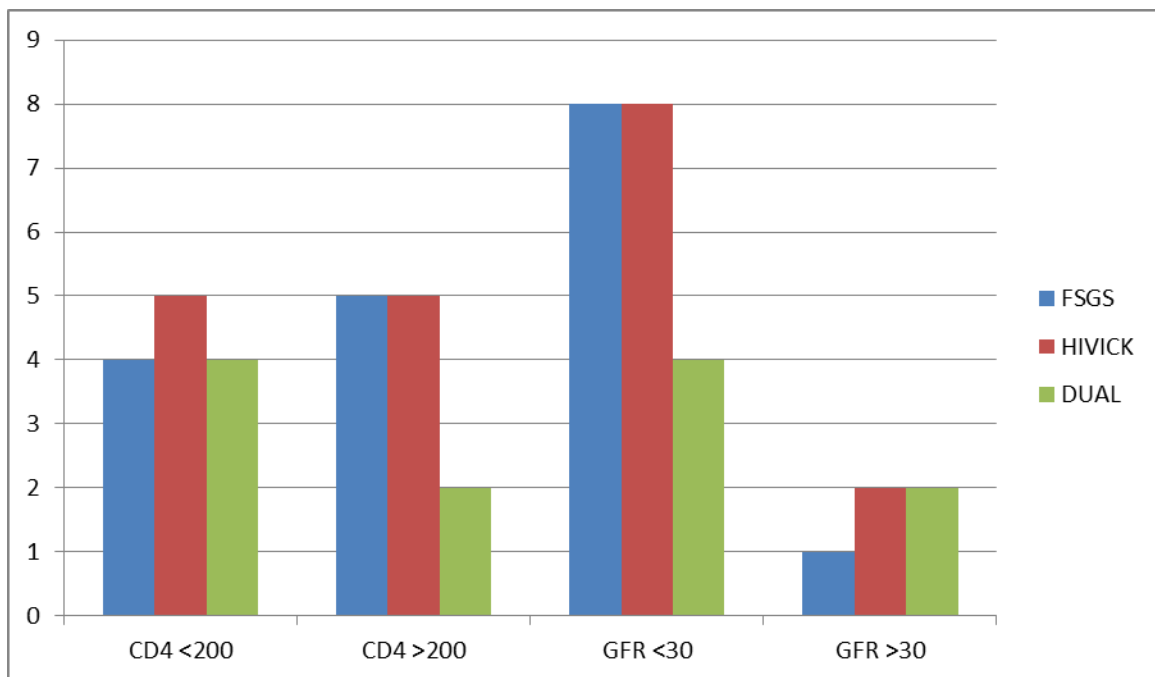
There was no significant difference in the clinical characteristics in the two main histological diagnoses as can be seen from the p values in Table 3.

The histological diagnoses in the remaining 12 patients were as indicated on page 14 in Figure 2; six had dual diagnosis of FSGS and HIVICK, 3 had minimal change disease and 3 were diagnosed as acute tubular necrosis. Of interest is that the 3 patients with ATN presented with sepsis. They however, had persistent proteinuria (of 3+ on urine dipstick) and the renal scan showed echogenic, normal sized kidneys. They were thus diagnosed as acute on chronic kidney disease clinically before renal biopsy was performed.

The clinical characteristics of the patients that had a dual diagnosis of FSGS/HIVICK did not differ clinically from the patients who had either FSGS or HIVICK as a single diagnosis. The severity of renal dysfunction and immunosuppression were not different in these patients. Figure 3 shows this distribution.

**Figure 3**

**Distribution of three main histological diagnoses in the study based on severity of renal dysfunction and immunosuppression (Total number 25)**



The patients with the commonest diagnoses did not differ in their clinical characteristics. However they had some differences in their overall histological descriptions.

Of the nine patients that had the diagnosis of FSGS, the commonest histologic variant was the Tip variant accounting for 55%. One patient (12%) had the cellular variant while 33% had the perihilar variant. This description is seen in Table 4.

