HUMAN IMMUNODEFICIENCY VIRUS (HIV)-ASSOCIATED NEPHROPATHY AMONG ANTIRETROVIRAL NAÏVE ADULTS WITH PERSISTENT PROTEINURIA AT THE MOI TEACHING AND REFERRAL HOSPITAL (MTRH)

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Human Immunodeficiency Virus(HIV)-associated nephropathy among antiretroviral naïve adults with persistent proteinuria at the Moi Teaching and Referral Hospital (MTRH)

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DECLARATION

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I declare that this research thesis is my original work and that it has never been presented for a degree in any other university. No part may be reproduced without prior permission of the author or Moi University.

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TABLE OF CONTENTS

TABLE OF FIGURES

ABBREVIATIONS

ACE	Angiotensin converting enzyme
ACEI	Angiotensin converting enzyme Inhibitor
AIDS	Acquired Immunodeficiency Syndrome
AIN	Acute interstitial nephritis
AMPATH	Academic Model Providing Access to Healthcare
APIGN	Acute postinfectious glomerulonephritis
APOL1	Apolipoprotein L1
ART	Antiretroviral therapy
ARV	Antiretroviral (drugs)
BP	Blood pressure
CBC	Complete blood count
CD4	Cluster of differentiation 4
CDC	Centers for Disease Control
CIN	Chronic interstitial nephritis
CKD	Chronic kidney disease
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
eGFR	Estimated glomerular filtration rate
ESRD	End-stage renal disease
FSGS	Focal segmental glomerulosclerosis
GFR	Glomerular filtration rate
HAART	Highly active antiretroviral therapy
HBV	Hepatitis B Virus

HCV	Hepatitis C Virus
H-ESKD	Hypertension-attributed end-stage kidney disease
HIV	Human Immunodeficiency Virus
HIVAN	HIV-associated nephropathy
HIVICK	HIV immune complex kidney disease
HTN	Hypertension
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IREC	Institutional Research and Ethics Committee
KAIS	Kenya AIDS Indicator Survey
MTRH	Moi Teaching and Referral Hospital
MYH9	Myosin, heavy chain 9, non-muscle
RNA	Ribonucleic acid
RRT	Renal replacement therapy
SNP	Single nucleotide polymorphism
TRI	Tubuloreticular inclusion
UACR	Urinary albumin-to-creatinine ratio
UTI	Urinary tract infection
WHO	World Health Organization

DEFINITIONS

Adult

We defined an adult as anyone with the age of 14 years and above.

Antiretroviral naïve

One who has never been exposed to antiretroviral medication.

HIV-associated nephropathy

HIV-associated nephropathy (HIVAN) is a unique kind of glomerular inflammation associated with HIV infection that presents with proteinuria, often in the nephrotic range, usually with no edema, a normal blood pressure and renal insufficiency that progresses rapidly to end-stage renal disease(ESRD). Its definitive diagnosis is made by renal biopsy which shows collapsing glomerular sclerosis with tubulointerstitial inflammation and fibrosis with tubular microcystic dilatation on light microscopy.

Microalbuminuria

Microalbuminuria is defined as the abnormal excretion of small amounts of albumin that are not detectable by urine dipstick analysis; this is typically between 30 and 300mg/day.

Proteinuria (macroalbuminuria)

Proteinuria is defined as the abnormal excretion of protein in urine as characterized by the presence of proteins on dipstick examination equal to or exceeding 1+, this corresponds to a protein (albumin) excretion of more than 300mg/day.

Persistent microalbuminuria/proteinuria

Persistent proteinuria, for the purposes of this study, is proteinuria that is notable on two occasions at least 2 weeks (14 days) apart, within a period of 3 months.
Persistent proteinuria is a marker of intrinsic kidney disease.
*For the purposes of this study microalbuminuria and macroalbuminuria were both considered as proteinuria.

Nephrotic range proteinuria

This is proteinuria exceeding 3.5g/day.

ABSTRACT

BACKGROUND: As the HIV/AIDS pandemic continues to affect millions in sub-Saharan Africa, more will fall victim to its systemic complications. Among African Americans, HIV-associated nephropathy (HIVAN)has been noted to be of particular predilection. Very little is known about this form of kidney disease in sub-Saharan Africa even though potentially life-saving antiretroviral drugs are increasingly available and are useful in the treatment of this serious renal disease.
METHODS: We conducted a cross-sectional study and screened HIV-infected non-febrile, non-hypertensive and non-diabetic, antiretroviral naïve adults attending a large HIV care program in Western Kenya for the presence of persistent microalbuminuria/macroalbuminuria (proteinuria). Those with persistent proteinuria, subject to consent, underwent a renal biopsy. Subjects were interviewed, examined and blood taken for laboratory analysis. Data on demographics, clinical presentation, CD4 counts, serum creatinine, urine dipstick protein, urinary albumin to creatinine ratio (UACR) and biopsy diagnosis were collected and analyzed. Proportions and means were calculated and inferential statistics was done using the exact method of chi-squared test.
RESULTS: 523 subjects were screened between December 2010 and May 2011. 85/523 (16.3%) had spot proteinuria; 53/85 (62.4%) came back for follow-up, 32/53 (60.4%) had had persistent proteinuria and by imputation 9.8% of all screened had persistent proteinuria. 27 subjects with persistent proteinuria underwent biopsy, 17/27 (63%) were female. The mean age was 36.6 years (range 23-65 years).14/27 (52%) reported being on medications,21/27 (78%) reported no symptoms and 23/27 (85%) had a normal physical exam. 24/26 (92%) had normal renal function.24/26 (92%) had UACR less than 1 g/g, mean UACR was 384.2 mg/g (range 5-1384 mg/g). Mean CD4 count was 340.7 cells/μL, median 369 cells/μL (range 3-1060 cells/ μL). None of them had histologic features consistent with HIVAN. 11/27 (41%) had a primary diagnosis of acute interstitial nephritis (AIN), 9/27 (33%) had non-specific findings, 2/27 (7.4%) arteriosclerosis, while focal segmental sclerosis (FSGS), acute postinfectious glomerulonephritis (APIGN), chronic interstitial nephritis (CIN), pyelitis and papillary sickling were 1/27 (3.7%) each. No association was found between age, sex, tribal lineage, UACR and CD4 with any particular diagnosis.
CONCLUSIONS: The prevalence of HIVAN among antiretroviral naïve adults with persistent proteinuria at the Moi Teaching and Referral Hospital (MTRH) is low. AIN is the most common cause of persistent proteinuria in this setting.

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CHAPTER ONE

INTRODUCTION

1.1 Background

HIV infection, now a pandemic, is a major problem all over the world and particularly in sub-Saharan Africa.[1] In Kenya HIV affects 6.7% of the population. The Rift Valley province alone is home to 304,000 HIV-infected adults.[2] The AMPATH program cares for close to 100,000 HIV-infected patients spanning Western Rift Valley and parts of Western province. It enrolls up to 200 new clients at its MTRH clinics every month. As of January of 2010, there were close to70,000 active patients with close to 40,000 on antiretroviral treatment (*AMPATH unpublished data*).

Many forms of kidney disease occur among the HIV-infected, ranging from urinary tract infections, acute and reversible renal failure, drug related kidney injury, glomerulonephritides, thrombotic microangiopathies to neoplastic lesions.[3-5] HIV-associated nephropathy (HIVAN) is the single most common identified cause of nephropathy among the HIV-infected, specifically in people of African ethnicity.[6-9]

HIVAN was first described among the HIV-infected African-American population in the United States.[10,11] It presents with proteinuria and renal insufficiency that progresses rapidly to end-stage renal disease (ESRD). In the pre-HAART era, it typically led to ESRD in 1 to 4 months with a mortality of close to 100% in 6 months.[8,10,12] It is characterized morphologically by segmental glomerulosclerosis with glomerular collapse, tubular microcysts, interstitial fibrosis, and inflammation and the presence of tubuloreticular inclusions (TRI) on electron microscopy.[8,13-15] It is the leading cause of chronic kidney disease among patients with HIV infection and in

the United States is the third leading cause of end-stage renal disease among the black population between the ages of 20 and 64 years.[8,9,16] It is important to note however that in patients presenting with typical clinical features of HIVAN, renal biopsy findings of HIVAN may not be present in up to 40-50% of patients.[17] The other lesions include membranoproliferative glomerulonephritis, minimal change disease, membranous glomerulopathy, amyloidosis, immune-complex glomerulonephritis, and IgA nephropathy.[6,10,18,19]

HIVAN has been described to be more prevalent among the black population with reports of as much as 97% of HIVAN patients being black. Males have a higher risk of HIVAN than females and most patients with HIVAN have high viral loads and lower CD4 T-cell count.[20] HIVAN can manifest in earlier stages of HIV, even before seroconversion and in these cases there is evidence that the initiation of HAART leads to dramatic improvement.[21]

With the increasing prevalence of HIV/AIDS, it is anticipated that the prevalence of HIVAN will increase. Some beneficial effect on disease progression can be achieved with the use of highly active antiretroviral therapy (HAART), angiotensin-converting enzyme (ACE) inhibitors and to some extent steroids.[22]

Worldwide, data on HIVAN is scarce. HIVAN is estimated to affect between 3.5-12% of HIV-infected persons in the United States[17] with the lower figure recorded in a cohort of HIV-1 seropositive patients screened for proteinuria in a primary care setting[23] and 12% from an autopsy series.[24] Very little is known about the prevalence of HIVAN in African populations. Studies differ in many settings; one study from

South Africa documented a 6% rate of proteinuria, with HIVAN being the most frequent diagnosis (83%) among those who underwent biopsy[19].

A study on Ethiopian Israelis on the other hand showed no clinical evidence of HIVAN among a cohort of 176 patients.[25] Similarly, in Uganda, a study on 299 HIV-infected patients found only 1 (0.3%) to have HIVAN.[26] This is despite overwhelming evidence that HIVAN is a disease with predilection for the black race. The possibility of regional differences or ethnic variations is therefore unexplored.

A study done at AMPATH, although not designed to evaluate the prevalence of HIVAN, reported a 6.2% rate of proteinuria as defined as a urine dipstick protein of equal to or greater than 1+[27]. This was however not persistent proteinuria. It is necessary to determine the prevalence of persistent proteinuria as this is more reflective of renal disease.[28] A study has shown that even with microalbuminuria, the renal histological changes are quite advanced.[19] This advocates for doing urinalysis to identify patients early enough to influence management.

1.2 Problem Statement

HIV complications affecting the kidney have been recognized and outlined. Of note is HIVAN which predominantly affects people of African ancestry and has a very aggressive clinical course. It can be easily detected by urinalysis, yet urinalysis is not part of the routine investigations for HIV-infected patients in Kenya.

We have no concrete information about the epidemiology, the histopathological spectrum and the outcomes of HIV-associated kidney diseases in our population. We

need to find out how big a problem or not HIVAN and other HIV-associated renal diseases are and based on this information, set up strategies and policies to deal with it.

1.3 Justification

HIVAN is an aggressive condition that confers significant morbidity and mortality to the HIV-infected. The impact of HIVAN is not known in our population as it is never looked for, yet it is a WHO stage 4 criterion. HIVAN leads to end-stage renal disease (requiring dialysis) within weeks to months. Considering that dialysis services have been prohibitively expensive and largely inaccessible even in the non HIV-infected population, HIVAN has the potential to pose serious challenges once these patients progress to ESRD. We need to focus on the early detection of this disease and offer treatment to forestall its progress to better the quality of lives of our HIV-infected patients and secondarily save up on resources that are otherwise scarce.

The only reliable way to establish HIVAN is by renal biopsy. This is because there are a host of other kidney diseases that may mimic HIVAN in clinical presentation but have entirely different modes of management. In the absence of platelet and coagulation disorders a renal biopsy is equally as safe in the HIV-infected as in the non HIV-infected. This makes it justifiable to perform renal biopsies on patients suspected to have HIVAN.

There is evidence to show that prompt initiation of HAART halts and even reverses HIVAN and greatly improves survival of patients with HIVAN especially if found early. HIVAN is classified by the WHO as an AIDS defining illness; a patient with

HIVAN is at clinical stage 4 of HIV infection. This means a diagnosis of HIVAN necessitates the initiation of HAART.

The hospital has a trained nephrologist now, biopsies are currently being done on a weekly basis, and there have been no adverse events so far due to necessary precautions and expertise in performing the procedure.

In the scientific field, this study will inform and guide further research into HIVAN and other HIV-associated renal diseases especially in the East African region where very few publications exist on this subject. It will also inform decision making and planning in HIV care on the extent of this problem.

1.4 Objectives

1.4.1 Main Objective:

To determine the prevalence of HIVAN among HIV-infected antiretroviral naïve adults with persistent proteinuria at MTRH.

1.4.2 Secondary Objectives:

1. To determine the prevalence of persistent proteinuria among HIV-infected antiretroviral naïve adults at MTRH.
2. To determine other histological variants of renal disease among HIV-infected antiretroviral naïve adults with persistent proteinuria.
3. To estimate the prevalence of HIVAN among HIV-infected antiretroviral naïve adults at MTRH.

1.5 Research Questions

This study aims to answer the following questions:

1. What is the prevalence of HIVAN among antiretroviral naïve adults with persistent proteinuria at MTRH?
2. What are the other renal histopathological findings among antiretroviral naïve adults with persistent proteinuria at MTRH?

CHAPTER TWO

LITERATURE REVIEW

HIVAN was first recognized in 1984 with initial reports of HIV-infected people presenting with nephrotic range proteinuria and progressing to end-stage renal disease(ESRD) within 8 to 16 weeks.[10,11] Since then there has been tremendous progress in the understanding of this unique condition.

