PREVALENCE OF CHRONIC KIDNEY DISEASE AMONG AMBULATORY HUMAN IMMUNODEFICIENCY VIRUS/ACQUIRED IMMUNODEFICIENCY SYNDROME PATIENTS ON ANTIRETROVIRAL THERAPY AT THE KENYATTA NATIONAL HOSPITAL

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A dissertation submitted in part fulfillment of the requirements for the degree of master of medicine in internal medicine university of Nairobi.

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DECLARATION
I certify that this is my original work and has not been presented for a degree at any other university.

Signature…………………………………………………Date: ……………………………

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ACKNOWLEDGEMENTS

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I am grateful to my supervisors for their invaluable input.

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<th>Description</th>
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<tr>
<td>AIDS</td>
<td>Acquired Immunodeficiency syndrome</td>
</tr>
<tr>
<td>ART</td>
<td>Anti Retroviral therapy</td>
</tr>
<tr>
<td>ACE—I</td>
<td>Angiotensin converting enzyme inhibitor</td>
</tr>
<tr>
<td>ARB</td>
<td>Angiotensin receptor blocker</td>
</tr>
<tr>
<td>ARV</td>
<td>Anti retroviral drugs</td>
</tr>
<tr>
<td>CCC</td>
<td>Comprehensive care centre</td>
</tr>
<tr>
<td>CD4+</td>
<td>Cluster of Differentiation 4+</td>
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<tr>
<td>CKD</td>
<td>Chronic kidney disease</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>EGFR</td>
<td>Estimated glomerular filtration rate</td>
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<tr>
<td>ESRD</td>
<td>End stage renal disease</td>
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<tr>
<td>HIVICK</td>
<td>HIV-associated immune complex kidney disease</td>
</tr>
<tr>
<td>HAART</td>
<td>Highly active antiretroviral therapy</td>
</tr>
<tr>
<td>HIVAN</td>
<td>HIV associated nephropathy</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency virus</td>
</tr>
<tr>
<td>KDOQI</td>
<td>Kidney disease outcome quality initiative</td>
</tr>
<tr>
<td>KNH</td>
<td>Kenyatta National Hospital</td>
</tr>
<tr>
<td>MDRD</td>
<td>Modification of Diet in Renal Disease</td>
</tr>
<tr>
<td>NNRTI</td>
<td>Non nucleoside reverse transcriptase inhibitors</td>
</tr>
<tr>
<td>NRTI</td>
<td>Nucleoside reverse transcriptase inhibitors</td>
</tr>
<tr>
<td>NSAID</td>
<td>Non-steroidal anti-inflammatory drugs</td>
</tr>
<tr>
<td>PI</td>
<td>Protease Inhibitor</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>-----------</td>
<td>------------------------------------</td>
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<tr>
<td>PLHA/PLWHA</td>
<td>People living with HIV/AIDS</td>
</tr>
<tr>
<td>TDF</td>
<td>Tenofovir Disoproxil fumarate</td>
</tr>
<tr>
<td>UPCR</td>
<td>Urine protein creatinine ratio</td>
</tr>
<tr>
<td>UoN</td>
<td>University of Nairobi</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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</table>
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ABSTRACT

Background: Availability of antiretroviral therapy has had a profound positive effect on HIV/AIDS morbidity and mortality. Renal dysfunction is increasingly being recognized amongst antiretroviral exposed and naïve people living with HIV/AIDS. Renal dysfunction is multifactorial and result from direct effects of the virus, some antiretroviral agents and drugs used for prophylaxis and treatment of opportunistic infections. Chronic kidney disease is expensive to treat besides being a strong independent risk factor for cardiovascular diseases. CKD is associated with higher mortality and morbidity in people living with HIV/AIDS.

Objective: To determine the prevalence of chronic kidney disease among ambulatory HIV/AIDS patients on antiretroviral therapy at Kenyatta National Hospital.

Methodology: This was a Cross sectional observational study conducted between July and September 2014 that enrolled patients who were 18 years and above on HAART for at least one year. We used a structured questionnaire for clinical and demographic data. Urine and blood were collected from patients. Dipstick analysis and urine albumin and creatinine assay were done in urine and creatinine assay in blood. Serum creatinine was used to calculate estimated glomerular filtration rate using modified diet in renal disease formula. Association between presence of chronic kidney disease and preselected risk factors such as hypertension, diabetes mellitus, CD4+ count, age, HIV stage, prior tuberculosis treatment, duration of illness and treatment.

Results: One hundred and fifty eight (158) patients were recruited. Average age was 43.3 years with a range of 24-69 and 78.5% of the patients were female. Mean baseline CD4+ count was 212 cells/ml (SD +/-174) and the most recent CD4+ was 496 cells/ml (SD +/-242). Average duration since diagnosis of HIV was 5.7 years and that from ART initiation was 4.8 years. Diabetes mellitus and hypertension was seen in 5.7% and 12% of patients respectively. Evidence of chronic kidney disease was seen in 88% of patients; 17.6% had estimated glomerular filtration rate less than 60 mls/min 1.73 and 86.3% had albuminuria. No preselected risk factors were found to have significant association with presence of CKD on univariate analysis.
Conclusion: There was a very high prevalence of CKD in patients on antiretroviral therapy at. Majority of the patients had proteinuria. Prevalence of Reduced glomerular filtration was high. No significant association was found between CKD and known risk factors assessed.
CHAPTER 1

1.0 INTRODUCTION

HIV/AIDS pandemic is still a big public health concern. Globally there were estimated 35.5(32.2-38.8) million people living with HIV/AIDS in 2012 [1]. The Kenya Aids Indicator Survey (KAIS) 2012 found HIV prevalence of 5.6% which corresponds to approximately 1,192,000 people living with HIV infection [2]. The economic and demographic impact of this is profound as majority of those affected are at their most productive age. This erodes the health and economic advances made in the last few decades in most developing countries where the burden of HIV/AIDS is highest.

Use of HAART has led to reduced mortality and morbidity in PLHA (3, 4, 5). This improved survival has spurred interest in chronic diseases like CKD. The PLHA are now exposed to established risks factors of CKD like age, hypertension diabetes mellitus and adverse effects of drugs such as tenofovir and Indinavir.

Kidney dysfunction is an independent predictor of mortality in PLHA in whom isolated proteinurias as well as impaired renal functions have been associated with faster progression to AIDS and death [6, 7]. Whereas early Kidney disease is asymptomatic and reversible, late disease is irreversible [8]. Effective strategy should aim at earlier identification and (where possible) prevention of progression of disease. The challenge is how to identify people with or at risk of developing CKD and its complications. Renal failure treatment requires dialysis or transplantation both of which are very expensive [3].

Renal function has serious implications regarding drug choice and drug dosing. Many ARVs are eliminated either in part or entirely by the kidneys and require dose adjustment in patients with reduced EGFR. Most nucleoside reverse transcriptase inhibitors are excreted unchanged in the urine and require dose adjustment except zidovudine and abacavir which require little or no dose adjustment. Protease inhibitors, non-nucleoside reverse transcriptase inhibitors, entry or fusion inhibitors and integrase inhibitors don’t require dose adjustment in people with decreased renal function [9].

Due to resource limitation in Sub-Saharan Africa particularly access to renal replacement; screening, early detection and treatment of CKD is the best intervention.
1.2 LITERATURE REVIEW

1.2.1 PREVALENCE OF RENAL DYSFUNCTION IN PLHA

Chronic kidney disease is defined as either a reduction in EGFR and or structural change in the kidney as evidenced by proteinuria or haematuria or pathological changes on renal biopsy.

Incidence and prevalence of renal dysfunction in PLHA is different in various parts of the world probably influenced by effects of other risk factors of renal dysfunction or ethnicity/race of population studied. This means that studies done in one demographic group may not apply in another.

Locally Wools-Kaloustian et al in a study of medically stable, HIV positive anti retroviral naïve patients found prevalence of CKD of 11.5% [11]. Studies employing varying criteria for diagnosis of kidney disease have reported a variable prevalence of kidney disease in patients with HIV in sub-Saharan Africa: 6-48.5% (12-18) Data on CKD in PLHA from the West and Asia also show varying prevalence 8.8-39% (21-25)

There’s a big difference in country prevalence rates which could be due to different criteria employed in the definition of CKD, different demographics of participants and whether or not proteinuria was used in assessment of renal dysfunction.