**Table 4****Histopathological Description of FSGS**

<b>Cortico-medullary junction</b>	<b>Number of Glomeruli</b>	<b>% of glomerular sclerosis</b>	<b>Tubulointerstitial involvement</b>	<b>Histologic Variant of FSGS</b>
Present	18	47	Multifocal chronic interstitial nephritis of moderate intensity	Tip variant
Absent	27	50	Diffuse chronic interstitial nephritis of moderate intensity	Cellular variant
Present	55	33	Diffuse chronic interstitial nephritis of marked intensity	Perihilar
Absent	12	25	Multifocal active chronic interstitial nephritis of mild intensity	Tip
Absent	18	32	Multifocal active chronic interstitial nephritis of mild to moderate intensity	Tip
Present	36	29	Diffuse chronic interstitial nephritis of moderate intensity, mild activity	Perihilar
Absent	8	24	Active chronic interstitial nephritis with mild intensity	Tip
Present	12	25	Diffuse chronic interstitial nephritis with moderate intensity	Tip
Present	10	5	Patch chronic interstitial nephritis of mild intensity	Perihilar

The 10 patients that had HIVICK as the histological diagnosis had HIVICK not otherwise specified (NOS) as the commonest subtype. Table 5 shows this distribution.

**Table 5****HIVICK subtypes**

<b>Subtype</b>	<b>Number</b>	<b>Interstitial involvement</b>
HIVICK with global sclerosis (NOS)	3	Patchy active chronic interstitial nephritis mild intensity involving 20-25% cortical volume. Tubulo-interstitial nephritis with tubule microcystic dilatation changes.
		Multifocal active chronic interstitial nephritis of moderate intensity accounting for 25-30% of cortical volume
		Diffuse active interstitial nephritis of marked intensity accounting for 35-40% cortical volume
Membranous glomerulonephritis	2	Active chronic interstitial nephritis patchy in distribution and moderate intensity accounting for 10-15% of cortical volume. There were also basement membrane holes noted.
		Active chronic interstitial nephritis patchy in distribution with mild intensity accounting for 20-25% cortical volume
HIVICK with minimal change disease	2	Mild and patchy active chronic interstitial nephritis of mild intensity
		Moderate tubulo-interstitial nephritis composed of lymphocytes and plasma cells
HIVICK with Glomerulopathy with organised deposits and immunotactoid glomerulopathy	1	Mild and global active chronic interstitial nephritis of moderate intensity accounting for 30% of cortical volume. Also global mesangiopathic alterations
HIVICK with Immune complex mediated membranous glomerulopathy	1	Active chronic interstitial nephritis which is patchy to diffuse of moderate intensity
HIVICK with membranoproliferative glomeronephritis	1	Mild and patchy active chronic interstitial nephritis of mild intensity

The patients in this study had severe immunosuppression as can be seen from the mean CD4 cell count of 197 cells/mm<sup>3</sup>. Putting, these patients in the various strata of CD4 cell counts; of the 31 study patients, 17 had CD4 cell counts of <200 per microliter. Seven of these patients had CD4 count less than 100 cells/mm<sup>3</sup>. Most of these patients with severe immunosuppression were not on cART. Of the 17 patients with severe immunosuppression, only six were on combination antiretroviral therapy (ART). The duration of ART ranged from 6 weeks to 9 years.

There was no overall difference in the histological diagnoses of those patients with CD4 cell counts <200 cells/mm<sup>3</sup>. The distribution of the histological diagnoses in these was; four had a dual diagnosis of HIVICK and FSGS, four had FSGS, five had HIVICK, and two each for MCD and ATN. Of the 14 patients in the study that had CD4 cell count ≥200 cells/mm<sup>3</sup>, five each had HIVICK and FSGS while two had a dual diagnosis of HVICK/FSGS, and one each for MCD and ATN. The main histological diagnosis distribution based on level of immunosuppression is as shown in Figure 3 on page 17.

Abdominal ultrasound scans were done in all the patients that were enrolled in the study in order to get a description of the structure of the kidneys and also to rule out any structural abnormalities in the kidneys before biopsy. The reports of the scans showed mostly hyperechoic, normal sized kidneys unlike the traditional description of large sized kidneys previously seen in HIVAN. Even the patients that were found to have ATN were found to have similar description of the kidneys on ultrasound. There was no significant difference in the description and appearance of the kidneys on ultrasound in most of the patients. There was only one patient who had bilateral enlarged kidneys on ultrasound (14×6.5×4.8 cm on the right and 15.3×6.3×4.3 cm on the left). This patient had FSGS with the perihilar variant. As indicated this patient had heavy proteinuria of 3+ on urine dipstick, severe immunosuppression and was on cART for two months at the time of kidney biopsy. This patient would have been postulated to have classical HIVAN in the absence of histology.

The patients in this study had massive proteinuria of 3+. The reading was being done by one research assistant and the results sent to the laboratory to confirm the readings. However protein in urine was not quantified. Patients with massive proteinuria in HIV were generally thought to have HIVAN. In this study however we did not see HIVAN.