2.1 Epidemiology of HIVAN

The prevalence of HIVAN varies widely. Most of the figures quoted are based on studies in the United States. HIVAN prevalence is estimated to vary between 1 and 10% of the HIV-infected population in different geographic locales.[9] A study on a cohort of 557 HIV-1 seropositive outpatients, half of whom were black, screened for proteinuria by urinalysis. Those with more than 1.5g/day of proteinuria underwent renal biopsy, and 3.5% of black patients were diagnosed with HIVAN.[23] Another recent study determined the prevalence of HIVAN from autopsy data of HIV-infected patients, they reported an overall prevalence of 6.9% with a majority (93%) being black. The prevalence among the blacks was 12%.[24] It is estimated in the United States that HIVAN is the leading cause of chronic kidney disease among the HIV-infected and is the third leading cause among blacks between the ages of 20 and 64 years.[8,9,16]

There is a paucity of published data on the epidemiology of HIVAN in Africa. Two studies in 2006 in South Africa are notable. Han *et al* in KwaZulu Natal screened 615 HIV-infected patients for proteinuria, of these, 38(6%) had proteinuria, 7 of 90 volunteers tested for microalbuminuria had persistent microalbuminuria. 30 with

varying degrees of proteinuria underwent renal biopsy and 25(83%) had HIVAN. Of note in this study is that they also did biopsies on patients with microalbuminuria and 6 of the 7 had features of HIVAN, a significant finding; it suggests that early diagnosis of HIVAN can be made on the basis of microalbuminuria.[19] Another study by Gerntholtz *et al* in Soweto conducted in retrospective fashion reviewed renal biopsies of HIV-positive black African patients to determine the prevalence of both 'classic HIVAN' and non-HIVAN pathologies. They documented 27% HIVAN and 21% HIV immune complex kidney disease(HIVICK).[29]

In Uganda, a study at Mbarara University Teaching Hospital on 299 HIV-infected adults found a very low rate of HIVAN. 1/299 (0.3%) had HIVAN.[26]

In Kenya, we have little information on HIVAN. In a review, McLigeyo also quotes a prevalence of 1 to 10 % in different geographic locales.[30] Two local studies so far have been done to assess kidney diseases in HIV. A study in 2004 at AMPATH assessed 373 antiretroviral naïve HIV-infected adults without diabetes or hypertension and documented a 11.5% rate of renal insufficiency as defined by a creatinine clearance of<60ml/min. Proteinuria as defined by a dipstick protein of equal to or greater than 1+ was detected in only 6.2%.[27]An unpublished study at the Kenyatta National Hospital(KNH) in 2004 on 56 HIV-infected subjects with proteinuria found 7(12.5%) to have proteinuria exceeding 1g of protein/g of creatinine by urinary albumin to creatinine ratio(UACR). 6 underwent biopsy and 5 of them had histological features suggestive of HIVAN.[31]

2.2 Clinical presentation of HIVAN

Most patients presenting with HIVAN are of African descent, presenting in late stages of HIV.[32] In most cases they have been diagnosed with HIV for long but there are reports of HIVAN presenting during HIV seroconversion.[21,33,34] These patients commonly present with nephrotic range proteinuria although not always and despite this they do not have significant peripheral edema.[18] This has been suggested to be because these patients being HIV-infected have high globulin levels and high total protein levels in serum despite losing protein (mostly albumin) in urine. They also present with advanced renal failure but are not hypertensive.[12,20,32] Most black patients with renal insufficiency due to other causes have been known to be hypertensive.[35] This is a bit surprising, but patients with HIVAN have been known to have a decreased ability to conserve sodium[36] and this could at least partly explain this.

2.3 HIVAN and racial predilection

HIVAN has been noted severally, at least in the United States, to have a predilection for people of African descent.[37,38] A study in the United States based on the US Renal Data System analyzed 375,152 patients started on dialysis between 1992 and 1997, 3653(0.97%) had HIVAN, and among those with HIVAN, 87.8% were African American. On logistic regression analysis, HIVAN had the strongest association (odds ratio 12.2) with African American race compared with other causes of renal failure except sickle cell anemia.[39] Similar results have been reported in France[20] where a retrospective review of 102 patients with biopsy-proven HIVAN showed 97% of them to be black. In London[40] a retrospective review of 17 patients with renal disease confirmed by antemortem percutaneous renal biopsy or necropsy resulted in 7(41%) having HIVAN, all of whom were black.

This strongly suggests a racial link between HIVAN and genetic predisposition. This is further reinforced by the fact that African Americans with ESRD due to HIVAN are 5.4 times more likely to have first-or second-degree relatives with ESRD (not caused by HIVAN) than are HIV-infected black people without renal disease.[41] Earlier studies had suggested a role for the nonmuscle myosin heavy chain type II isoform A (MYH9) gene.[42,43] For a while there wasn't any obvious causal link between MYH9 and primary focal segmental glomerulosclerosis(FSGS) as there was no identifiable causal sequence variation. Further research yielded fruit with a landmark paper in 2010 that found an association with a gene ~20kb upstream of MYH9 called APOL1 (apolipoprotein L1). It showed that in African Americans, FSGS and hypertension-attributed end-stage kidney disease(H-ESKD) are associated with two independent sequence variants in the APOL1 gene on chromosome 22 with odds ratio, OR of 10.5(95% confidence interval, CI 6.0-18.4) and 7.3(95% CI 5.6-9.5) respectively.[44] Furthermore, they found that the two APOL1 variants are common in African chromosomes but absent from European chromosomes and that both reside within haplotypes that harbor signatures of positive selection. APOL1 is a gene whose product has been shown to confer protection from African trypanosomiasis by causing lysis of the parasite. Only the APOL1 variants associated with kidney disease were shown in subsequent *in vitro* assays to lyse *Trypanosoma brucei rhodesiense.* It is now thought that evolution pressure that conferred protection from this parasite conversely predisposed to kidney disease.

2.4 Pathogenesis of HIVAN

Evidence now points to direct infection by HIV of the renal parenchymal cells as being important in the pathogenesis of HIVAN. Initial studies were done with animal

models, and Dickie *et al*[45] reported on an HIV-1 transgenic mouse model with *gag*/*pol* deleted defective HIV provirus. The mice manifested renal disease associated with proteinuria, a high mortality rate and HIV-specific gene expression in the kidney. The mice also had lesions identical to HIVAN on histopathological examination. A study using reciprocal transplantation techniques confirmed that it is the HIV-1 gene expression rather than dysregulated cytokines secondary to HIV infection that causes HIVAN. Bruggeman *et al*[46] transplanted kidneys between normal and transgenic mice, HIVAN developed in transgenic kidneys transplanted into nontransgenic littermates, normal kidneys remained disease free when transplanted into transgenic littermates.

In 2000, Bruggeman *et al*[47] further confirmed HIV-1 infection and expression in renal parenchymal cells; renal glomerular and tubular epithelial cells were found to contain HIV-1 mRNA and DNA indicating infection by HIV-1. They also found circularized viral DNA in the biopsies suggesting active viral replication in renal tissue. Further studies have confirmed HIV-1 infection in other parts of the nephron reflecting the pattern of disease as appears on histological examination.

Kidney diseases that manifest with proteinuria are typified by abnormalities in podocyte structure and function. Podocytes are infected by HIV-1 in HIVAN.[47] It has also been reported that in collapsing forms of FSGS including idiopathic FSGS and HIVAN, podocytes undergo characteristic and irreversible ultrastructural changes with loss of specific podocyte markers defining a novel dysregulated podocyte phenotype.[48] This similar podocyte phenotype has been observed in HIV-1 transgenic mice.[49]

Tubular epithelial abnormalities have also been documented in HIVAN. There is increased proliferation and apoptosis, microcystic dilatation, flattening and atrophy of epithelial cells with dedifferentiation and abnormal polarization of sodium-potassium ATPase.[13,46,48-51]

Based on studies in transgenic mice it is thought that expression of viral *gag* and *pol* genes is not essential for causation of HIVAN[45], the expression of *nef* gene is probably the major determinant in the pathogenesis of HIVAN.[52]

2.5 Diagnosing HIVAN

The diagnosis of HIVAN is made by histological examination of renal biopsy tissue.[53] The findings include collapsing glomerulosclerosis due to podocyte proliferation, with tubular microcystic dilatation, atrophy and flattening of tubular epithelial cells and interstitial inflammation and fibrosis. On electron microscopy, the presence of tubuloreticular inclusions (TRIs) and podocytes dedifferentiation is common.[13,14,48]

A kidney biopsy is a relatively safe procedure, especially with the improvements in imaging and biopsy techniques. In the absence of platelet and coagulation disorders, patients with HIV infection are not at a greater risk of complications during renal biopsy than the general population.[3]

2.6 Treatment of HIVAN

The literature so far lacks randomized controlled trials aimed at defining the benefits of any form of therapy for HIVAN. Currently it is reasonable to direct treatment towards the goals of reducing HIV replication, slowing the progression of renal

disease and when necessary, preparing for ESRD therapy-renal replacement therapies(RRTs): dialysis or transplantation.[54]

There are several case reports of dramatic clinical and histological improvement of renal disease with the use of HAART.[21,55,56]

Angiotensin-converting enzyme (ACE) inhibitors have been shown to prevent or slow progression of renal failure in various proteinuric renal diseases.[57-59] Kimmel *et al* reports on 18 patients with biopsy-proven HIVAN, 9 treated with captopril and 9 not treated (matched controls). Renal survival was enhanced in the treatment groups compared to controls.[60] This similar result is reported by Wei *et al*[61] in a longer term study of 44 patients with biopsy-proven HIVAN. These studies, although limited by design and the small number of patients, suggest a benefit of ACE inhibitors in HIVAN prior to the onset of severe renal disease.

The role of steroids in HIVAN is currently not established. Some authorities do not recommend its use except for patients with HIVAN who have no active infectious complications where there may be a short term benefit while HAART is being titrated to achieve maximal suppression of HIV.[62]

Once in ESRD, options include dialysis and kidney transplantation. Dialysis has been shown to improve survival in patients in ESRD on HAART.[63] Regarding transplantation, little data is available so far. There are reports from various transplant centers in the US of good outcomes with transplantation of HIV patients with CD4 counts more than 200cells/mm^3 and undetectable viral loads on HAART.[64]A 2010

publication concluded that with careful selection, high patient- and graft-survival rates could be achieved with no increases in complications associated with HIV infection. However they found unexpectedly high rejection rates that warrant the search for better immunotherapy.[65] There is some suggestion on even offering HIV-positive donor kidneys to HIV-infected renal failure patients.[66]

2.7 Prognosis of patients with HIVAN

Unfortunately, most patients with HIVAN present late and usually will have advanced renal failure.[32] Without prompt treatment with HAART, ACE inhibitors or steroids, most progress to ESRD in 1-4 months.[60,67-69] This can be dramatically different when patients are put on treatment.

Factors that are associated with increased risk of progression to ESRD include: high serum creatinine,[20,70,71] low CD4 cell count,[20,71] higher levels of proteinuria,[20] higher viral load,[71] and prior antiretroviral therapy.[20] There is however evidence that in the HAART era, remarkable improvements in survival have been achieved.

It is important to note that early referral to a nephrologist is important since early intervention in any form of renal disease has a significant bearing on its outcome. Late nephrologist referral is an independent risk factor for early death on dialysis.[72]

CHAPTER THREE

METHODOLOGY

3.1 Design

The study was a cross-sectional descriptive study. Although patients were seen on two occasions (14 days apart), it was part of the process of screening, to ensure proteinuria was persistent before subjects were recruited. Only subjects with persistent proteinuria were recruited into the study.

3.2 Study site

The AMPATH clinics at MTRH. AMPATH was chosen as the study site because of its high recruitment rate based on a wide catchment area.

3.3 Study population

All HIV-infected adults attending AMPATH who were not on antiretroviral treatment.

3.4 Sampling and recruitment

Sampling was done conveniently; subjects who were found at the clinic were screened.

Subjects coming to AMPATH between Monday and Friday from 8am to 5pm with known diagnosis of HIV infection (newly diagnosed or on follow-up) but not on any antiretroviral treatment were screened for proteinuria.

They were approached and informed about the concern for HIVAN by virtue of being HIV-infected. They were informed that they were to be screened for proteinuria and if found to be proteinuric, they would have a further check for proteinuria at their return visit in at least 14 days. Those satisfying the inclusion criteria were reviewed by a clinician as per standard practice and conditions included in the exclusion criteria if found were noted, and the patient excluded. Those not excluded were initially screened for hypertension; only patients with systolic blood pressure less than 140mmHg and diastolic blood pressure less than 90mmHg were included in the study (see Appendix II for details). Those who were normotensive were next screened for diabetes. A blood sugar was taken using a glucose meter (see Appendix IV for details). Those that had high blood sugar (fasting blood sugar exceeding 7.0mmol/L, or random blood sugar exceeding 11.1mmol/L) were excluded. The remaining subjects underwent standard lab tests: complete blood count (CBC), serum creatinine and CD4 counts and provided a urine sample for dipstick analysis (see Appendix V, VI, VII and VIII for details).

Those who had no macroalbuminuria (routine dipstick protein nil) were further tested for microalbuminuria, those who turned up negative for the two tests were excluded at this point; those that had micro/macroalbuminuria (proteinuria) were booked for further urine dipstick evaluation at a return visit not earlier than 2 weeks (14 days).

At the two weeks return, a repeat dipstick for macroalbuminuria and for microalbuminuria was performed. Those that had no micro/macroalbuminuria at this point were excluded, those that still had micro/macroalbuminuria had their urine assayed to quantify 24hr proteinuria using spot urine albumin-to-creatinine ratio

(UACR); they were evaluated and informed of the concern for HIVAN and informed written consent was sought for renal biopsy. Candidates for biopsy were screened for a bleeding tendency by testing the bleeding time and if normal a renal ultrasound was done to ensure they had two native kidneys. If fit, they underwent a percutaneous renal biopsy (done by the investigator with supervision of a trained nephrologist under ultrasound guidance, see Appendix X and XI for details).

Study subjects were recruited based on inclusion and exclusion criteria.

3.4.1 Inclusion criteria

a. HIV-1 infected, antiretroviral naïve
b. Aged 14 years and above
c. Persistent proteinuria(proteinuria of 1+ or more on routine dipstick or more than 30 mg/g by quantitative dipstick done on two occasions at least 14 days apart)
d. Consent given by patient or parent/guardian for those under 18 (with their assent)

3.4.2 Exclusion criteria

a. Previous or current use of antiretroviral drugs
b. Evidence of urinary tract infection, concurrent febrile illness, diabetes, hypertension, heart disease or other known causes of chronic kidney disease
c. Transient proteinuria
d. Contraindications for renal biopsy (solitary native kidney, uncontrollable bleeding diathesis, small kidneys < 8cm long, uncooperative patient)
e. Documented end-stage renal disease (ESRD)

See Figure 1 for a recruitment schema.