1.3 CAUSES OF CHRONIC KIDNEY DISEASE IN HIV/AIDS

Many adverse factors affect the kidneys in PLHA. They arise from direct effects of HIV e.g. HIV associated nephropathy (HIVAN) and immune complex disease or indirectly from opportunistic infection and drugs. The pathogenesis of renal dysfunction is multifactorial. Some of the common causes are glomerulonephritis, acute or chronic tubulointerstitial nephritis, prerenal azotemia, acute or chronic tubular necrosis and post renal obstruction.

A systemic review and metanalysis of relative risk of renal disease among populations of people living with HIV reported in studies from peer reviewed literature done by Islam MF et al (26) and published in 2012 found the following risk factors.
Table 1. Relative risk of renal disease among people living with HIV: a systematic review and metanalysis. Islam F et al. (26)

<table>
<thead>
<tr>
<th>RISK FACTOR</th>
<th>NUMBER OF STUDIES</th>
<th>POOLED RELATIVE RISK</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLHIV vs. HIV negative</td>
<td>3</td>
<td>3.87(2.18-6.85)</td>
</tr>
<tr>
<td>ART- experienced vs. ART-naïve</td>
<td>5</td>
<td>0.54(-0.99)</td>
</tr>
<tr>
<td>TDF based ART vs. non TDF</td>
<td>4</td>
<td>1.56(0.83-2.93)</td>
</tr>
<tr>
<td>Treatment duration</td>
<td>3</td>
<td>1.29/year of exposure to TDF</td>
</tr>
<tr>
<td>CD4+,+- T cell count</td>
<td>9 Heterogeneous</td>
<td>negative correlation</td>
</tr>
<tr>
<td>Late stage HIV vs. non-AIDS</td>
<td>3</td>
<td>3.32(1.85-5.93)</td>
</tr>
<tr>
<td>AGE</td>
<td>5</td>
<td>1.54/10 year increase in age</td>
</tr>
</tbody>
</table>

1.3.1 Antiretroviral drugs as cause of kidneys dysfunction

The role of long-term exposure to ARVS in chronic kidney disease causation is not fully understood. There are few studies to evaluate the long term relationship between ART and renal disease. For example more data is needed on tenofovir and long term renal safety because of its association with renal diseases.

Rasch MG et al [27] in a study to assess renal function and incidence of chronic kidney disease in HIV patients in Denmark found that HAART and in particular use of TDF and Indinavir were associated with increased risk of CKD with adjusted incidence risk rate of 6.08 and 26.75 respectively.
Kalayjian R C et al [28] assessed risk factors for chronic kidney disease in a large cohort of HIV-1 infected individuals initiating antiretroviral therapy in routine care. They found ART was associated with reduced CKD risk in association with CD4+ cell restoration and plasma viral load suppression.

In a large observational cohort study of the John Hopkins HIV clinical cohort done to evaluate changes in renal function associated with TDF treatment compared with NRTI treatment found both were associated with modest decline in EGFR. Tenofovir was associated with a reduction in creatinine clearance of minus (-) 13.3 ml/min against minus (-) 7.5 ml/min for NRTI. Other associations included a lower CD4 cell count, decreased renal function at baseline, and diabetes. Hypertension, age and use of protease inhibitors were not associated with significant decline [29].

In a retrospective chart review of data of outpatients attending an infectious disease clinic in New York, between December 1998-2008 Monteagudo-Chu et al [30] found a reduction in EGFR compared with baseline with TDF and abacavir use. TDF was associated with EGFR decline of minus (-) 22.4 mls/min per 1.73 m² and abacavir group minus (-) 7.5 mls/min per 1.73m² respectively. This is a very rapid decline in EGFR compared to the expected loss of 1ml/min 1.73m² annually. Study of a Swiss cohort found prolonged ART exposure led to reduced EGFR with Indinavir and tenofovir having significant association [31].

1.3.2 HIV-associated nephropathy

HIV-associated nephropathy [HIVAN] is the most common form of glomerulonephritis associated with HIV [32, 33]. It’s a focal segmental glomerulosclerosis of the collapsing type affecting majorly black people with prevalence among black Americans of 3-10% [34,35] in ambulatory cohort and 12% in an autopsy series in the 1990s [36]. Renal biopsy in the USA suggest 45-50% of CKD in HIV infected patients is due to HIVAN (37,38). In South Africa a retrospective review of renal biopsies in HIV-positive Black African patients to determine the prevalence of both ‘classic HIVAN’ and non-HIVAN pathologies and compare it to a cohort of HIV negative individuals who underwent renal biopsy from 1st January 2003 to 31st December 2004 was carried out. The histological findings in HIV positive cohorts were ‘classic HIVAN’ (27%) and HIV immune complex kidney disease (21%). Other glomerulonephritis included membranous, post-infectious disease, mesangial hyperplasia, and immunoglobulin A nephropathy. In contrast those who
Prevalence of CKD in PLHA on HAART

were HIV negative had no histological findings of collapsing focal segmental glomerulosclerosis or non specific immune complex disease; instead there were increased numbers of minimal change and membranoproliferative disease [39].

Koech et al in a study conducted at Kenyatta National Hospital found features suggestive of HIVAN in five of six biopsies done to evaluate causes of renal disease in patients with proteinuria [40].

HIVAN had become the most rapidly increasing cause of end stage renal failure becoming the third leading cause of end stage renal disease in African American between the age of 20-64 by 1990s after diabetes mellitus and hypertension [10]. If the prevalence of HIVAN reported in American studies were to be extrapolated to sub-Saharan Africa where more than 2/3rd of PLHA reside then the numbers would be astronomical.

HIVAN is characterized by nephrotic range proteinuria i.e. >3g/24hr, minimal edema, worsening azotemia with echogenic normal sized or large kidneys on imaging confirmed only through renal biopsy. Risk factors of HIVAN include black race, Low socio economic status, viral load >100000copies/ml, Low CD4+ count, Male gender, family history of renal disease and intravenous drug use [41]. ART has led to lower prevalence of HIVAN [32, 42]. Initiation of HAART has survival benefit and can reverse pathologic changes and improve renal functions. HAART is protective and HIVAN is rare in those with suppressed infection [43].

1.3.3 HIV-associated immune complex diseases

HIV Associated immune complex disease is mostly reported in Europe, almost all cases in Caucasians. HIVAN is rare in this population. Lescure FX et al [44] in a retrospective analysis of 88 patients with biopsy proven glomerular disease found that 97% of patients with HIVAN were black with low CD4+ count. This is in contrast to those with classic FSGS who were less often black (p<0.01)

A proposed mechanism for the development of immune complex-mediated renal disease in HIV-infected patients is the deposition of circulating immune complexes or polyclonal B-cell activation against an HIV-related antigen in renal tissue. HIV DNA has been identified in renal tissue, and HIV P24 antigen has been shown in biopsy specimen suggesting that there may be cellular incorporation of HIV genome products and the subsequent deposition of
antibody or circulating immune complexes. Interstitial inflammatory cells are present in HIVIKS and HIVAN. These cells are perhaps important in the pathogenesis of both entities due to their elaboration of various cytokines. The majority of cells in HICIDS are B cells unlike HIVAN where the majority cells are macrophages.

### 1.3.4 Nephrotoxic drugs

A number of other drugs that are commonly used in people living with HIV/AIDS are harmful to the kidneys. Apart from ARVs other commonly used drugs in PLHA are for prophylaxis and treatment of opportunistic infections.

Amphotericin B is used in the treatment of cryptococcal meningitis a fungal opportunistic infection in HIV/AIDS particularly affecting people not on ART with low CD4+ count. It causes renal damage in two ways; a pre-renal reversible component which is associated with decreased renal perfusion and an irreversible component due to renal tubular injury and subsequent dysfunction. A prospective study by Ochieng P O et al done in KNH found incidence of amphotericin B induced nephrotoxicity at 58.6% [45]

Masese et al in his work comparing TDF and stavudine nephrotoxicity found use of amphotericin B increased risk of nephrotoxicity 20 fold [46]. Other potentially nephrotoxic drugs are commonly used in PLHA are acyclovir, foscarnet, cotrimoxazole aminoglycosides, prolonged NSAIDS use amongst others.

### 1.3.5 Interstitial nephritis

Interstitial nephritis may lead to chronic kidney disease. Drugs and infectious agents are the main causative agents.

Drugs lead to interstitial nephritis via hypersensitivity reaction [39]. In the setting of HIV/AIDS some of these drugs include cotrimoxazole commonly used in prophylaxis and treatment of opportunistic infections. Other drugs are rifampicin, and less commonly abacavir, ritonavir and atazanavir.