## CHAPTER SIX

### 6.0 DISCUSSION

In this study, of the 31 renal biopsies performed, there was no case of classical HIVAN found based on histological description. This is in contrast to studies conducted previously in Africa (mostly in South Africa) and the United States of America where HIVAN was the commonest diagnosis in individuals of African descent (2, 3, 4, 6, 19). This could be due to our small sample size and fact that our study did not have follow up biopsies.

The commonest diagnoses in our study were HIVICK (32%), focal segmental glomerulosclerosis (29%) and the dual diagnosis of FSGS/HIVICK (19%). All the patients had massive proteinuria (grade 3+ on urine dipstick) and severe renal dysfunction with mean GFR of 24 ml/min/m<sup>2</sup>. One study in South Africa found a similar percentage of HIVICK in individuals of African descent (14).

The severe renal dysfunction that patients with HIVICK presented with is in agreement with some studies (14, 34) which indicate that patients with HIVICK tend to have severe renal dysfunction. These patients, however, do not show the rapid decline in renal dysfunction seen in patients with HIVAN (34, 35). Most patients with HIVICK in this study were not on HAART and had severe immunosuppression as can be seen from the mean CD4 cell count of 211 cells/mm<sup>3</sup>. Previous studies though have shown that patients with HIVICK usually have had more exposure to ARVs (34). All the patients in this study had massive proteinuria on urine dipstick, including those patients that were diagnosed as HIVICK. This is in contrast to what was observed in a study where patients with HIVICK had proteinuria less than 1+ on urine dipstick (34). Some studies though are in agreement with this observation of massive proteinuria in that patients with HIVICK tend to have massive proteinuria (14).

In this study, there were only five patients, out of 31 (16.1%) who were hypertensive and on medication for hypertension. Two of these patients had a dual diagnosis of HIVICK/FSGS and two had HIVICK. This is in agreement with what has been observed in other studies which show that HIVICK is more prevalent in patients that have comorbid conditions like hypertension and diabetes (34). In our study however, there was none among the patients enrolled who had diabetes as a comorbid condition.



The patients with HIVICK had global sclerosis not otherwise specified (NOS) as the commonest subtype. The only patient who had hepatitis B had membranoproliferative glomerulonephritis. This patient was on tenofovir based ART regimen which was adjusted based on the creatinine clearance that was calculated. In previous studies, Hepatitis C infection has shown to be strongly associated with the development of HIVICK (36, 37). We did not however see a patient with Hepatitis C in our study.

Focal segmental glomerulosclerosis was the second commonest diagnosis in this study. This is in accordance with what has been found in other studies which indicate that FSGS is a very common histological finding in patients of African descent that have HIV infection (38). We however did not observe any collapsing histologic variant of FSGS. There was also no microcystic tubular dilatation, podocyte proliferation and effacement of the foot processes to confirm the diagnosis of classic HIVAN. The most common histologic variant of FSGS was the tip variant accounting for 55.5%. The second commonest variant was the perihilar variant (33.3%). Studies elsewhere have shown varying frequencies of these histologic variants of FSGS (39-42). Most of these studies have shown that the histologic variant NOS (not otherwise specified) is the commonest.

There was major involvement of the interstitium in FSGS with most patients having chronic interstitial nephritis with moderate to marked intensity. The study from Pakistan found that there 93% interstitial involvement in the patients with FSGS (43). Of the nine patients with FSGS, only one had secondary FSGS. This patient had also been treated for hypothyroidism and nephrotic syndrome in the younger years (about 10 years before recruitment in the study).

The patients in this study had severe immunosuppression with mean CD4 cell count being 197 cells/mm<sup>3</sup> overall, 175 in those patients who were not on cART and 236 in those patients on cART. The patients that had FSGS 66.6% (6 out of 9) were on cART with a mean duration of 2 years. Thirty percent of the patients with a diagnosis of HIVICK were on cART; while the majority of the patients (70%) were not yet on cART. This is contrast with literature that indicates that patients with HIVICK tend to have more exposure to drugs like HAART (34).

In this study there were no patients with microangiopathies. Most of the patients that came in with acute kidney injury had recovery of their kidney dysfunction on conservative treatment and they thus did not undergo renal biopsy. The other reason could be that most of the patients who came in very sick and had serious comorbid conditions were excluded from the study and that the patients who had severe renal dysfunction at presentation actually had chronic kidney disease.