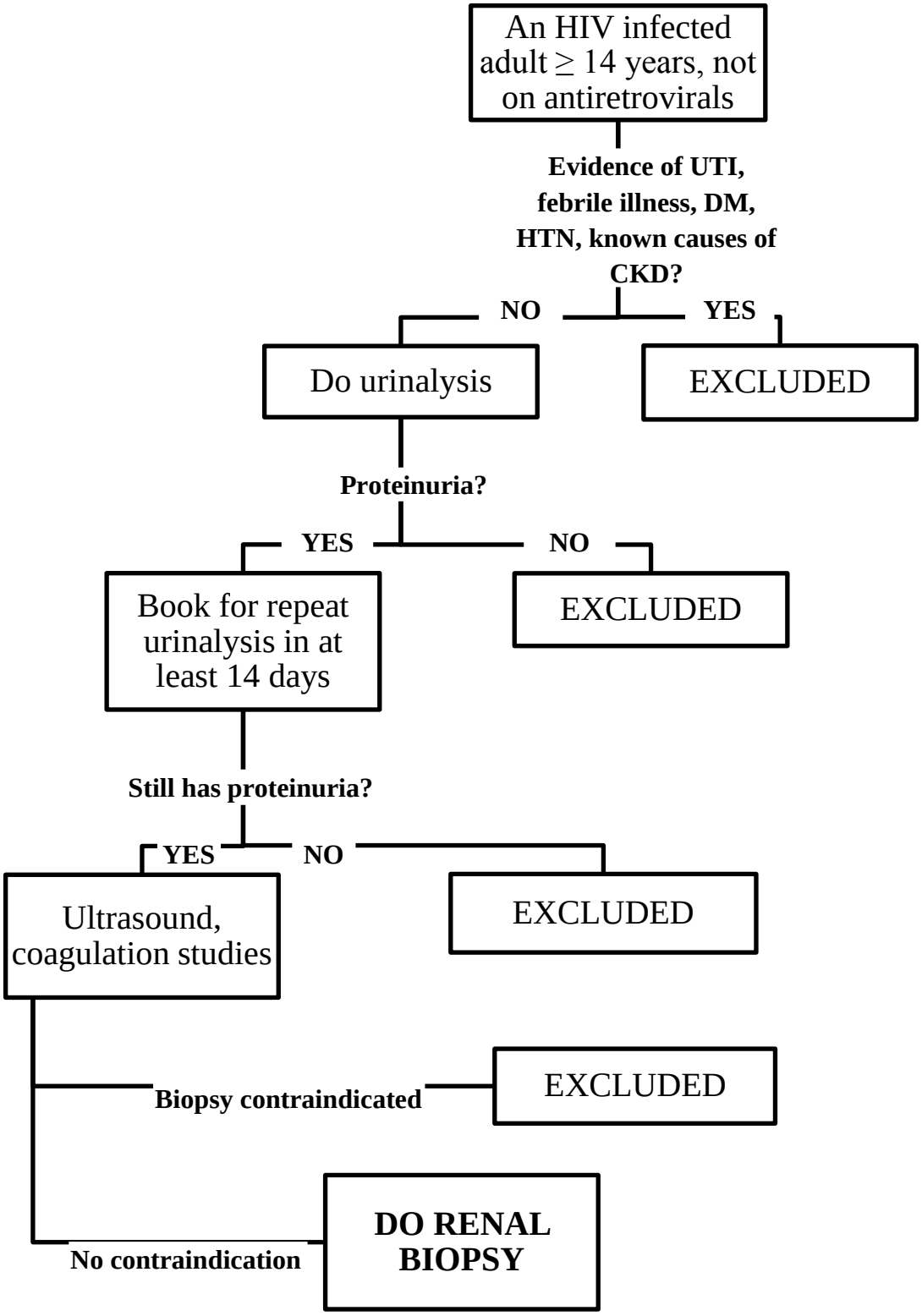

Figure 1: Recruitment schema

3.4.3 Sample size

This was done using the equation:

$$n = \frac{Z^2_{(1-\alpha/2)} \cdot p(1-p)}{D^2}$$

Where n = sample size; Z = the z-value corresponding to 95% confidence (1.96);

α = significance level (5% i.e. 0.05); p = estimated prevalence; D = precision

Based on an earlier study done in our setup[27], 6.2% of HIV-infected antiretroviral naïve adults had proteinuria. Based on this, in the above equation; p = 6.2% (0.062) with D = 10% (0.1)

$$n = \frac{1.96^2 \times 0.062(1-0.062)}{0.1^2} = \frac{3.8416 \times 0.062 \times 0.938)}{0.01}$$

$$= 22.34$$

Therefore, at least **23subjects needed to be biopsied** in this study.

3.5 Procedures

Procedures performed on the patients included blood sugar measurement, measurement of blood pressure, collection of urine for urinalysis and quantification of proteinuria, blood sampling for full blood counts, CD4 counts and serum creatinine and for those to be biopsied, a renal ultrasound scan and bleeding time test. All those who were suitable underwent a renal biopsy.

Formalin-fixed biopsy samples were embedded in paraffin and sent for processing and analysis at the Glomerular Institute of Columbia University, New York, USA. All

tissues were stained with Hematoxylin and Eosin (H&E), Periodic acid-Schiff (PAS), trichrome and Jones' methenamine silver (JMS) stain. The slides were reviewed by a team of pathologists under Dr. Vivette D'Agati at Columbia University. After review, the reviewing pathologist didn't see a need to do further immunohistochemistry staining.

These procedures are detailed in appendices III to XII.

3.6 Quality control

All lab tests were done by the AMPATH reference lab that has internal quality controls. These are done daily as per standard protocol (see appendices for specific details).

The histological specimens were processed and read by renal pathologists at a major center specializing in glomerular diseases at Columbia University, New York, USA. The team was led by Dr. Vivette D'Agati; she reviewed all the slides.

3.7 Data collection and management

Data was collected on a form and later transferred to a computer database; double entry was used to ensure accuracy of the data. All patient details were kept confidential and data was only available to the investigator and the supervisors via password protection. Patients were provided with a copy of their results and they have autonomy over who else can view their test result(s). Copies have also been sent to AMPATH for inclusion in the clinical record.

In consultation with the Department and the School, the results may be published in a peer-reviewed journal.

Data collected was analyzed for means, proportions and were subjected to a chi-squared test using the exact method for inference.

3.8 Ethical considerations

Approval to carry out the study was sought from the Institutional Research and Ethics Committee (IREC). All patients were informed about HIVAN, how it comes about, what it means to their health and how the investigator was to go about finding out if they had HIVAN. They were informed about the procedures involved in the study and the possible benefits and harm to them, the patients were informed that the procedure is generally safe but has potential risks. Patients were informed that all study related costs were not to be charged on them. Informed consent was emphasized (see Appendix I for details). All patient records were kept confidential and all patients received medical attention as necessary regardless of their willingness/unwillingness to participate in the study. No incentives or inducements were used to convince patients to participate in the study. Patients were informed of their results and appropriate standard treatment given. Laboratory results have been availed to the clinicians who routinely take care of the patients who participated in this study.

CHAPTER FOUR

RESULTS

The study was conducted between December 2010 and May 2011. 534subjects were screened, 32 had persistent proteinuria and 27 underwent renal biopsy. See Figure 2 below.

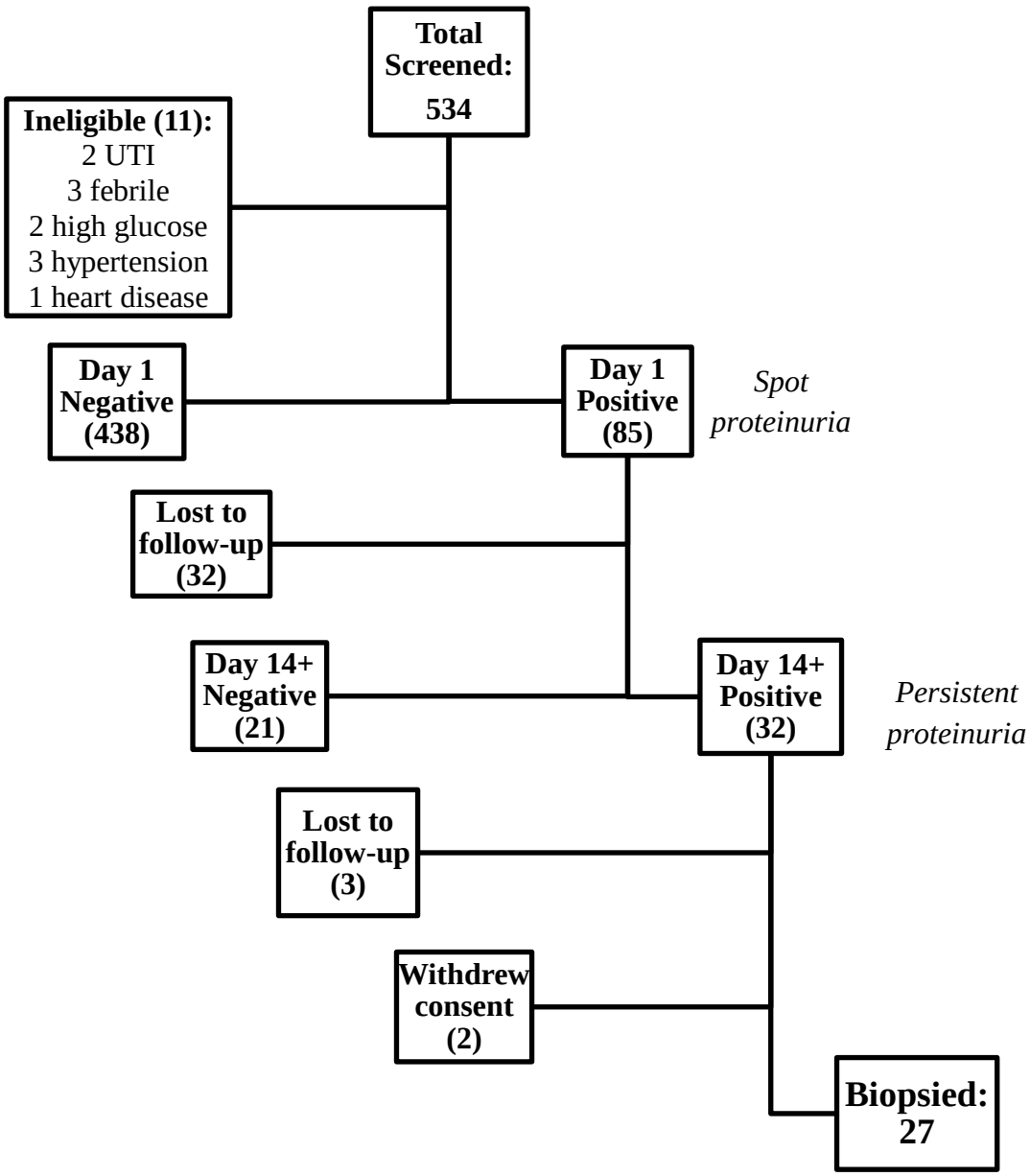

Figure 2: Flow diagram of screening and recruitment

4.0 Results Summary

	Number(range)	Percentage
Screened (eligible)	**523**	
Spot proteinuria	85	16.3%
Persistent proteinuria	32	9.8%
Biopsied	**27**	
Age, mean (range) in years	36.6 (23-65)	
Sex, Females	17/27	63.0%
Black race	27/27	100%
Symptomatic	6/27	22.2%
Family history of kidney disease	1/27	3.7%
On medications	14/27	51.9%
Abnormal physical exam	4/27	14.8%
CD4 cell counts, mean (range) in cells/μL	340.7 (3-1060)	
CD4 cell count < 200 cells/μL	9/27	33.3%
Creatinine clearance, mean (range) in	103.8 (30.1 – 211.9)	
Creatinine clearance < 60 ml/min	2/26	7.7%
UACR, mean (range) in mg/g	384.2 (5-1384)	
UACR > 1 g/g	2/26	7.7%
HIVAN	**0/27**	**0%**
AIN	11/27	40.7%
Non-specific findings	9/27	33.3%
Arteriosclerosis	2/27	7.4%
Others(FSGS, APIGN, CIN, pyelitis, papillary	5/27	18.5%

4.1 Demographics

Of the 27 subjects with persistent proteinuria who underwent biopsy, 10(37.0%) were male, 17(63.0%) were female.

Overall mean age was 36.6 years, with a median age of 34 years and range of 23-65 years. Among males the mean age was 38years, median age 38 years and a range of 32-45 years; for the females the mean was 35.8 years, median age 33 years and a range of 23-65 years.

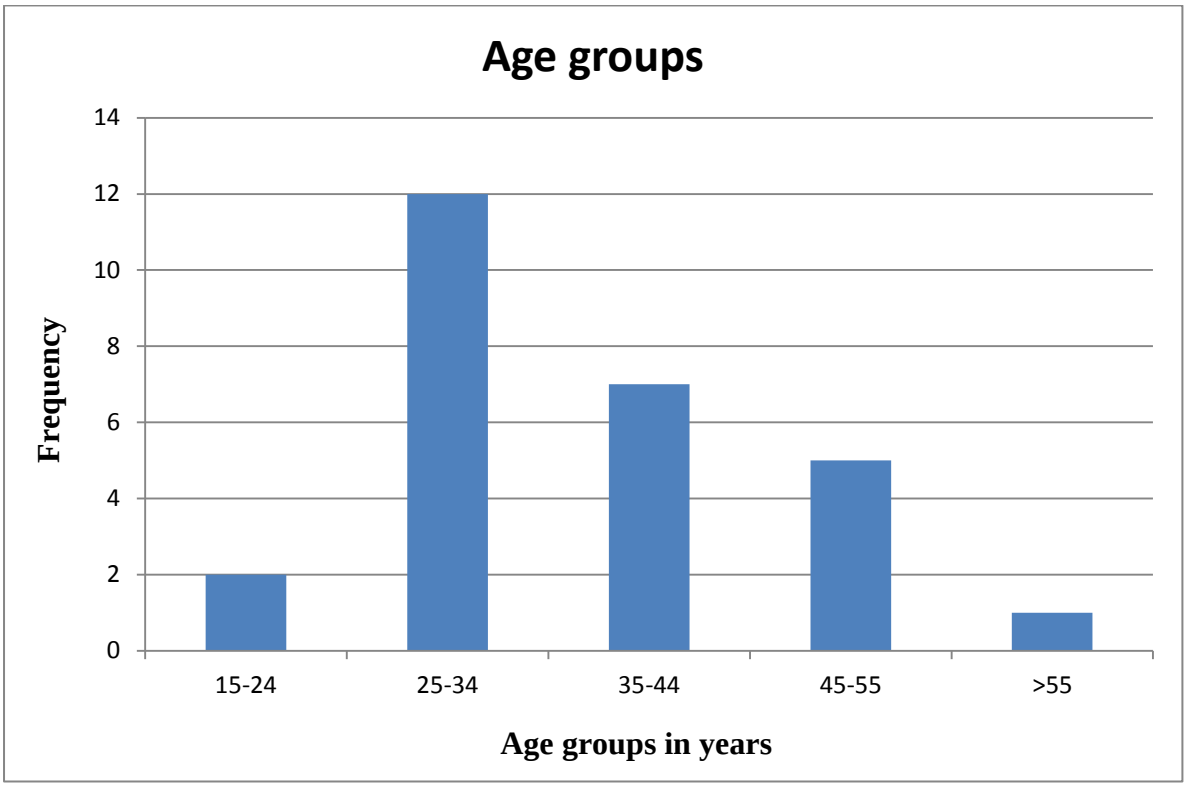

Figure 3: Age distribution, grouped

All subjects were of black race/indigenous African.