In advanced disease, opportunistic genitourinary pathogens (parvovirus B19, herpes virus, cytomegalovirus, polyomavirus, Candida species, aspergillus species and mycobacterium avium complex) do cause interstitial nephritis [47].
Interstitial nephritis may be acute or chronic. In the acute form withdrawal of the offending agent leads to recovery of renal function. Chronic interstitial nephritis is not reversible.

1.3.6 Urinary tract obstruction

In the setting of HIV/AIDS a number of commonly used drugs can cause urinary tract obstruction. This include acyclovir, indinavir and sulfadiazine that can form crystals.

Indinavir, a protease inhibitor is associated with crystalluria and nephrolithiasis in up to 20% of patients [31].

1.3.7 Non-HIV related causes of CKD

Other established risk factors for renal disease such as diabetes mellitus Hypertension, aging, smoking and genetic predisposition compound HIV related causes. Diabetes mellitus and HIV have been shown to have additive effects in promoting CKD (47).

1.4 ASSESSING FOR RENAL DYSFUNCTION

The guidelines of the Infectious Diseases Society of America for the management of CKD in HIV-infected patients recommend screening for kidney disease at the time of HIV diagnosis. Tests should include:-

i. Urinalysis for hematuria and proteinuria

ii. A measure of kidney function (creatinine to estimate GFR)

If there is initial evidence of kidney disease, screening should be repeated annually. Semi-annual monitoring of kidney function and urinary markers of kidney damage is recommended for those receiving long-term drug therapies with toxicity to the kidney [48]

1.5 PROTEINURIA

Most renal diseases present with varying degrees of proteinuria. Normal daily protein excretion is less than 150 mg. Glomerular dysfunction is associated with large molecules proteinuria majorly albumin whereas tubular dysfunction is associated with low molecule proteinuria e.g. retinol binding proteins.. Early renal disease is reflected by lesser degrees of proteinuria, particularly increased amounts of albuminuria (microalbuminuria).
1.5.1 Dipstick

Standard urinary dipstick measures albumin concentration via a colorimetric reaction between albumin and tetrabromophenol blue producing different shades of green according to the concentration of albumin in the urine sample and reported as follows:-

- **Negative**
- **Trace** — between 15 and 30 mg/dL
- **1+** — between 30 and 100 mg/dL
- **2+** — between 100 and 300 mg/dL
- **3+** — between 300 and 1000 mg/dL
- **4+** — >1000 mg/dL

Dipstick is a simple cheap convenient and useful screening tool more so in resource poor setting. It is highly sensitive (>90%) and specific (95%) for detection of albuminuria of more than 250mg/day. False positive results may result due to urinary tract infection or other reducing substances in urine.

1.5.2 Urine albumin/creatinine ratio (UACR)

The gold standard for measurement of protein excretion is a 24 hour urine collection, with the normal value being less than 150 mg/day.

UACR Utilizes spot sample of urine to quantify proteinuria and has been shown to strongly correlate with 24hour collection for proteinuria (49, 50) with the added advantage of being more convenient, less cumbersome and less prone to errors of collection.

UACR is a good marker of renal disease that correlates well with rate of loss of renal function. It is a risk factor for progression to ESRD and cardiovascular disease [51].

Urine albumin creatinine ratio is an acceptable tool for assessing proteinuria for diagnosis of renal disease [52]. UACR >30mg/g is considered abnormal.
1.6 ESTIMATED GLOMERULAR FILTRATION
The normal value for GFR depends on age, gender, and body size, and is approximately 130 and 120 mls/min/1.73 m2 for men and women, respectively with inter individual variation. A reduction in EGFR implies reduced kidney function.

Modification of Diet in Renal Disease (MDRD)[53] and Cockcroft-Gault[54] formula are two of the commonly used estimation equations based upon the serum creatinine . Compared to gold standard they have shown satisfactory good correlation [55].
In people with low muscle mass this formulae might underestimate the EGFR.

1.6.1 MDRD equation

\[(\text{eGFR}) \text{ in mls/min} = 175 \times (\text{serum creatinine in mg/dl})^{-1.154} \times (\text{years})^{0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if African-American}).\]

1.7 CKD STAGING
The national kidney foundation, kidney disease outcome quality initiative categorizes kidney dysfunction into five stages as follows. [52]
A collaborative meta-analysis was conducted to examine the relationship between eGFR and albuminuria to mortality and kidney disease outcomes (52).

This helped stratify patients by prognosis as low risk, moderate risk, high risk and very high risk. They are represented by colour codes green, yellow, orange and red respectively in table 3 below. Every drop in creatinine clearance is associated with increased mortality and morbidity. As the EGFR drops known renal disease complications like anemia, bone mineral disease, malnutrition, susceptibility to infections etc increase. Reduced EGFR and albuminuria are strong cardiovascular risk factor (56, 57).
Figure 2: Staging and prognosis of CKD by eGFR and albuminuria categories: KDIGO 2012

<table>
<thead>
<tr>
<th>Persistent albuminuria categories</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
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<tbody>
<tr>
<td>Normal to mildly increased</td>
<td>&lt;30mg/g</td>
<td>Moderately increased 30-300mg/g</td>
<td>Severely increased &gt;300mg/g</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GFR categories (ml/min/1.73m²)</th>
<th>Normal or high</th>
<th>Mildly increased</th>
<th>Mildly to moderately increased</th>
<th>Moderately to severely decreased</th>
<th>Severely decreased</th>
<th>Kidney failure</th>
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<td>&gt;90</td>
<td>60-89</td>
<td>45-59</td>
<td>30-44</td>
<td>15-29</td>
<td>&lt;15</td>
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<td>G5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Low risk  Moderate risk  High risk  Very high risk
CHAPTER 2: STUDY JUSTIFICATION

HIV remains an important cause of morbidity and mortality in our setup with a prevalence of more than 5%. The survival of human immunodeficiency virus (HIV)-infected patients has increased significantly since the introduction of combination antiretroviral therapy leading to the development of important long-term complications including cardiovascular disease (CVD) and renal disease. Microalbuminuria, an indicator of glomerular injury, is associated with an increased risk of progressive renal deterioration, CVD and mortality. However, the prevalence of microalbuminuria has barely been investigated in HIV-infected individuals.

Chronic kidney disease is associated with increased mortality in PLHA, limits drug use due to reduced excretion and increases overall cost of care. Research in other population has shown high prevalence of CKD in both ART naïve as well as ART experienced PLHA. Currently there is no published data on prevalence and determinants of CKD in PLHA on HAART in Kenya. This study aims to provide baseline information and heighten the awareness of health providers and policy makers about this important subject. The best intervention for CKD is periodic assessment of renal function since early diagnosis will lead to treatment being initiated in the early reversible phase.

2.1 RESEARCH QUESTION
What is the burden of chronic kidney disease among ambulatory HIV/AIDS patients on antiretroviral therapy at the Kenyatta National Hospital?

2.2 RESEARCH OBJECTIVES

2.2.1 BROAD OBJECTIVE
To determine the prevalence of CKD in people living with HIV on antiretroviral therapy

2.2.2 PRIMARY OBJECTIVES

i. To determine prevalence and stage of chronic kidney disease in people living with HIV on antiretroviral therapy

ii. To describe the sociodemographic and clinical characteristics of this cohort
2.2.3 SECONDARY OBJECTIVE

i. To explore association between pre-selected independent variables i.e age, CD4+ count, hypertension, concomitant use of other nephrotoxic drugs, diabetes mellitus, and previous treatment for tuberculosis and CKD in this cohort
CHAPTER 3

3.1 MATERIALS AND METHODS

3.1.1 STUDY DESIGN

Hospital based cross sectional observational study.

3.1.2 STUDY SITE

This study was conducted at the Kenyatta National Hospital comprehensive care centre. This is a dedicated outpatient HIV/AIDS which is the reference centre for Kenyatta National Hospital (a tertiary teaching and referral hospital) and its environ. The patients on follow up come from KNH wards, KNH emergency department, various HIV testing sites, local correctional facilities and self referral. Majority of the patients are residents of Nairobi a cosmopolitan city and surrounding counties of Kiambu and Kajiado. Currently there are more than ten thousand patients on follow-up.

3.2 STUDY SUBJECTS

3.2.1 Study population

People living with HIV/AIDS who are 18 years and above on antiretroviral therapy for a period of at least one year.