There was no major post kidney complication that was recorded in the study. This was as a result of careful selection of patients before performing the biopsy. This is in agreement with studies conducted in Europe which did not record major adverse events even in critically ill patients. This study has strengthened the fact that a percutaneous kidney biopsy is an important and necessary procedure to diagnose kidney diseases and guide therapy in the process (44, 45).

Kidney biopsy, as has been shown in this study, is an important procedure for diagnosis and subsequent management of patients especially in this study population. Without biopsy, most of the patients in this study could have been wrongly diagnosed as HIVAN.

## **CHAPTER SEVEN**

### **7.0 CONCLUSION**

HIVAN was not the commonest histological diagnosis in the HIV positive black Zambian patients with renal failure undergoing renal biopsy at the University Teaching Hospital in Lusaka. HIVICK and FSGS were the commonest diagnoses in this patient population. The patients had severe renal dysfunction and advanced HIV disease. Among the patients that had FSGS, the tip variant was the commonest histologic variant. We did not see the collapsing variant that has commonly been associated with HIV.

### **7.1 Study limitations**

- Electron microscopy and immunohistochemistry was not done on all the biopsy tissues to conclusively diagnose the various subtypes of HIVICK.
- HIV viral load data was not available to see if the level of viremia matched the level of immunosuppression as indicated by the low CD4 cell counts seen in the study patients.
- Shortage of biopsy needles. Number of patients biopsied was severely limited by number biopsy needles available. Study was also hindered because of the delay in purchase of the same biopsy needles.
- We did not biopsy very sick patients in this study. Therefore we did not conclusively see the entire spectrum of kidney diseases that is seen in HIV positive patients.
- The small number of patients that underwent kidney biopsy in the study, and the fact that this was a single centre study, makes it difficult to draw definite and strong conclusions.

## 7.2 Recommendations

- There is need to do a multicentre study with a bigger sample size and increase the scope of the inclusion criteria for us to draw strong conclusions about this patient population. These patients should be followed up so that we can assess the outcomes over time.
- Kidney biopsy is mandatory and should be performed in all patients that present with renal dysfunction as it gives a definite diagnosis and thus guides therapy and improves outcomes. Adequate stocks of these needles should be made available to make a confirmatory diagnosis.
- All HIV positive patients need to have their kidney function evaluated at each visit by way doing urinalysis and serum creatinine so that those that show derangements in kidney function can be evaluated further in order to optimise their clinical care

## CHAPTER EIGHT

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## CHAPTER NINE

### 9.0 APPENDICES

#### 9.1 Patient Information

Thank you for your interest in this study.

My name is Dr Chansa Abidan of Lusaka Woodlands extension plot number 85356. I am a Master of Medicine student in the Department of Internal Medicine at University Teaching Hospital under the University of Zambia.

You are invited to participate in this research study which is part of fulfilment of my research in the Master of Medicine in Internal Medicine, postgraduate training.

**STUDY TITLE: Prevalence, histopathological and clinical presentation of HIVAN in adults presenting with renal insufficiency to UTH**

This study is looking at the number of, and ways in which or problems that patients with kidney diseases present with to the hospital. The study also entails to identify the various forms of kidney diseases that patients with HIV infection have.

The study will be conducted by getting information about the patient and the complaints that they present with to the hospital. Then some blood and urine tests including a biopsy of the kidney will be performed. The blood samples will be collected in three small specimen bottles. You will be requested to submit two urine samples two weeks apart for analysis. You will also have an ultrasound scan of your abdomen done to look at the kidneys.

Biopsy of the kidney is a scientific procedure where a biopsy needle is used to get a tiny piece of the kidney. This procedure is done under local anaesthesia (that is to make the area where the needle will be placed during the time of the procedure numb). The patient is awake during the time of the procedure.

Once you join in this study, your participation in the study will be confidential and your personal information will not be disclosed as you will be accorded a study identification code.

**Who is eligible to be recruited in the study?**

Patients who are 18 years of age and above, are HIV positive, have kidney dysfunction, willing to undergo study procedures and have signed informed consent.

**What are the benefits of you joining this study?**

By joining in this study, more tests than those that are normally done, will be conducted on you and a definitive diagnosis will be made. When you undergo renal biopsy a definitive diagnosis of your kidney disease will be reached at. This has been proven to guide treatment as the cause of the disease is found through doing this procedure. This will greatly assist in giving you the exact treatment tailored to the disease entity identified rather than the empirical treatment that is normally given. Additionally, all tests will be conducted free of charge. This study will also enhance scientific knowledge such that the general public will also benefit.