In terms of tribal lineage, 10 subjects (37.0%) were of the Luo tribe, 8(29.6%) being Kalenjin, 5(18.5%) Kikuyu and 4(14.8%) Luhya.

4.2 Clinical presentation

4.2.1 Symptoms

The majority of subjects, 21(78%) were asymptomatic. Only 6(22%) reported symptoms. 2(7.4%) reported occasions of leg swelling, 2(7.4%) reported reduced urination, 1(3.7%) reported increased frequency of urination and 1 (3.7%) darkening of urine.

4.2.2 Family History

Only 1 subject (3.7%) reported a family history of kidney disease.

4.2.3 Medication usage

Thirteen subjects (48%) reported no current medication usage. Among those on medications, the commonest medication was cotrimoxazole - 5 (24%), amoxicillin - 5 (24%), anti-tuberculosis drugs - 2 (14%), multivitamins - 2 (9.4%) and 1 - (4.7%) each for dapsone, paracetamol, ibuprofen, indomethacin, metronidazole and fluconazole.

4.2.4 Examination findings

A majority of the subjects, 23(85%) had a normal physical exam. Abnormal physical exam was documented as oral thrush in 1 subject (4.7%), wasting in 1 subject (4.7%), and angular cheilosis in 1 subject (4.7%); 1 had a combination of oral sores, bilateral lower limb furunculitis and 20-week fibroids.

4.3 Labs

4.3.1 CD4 counts

The mean CD4 counts for the subjects with persistent proteinuria who underwent biopsy was 340.7 cells/µL, median count was 369 cells/µL with a range of 3 to 1060 cells/µL.

Nine (33%) of the subjects had CD4 counts of less than 200 cells/µL, 4 (14.8%) between 200 and 350 cells/µL, 7 (26%) between 350 and 500 cells/µL and 7 (26%) above 500 cells/µL.

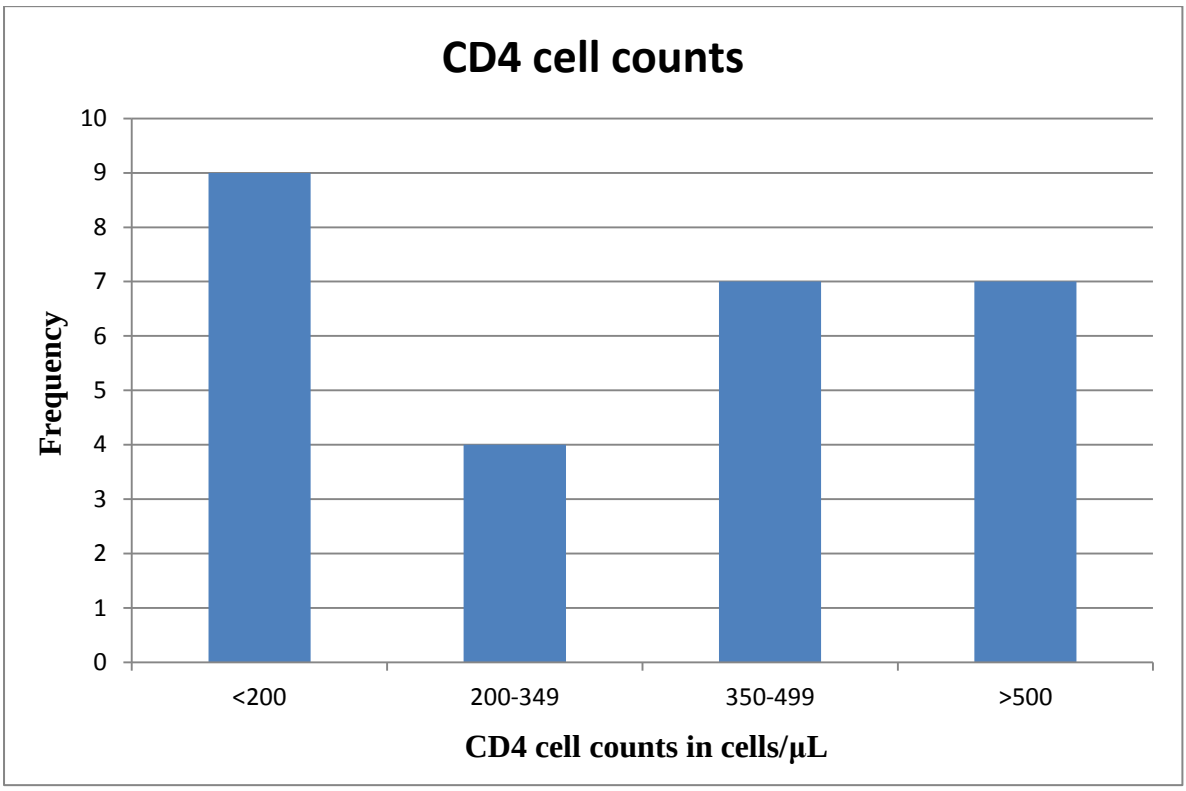

Figure 4: CD4 cell counts, grouped

4.3.2 Renal function

Twenty four subjects, 24/26 (92%) had serum creatinine within normal limits. The estimated glomerular filtration rate (eGFR) by Cockroft-Gault formula was on average 103.8 ml/min, median 97.1 ml/min with a range of 30.1 to 211.9 ml/min. eGFR was normal (more than 60ml/min) for still 24/26 (92%) of the subjects.

4.3.3 Urinary findings

Of all 523 subjects screened, 85(16.3%, 95% CI 13.3-19.7) had spot proteinuria (dipstick proteinuria of more than 1+ or more than 30mg/g using quantitative dipstick). When further assessed after a minimum of 14 days from the first test, only 32 of 53(60.4%) subjects who returned for follow-up continued to have a positive test. This works out to a 9.8% overall rate of persistent proteinuria by imputation.

Among the subjects with persistent proteinuria who underwent biopsy, 25(93%) had proteinuria detectable only by microalbumin strips or 1+ by routine (macroalbumin) strips, with 1 each having dipstick proteinuria of 2+ and 3+ respectively.

Quantification of 24-hour proteinuria was estimated using urinary albumin to creatinine ratio (UACR). 24/26 (92%) of the subjects had proteinuria less than 1g/g. 2 (7.7%) had normal albuminuria (less than 30mg/g), 11 (42%) had between 30 and 300mg/g, 11 (42%) ranged between 300 and 1000mg/g and 2 (7.7%) above 1000mg/g.

The average UACR was 384.2 mg/g with a median of 257.5 mg/g and a range of 5-1384 mg/g.

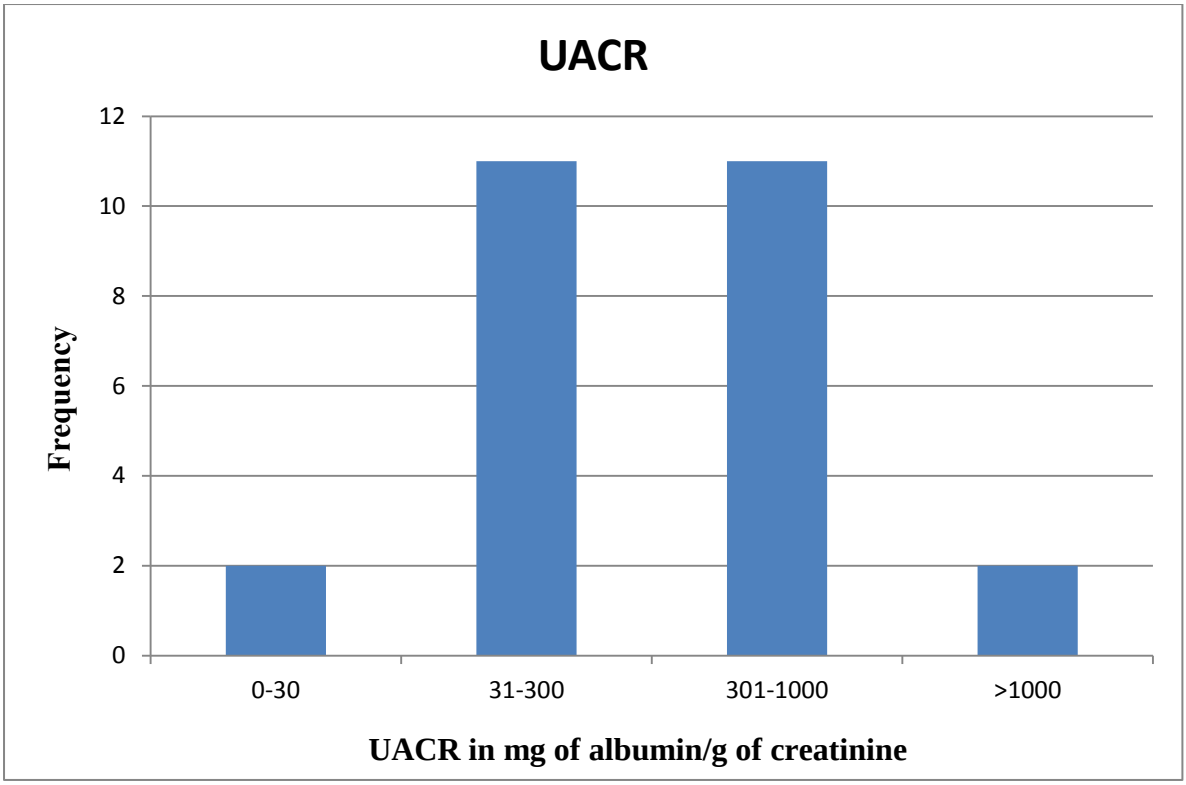

Figure 5: UACR distribution

4.4 Biopsy findings

Of the 27 biopsies taken, none had features consistent with HIVAN.

The commonest diagnosis was acute interstitial nephritis (AIN) – 11 (40.7%), followed closely by non-specific findings – 9 (33.3%).

Other diagnoses include arteriosclerosis – 2 (7.4%), focal segmental glomerulosclerosis (FSGS) – 1 (3.7%), acute post-infectious glomerulonephritis(APIGN)– 1(3.7%), chronic interstitial nephritis(CIN)– 1(3.7%), pyelitis– 1 (3.7%), and papillary sickling – 1 (3.7%).

The findings are summarized in the chart below:

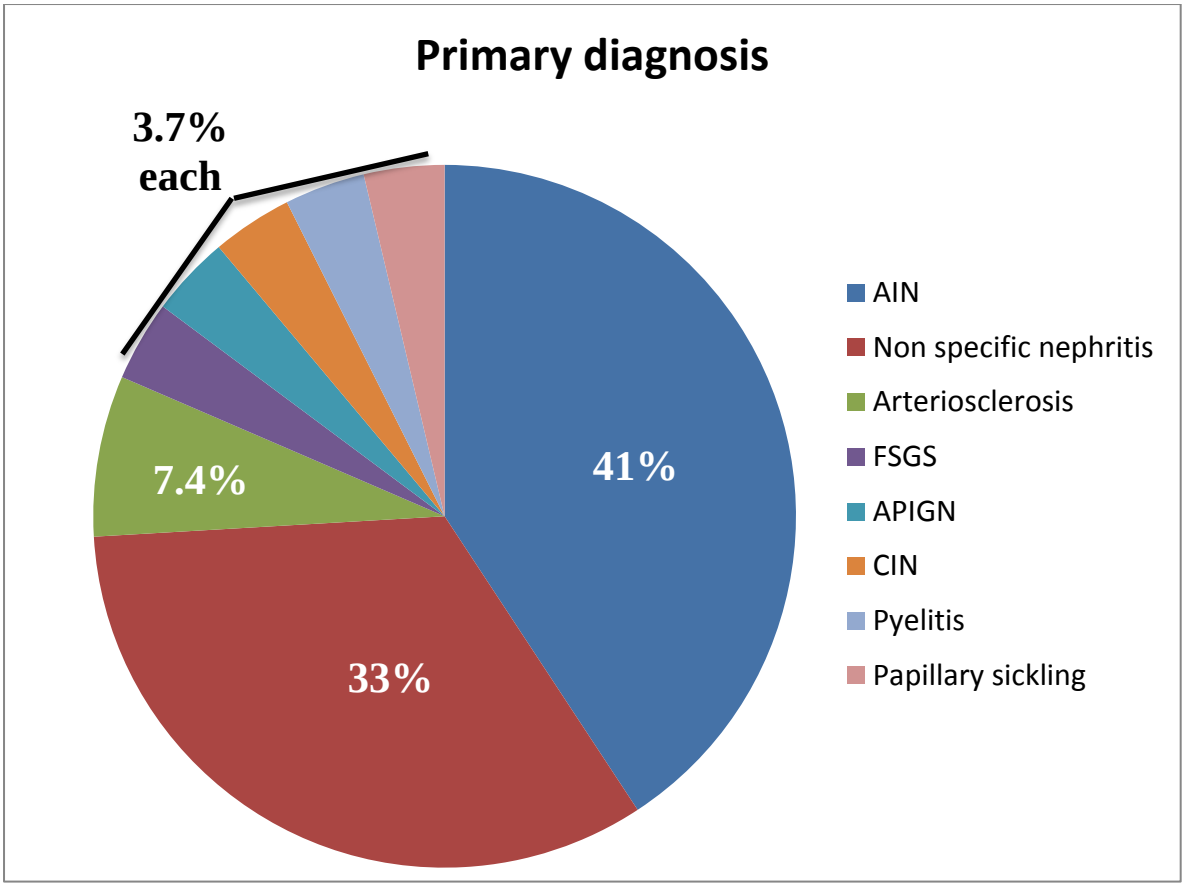

Figure 6: Primary diagnosis

Arteriosclerosis (5 biopsies) and papillary sickling (2 biopsies) are diagnoses that frequently overlapped. In addition, mild or isolated arteriosclerosis was also frequently observed among subjects with non-specific findings.

The table below summarizes all occurrences of each of the diagnoses above.