3.2.2 Inclusion criteria

i. Patients who have been on ART continuously for one year and above
ii. Patients who are 18 years and above
iii. Patients willing to provide written consent to take part in the study

3.2.3 Exclusion criteria

i. Patients who are HAART-naïve
ii. Patients who have been on HAART for less than one year
3.3 SAMPLE SIZE CALCULATION
Our minimum sample size was 157 as determined by the formula for prevalence studies by Daniel et al (1999).

\[ n = \frac{z^2 \cdot p \cdot (1-p)}{d^2} \]

Where \( n \) = desired minimum sample size;
\( z \) = standard normal distribution value (1.96)
\( p \) = known prevalence rate for the factor of interest under study
\( d \) = the level of desired precision (0.05).

Prevalence of renal insufficiency in HAART naïve patients at 11.5% found by Kara Wools Kaloustian et al in western Kenya was used.

\( z = 1.96, \ p = 11.5\%, \ d = 0.05 \)

3.4 SAMPLING METHOD
Simple random sampling method was used

Files of people who fit the inclusion criteria were identified and assigned consecutive numbers starting from one (1) to the last number which was between 100-120, the average number of patients seen per day.

This numbers were then input into a Microsoft excels spreadsheet for computer generated random sample of nine patients per day.

This was repeated every day during study duration until we achieved the desired sample size.

3.5 STUDY PARTICIPANTS RECRUITEMENT AND CONSENTING PROCEDURE
All patients at time of enrolment into care at the CCC undergo full evaluation including history and physical examination, baseline renal function and CD4+ count. All this information is stored in a computer storage system and is retrievable. We retrieved all requisite data as required in our data collection tool (APPENDIX I)

Each morning the principal investigator and his two assistants visited the KNH CCC and went through the records of patients due for clinic visits.
Sampling was done as outlined above after which the patient were called in to the doctors consultation room. The principal investigator or the research assistants explained to the patient in details the study details.

Those who gave written informed consent (as appears in appendix IV) were enrolled into the study.

The following data was collected in a predesigned data collection tool (appendix I) by interviewing the participants and chart abstraction from CCC records.

a) Age, gender
b) HIV clinical history (duration of HIV infection, WHO stage, type and duration of specific antiretroviral)
c) History of other co morbidities like diabetes mellitus, hypertension
d) Positive exposure to Amphotericin B, acyclovir, cotrimoxazole, NSAIDS, complementary medicines
e) Current or previous treatment for tuberculosis
f) Laboratory studies such as baseline renal function test, CD4+

The participant’s blood pressure was then taken as outlined below and thereafter blood and urine collected as also outlined below.

3.6 CLINICAL METHODS

3.6.1 Blood Pressure

Blood pressure was measured using mercury sphygmomanometer as per WHO recommendation with patient seated using a cuff that covered 2/3rd of the arm. [58]

The patient was allowed 15 minutes rest upon arrival in the examination room.

Systolic blood pressure was based on the appearance of 1st Korotkoff sound (phase 1) and diastolic pressure on disappearance of the korotkoff sounds (phase 5) measured to the nearest two (2) mmHg. The blood pressure was repeated after 10 minutes and the average of the two used.

A diagnosis of hypertension was based on systolic blood pressure of more than 140mmHg systolic and or diastolic blood pressure of more than 90mmHg, history of diagnosis of hypertension by a clinician or current use of antihypertensive drugs.
3.7 LABORATORY METHODS

3.7.1 Urine

Participants were requested to give ten milliliters of spot urine sample collected in a sterile urine container and sent for dipstick analysis and for evaluation of the protein/creatinine ratio in the KNH renal laboratory.

3.7.2 Blood

(i) The procedure was explained to the study participants by principal investigator or his trained assistants.

(ii) A suitable vein was then identified in the antecubital fossa of the participant’s hand of choice after tourniquet was applied proximal to the elbow joint.

(iii) Puncture site was aseptically cleaned using cotton wool soaked in methylated spirit

(iv) Using a sterile needle and syringe four milliliters of blood was drawn and put into a plain blood vacuitaner.

(v) The blood was then taken immediately to the KNH renal laboratory where creatinine levels was determined using the Mindrayr clinical chemistry analyze

3.8 STUDY VARIABLES

3.8.1 Case definition of CKD

i. EGFR of less than 60ml/min 1.73m2

ii. Proteinuria of 1+ and above using dipstick or UACR >30mg/g

3.8.2 Dependent variables

1. Proteinuria defined as either;

   - Dipstick proteinuria of 1+ and above or

   - Urine protein creatinine ratio >45mg/mmol

2. Estimated Glomerular filtration rate Using MDRD formula outlined previously
3.8.3 Independent variables

1. Current age (years)

2. Gender (male or female)

3. Hypertension (BP >140/90), current antihypertensive drug use or history of diagnosis of hypertension by a clinician.

4. WHO stage at diagnosis of HIV

5. Positive history of or treatment of Diabetes mellitus

6. CD4+ done closest to date of study

7. Cumulative duration of use of each antiretroviral

8. Positive exposure to other nephrotoxic agents; prolonged use of NSAIDS for more than a month, amphotericin B, septrin, alternative medicine

9. Current or previous treatment for tuberculosis

3.9 QUALITY ASSURANCE MEASURES

Research assistants were registered clinical officers. A week prior to conducting the study the principal investigator took the research assistants through the process of proper administration of consent and data collection to ensure proper adherence to laid out procedure.

The principal investigator provided direct supervision throughout the study period. Recommended procedure of specimen collection in an aseptic technique, proper labeling and storage was adhered to. Laboratory tests were done in KNH CCC and renal unit laboratories which have put in place the following quality assurance processes;

i. Qualified staff duly licensed by the Kenya Medical Laboratory Technicians and Technologist Board

ii. Carry out periodic internal audits and reviews

iii. All machines and procedures have operation manuals accessible for quick reference
iv. The chemistry analyzer and other machines undergo periodic calibrations as per manufacturer’s instructions

v. The laboratory runs daily quality control tests before samples are analyzed

vi. The laboratory is ISO 9001:2008 certified

3.10 ETHICAL CONSIDERATION
Proposal was reviewed by the KNH/UoN ethics and research committee and was conducted after ethical approval was granted. We sought informed consent from all study participants and those who declined were excluded without any prejudice to their care. Blood and urine samples taken were discarded as per the KNH CCC and renal laboratory protocols. Where need arose we made available adverse results to the primary care giver for appropriate treatment.

No patient adverse events were reported as a result of participating in this study. Findings of this study have been handed over to UoN department of internal medicine as well as KNH CCC for further dissemination and to improve practice. Efforts at publishing in relevant accredited journals and presentation in appropriate scientific meetings will be made as a way of adding to available information. Confidentiality was maintained at all times by use of unique identifiers only known to principal investigator in place of patient’s names.

3.11 DATA MANAGEMENT AND STATISTICAL ANALYSIS
Data from the research questionnaire was verified and cleaned before being entered into a predesigned pass word protected Microsoft Access data base. Analysis was done with the input of a statistician using STATA version 12. The study population was described by summarizing categorical data such as gender, WHO HIV/AIDS stage etc into proportion Continuous data such as age, CD4+ count, and duration on ART will be summarized into means and medians.

Prevalence of renal dysfunction was calculated as percentage of participants with EGFR of 60mls/min and below and or proteinuria within 95% Confidence intervals. Association between CKD and independent continuous variable of age, CD4+ count hypertension duration on HAART was done using students t test and that with independent categorical data like diagnosis of diabetes mellitus, gender, WHO stage of HIV and use of other nephrotoxic drugs explored using Chi square test. Logistic regression method used to determine predictors of CKD. Statistical significance was defined as a p-value < 0.05.
3.12 DATA STORAGE
All the raw data in this study was filed in a box file which and stored in a lockable cabinet accessible only to the principal investigator. All the sheets were checked to confirm completeness before filing. This data was only accessible to the principal investigator.
CHAPTER 4

4.1 RESULTS AND ANALYSIS
During the study period extending from July to September 2014, records of 724 ambulatory HIV patients seen in KNH CCC were perused and after random sampling 175 considered for enrolment into the study. Out of these 175 patients, 158 met the inclusion criteria and were enrolled while were 17 excluded (flow chart 1). The main reasons for exclusion were: short duration of ART treatment (less than 1 year, n = 13) while four eligible patients refused to give consent for study enrollment.