**Are there any risks involved in your participation in this study?**

There are very minimal risks involved in your participation of this study. These include, the discomfort of a needle prick and bruising that come with collecting blood samples. And also the pain that comes after the kidney biopsy. These are however minimized by qualified and experienced personnel doing the procedures. Also pain relief will be offered where the pain becomes unbearable.

**What will happen to you in case you decide to leave the study?**

You are free to leave the study at any point without suffering neglect or compromising your clinical care. You will not be discriminated against by any member of the medical staff in any way.

**Who do you contact in case of any questions or clarifications regarding the study?**

You can contact me, Dr Chansa Abidan, on mobile phone number +260 969119118 or e mail address: [chansachilupe@yahoo.com](mailto:chansachilupe@yahoo.com)

My supervisor, Dr Aggrey Mweemba on mobile number +260 976853505 or e mail address: [aggmw@yahoo.com](mailto:aggmw@yahoo.com)

My co-supervisor, Dr Shabir Lakhi, on mobile number +260 0979709010 or e mail address:  
[slakhi2009@gmail.com](mailto:slakhi2009@gmail.com)

ERES CONVERGE on mobile +260955155633, e mail: [eresconverge@yahoo.co.uk](mailto:eresconverge@yahoo.co.uk)

## 9.2 Consent form

I,....., hereby confirm that I have been sufficiently explained to about the nature, conduct, benefits and risks of this clinical study. I have also received, and/or read and understood the above written information about the study. I am aware that my personal details will be anonymously processed into the research report. I have understood that I may voluntarily, at any point, withdraw my participation in the study without suffering any consequences. I have been given sufficient time to ask questions and seek clarifications, and of my free will declare my participation into the research study.

I have received a signed copy of this agreement.

Participant's signature or thumbprint                      Date

Person obtaining informed consent                      Date

Witness    Date

### 9.3 Data Collection Tool

#### Eligibility

##### Inclusion criteria

HIV positive	Yes	No
Age 18-65	Yes	No
Renal insufficiency defined as creatinine >146umol/L and/or persistent proteinuria or microalbuminuria of 1+ and above	Yes	No
Willingness to undergo study procedures	Yes	No
Signed informed consent	Yes	No

##### Exclusion criteria

Shrunken kidneys or single kidney on abdominal US scan	Yes	No
Patient with overt heart failure	Yes	No
Moribund patients	Yes	No

Patients with uncontrolled hypertension	Yes	No
Patients with bleeding diathesis	Yes	No
Pregnancy	Yes	No
Patients on aspirin, clopidogrel, heparin and warfarin	Yes	No

**Patient demographics**

Name of patient	
Contact number	
Next of kin	
Residential address	

**History**

Age:

Sex:

**Symptoms**

<b>Symptom</b>	Yes	No
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Passing urine		
Fever		
Nausea/vomitting		
Shortness of breath		
Easy fatiguability		

**Past medical history**

	<b>Yes</b>	<b>No</b>
Diabetes mellitus		
Hypertension		
Past kidney disease		
Hepatitis B		
Bleeding tendencies		
Patient on ARVs		
Is patient pregnant		

If patient is on ARVs, 1. For how long.....

2. What regimen.....

**Social history**

Intravenous cocaine use	Yes	No
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## Physical examination

### Vitals

Temperature	
Respiratory rate	
Pulse rate	
Blood pressure	

### Urinalysis

Proteinuria	Yes	No
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Level of proteinuria	1+	2+	3+
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### Systemic examination

	Normal	Abnormal
Chest findings		
Cardiovascular		
Abdominal findings		
Pedal oedema		



## Results of investigations

Hemoglobin	
Platelet count	
Creatinine	
Urea	
Hepatitis B surface antigen	
CD4+ cell count	
Bleeding time	

Summary of abdominal ultrasound findings:

Histopathological diagnosis:

## **9.4 Summary of Kidney Biopsy Tissue Preparation**

The kidney biopsy liquid filled containers were labelled with the patient's name, age and date of biopsy.

After biopsy, the tissues were put in the containers and the tops secured, placed inside the Ziploc specimen biohazard bag and absorbent sheet and seal.

Only light microscopy was done on the tissues. Thus the cores of biopsy tissues were put in saline solution in a petri dish. Tissues were then cut using a microtome or dissecting microscope.

The specimens were then fixed with 10% formalin neutral-buffered formalin at 4 °C.

After fixation, a qualified pathologist examined the slides and provided a histopathology report.