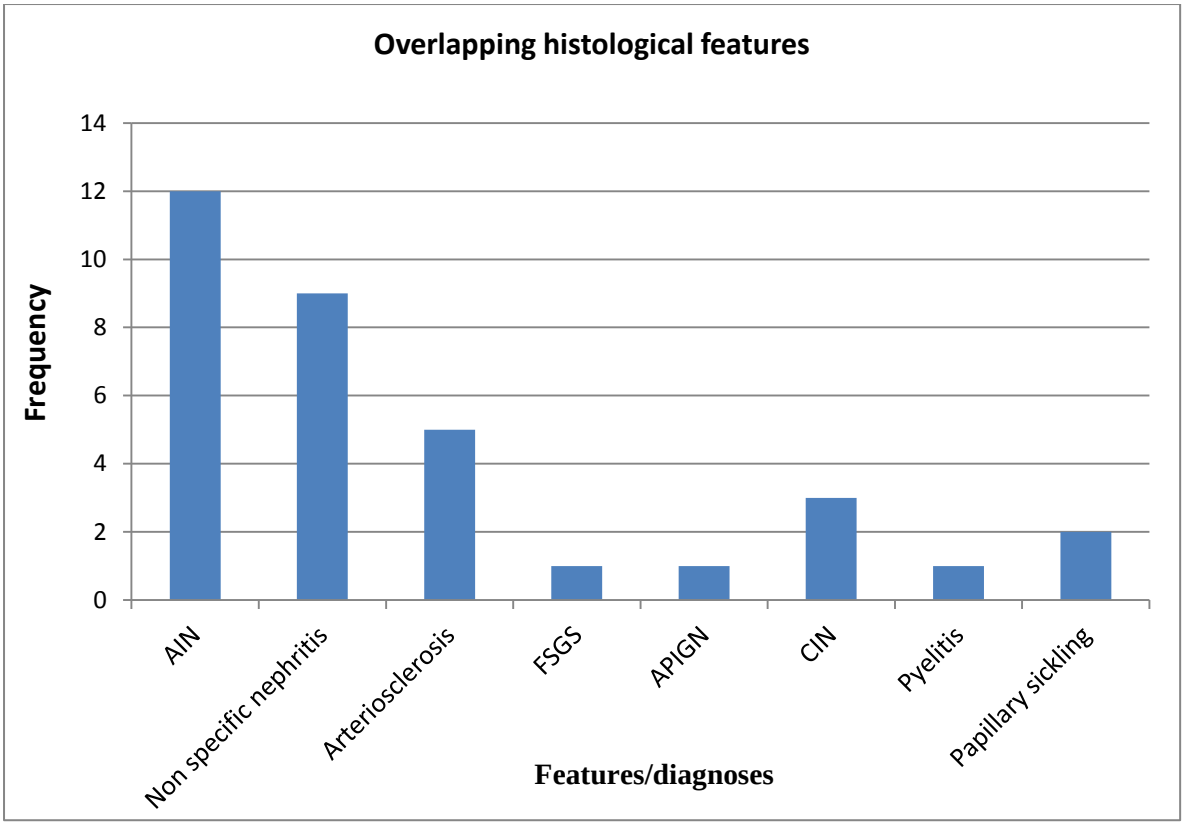

Figure 7: Overlapping histological features

Here are some of the representative images:

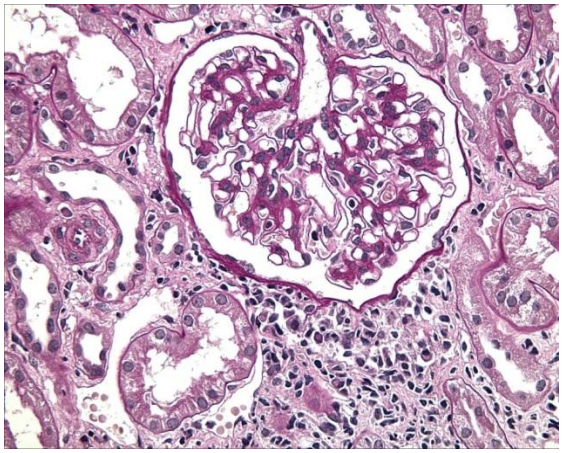

Figure 8: AIN with periglomerular inflammation PAS x400

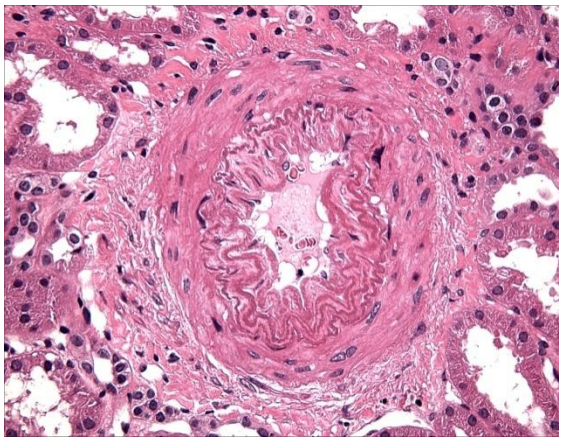

Figure 9: Arteriosclerosis H&E x400

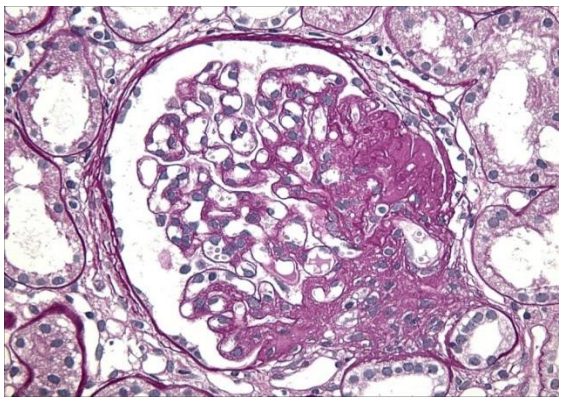

Figure 10: FSGS perihilar variant PAS x400

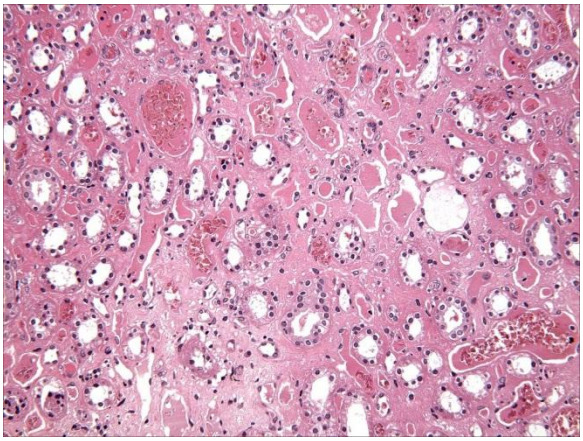

Figure 11: Papillary necrosis and sickling H&E x200

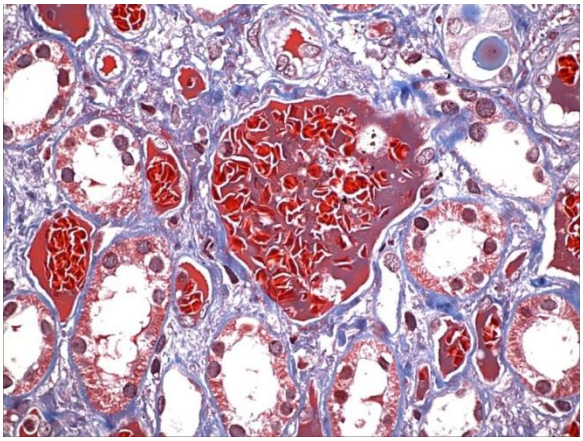

Figure 12: Sickled vasa recta Trichrome x600

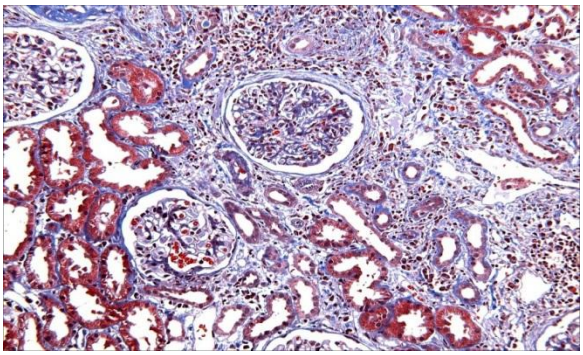

Figure 13: CIN Trichromex200

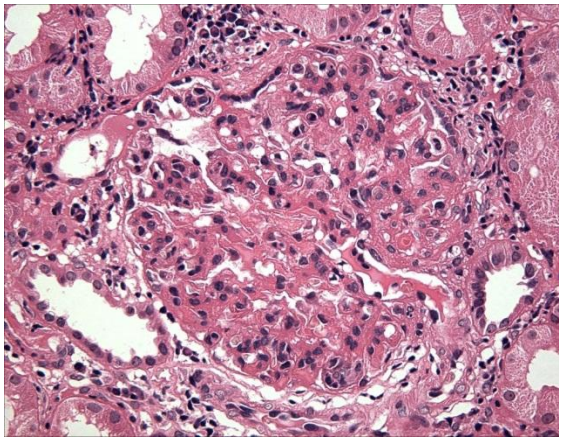

Figure 14: AGN H&E x400

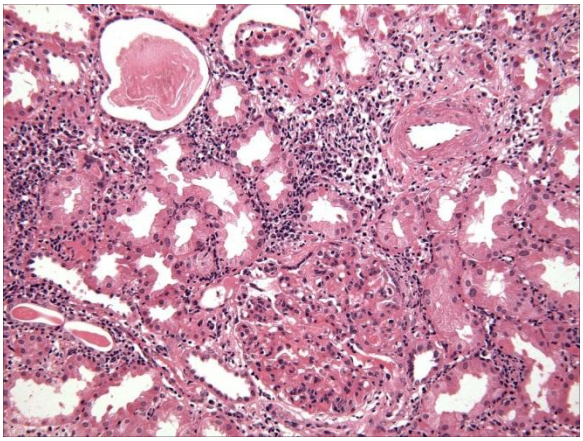

Figure 15: AGN and AIN H&E x200

4.5 Inferential statistics

Using chi-squared tests, no association was found between age and the respective diagnoses ($p = 0.757$ by exact method); sex ($p = 0.885$ by exact method); tribe ($p = 0.823$by exact method); UACR ($p = 0.679$ by exact method) and CD4 counts ($p = 0.450$ by exact method).

CHAPTER FIVE

DISCUSSION

A predominant number (63%) of study subjects were female. This is in keeping with national statistics that indicate a higher incidence of HIV among females as compared to males.[2] This finding is similar to that of a study by Wools-Kaloustian *et al* done in the same setting(AMPATH) in 2005; of 373 subjects screened for proteinuria, females constituted 67.9%.[27] So too did a study by Andia *et al* in Uganda in 2003; of 299 screened, 65% were females.[26] A study similar to this one by Han *et al* in South Africa in 2004[19]documented a 71% female predominance too. There is a possibility than more females come in contact with HIV testing by virtue of antenatal testing and other women-only testing services e.g. maternal and child health clinics explaining the higher rates of testing observed among females. Other cultural factors are at play too in maintaining a higher rate of HIV infection among the female population for instance by virtue of their gender, women have less bargaining power over their sexuality and may be more likely to engage in high risk lifestyles like commercial sex that leads to differential vulnerability to HIV.[73,74]

The study population of this study consisted mostly of a younger age group. The mean age of the subjects biopsied was 36.6 years (35.8 for females, 38 for males). This is also consistent with our national HIV data. Most of Kenya's HIV population is young, the peak age for females is 30-34 years and for males 40-44 years.[2] The study by Wools-Kaloustian *et al* done in this same setting had a similar mean age of 35.0 years.[27] Andia *et al* in Uganda reported a mean of 36 years[26] and Han *et al* in South Africa 32 years.[19] This trend reflects a higher transmission rate among the younger age group; this is possibly due to cultural/behavioral factors

All subjects understandably were African. By tribe: Luo (37.0%), Kalenjin (29.6%), Kikuyu (18.5%) and Luhya (14.8%). The area around Eldoret where AMPATH serves, being cosmopolitan, is reflected well in this distribution.

A majority of subjects (78%) reported no symptoms. The study was based on an outpatient population of HIV-infected adults on follow-up. At screening, any subjects with fever, hypertension, diabetes or heart disease were specifically excluded because of the association of these states with proteinuria, usually transient. So the population selected consisted mostly of well people unlikely to have any medical complaints.

Only 3.7% reported a family history of kidney disease. Although the study sample was small, it compares quite well with what was documented in an earlier study in the same setting where 6.9% reported a family history of kidney disease.[27]

A relatively fair proportion of our study subjects were on medications(52%).Of this, cotrimoxazole usage was at 12%. Cotrimoxazole is a known potential nephrotoxin, the sulfamethoxazole component can cause crystalluria with acute renal failure, it may also cause interstitial nephritis usually with eosinophilia. Trimethoprim causes acute rises in creatinine by reducing creatinine secretion, there is therefore no actual reduction in glomerular filtration rate (GFR).[75]Its usage being low in the study means any abnormalities to be found are unlikely to be attributable to cotrimoxazole. Dapsone (4.7%) is rarely associated with nephrotoxicity. Amoxicillin is also a potential nephrotoxin. Penicillins are commonly associated with acute (allergic) interstitial nephritis usually with eosinophilia and rash.[76] Its usage was at 12%, again a possible contributor, but quite unlikely.

Non-steroidal anti-inflammatory drugs (NSAIDs) are known to cause ARF and nephrotic syndrome but their usage in this study was low: ibuprofen (4.7%) and indomethacin (4.7%).

A majority of subjects had a normal physical exam and those who had abnormal findings had mostly dermatological and mucosal findings of advanced HIV. None had clinical features suggestive of kidney disease. This is not unexpected, most kidney diseases present with no obvious clinical findings. Because this study was based on screening of an untreated and probably relatively healthy lot, it would be expected that few if any would have positive clinical findings.

The mean CD4 of the subjects was 340 cells/μL with a pretty even distribution between the categories thus: less than 200(33%), 200 to 349(14.8%), 350 to 499(26%) and more than 500(26%).This is close to the figure of 391 cells/μL seen by Wools-Kaloustian *et al* in an earlier study in the same setting, AMPATH.[27] Han *et al* in South Africa found a mean of 264±46 cells/μL.[19] This current study was based on an outpatient population and the requirement to exclude patients with fever may explain why most of these patients had CD4 counts at this level. The South African study too excluded subjects with fever; it was however based on an inpatient (2%) and outpatient (98%) setting and could to some degree explain the lower CD4 counts.