Figure 1: Flow chart: Study enrollment of ambulatory HIV/ AIDS patients at KNH

4.2 PARTICIPANTS DESCRIPTION

4.2.1 Socio- demographic characteristics
The average age of patients in this cohort was 43.1 years (SD 9.2) with a range from 24 to 69 years. The modal age group was 40-44 years, n = 36 (22.8%) followed by the age group 45-49 years, n = 32 (20.3%), table 1. Female patients accounted for 78% of the participants yielding a Male-to-Female ratio of 1: 3.5.
Table 3: Socio-demographic characteristics of study participants

<table>
<thead>
<tr>
<th></th>
<th>Frequency (n)</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients' age(years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-29</td>
<td>9</td>
<td>5.7</td>
</tr>
<tr>
<td>30-34</td>
<td>19</td>
<td>12.0</td>
</tr>
<tr>
<td>35-39</td>
<td>27</td>
<td>17.1</td>
</tr>
<tr>
<td>40-44</td>
<td>36</td>
<td>22.8</td>
</tr>
<tr>
<td>45-49</td>
<td>32</td>
<td>20.3</td>
</tr>
<tr>
<td>50-54</td>
<td>19</td>
<td>12.0</td>
</tr>
<tr>
<td>Over 55</td>
<td>16</td>
<td>10.1</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>34</td>
<td>21.5</td>
</tr>
<tr>
<td>Female</td>
<td>124</td>
<td>78.5</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>39</td>
<td>24.7</td>
</tr>
<tr>
<td>Widowed</td>
<td>43</td>
<td>27.2</td>
</tr>
<tr>
<td>Divorced</td>
<td>4</td>
<td>2.5</td>
</tr>
<tr>
<td>Separated</td>
<td>13</td>
<td>8.2</td>
</tr>
<tr>
<td>Married</td>
<td>59</td>
<td>37.3</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>2</td>
<td>1.3</td>
</tr>
<tr>
<td>Primary</td>
<td>36</td>
<td>22.8</td>
</tr>
<tr>
<td>Secondary</td>
<td>71</td>
<td>44.9</td>
</tr>
<tr>
<td>Tertiary</td>
<td>49</td>
<td>31.0</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formal employment</td>
<td>30</td>
<td>19.0</td>
</tr>
<tr>
<td>Self employed (formal)</td>
<td>20</td>
<td>12.7</td>
</tr>
<tr>
<td>Self employed (casual)</td>
<td>73</td>
<td>46.2</td>
</tr>
<tr>
<td>Housewife/ unemployed</td>
<td>33</td>
<td>20.9</td>
</tr>
<tr>
<td>Student</td>
<td>2</td>
<td>1.3</td>
</tr>
</tbody>
</table>
4.2.2 Clinical characteristics of study participants

Table 4 summarizes the clinical characteristics of HIV patients in the study. The mean duration between HIV diagnosis and enrollment in the study was 5.8 years (SD 3.2), range 1-19 years. On average the duration of patient ARV treatment was 4.8 years (SD 2.8). At the time of diagnosis with HIV infection mean CD4 count was 212 cells/ml, range 4-1000, compared to the latest mean CD4 count of 496 cells/ml, range 4-1428 with a mean difference 283.6 (95% CI 244.1 to 323.1), p value < 0.001. BMI measures ranged from 19 to 43 with a mean value of 25.2.

| Table 4: Clinical characteristics of ambulatory HIV patients on treatment at KNH |
|---------------------------------|----------|-----|--------|--------|
|                                | n        | Mean | SD     | Minimum | Maximum |
| BMI                             | 157      | 25.2 | 4.6    | 19      | 43      |
| Baseline creatinine(micromol)   | 82       | 89.0 | 31.8   | 44      | 293     |
| Current creatinine              | 158      | 90.0 | 32     | 50      | 300     |
| Years since HIV diagnosis       | 158      | 5.8  | 3.2    | 1       | 19      |
| Baseline CD4 count(cells/ml)    | 158      | 212.8| 174.3  | 4       | 1000    |
| Latest CD4 count(cells/ml)      | 158      | 496.4| 242.0  | 6       | 1428    |
| Duration on ART(years)          | 158      | 4.8  | 2.8    | 1       | 12      |

We found 27% of patients had history of tuberculosis treatment. There was a small increase of average creatinine levels from 89 micromol/l at baseline to 90 micromoles/l at study time. Hypertension and diabetes mellitus were co morbidities in 12 and 5.7% of patients respectively. One hundred and thirteen of 158 (71.5%) participants were on Tenofovir, 24.7% on Zidovudine and 3.8% on Abacavir based regimens. All study participants were on pneumocysticis jirovecii prophylaxis; 94.2% on cotrimoxazole and 3.8% on dapsone.
4.3 PREVALENCE OF CKD
In this study 88% i.e. 139 of the 158 patients recruited had chronic kidney disease using our case definition (page 28). Albuminuria (dipstick proteinuria of 1+ and above or UACR of >30mg/g) was present in 136 of 158 (86.1%) of the patients. Only 19 of 158 (12%) patients had a positive dipstick for proteinuria all of whom had elevated UACR.

Conversely all those classified as having albuminuria had an elevated UACR.

Table 5 CKD case definition variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>n=158</th>
<th>positive</th>
<th>negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR &lt;60mls/min</td>
<td></td>
<td>28(17.8%)</td>
<td>130(82.2%)</td>
</tr>
<tr>
<td>UACR &gt;30mg/g</td>
<td></td>
<td>136(86.1%)</td>
<td>22(13.9%)</td>
</tr>
<tr>
<td>Dipstick proteinuria 1+ or above</td>
<td></td>
<td>19(12%)</td>
<td>139(88%)</td>
</tr>
</tbody>
</table>

4.4 CKD STAGING AND PROGNOSIS
In regard to estimated risk of concurrent complications and future outcomes (prognosis), 5.1% of the patients were categorized as being at very high risk, 14.6% as high risk 67.4% as medium risk and the remaining 12.1% had low risk.
Figure 3: KDOQI CKD stage and prognosis among ambulatory HIV patients on ARV therapy (n=158)

<table>
<thead>
<tr>
<th>Persistent albuminuria categories</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal to mild</td>
<td>24.7%</td>
<td>4.4%</td>
<td>4.4%</td>
<td>31.6%</td>
</tr>
<tr>
<td>Moderately</td>
<td>2.5%</td>
<td>24.7%</td>
<td>4.4%</td>
<td>31.6%</td>
</tr>
<tr>
<td>Severely</td>
<td>30mg/g</td>
<td>300mg/g</td>
<td>&gt;300mg/g</td>
<td>31.6%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GFR categories (ml/min/1.73m²)</th>
<th>G1</th>
<th>G2</th>
<th>G3a</th>
<th>G3b</th>
<th>G4</th>
<th>G5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal or high</td>
<td>&gt;90</td>
<td>60-89</td>
<td>45-59</td>
<td>30-44</td>
<td>15-29</td>
<td>&lt;15</td>
<td></td>
</tr>
<tr>
<td>Mildly</td>
<td>4.4%</td>
<td>7.6%</td>
<td>1.9%</td>
<td>1.9%</td>
<td>-</td>
<td>-</td>
<td>13.9%</td>
</tr>
<tr>
<td>Moderately</td>
<td>4.4%</td>
<td>41.8%</td>
<td>10.8%</td>
<td>1.9%</td>
<td>-</td>
<td>-</td>
<td>79.1%</td>
</tr>
<tr>
<td>Severe</td>
<td>2.5%</td>
<td>1.3%</td>
<td>3.2%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.0%</td>
</tr>
<tr>
<td>Kidney failure</td>
<td>2.5%</td>
<td>1.3%</td>
<td>3.2%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.0%</td>
</tr>
</tbody>
</table>

Low risk | Moderate increased risk | High risk | Very high risk
4.5 ASSOCIATION BETWEEN CKD AND PRE-SELECTED CHARACTERISTICS
T-tests for association between preselected clinical characteristics and presence of CKD found no association (table 6)