Most (92.6%) of the subjects had normal renal function based on normal levels of serum creatinine and also by estimated glomerular filtration rate(eGFR) of more than 60ml/min using the Cockroft-Gault formula. Wools-Kaloustian *et al* documented a 11.5% rate of abnormal eGFR.[27] The 7.4% of this study and 11.5% are probably comparable considering that this study analyzed 27 subjects only. Han *et al*

documented a mean creatinine clearance of 77±35 ml/min among the subjects with persistent proteinuria.[19] This however was a different population by demography and geography.

This study found a spot proteinuria rate of 16.3%.Anearlier study in the same setting had found a rate of 6.2%.[27] The differences in methodology largely account for this difference, the study in 2004 by Wools-Kaloustian used only urinary dipsticks to characterize proteinuria, these sticks have a low sensitivity for lower degrees of proteinuria.[77] In this study dipsticks were used first and a negative result rechecked using quantitative microalbumin dipsticks that extended the range of sensitivity. This figure is also higher than that among non-pregnant Rwandan women infected with HIV;8.7% had proteinuria 1+ or more by dipstick.[78] The study by Han *et al* in South Africa tested microalbuminuria in a subset of subjects and macroalbuminuria (proteinuria)in all subjects. They found spot microalbuminuria at 32% and persistence at 8%, macroalbuminuria was 6.2%. Their overall persistent microalbuminuria/proteinuria came to 9.8%.[19]This study's rate is appears to be within the range of variability observed in these studies.

Persistent proteinuria was emphasized in this study because it is an indicator of serious renal disease; only subjects with persistent proteinuria underwent biopsy. Microalbuminuria was included as part of screening because of earlier findings by Han *et al* that HIVAN was manifest in subjects with persistent microalbuminuria suggesting that microalbuminuria may be an early marker of HIVAN.[19]

Notably, of the 85 who had spot proteinuria, only 53 returned for urine recheck, giving a return rate of 62.4%. From this, an imputed value of persistent proteinuria

was calculated. There are various reasons for these recheck losses. Most of them had no phone contacts or were not reachable by phone. Some had return visits to the HIV clinic that were beyond the study period; they also lived far and didn't have the capability of coming back earlier. Since the study ran for 6 months only, some of these subjects ended up being lost. There also are a few who were reported dead afterwards. It is possible that these losses may alter the exact numbers in the results of this study although probably not to a significant extent. It was assumed that these losses were random and not associated with the outcome of interest i.e. HIVAN.

24-hour protein excretion as estimated using urinary albumin to creatinine ratio (UACR) was on average 384.2 mg/g and the majority (92%) of subjects had 24-hour proteinuria less than 1 g/g. This is in clear contrast with Han *et al*'s study in South Africa that had an average UACR of 6±6 g/24hr.[19] This is most probably accounted for by the different demographic, environmental and probably genetic differences among the two populations.

This study set out to find out if HIV-associated nephropathy (HIVAN) was present in the study population. There were no features consistent with a diagnosis of HIVAN in this sample. The most common histological diagnosis was acute interstitial nephritis (AIN) at 40.7%. No eosinophils were noted so it was reported as probably infection related.

33.3% of subjects had non-specific findings which refers to the presence of histological abnormalities that do not meet a sufficient threshold for any particular renal disease entity. The findings noted in this group included mild(1+ on a scale of 0 to 3+) arteriosclerosis seen in 7 out of 9(78%), minor degrees (up to 10%) of

interstitial fibrosis and tubular atrophy(IFTA) seen in 5(56%) and minor degrees(up to 10%) of interstitial inflammation in 5(56%).Other findings include mesangial proliferation(33%), glomerulomegaly(33%), endocapillary proliferation(11%) and podocyte swelling(11%).

Other diagnoses included arteriosclerosis (7.4%), FSGS (3.7%), APIGN (3.7%), CIN (3.7%), pyelitis (3.7%) and papillary sickling (3.7%).

The definition of HIVAN requires the demonstration of collapsing glomerular sclerosis, tubulointerstitial inflammation and fibrosis, and the presence of tubular microcystic dilatation. None of the reviewed slides had features consistent with this definition.

The fact that no HIVAN was found in this sample is of significance because prior studies had suggested it is common among people of African descent infected with HIV. However this negative find is not entirely surprising because an earlier study on native Ethiopians in Israel could not find clinical features to suggest HIVAN in a cohort of 176.[25] Another study in Uganda on 299 subjects had also found a very low rate of HIVAN(0.3%).[26]Studies in South Africa too seem to differ a lot depending on geographic location. In KwaZulu- Natal, a prospective study on a large cohort found 83% of those biopsied had HIVAN.[19] In Witwatersrand a retrospective study of renal biopsies on HIV-infected patients found HIVAN to be 27% with HIV-immune complex kidney disease(HIVICK) at 21% and other diagnoses that included membranous glomerulopathy, post-infectious glomerulonephritis, IgA nephropathy and various overlapping diagnoses.[29] A recent prospective study at Witwatersrand found 5% HIVAN, 40% HIVICK and 45% that had mesangial and interstitial changes

that did not meet current histological criteria for HIVAN.[4,79] In a study in Nigeria, of 10 patients with proteinuria who got biopsied, 7 had FSGS with partially collapsed glomeruli, microcystic tubular dilatation and mononuclear cell infiltration, features consistent with HIVAN.[80]

The unpublished KNH study[31] is the only one in our region that found significant HIVAN among its subjects. 56 patients with proteinuria were screened, 7 (12.5%) had proteinuria more than 1g/g by UACR and 6 of them underwent biopsy with 5 being found to have histological features suggestive of HIVAN. Biopsies were performed only on the ones with proteinuria exceeding 1 g/g. This could be part of the reason they found HIVAN. Our study had only two patients with proteinuria exceeding 1 g/g who didn't have HIVAN. It is notable however that we screened a large number of HIV-infected subjects, we found few to have heavy proteinuria. The population under study at KNH was different geographically from the population of this study and may explain this difference.

It is possible that our study population was not in very advanced stages of HIV (based on CD4), had less proteinuria and had more females than males (HIVAN is thought to affect males more)[20] that may explain this low prevalence of HIVAN.

It is worth noting that in this study we excluded subjects with fever, urinary tract infection, diabetes, hypertension and heart failure. These conditions are associated with transient proteinuria. Even though biopsy would have distinguished clearly between HIVAN and these conditions, it would not have been prudent to subject these subjects to unnecessary biopsy and its attendant risk. The study by Han *et al* in South Africa[19] also excluded subjects with these conditions and thus it is unlikely that these

exclusions explain why this study found no HIVAN. Moreover, the number excluded (11/534) is not very significant.

From these studies, it seems that differences in the presence of HIVAN exist across geographical locales. Of note especially is the low rate of HIVAN in East Africa (Uganda and Ethiopia studies) while it is variable in South Africa and seems to be quite common in West Africa.

Based on studies in the US and the discovery of a link between patients with HIVAN and a family history of ESRD, scientific inquiry has pointed to the possible role of the gene APOL1 in predisposition to HIVAN, primary FSGS and H-ESKD.[44] It is hypothesized that the evolutionary pressure due to high trypanosome transmission in West Africa and probably parts of Southern Africa led to selection of variants of APOL1 that are protective against trypanosomiasis but unfortunately confer excess risk of primary FSGS, H-ESKD and HIVAN. Possibly our populations in East Africa, have less of these variants in the population and thus are less likely to develop these diseases.

AIN was the major diagnosis in this study. The etiologies of AIN are: drugs (71%), infections(16%), idiopathic (8%), tubulointerstitial nephritis and uveitis syndrome (TINU) – 5% and sarcoidosis (<1%).[81]52% of subjects reported using drugs. Common drugs that cause AIN include penicillins, sulfonamides and analgesics; these are the commonest medications reported by subjects. However, in the biopsies examined, infection was reported the most likely cause since none had eosinophilia, a feature of drug-induced AIN. It is a possibility that these cases of AIN could have been caused by infections with either *Legionella*, *Leptospira*, *Staphylococci*, *Streptococci*,

Brucella, syphilis, *Mycobacterium tuberculosis*, *Mycoplasma*, HIV, cytomegalovirus, *Toxoplasma*, *Leishmania*, among many others.[76]

Another possibility is the use of herbal medicines. Among other renal toxicities, herbal medicines cause interstitial nephritis. In developing nations usage of traditional medicine for primary healthcare approaches 80%.[82] HIV is a chronic disease with no known cure and subjects might have been using herbal remedies on a chronic basis that might have contributed to these cases of AIN.

5.1 Limitations of this study

The study could not elucidate further on the probable causes of the diagnoses seen at biopsy e.g. acute interstitial nephritis because it was designed to look for HIVAN.

Renal pathology was limited to light microscopic examination only.

Quite a number of subjects were lost in between urine checks.

We had a limited number of subjects with heavy proteinuria.

5.2 Conclusions

The prevalence of HIVAN among HIV-infected antiretroviral naïve adults with persistent proteinuria at the Moi Teaching and Referral Hospital is very low.

The prevalence of persistent proteinuria among HIV-infected antiretroviral naïve adults at MTRH is 9.8%.

HIV-infected antiretroviral naïve adults with persistent proteinuria at the MTRH are likely to have acute interstitial nephritis (AIN), non-specific findings or arteriosclerosis. Other rare possibilities include focal segmental glomerulosclerosis

(FSGS), acute post-infectious glomerulonephritis (APIGN), chronic interstitial nephritis (CIN), pyelitis or sickle cell nephropathy.

There is probably a very low prevalence of HIVAN among all HIV-infected antiretroviral naïve adults at MTRH.

Potentially, genetic differences between our population and other populations mean that our kidney disease epidemiology in our setting is not necessarily similar to other settings.

5.3 Recommendations

Biopsies are probably not very critical in diagnosing HIV-infected patients presenting with features of kidney injury at MTRH especially if they have low degrees of proteinuria (less than 1 g/g by UACR).

The routine use of urinary microscopic examination and dipstick analysis needs to be utilized to identify patients with proteinuria who likely have potentially treatable kidney disease like AIN. This is a cheaper than renal biopsy and the potential sequelae of these diseases i.e. chronic renal failure and dialysis.

Further studies need to be done to ascertain the cause of AIN and non-specific renal inflammation that is common among HIV-infected patients with persistent proteinuria.

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APPENDICES

APPENDIX I: Consent form

A. ENGLISH:

My name is Dr. Mathew K. Koech. I am a qualified doctor, registered by the Kenya Medical Practitioners and Dentists Board. I am currently pursuing a Master's degree in Internal Medicine at Moi University. I would like to recruit you into my research which is to study whether HIV-infected adults, who are HAART naïve, attending the AMPATH clinics, could have a condition called HIV-associated nephropathy (HIVAN).

ABOUT HIV-ASSOCIATED NEPHROPATHY

HIVAN is a disease that affects the kidneys of some people with HIV infection. It has been shown to be an aggressive condition that causes abnormal loss of protein in urine and leads to failure of function of the kidneys if not treated. It is caused by the HIV virus getting into the kidneys and damaging them hence impairing its normal functioning. It is not a very common condition but it is important to know whether one has it since treatment with antiretroviral drugs and other medicines has been shown to be beneficial in halting and even reversing its effects on the kidneys.

We shall screen you for other renal diseases, high blood pressure, diabetes mellitus, heart disease and any infectious illnesses that may present as fever and if you have any of those conditions you shall be excluded from the study although you will be appropriately offered treatment for your respective condition.

For us to know whether you have HIVAN, we will request you to undergo some tests preceding which you shall have to answer a few questions on your pertinent medical history. We will take a urine sample to check whether you are losing abnormal amounts of protein in your urine, if you do not, we will safely assume you are

unlikely to have HIVAN and hence exclude you from this study. If you are losing abnormal amounts of protein in your urine, we will need to follow you and have a repeat check on your urine after 14 days to ascertain that you are actually losing abnormal amounts of protein in urine. If so, we recommend that we do a kidney biopsy, that is, we use a small needle to take a small piece of your kidney to be examined under a microscope to find out whether or not you have HIVAN. Before we do a biopsy, we will need to do some tests to ensure that you have two kidneys using non-invasive sonographic evaluation and serological assays to screen your kidney functions. Baseline blood tests shall be run on you in assessment of the presence of infections, anemia and to evaluate your blood clotting system. Your CD_4 cell counts will be measured to define how advanced your HIV infection is. After we have done the above tests, we shall admit you to the kidney specialist's ward and perform a kidney biopsy on you.

A kidney biopsy will be done by a specialist doctor called a consultant nephrologist. He will perform it with the guidance of images from an ultrasound machine. He will perform it while you are conscious and awake but under pain relieving medicines (local anesthesia). The procedure is done while you lie on your belly and you may feel some pain when the first injection of anesthesia is being administered. It takes between 5 minutes and 15 minutes on average to perform and then you will need to be admitted for a period of 24 hours for observation and to make sure you do not develop complications. You may have episodes where you pass out blood stained urine which fades with time.

It is generally a safe procedure but it has some risks to it. You may have some pain on your back at the site of the biopsy. In rare cases, the procedure may complicate and cause severe pain and swelling of the kidney, if that happens, we will consult another

specialist doctor called a urologist and we will administer medicines to ease the pain. In very rare cases it may necessitate the removal of your kidney if bleeding occurs that cannot be controlled by the doctors. But when done carefully and after the tests above, these complications are not expected.

We will keep all your test results in confidence and keep you informed of the results and what they mean. Treatment does not depend on your participation in this study. We will offer appropriate treatment for any condition that we find from assessing you and from your test results.

This study has been approved by the Institutional Research and Ethics Committee (IREC) of Moi University/Moi Teaching and Referral Hospital.

If you need further clarifications please contact IREC using the address below.

The Chairman IREC,

Moi Teaching and Referral Hospital,

PO Box 3,

Eldoret.

Tel: 33471/2/3

My cell phone number is: 0722 915 159

YOUR CONSENT:

Adults above 18 years of age

I have been adequately informed that I am being recruited in a study to find out if I have HIV-associated nephropathy. The investigator has also informed me that my participation in this study is voluntary and will not exclude me from my routine care even if I were to opt out. He has also informed me that I'll not be required to pay for the tests done for the purposes of this study.

Sign: ……

Name: ……

Date: ……

YOUR CONSENT:

Patients below 18 years of age

I have been adequately informed that my son/daughter is being recruited in a study to find out if he/she has HIV-associated nephropathy. The investigator has also informed me that his/her participation in this study is voluntary and will not exclude him/her from their routine care even if he/she were to opt out. He has also informed me that I'll not be required to pay for the tests done for the purposes of this study.

PATIENT'S PARENT/GUARDIAN:

Sign: ……

Name: ……

Date: ……

B. KISWAHILI:

Jina langu ni Daktari Mathew K. Koech. Mimi ni daktari aliyefuzu nakusajiliwa na bodi ya madaktari wa Kenya (Kenya Medical Practitioners and Dentists Board). Mimi ni msomi wa shahada ya juu (Masters) ya udaktari (Internal medicine)katika chuo kikuu cha Moi University. Nimekuona leo kwa sababu ninafanya uchunguzi kujua kama watu wazima walioambukizwa na viini vya HIV ambao hawatumii dawa za virusi (antiretrovirals, ARV) wanaohudumiwa na kliniki za AMPATH huenda wakawa na ugonjwa wa figo utokanao na viini vya HIV, ugonjwa huu kwa kiingereza unaitwaHIV-associated nephropathy (HIVAN).

KUHUSU HIV-ASSOCIATED NEPHROPATHY

HIVAN ni ugonjwa unaodhuru figo ya watu kadhaa walioambukizwa na viini vya HIV. Ugonjwa huu unajulikana kuwa na mwendo wa kasi, husababisha mtu kukojoa makojoo yenye protini kuzidi kawaida kwa sababu inadhuru utendaji kazi wa figo. Ugonjwa huu unaweza kusababisha figo kufeli isipotibiwa.Unasababishwa na viini vya HIV kuingia kwenye figo na kuidhuru hivyo kusababisha isitende kazi iliyoumbiwa ipasavyo.Ugonjwa huu haupatikani kwa kila mtu mwenye viini lakini ni muhimu kujua kama mtu yeyote anayo ugonjwa huu kwa maana matibabu na ARV na madawa mengine umeonyeshwa kuwa unakomesha na kuponyesha madhara yanayoletwa na ugonjwa huu kwenye figo.

Ili kufanya uchunguzi huu, tutahakikisha kuwa huna magonjwa mengine ambayo yanajulikana kusababisha ugonjwa wa figo, tukipata kuwa unayo mojawapo wa magonjwa yoyote hayo, hutahitajika kushiriki katika uchunguzi huu, lakini utahudumiwa na kutibiwa ugonjwa huo vilivyo. Magonjwa hayo ni kama: ugonjwa wa damu kukimbia (pressure), ugojwa wa sukari (diabetes), na ugonjwa wa roho.Tutahakikisha pia kuwa huna ugonjwa wowote usababishao homa au joto jingi

mwilini. Tutakuuliza maswali kuhusu magonjwa yoyote mengine unayoweza kuwa nayo au unayotibiwa, historia ya jamii yako, madawa yoyote unayotumia na kisha tutakupima.

Ili tujue kama unayo ugonjwa huu wa HIVAN, tutakuomba tufanye uchunguzi kadhaa. Mwanzo tutahakikisha huna ugonjwa wa pressure, hivyo tutapima pressure ya damu yako. Tutakupima tuone kama unayo ugonjwa wa sukari, tutachukua damu tupime sukari. Tutafanya uchunguzi wa hesabu ya damu yako: tutapima white blood cellskuona kama unamiambukizo, red blood cellsili kujua kama una damu ya kutoshanaplateletskujua vile damu yako inashikana, tutapima pia sehemu ya damu ya CD4 ambayo inatujulisha vile ugonjwa wa ukimwi umekudhuru. Tutachukua damu kupima vile figo yako inafanya kazi. Tutachukua mkojo wako tupime kama protini inamwagika kupita kiasi cha kawaida, kama sivyo, tutafikiria kama huna HIVAN. Kama mkojo wako unamwaga protini kupita kiasi, tutahitaji tupime mkojo wako kwa mara nyingine tena katika muda usiopungua siku kumi na minne (wiki mbili) ili tuwe na hakika kuwa ni kweli unapoteza protini kuliko kawaida.Siku utakaporudi tutapima mkojo wako tena, kama bado utakuwa unapoteza protini kupita kiasi, tutakushauri kuwa utahitaji kufanyiwa uchunguzi zaidi wa biopsy ya figo. Hii ni kusema tutatumia shindano kuchukua kipande kidogo cha figo yako kifanyiwe uchunguzi kwenye mahabara kuona kama unayo ugonjwa wa HIVAN. Kabla tufanye biopsy, tutapiga picha kuona kama unayo figo mbili na kuhakikisha damu yako inashikana vilivyo ili tufanyapo biopsy damu haitatiririka kupita kiasi. Baada ya hayo, tukiona wafaa kufanyiwa biopsy tutakutuma kwenye wodi ya daktari mkuu wa figo ili afanye hiyo biopsy.

Biopsy hufanywa na daktari mkuu mwenye ujuzi na utaalamu wa magonjwa ya figo. Yeye atafanya uchunguzi huu akitumia usaidizi wa picha maalumu ya ultrasound.

Yeye atafanya biopsyukiwa unafahamu, hutalalishwa lakini atatumia madawa ya kuzuia uchungu kwenye upande ambapo biopsyutafanywa. Utakuwa umelala kifudifudi (kwa tumbo yako) na hutasikia uchungu wowote ila wakati ule atakuwa akiweka dawa za kumaliza uchungu. Uchunguzi huu huchukua kati ya dakika tano hadi kumi na mitano, kisha itabidi ulazwe kwenye wodi kwa muda wa masaa ishirini na manne ili utiliwe makini na kuhakikisha huna matatizo yoyote yatokanayo na uchunguzi huu. Waweza kusikia uchungu kidogo baadaye lakini huwa inakwisha baada ya siku chache, waweza pia kuwa na nyakati utakojoa mkojo ulio na damu kidogo, hiyo pia huenda.

Uchunguzi huu huwa salama lakini inaweza kuwa na madhara. Waweza kuwa na uchungu mgongoni pahali ambapobiopsyulifanywa. Wakati nadra sana uchunguzi unaweza kuleta uchungu sana na kufura kwa figo, iwapo tukio hiIi litendeke tutauliza ushauri wa daktari mwenye ujuzi wa upasuaji wa magonjwa ya figo na tutakupa madawa za kutuliza uchungu.Kwa wakati nadra sana inaweza ikabidi figo itolewe ikiwa damu itiririke kuzidi uwezo wa madaktari. Lakini biopsy ukifanywa kwa makini na kufuatilia majibu ya awali madhara haya hayatatarajiwa.

Tutayaweka matokeo yako kwa njia ya kuheshimu haki yako ya kutojulisha yeyote. Tutakujulisha kuhusu matokeo yako na maana kwa afya yako. Hatutakataa kupa matibabu iwapo utachagua ama usichague kushiriki katika uchunguzi huu.Tutakupa matibabu yafaayo kwa magonjwa yoyote tupatayo tukikuchunguza na yatakayopatikana kwa matokeo yako.

Uwe huru kuuliza maswali yoyote.Uchunguzi huu umehidhinishwa na kamati ya kusimamia machunguzi ya wasomi na haki ya wanaochunguzwa (Institutional Research and Ethics Committee-IREC) katika chuo kikuu cha Moi University na hospitali kuu ya Moi Teaching and Referral.

Iwapo unahitaji maelezo zaidi tafadhali wasiliana na IREC kwa kutumia anwani ifuatayo.

Mwenyekiti IREC,

Moi Teaching and Referral Hospital,

S. L. P.3,

Eldoret.

Simu: 33471/2/3

Nambari yangu ya simu ya rununu ni: 0722 915 159

IDHINI YAKO:

Walio na miaka 18 na zaidi

Nimeelezwa ipasavyo ya kwamba ninashiriki katika uchunguzi wa usomi utakayo chunguza iwapo ninaugua ugonjwa wa figo utokanao na viini vya HIV(*HIV-associated nephropathy*), ugonjwa ambao mimi kwa ajili ya kuwa na viini vya HIV ninaweza kuwa nayo. Mchunguzi pia amenieleza kuwa sitakosa matibabu yangu ya kawaida iwapo nishiriki katika uchunguzi au nisiposhiriki. Pia nimeelezwa kuwa sitahitajika kulipia chochote kinachohusiana na uchunguzi huu.

Sahihi: ……

Jina: ……

Tarehe: ……

IDHINI YAKO:

Walio na miaka chini ya 18

Nimeelezwa ipasavyo ya kwamba mwana wangu anashiriki katika uchunguzi wa usomi utakayo chunguza iwapo anaugua ugonjwa wa figo utokanao na viini vya HIV (*HIV-associated nephropathy*), ugonjwa ambao yeye kwa ajili ya kuwa na viini vya HIV anaweza kuwa nayo. Mchunguzi pia amenieleza kuwa yeye hatakosa matibabu yake ya kawaida iwapo atashiriki katika uchunguzi au asiposhiriki. Pia nimeelezwa kuwa sitahitajika kulipia chochote kinachohusiana na uchunguzi huu.

MZAZI AMA MLINZI:

Sahihi: ……

Jina: ….……

Tarehe: ……

APPENDIX II: Data collection form

DEMOGRAPHICS

Date: ……. Medical Record Number: ……

Age: …… Sex: Male ☐ Female ☐

Race/Ethnicity: ……..

HISTORY

Do you have any of these symptoms?

Body swelling	Yes	☐	No	☐
Reduced urination	Yes	☐	No	☐
Darkening of urine(‘cola’ colored urine)	Yes	☐	No	☐

Are you known to have any of these illnesses?

High blood pressure (hypertension)	Yes	☐	No	☐
High blood sugar (diabetes)	Yes	☐	No	☐
Kidney disease	Yes	☐	No	☐
Heart disease	Yes	☐	No	☐

Is there anyone in your family with kidney disease?

Yes ☐ No ☐ **If so, who?** Nuclear family ☐ Extended family ☐

Are you on any of these medicines? Have you been on any of these recently?

Septrin	☐	Paracetamol	☐	Aspirin	☐
Ibuprofen	☐	Diclofenac	☐	Other NSAIDs	☐
Gentamicin	☐	Other aminoglycosides	☐	Don’t know	☐

None at all ☐

Others……..….

If female, LMP:……

EXAMINATION

General:

Normal	☐	Pallor	☐	Jaundice	☐
Dehydration	☐	Lymphadenopathy	☐	Oral Thrush	☐
KS	☐	Wasting	☐	Other……	

Height: ……..cm **Weight:** ……..kg

Vital signs:

BP: ……/……mmHg Pulse: ……../min

Temp: ……^{o}C $SpO_{2:}$ ……..%

Chest examination:

Normal ☐ Abnormal ☐

…….……

Heart examination:

Normal ☐ Abnormal ☐

…….……

Abdominal examination:

Normal ☐ Abnormal ☐

…….……

Nervous system examination:

Normal ☐ Abnormal ☐

…….……

Other findings

……

……

……

LABORATORY RESULTS

TEST	Result	Date
CBC: Twbc		
Hb		
Plt		
Serum creatinine		
Blood sugar:Random		
Fasting		
Urinalysis 1		
Urinalysis 2		
UACR		
CD4 cell counts		
Bleeding time		
Coagulation profile		
Renal ultrasound		
Renal biopsy		

APPENDIX III: Procedure for measuring blood pressure

Blood pressure will be taken using an Omron M2 compact upper arm blood pressure monitor (**Omron Healthcare, Inc., 1200 Lakeside Drive, Bannockburn, Illinois 60015**).

The patient should be in a quiet place, in a relaxed sitting position with no tight fitting clothing on the upper arm, or any thick clothing such as a sweater.

The patient sits upright with the back straight and places the arm on the table so that the cuff is on the same level as the heart. The cuff is wrapped on the right arm such that the bottom of the cuff is at least 1cm above the elbow. It is then fastened snugly. The start button on the machine is then pressed and automatically the cuff begins to inflate and the machine takes a reading. The blood pressure results as well as a heart rate reading are then displayed on the screen.

Should an error occur, the cuff is deflated and the process is repeated. High blood pressure readings are confirmed manually using a mercury sphygmomanometer.

The blood pressure machines are calibrated every week.

APPENDIX IV: Procedure for measuring blood sugar

Blood sugar will be measured using the finger-prick method.

The procedure will be explained to the patient and verbal consent then obtained.

Universal precautions will be observed.

Gloves are worn. The patient's right (or left) index finger is cleaned with an alcohol swab and allowed to dry. An Optium Xceed™ blood glucose meter (**Abbott Diabetes Care Ltd., Range road, Witney, Oxon, OX29 0YL, UK**) will be used. A new blood measuring strip (manufacturer specific) is inserted which switches it on and configures it to take a blood sugar test. The glucose meter is checked to ensure it is ready.

The machine works on the principle that glucose in the patient's blood reacts with chemicals in the test strip to produce a current that the machine measures and converts it to a glucose level reading.

Using a sterile lancet, a nick is made at the tip of the patient's finger such that some bleeding occurs. A drop of blood is applied at the test pad of the blood measuring strip. The machine takes a while before displaying a result. In case of error, the process is repeated. The machine displays the result in mmol/L.

APPENDIX V: Procedure for drawing blood

The procedure is explained to the patient and verbal consent sought.

Universal precautions will be observed.

A tourniquet is applied at a distal site about 5cm proximal to the selected site of venipuncture. The patient makes a fist without pumping the hand. The phlebotomist puts on a pair of clean gloves. The selected site is cleaned thoroughly with methylated spirit or povidone Iodine starting with the center and working outward. It is then allowed to dry.

The patient's arm is grasped firmly using the thumb to keep the skin taut and to anchor the vein. A sterile Vacutainer® system (**Becton, Dickinson and Company, 1 Becton Drive, Franklin Lakes, NJ USA 07417**) is opened and the blood collection needle inserted gently into the lumen of the vein at an angle of 15- 30^{o}, then the other end is attached to a Vacutainer® blood collection bottle. Blood flows freely into the bottle due to negative pressure.

The plain bottle is first; 2ml of blood for creatinine determination is collected. EDTA-containing bottles follow; blood for cell counts are collected in 2 different bottles, in one bottle 2ml will be used for CBC, another 2ml bottle will be sent for CD4 cell count. Blood for coagulation profile is taken in a 4ml Vacutainer® bottle containing sodium citrate. Additive-containing bottles are gently swirled to ensure uniform mixing.

After adequate blood has been collected, the tourniquet is released then the Vacutainer® needle is removed gently and an alcohol impregnated swab is applied at the site under pressure. Pressure is applied for a whole minute then the site is reassessed for continued bleeding. The area is dressed with a dry gauze and tape.

APPENDIX VI: Procedure for determining blood counts

Blood in EDTA-containing Vacutainer® bottles are taken to the lab for cell counts.