Table 5: Patient age, CD4 and creatinine’s association with CKD

<table>
<thead>
<tr>
<th></th>
<th>CKD(n=139)</th>
<th>No CKD(n=29)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age</td>
<td>42.7± 9.2</td>
<td>46.3 ± 8.6</td>
<td>0.11</td>
</tr>
<tr>
<td>Baseline creatinine</td>
<td>89.3 ± 32.9</td>
<td>86.5 ± 21.3</td>
<td>0.81</td>
</tr>
<tr>
<td>Previous year creatinine</td>
<td>94.1 ± 56.4</td>
<td>93.5 ± 20.1</td>
<td>0.91</td>
</tr>
<tr>
<td>Baseline CD4</td>
<td>218.4 ± 181.8</td>
<td>171.7 ± 97.8</td>
<td>0.27</td>
</tr>
<tr>
<td>Latest CD4</td>
<td>497.6 ± 244.8</td>
<td>487.3 ± 224.6</td>
<td>0.86</td>
</tr>
<tr>
<td>Duration on treatment</td>
<td>4.8 ± 2.8</td>
<td>4.8 ± 2.6</td>
<td>0.93</td>
</tr>
</tbody>
</table>

No significant association was found between diabetes mellitus, hypertension, exposure to tenofovir, previous treatment of tuberculosis, and WHO HIV stage at diagnosis and CKD (table 7,8)
### Table 6: TDF use, previous tuberculosis treatment and HIV stage association with CKD (n=158)

<table>
<thead>
<tr>
<th>ART regimen</th>
<th>CKD</th>
<th>Chi statistic</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>YES</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDF based</td>
<td>102 (90.3)</td>
<td>11 (9.7)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Other regimen</td>
<td>37 (82.2)</td>
<td>8 (17.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous TB treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>99 (89.2)</td>
<td>12 (10.8)</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Yes</td>
<td>40 (85.1)</td>
<td>7 (14.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHO AIDS staging</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIDS</td>
<td>81 (92.0)</td>
<td>7 (8.0)</td>
<td>3.1</td>
<td>1</td>
</tr>
<tr>
<td>Non AIDS</td>
<td>58 (82.9)</td>
<td>12 (17.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 7: Diabetes mellitus and hypertension association with CKD (n=158)

<table>
<thead>
<tr>
<th></th>
<th>CKD</th>
<th>Chi statistic</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>YES</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DIABETES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>130 (88.4)</td>
<td>17 (11.6)</td>
<td>0.4</td>
<td>1</td>
</tr>
<tr>
<td>Yes</td>
<td>9 (81.8)</td>
<td>2 (18.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HYPERTENSION</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>122 (87.1)</td>
<td>18 (12.9)</td>
<td>0.8</td>
<td>1</td>
</tr>
<tr>
<td>Yes</td>
<td>17 (94.4)</td>
<td>1 (5.6)</td>
<td></td>
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</tr>
</tbody>
</table>
Table 8: Logistic regression analysis of predictors of CKD in ambulatory HIV patients on ART (n=158)

<table>
<thead>
<tr>
<th></th>
<th>Odds Ratio</th>
<th>P</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comorbidity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co morbid illness absent</td>
<td>1.00 (Reference)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co morbid illness present</td>
<td>2.84</td>
<td>0.066</td>
<td>0.93</td>
</tr>
<tr>
<td>Patients age (in years)</td>
<td>1.03</td>
<td>0.405</td>
<td>0.97</td>
</tr>
<tr>
<td>Creatinine level (baseline)</td>
<td>1.07</td>
<td>&lt;0.001</td>
<td>1.04</td>
</tr>
<tr>
<td>Baseline CD4 count</td>
<td>1.00</td>
<td>0.817</td>
<td>1.00</td>
</tr>
<tr>
<td>Latest CD4 count</td>
<td>2.06</td>
<td>0.406</td>
<td>0.38</td>
</tr>
<tr>
<td>WHO staging</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-AIDS</td>
<td>1.00 (Reference)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIDS</td>
<td>0.52</td>
<td>0.291</td>
<td>0.16</td>
</tr>
<tr>
<td>ART regimen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDF based</td>
<td>1.00 (Reference)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other regimen</td>
<td>0.94</td>
<td>0.915</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Logistic regression analysis showed that previous creatinine levels were an important independent predictor of CKD in HIV positive ambulatory patients on ART (table 9). The odds of CKD diagnosis increased by 1.07 (95% CI 1.04-1.1) for each unit increase in creatinine levels based on previous creatinine measurements. There was evidence of higher likelihood of CKD in patients with co morbid illnesses (OR = 2.84; p = 0.066) but this was not statistically significant. The remaining factors included in the analysis including patient age, baseline and latest CD4 counts, WHO staging of HIV infection and ART regimen were not significantly associated with CKD and therefore were not independent predictors of the risk of CKD in ambulatory HIV patients.
CHAPTER 5

5.1 DISCUSSION

The survival of human immunodeficiency virus (HIV)-infected patients has increased significantly since the introduction of combination antiretroviral therapy. This has in turn led to the development of important long-term complications including cardiovascular disease (CVD) and renal disease (3-5).

Ours was the first study done in this population of patients at KNH and we found a very high CKD prevalence of 88%. This high prevalence is not unusual as it is close to the 85% and 88% in Tanzania and Japan respectively (25, 60). In the Tanzanian study they enrolled ART naïve patients and excluded patients with other risk factors for CKD like hypertension. The Japanese study was a retrospective chart review of patients on ART. CKD Prevalence is high in PLHA regardless of ART status. Exclusion of traditional CKD risk factors did not change prevalence significantly. HIV seems to be the major determinant of CKD. Incorporation of albuminuria as assessed using UACR a very sensitive marker of renal dysfunction may have contributed to the high CKD prevalence in our study.

We found that 86.7% of our patients had albuminuria. Albuminuria is a marker of endothelial dysfunction and is both a risk factor for cardiovascular diseases as well as a marker of renal dysfunction. Dipstick urinalysis was only able to detect albuminuria in 12% of patients meaning that a routine dipstick is likely to miss many patients with mild renal dysfunction. Poor sensitivity of dipstick proteinuria in PLHA has been shown in other studies (61, 62). Despite this low sensitivity, dipstick will continue being an important tool in screening in poor resource setting due to its affordability and convenience. In facilities like KNH with capability to do UACR, it should be considered as a screening tool taking into account cost-benefit ratio.

Msango et al in Tanzania (60) in a study of ART naïve HIV infected patient reported an equally high prevalence of renal dysfunction at 85.6% despite excluding patients with comorbidities like hypertension, diabetes mellitus etc that also affect the kidney. Microalbuminuria was present in 71% of their patients. From the high prevalence of microalbuminuria in ART naïve patients it is plausible to then attribute the microalbuminuria to direct effects of HIV. Experimental studies demonstrate a direct impact of viral components (gp120, TAT) on the endothelium, as they lead to the expression of adhesion molecules (intercellular adhesion molecule [ICAM], E-selectin), a prothrombotic state
Prevalence of CKD in PLHA on HAART

(increase of von Willebrand factor, plasminogen activator inhibitor-[PAI]-1, tissue plasminogen activator [t-PA], tissue factor). Our cohort had fewer patients with the traditional risk factors for CKD/CVD. These results suggest that microalbuminuria may be a sign of current endothelial dysfunction and microvascular disease rather than of advanced HIV infection since more than 90% of our patients had CD4+ more than 200 cells/ml. This portends substantial risk of future cardiovascular disease events. Possible contributing factors include early kidney disease such as HIV-associated nephropathy, a marker of end organ damage related to comorbidities of diabetes mellitus or hypertension, or more diffuse endothelial cells dysfunction.

In the Tanzanian study by Msango microalbuminuria was significantly more common in female and this may explain the high prevalence of the same in our study that was predominantly female (60). We did not find significant association between gender and CKD but it’s an area that should be studied further.

Telmisartan has been shown to improve blood pressure and albuminuria in PLHA with hypertension and microalbuminuria (63). Though not set out as part of our study objective we found most of our patients who were hypertensive were on calcium channel blocker not ARB or ACE-inhibitor. With such high prevalence of albuminuria all patients with HIV and hypertension should be on an ACE-inhibitor or ARB drugs. About 20% of our patients were categorized as having either high or very high mortality risk. For a fairly young population with an average age of about 43 this is worrying. Two third (2/3) were in moderate risk category and the rest low or no risk.

Using EGFR alone, 17.6% of our patients had CKD (EGFR less than 60 mls/min 1.73 m2). This prevalence falls within what has been reported by others. Wools –Kaloustian and others found a prevalence of 11.5% in Kenya (11), Msango and co-workers had 24.3% of their patients having EGFR less than 60 mls/min 1.73 m2 (60). Other studies found prevalence of 2% in Burundi, (20) 8.8% in Brazil and 17.6% in Japan (20,21,25). Wide variations exist in published data on prevalence depending on method of EGFR estimation used, whether patients with co morbidities were included, HAART naïve or experienced among other factors.
Wools-Kaloustian and co-workers conducted their study in a cohort of ART naïve patients without other co morbidities and found prevalence of 11.5%. Our higher prevalence is possibly due to the fact that we did not exclude patients with other co morbidities known to cause renal dysfunction like diabetes mellitus and hypertension. ART drugs may have contributed to the kidney disease in our study. Our findings are more likely to be a reflection of the actual prevalence of CKD in this population.