1. Complete blood counts

Blood for complete blood counts are taken to the lab immediately. Blood should be stored at room temperature for not more than 24 hours before being analyzed. Vacutainer® bottles are cross-checked with request forms. Patient's details are entered onto the machine. Sample tubes are rocked gently to mix blood and anticoagulant. The tubes are examined to ensure blood is not clotted or hemolyzed. The Vacutainer® bottle is uncapped and presented to the aspirator probe of the Ac·T™ 5 diff OV (Open Vial) Hematology Analyzer (COULTER®) (**Beckman Coulter, Inc., 250 S. Kraemer Boulevard, P.O. Box 8000, Brea, CA 92822-8000, USA**). The 'aspirate' button is pressed; the machine aspirates a sample of blood from the Vacutainer® bottle.

This is an autoanalyzing coulter counter; it does the cell counts based on the coulter principle. Cells are poor conductors of electricity and will interrupt current flow; impedance variations generated by passage of non-conductive cells through a small calibrated channel is used to determine the counts and size (volume) of particles passing through in a time period. The machine based on software calculates this and prints out the result on paper.

Quality control checks are run daily.

2. CD4 cell counts

Blood for CD4 cell counts are taken to the lab immediately, ideally within 24 hours. Blood samples are stored at room temperature and analyzed within 48 hours of collection.

Vacutainer® bottles are loaded onto a BD FACS Calibur™ flow cytometer (**BD Biosciences, 2350 Qume Drive, San Jose, California, USA, 95131**). This is a flow cytometer that quantifies absolute CD4 and %CD4 counts based on the use of monoclonal antibodies against the CD4 molecule. Stained cells pass under a laser beam which generates a light scatter and a fluorescence pattern. The amount and intensity of scattered light is proportional to the characteristics being measured. This is converted to electricity and the machine reports this in counts and scatter plots.

The samples in Vacutainer® bottles are transferred to BD TruCount® tubes and processed. They are then loaded onto the flow cytometer.

The system prints out the result on paper.

Quality control checks are run daily.

APPENDIX VII: Procedure for determining serum creatinine

Blood in plain Vacutainer® bottles are taken immediately to the lab. Serum may be stored for up to one day at 2 to 25^{o}C, up to seven days at 4 to 8^{o}C and up to six months at -20 to -80^{o}C.

The bottle is set onto a centrifuge and spun at 3000 rpm for 3 minutes to separate the serum from the cells. The supernatant (serum) is carefully suctioned using a micropipette and transferred to a sample cup.

The sample cups are systematically set on a rack that goes onto a Cobas Integra® 400 plus analyzer (**Roche Diagnostics, 9115 Hague Road, PO Box 50457, Indianapolis, IN 46250-0457**). This is an autoanalyzer that uses the Jaffe reaction to quantify creatinine; creatinine reacts with picric acid in the presence of an alkaline pH to produce a yellow-red complex that has a maximum absorbance at 512nm. The rate of dye formation is proportional to the level of creatinine in the sample. The analyzer reads out this absorbance and based on its software it calculates the serum creatinine. It prints out the result on paper.

The result is reported in µmol/L alongside reference serum creatinine levels.

Quality control checks are run daily.

APPENDIX VIII: Procedure for urinalysis

Fresh urine is collected from a patient using a clean, dry bottle.

Urine will be used for two tests, so it will be split into two samples.

1. Dipstick urinalysis

A Uristix® strip (**Siemens Healthcare Diagnostics, 1717 Deerfield Road, Deerfield, IL 60015-0778, USA**) is briefly immersed in the urine specimen, covering all reagent areas.

The edge of the Uristix® strip is run against the rim of the urine container to remove excess urine. The strip is held in a horizontal position.

The strip measures proteinuria based on 'protein error of pH indicators' principle. This principle states that at constant pH the development of any green color is due to the presence of protein. The result ranges from yellow for negative results to yellow green to green blue for positives. The test pad contains a pH dye indicator using bromphenol blue. Due to the negative charge of albumin, if protein (albumin) is present in urine, the pH increases, and a positive test result occurs.

The reactions are read visually. The strip test area is compared to that on the Uristix® color chart. This will be read after 60 seconds. The color at the center of the pad is compared to the corresponding color chart on the bottle label. 2 observers will read the color and a third person acts as a tie-breaker if there is disagreement.

The results are recorded, and the strip is discarded.

Normal and abnormal controls will be run daily to ensure validity of results.

2. <u>Urinary microalbumin</u>

A Clinitek 50® microalbumin analyzer (**Bayer HealthCare LLC, Subsidiary of Bayer Corporation, Elkhart, IN 46514 U.S.A.**) is used for this test.

A Clinitek Microalbumin 2® strip (**Siemens Healthcare Diagnostics, 1717 Deerfield Road, Deerfield, IL 60015-0778, USA**) is briefly immersed in the urine specimen, covering all reagent areas.

On the Clinitek 50® machine, the green key (START) is pressed at the same time as the strip is removed from the urine.

The strip is blotted by touching the edge only to a paper towel.

The reagent strip is placed with the reagent pads facing up, onto the instrument's test/feed table. The strip is slid along the table until it touches the end of the table.

The table is automatically pulled into the instrument, where the strip is identified and read. Results are displayed or printed as soon as they are available.

The results obtained are recorded; the strip is discarded into a suitable trash container.

The test table is wiped with a damp, lint-free tissue as often as needed to prevent urine from building up.

3. <u>Urinary albumin to creatinine ratio (UACR)</u>

Fresh urine is taken immediately after collection to the lab for assay.

Urine is assayed for the level of albumin and creatinine in the lab using the Cobas Integra® 400 analyzer (**Roche Diagnostics, 9115 Hague Road, PO Box 50457, Indianapolis, IN 46250-0457**).

This is an autoanalyzer that uses the Jaffe reaction to quantify creatinine; creatinine reacts with picric acid in the presence of an alkaline pH to produce a yellow-red

complex that has a maximum absorbance at 512nm. The analyzer reads out this absorbance and based on its software it calculates the urine creatinine.

It quantifies urinary protein by colorimetric method. Pyrogallol-red forms a complex with molybdate that is red in color with a maximum absorbance at 470nm. Binding of basic amino acids of proteins to this pyrogallol-red molybdate complex induces a spectral shift forming a blue-purple complex. The color intensity of this blue-purple complex is directly proportional to the level of proteins in the urine. The analyzer using software calculates this. It prints out the result on paper.

The results are reported in mg/dl for both creatinine and albumin.

Quality control checks are run daily.

The UACR is calculated thus:

$$UACR = \frac{Albumin(mg/dl)}{Creatinine(g/dl)}$$

The result from the above equation gives a fairly accurate estimate of the 24 hour albumin excretion in mg/day; this can be converted to g/day by dividing by 1000.

Normally there should be less than 30mg of albumin excreted per day, a value between 30 and 300mg/day is not detectable by dip stick and is called microalbuminuria, beyond 300 mg/day dipsticks detect it and is called macroalbuminuria (proteinuria).

APPENDIX IX: Procedure for coagulation studies

1. Platelet numbers

Platelet numbers are checked once a complete blood count (CBC) has been done, see Appendix V above.

2. Bleeding time

Bleeding time is a test used to assess for bleeding disorders caused by vascular defects or defects in platelet numbers or functioning. Since platelet numbers are already checked as above, it will serve to assess the platelet functioning.

Bleeding time will be assessed using the Ivy method.

Universal precautions will be observed.

The procedure is explained to the patient and then consent is obtained.

A sphygmomanometer cuff is secured on the upper arm. A suitable site is selected on the forearm-one that has no hair and has no superficial veins. It is gently cleaned with alcohol and allowed to dry.

The sphygmomanometer cuff is inflated to 40mmHg then allowed to equilibrate.

A lancet is used to make a cut on the forearm in a site where there is no hair and no superficial veins. A cut 10mm long and 1mm deep is made on the skin and a timer started. A filter paper is used every 30seconds to blot the blood without touching the wound. The test ceases when bleeding stops. The time is noted and recorded. The site is cleaned.

A normal bleeding time is 2-7 minutes.

APPENDIX X: Procedure for renal biopsy

A renal biopsy is performed by a trained nephrologist using ultrasound guidance (or by a trained physician under the supervision of a trained nephrologist).

The procedure is explained to the patient and consent obtained.

The patient lies prone (on the tummy).

The renal angle, the area below the ribs lateral to the spine is identified. The skin is then cleaned using methylated spirit and then povidone iodine is applied and allowed to dry. The area is draped in sterile towels.

An ultrasound probe is inserted into a sterile glove that has been partially filled with sonic gel; this is to keep the probe sterile. A trained ultrasonographer uses the probe to localize the kidney. A suitable site at the lower pole of the kidney is identified by the nephrologist with the ultrasound guidance.

The skin is anesthetized using lidocaine, infiltrated around the skin and further deep into the subcutaneous structures. Further anesthesia is applied to the deeper structures all the way to the capsule of the kidney using a long spinal needle under ultrasound guidance.

A 5mm nick in the skin is made to facilitate entry of the biopsy needle. A spring-loaded biopsy needle is then inserted under ultrasound guidance through the skin nick and gently advanced through to the capsule of the kidney.

Once a suitable position is assured, the gun is fired; the needle automatically takes a core biopsy of the kidney.

The needle is retracted gently and the gun reloaded, exposing the core of kidney parenchyma. This is gently eased off the needle into a receptacle containing formalin.

A second biopsy is done to obtain another core of tissue.

The ultrasonographer checks the kidney and surrounding structures for the presence of a hematoma, usually hematomas form, but are of no consequence if they are not large and the patient reports no pain.

The area is cleaned and sterile dressing applied.

The patient is allowed back to the ward with instructions for strict bed rest while supine for at least 6 hours. Urine is collected in a clear container measured and observed for presence of frank blood. The patient is instructed to report any pain or presence of blood in urine.

All patients are observed for 24 hours then discharged if no complications arise.

In case of complications, the nephrologist will review the patient and consult the urologist for further action if necessary.

A repeat ultrasound is done before the patient is let home to assess for the presence of significant hematoma formation and any other abnormalities.

APPENDIX XI: Handling complications related to renal biopsy

Common complications after renal biopsy include pain, hematuria and hypotension. Most case require monitoring under analgesia, some may require transfusion as additional management. In rare cases the patient may require surgery to stem continued bleeding or for evacuation of hematoma. Very rarely a patient may require nephrectomy.

All patients after renal biopsy need to be observed for a minimum 24 hours for complications. During this period, the must be bed-bound, lie supine for at least 6 hours. Vital signs-temperature, heart rate and blood pressure will be monitored closely, hourly for the first 6 hours and 4 hourly thereafter. All urine output must be collected in a transparent container and monitored for frank hematuria. The patient is instructed to report any pain on the biopsied side and especially if it is worsening. He/she is also to report on worsening hematuria, dizziness, orthostatic hypotension and any other complaints they may have.

The investigator will review the patient frequently and as needed.

All patients undergo a repeat renal ultrasound at 24 hours to screen for the presence of serious complications e.g. massive hematoma. An ultrasound will be done if necessary before the 24 hour period elapses if a patient has worsening pain or worsening hematuria.

Decisions will be made on a case by case basis by the nephrologist, it's anticipated that most cases will be minor; requiring analgesia or/and transfusion(s).

Following review, if indicated, a urologist is called to review the patient and decide on further management. In case of the need for surgical intervention, all costs will be borne by the investigator.

APPENDIX XII: Histological examination of renal biopsy tissue

Kidney biopsy specimens are prepared for examination using two techniques, light microscopy and immunohistochemistry or immunofluorescence.

Tissues received in the lab are mounted onto paraffin blocks and prepared for slicing. Consecutive serial 2-3µm sections are prepared for use in histological and immunohistochemical staining procedures. Sections are mounted on slides each carrying 1-6 sections.

For light microscopy (LM) slides are stained using Hematoxylin and Eosin (H&E), periodic acid-Schiff (PAS), silver stains and trichrome stains. For immunohistochemistry, slides are first subjected to proteolytic digestion to unmask antigenic epitopes and to remove plasma proteins. Antibodies against IgG, IgM, IgA, and complement factor C3 are applied on various slides.

A pathologist will view the slides and make conclusions from it. This will be done at the Glomerular Institute of Columbia University, New York, USA.

A report on kidney biopsies contains the following information:

i. Adequacy of specimen (number of glomeruli and arteries)
ii. Systematic description of morphological changes in each compartment i.e. glomeruli, tubules and interstitium.
iii. Results of immunohistochemistry and/or immunofluorescence
iv. Descriptive diagnosis(using the WHO scheme of classification)

Concluding remarks discussing potential diagnoses

APPENDIX XIII: Study Approvals

I. IREC

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471//2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET
Tel: 33471/2/3

Reference: IREC/2010/52
Approval Number: 000539

4th June, 2010

Dr. Koech K. Mathew,
P.O. Box 7287, 30100,
ELDORET, KENYA.

Dear Dr. Koech,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee has reviewed your research proposal titled:

"Human Immunodeficiency Virus (HIV) – Associated Nephropathy among Antiretroviral Naïve Adults With Persistent Proteinuria at the Moi Teaching and Referral Hospital (MTRH)"

Your proposal has been granted a Formal Approval Number: **FAN: IREC 000539** on 4th June, 2010. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 3rd June, 2011. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Yours Sincerely,

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
APPROVED
04 JUN 2010
P.O. Box 4606 ELDORET

DR. OMAR ALY
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc: Director - MTRH
Dean - SOM
Dean - SPH
Dean - SOD

II. MTRH

MOI TEACHING AND REFERRAL HOSPITAL

Telephone: 2033471/2/3/4
Fax: 61749
Email: director@mtrh.or.ke
Ref: ELD/MTRH/R.6/VOL.II/2007

P. O. Box 3
ELDORET

7th June, 2010

Dr. Koech K. Mathew,
P.O. Box 7287-30100,
ELDORET, KENYA.

RE: APPROVAL TO CONDUCT RESEARCH AT MTRH

Upon obtaining approval from the Institutional Research and Ethics Committee (IREC) to conduct your research proposal titled:

"Human Immunodeficiency Virus (HIV) – Associated Nephropathy among Antiretroviral Naïve Adults with Persistent Proteinuria at the Moi Teaching and Referral Hospital (MTRH)."

You are hereby permitted to commence your investigation at Moi Teaching and Referral Hospital.

PROF. H.N.K. Arap MENGECH
DIRECTOR
MOI TEACHING AND REFERRAL HOSPITAL

INSTITUTIONAL RESEARCH & ETHICS COMM.
APPROVED
07 JUN 2010
P.O. Box 4606 ELDORET

CC - Deputy Director (CS)
- Chief Nurse
- HOD, HRISM