A cross sectional study by Menesez et al (21) to determine prevalence of CKD in patients on treatment for at least one year with undetectable viral load found a prevalence of 8.8%. This is way below our finding of 17.2% despite targeting almost similar population in terms of treatment duration. The difference could be due to the fact that theirs was predominantly Caucasian population unlike ours which was all black. Black race is an established risk factor for CKD in PLHA due to apolipoprotein L1 (APOL1) mutation. Another possible explanation for our higher prevalence is effects of ART drugs. From this Brazilian study, TDF was shown to increase risk of CKD. More than 70% of our patients were on TDF.

The average age of our patients was 43.1 years, range of 24-69 and modal age 40-44 years n=36(22%). This represent a fairly young population which is consistent with the overall HIV demographics in Kenya as per KAIS report of 2012(2). This represents an older population compared to previous studies. Masese in 2010 and Macligeyo in 2012 found average an age of 37 and 40 respectively (46, 64). This is attributable to increasing life expectancy with treatment.

Majority, (78.5%) of the study participants were female. The gender disparity is in keeping with the current epidemiological profile of people infected with HIV/AIDS in the catchment area of the KNH CCC which encompasses Nairobi and parts of central Kenya where the ratio of males to females with HIV/AIDS is 1: 1.6 and 2.8 respectively KAIS 2012(2) Another contributing factor could be the fact that we targeted patients on HAART for one year and above. Ochieng et al (65) found that males had higher mortality and loss to follow up than females. Survival bias may thus explain the higher number of female in our study.

Education levels were high in our patients with 75% having secondary education and above. This study was conducted in an urban area where literacy levels are higher than rural areas. However despite the high education levels only about 30% were engaged in a formal form of
employment probably reflecting the fact that KNH is a public facility that caters mostly for people from lower socioeconomic state. CKD is an expensive disease to treat since patients will need dialysis and or renal transplant. Close monitoring of those at risk and withdrawal of adverse factors would be the best intervention for this population. Low socio economic status is a risk factor for HIVAN (41). We also explored the clinical characteristics of the patients in this study. These included the CD4+ count, duration since diagnosis of HIV and on treatment, body mass index, diagnosis of diabetes mellitus, hypertension, and previous treatment for tuberculosis.

The mean CD4 count at initiation of HAART of 212 cells/ml with about 53% of study participants having CD4+ of less than 200cells/ml. This reflects the fairly severe immunosuppression of our patients at diagnosis a finding replicated by kamano (66) *et al* who found 75% of those on treatment at AMPATH had CD4+ of less than 200cells/ml. Masese *et al* (46) found an average baseline CD4+ count of 85 cells/ml. There is a trend towards higher CD4+ at treatment initiation and this will be the case going forwards as the current guideline recommend initiating treatment at CD4+ of 500 and below from previous 350 and below. Immune restoration depends heavily on nadir CD4+ count. Advanced HIV/AIDS is by itself a risk factor for renal diseases including HIVAN (41). We found a higher likelihood of CKD with AIDS (WHO stage 3, 4) but this was not statistically significant.

The mean of most recent CD4+ count was 496 cells/ul reflecting the relatively long duration patients were on treatment. This compares favorably with findings of the DAD study, a large intercontinental study that found the average CD4+ count of more than seventeen thousands (17,000) patients at between 385-588 cells/ml (67). In Brazil (21) the average CD4+ on treatment was 568 cells/ml. Locally MacLigeyo found *et al* found an average CD4+ count on treatment of 330 cells/ml (64). As the CD4+ count at which treatment should be initiated goes up immune reconstitution will be more robust.

Average BMI was 25.2 kg/m2 which is slightly above normal reference range. We found 54% had normal BMI, 26% were overweight and 20% classified as obese. No participant was found to be underweight in this particular study. The high number of overweight and obese patients might be due to the metabolic changes caused by antiretroviral therapy use. Crum-Cianflone *et al* (68) found that patients on ART had 0.55 kg/m2 increase in BMI per year while on treatment and more than 60% of the patients had increase in weight with treatment. Tyler Tate *et al* (69) in a study to assess change of BMI with treatment found that 20% of
patients had moved from one BMI category a higher one after two years on treatment. Higher weight has been shown to be protective against kidney diseases but we did not find any association in our study. We found 27.6% of our patients had been treated for tuberculosis which is lower than the 42% findings by Kamano et al (66). Prior treatment for tuberculosis has been reported as a risk of kidney disease. In this study there was no significant association between prior treatment for tuberculosis and CKD.

Hypertension was a co-morbidity in 12% of our patients and another 19% classified as pre-hypertension. Diabetes mellitus was seen in 5.7% of our patients. This is compared to 8% of patients in the drug adverse outcome study (DAD) (67) who were hypertensive. Currently information is conflicting on whether ART increases risk of hypertension. Our prevalence of diabetes mellitus was higher than the 2.5% found in the DAD study. Known risk factors for DM are NRTI, PI, obesity, and advanced HIV disease factors that were common in our study (67) Hypertension and diabetes mellitus are established causes of renal disease in PLHA (21,25) We did not find a significant association between the two and CKD.

Tenofovir based therapy was used by 71% of the patients. This is in keeping with the Kenya ART guidelines which advocate for TDF based therapy as preferred regimen. Tenofovir has been shown to be safe with a small risk of CKD. We did not find significant association between TDF use and CKD but we are cognizant of the fact that we may not have been adequately powered to assess for this. More than 99% of the study participants were on drug for prophylaxis against opportunistic infection as per Kenya ART guideline. Cotrimoxazole was used by 96.4% of the patients. Cotrimoxazole can cause interstitial nephritis as well as crystal nephropathy.

Known risk factors for CKD in PLHA include TDF, advanced age, black race, advanced HIV disease, underlying renal dysfunction due to co morbidities e.g diabetes mellitus, hypertension, drugs like cotrimoxazole, rifampicin and opportunistic infections. In univariate analysis only advanced age was shown to have significant association with reduced EGFR. Overall there was no association found between known risk factors and CKD. Our study may not have been adequately powered for this secondary objective. Multivariate analysis conducted using logistic regression found out that the odds of CKD increased by 1.07 for each unit increase in creatinine levels based on baseline creatinine.
5.2 CONCLUSION
Chronic kidney disease is very common in PLHA on HAART at the KNH.

Univariate analysis did not find significant association between known risk factors assessed such as exposure to tenofovir, diabetes mellitus, hypertension, CD4+ count, age, WHO stage, prior treatment for tuberculosis and CKD.

Multivariate analysis conducted found out that the odds of CKD increased by 1.07 for each unit increase in creatinine levels based on baseline creatinine.

5.3 RECOMMENDATION
i. It is essential that all HIV/AIDS patients be screened for kidney diseases at initial visit and thereafter as necessary.

ii. Studies on therapeutic interventions like ARBs and ACE-I are vital for development of appropriate treatment

iii. More robust longitudinal studies to determine predictors of CKD in people with PLHA.

iv. Further tests to determine the specific causes of patients with persistent proteinuria including renal biopsy where applicable

5.4 LIMITATION
i. Cross sectional design of this study makes it difficult to establish temporal association of renal dysfunction with HIV/AIDS

ii. We did single measurement of creatinine and albuminuria and this may overestimate presence of CKD due to possibility of reversal causes.

iii. Our sample size was relatively small thus limiting the generalization of the results to the entire population of patients living with AIDS.


22. EK De’ti, R Thie´baut, F Bonnet: Prevalence and factors associated with renal impairment in HIV-infected patients, ANRS C03 Aquitaine Cohort, France. HIV Medicine 2010;11: 308–317


49. Ruggenenti P. Cross-sectional longitudinal study of spot morning protein: creatinine ratio, 24 hour urine protein excretion rate, Glomerular filtration rate, and end stage


69. Tate T, Willig A, Raper L et al. HIV and obesity: where did all the wasting go?. Antiviral Therapy 17.7(2012):1281
APPENDICES

APPENDIX I: DATA COLLECTION TOOL

PREVALENCE OF CHRONIC KIDNEY DISEASE AMONG PEOPLE WITH HIV/AIDS ON ANTIRETROVIRAL THERAPY AT KNH

<table>
<thead>
<tr>
<th>UNIQUE IDENTIFIER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of Assessment (DD-MM-YYYY):</td>
</tr>
<tr>
<td>Assessor’s Name:</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number</th>
<th>Question</th>
<th>Response/Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>QA1</td>
<td>Age</td>
<td>Male: 01 Female: 02</td>
</tr>
<tr>
<td>QA2</td>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>QA3</td>
<td>Marital Status</td>
<td>Single: 01 Widow: 02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Separated: 03 Divorced: 04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Married and living together: 05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Married but living alone: 05</td>
</tr>
<tr>
<td>QA4</td>
<td>Highest level of education completed?</td>
<td>No School: 01 Primary: 02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>secondary: 03 College/University: 04</td>
</tr>
<tr>
<td>QA6</td>
<td>Occupation</td>
<td>Formal employment: 01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Self-employed formal: 02</td>
</tr>
</tbody>
</table>
### SECTION B. BASELINE CLINICAL DATA

<table>
<thead>
<tr>
<th>QB1</th>
<th>Date of HIV diagnosis</th>
<th>Date</th>
<th>Month</th>
<th>Year</th>
</tr>
</thead>
</table>

| QB2 | initial CD4 count?(cells/mm3),date done | |

<table>
<thead>
<tr>
<th>QB3</th>
<th>Was a viral load done?</th>
<th>Yes</th>
<th>01</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>02</td>
</tr>
</tbody>
</table>

| QB4 | Initial viral load where done and date done | |

<table>
<thead>
<tr>
<th>QB5</th>
<th>WHO stage at diagnosis</th>
<th>Stage 1</th>
<th>01</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td>Stage 2</td>
<td>02</td>
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<tr>
<td></td>
<td></td>
<td>Stage 3</td>
<td>03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stage 4</td>
<td>04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not documented</td>
<td>99</td>
</tr>
</tbody>
</table>

| QB6 | Weight at HAART initiation(in Kgs) | |

| Blood pressure(mmhg) | |

### CO MORBIDITIES

<table>
<thead>
<tr>
<th>QB3</th>
<th>Diabetes mellitus</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypertension</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>TB</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Heart disease</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Others(specify)</td>
<td>4</td>
</tr>
</tbody>
</table>

[ ]
### DATA AS AT STUDY TIME

<table>
<thead>
<tr>
<th>QB4</th>
<th>a. Duration since diagnosis?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>b. Current CD4 count(cells/mm³), date done</td>
</tr>
<tr>
<td></td>
<td>c. Current viral load, date done</td>
</tr>
<tr>
<td></td>
<td>d. Blood pressure</td>
</tr>
<tr>
<td></td>
<td>e. Current weight</td>
</tr>
</tbody>
</table>

### SECTION C. CURRENT ART REGIMEN

<table>
<thead>
<tr>
<th>QC1</th>
<th>What HAART regimen is the patient currently on? (tick appropriately)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>TDF/3TC/EFV 0</td>
</tr>
<tr>
<td></td>
<td>TDF/3TC/NVP 1</td>
</tr>
<tr>
<td></td>
<td>TDF/ABC/LPV/r 2</td>
</tr>
<tr>
<td></td>
<td>TDF/3TC/LPV/r 3</td>
</tr>
<tr>
<td></td>
<td>TDF/AZT/LPV/r 4</td>
</tr>
<tr>
<td></td>
<td>d4t/3TC/NVP 5</td>
</tr>
<tr>
<td></td>
<td>AZT/3TC/NVP 6</td>
</tr>
</tbody>
</table>

- **QC2** Duration on HAART in years
- **QC3** Other drugs patient is taking and duration ( )

<table>
<thead>
<tr>
<th></th>
<th>Seprtin ( ) 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nsaisds ( ) 2,</td>
</tr>
<tr>
<td></td>
<td>Others, specify ( ) 3</td>
</tr>
</tbody>
</table>
APPENDIX II: LABORATORY PROCEDURES

SERUM CREATININE ESTIMATION (MINDRAY® CLINICAL CHEMISTRY ANALYZER)

Principle of the method:

Creatinine reacts directly with picrate ion under alkaline conditions to form a red-orange compound, called a Janovski complex, with an absorbance peak at 520 nm whose color intensity is directly proportional to the creatinine concentration in the sample. The analytical procedure will be fully automated.

Procedure:

1. Ten microlitre of sample will be mixed with 1500µl of working reagent and mixed well
2. The mixture will be incubated for 5 min at 37° C
3. Absorbance will be read at 520 nm
4. Serum creatinine concentration will be expressed in µmol/L

For quality control purposes daily controls are done to validate the results.

1. URINE ALBUMIN-CREATININE RATIO (CLINITEK® MICROALBUMIN ANALYZER)

Principle of the method:

Albumin: This test is based on dye binding using a high affinity sulfonephthalein dye. At a constant pH, the development of any blue colour is due to the presence of albumin.

Creatinine: This test is based on the peroxidase-like activity of a copper creatinine complex that catalyzes the reaction of diisopropyl-benzene dihydroperoxide and 3,3’,5,5’-tetramethylbenzidine.

Albumin is then recorded as concentration in mg/L, and creatinine in mmol/L. Albumin-creatinine ratio is then finally given in mg/mmol
3. DIPSTICK ALBUMINURIA

Done using urine dipstick Multistix™ (Bayer, Germany). Measures albuminuria via a colorimetric reaction between albumin and tetrabromophenol blue producing different shades of green according to the concentration of albumin in the sample. The dipstick strip is immersed in the urine for one minute then removed and left to stand for another minute for the reaction to occur. The colour that appears is compared against chromatic scale provided by the manufacturer.
APPENDIX III: CONSENT INFORMATION FORM

Study title: Prevalence of chronic kidney disease among ambulatory HIV/AIDS patients on antiretroviral therapy at Kenyatta National Hospital.

Investigator: Dr Kairu Brian Njogu

Supervisors: Prof Kayima, and Dr Omonge all from department of medicine and Therapeutics University of Nairobi.

INTRODUCTION

My name is Dr Kairu Brian a post graduate student in the department of internal medicine at the University of Nairobi. The aim of this form is to give you information about my study which I am conducting as part of my training to enable you make a decision on whether you will participate or not.

PURPOSE OF THE STUDY

The aim of my study is to assess kidney diseases in people living with HIV/AIDS on antiretroviral drugs. Anti retroviral drugs are the medicine given to reduce the HIV virus in your body. People living with HIV/AIDS have increased risk of kidney diseases due to the HIV itself, other infections and drugs.

You have been chosen to participate in this study because you fit into our inclusion criteria which is people living with HIV/AIDS on antiretroviral medications for a duration of more than one year.

BENEFITS FOR PARTICIPANTS

There are no monetary benefits you will derive from this study. However you will not be charged for the tests done. Information gathered will be shared with your primary physician for intervention if your test results so require.
RISKS

You will experience some pain and mild swelling where the blood sample is taken because of being pricked with the needle. No other risk is foreseen. In case of any other unexpected adverse events you are advised to seek treatment at the KNH casualty. This will be at your own cost.

PROCEDURE

If you agree to participate I will enroll you into the study, I will take a brief medical history about your illness and medications you are on as well as other illness that you may be having or have had in the past.

Two milliliters (2mls) of your blood will be drawn from a suitable vein and the sample used to assess for your kidney function.

I will also request you to collect 5 milliliters of urine for assessment of proteins in urine which may indicate kidney disease.

CONFIDENTIALITY

All information gathered will be confidential and will only be shared with your primary physician for the benefit of your care when necessary. Your name or file number will not appear on any data form or specimen as we shall assign you a unique number linked to your name.

REASSURANCE

Participation in this study is voluntary; you are free to decline participation or to withdraw at any time without any compromise to your continued care.
ETHICAL CONSIDERATION

This study is approved by the University of Nairobi/Kenyatta National Hospital ethics committee after review and having been satisfied that it will be conducted properly and the participant’s safety and rights will be respected. Study findings will be presented to the department of medicine and the CCC and any other scientific forum to improve knowledge on this condition. This can be verified via the address

The Chairperson, NH/UON-Ethics Review Committee, Kenyatta National Hospital, Hospital Rd, along Ngong Rd, P.O BOX 20723, Tel 726300-9, Fax 725272

You can also contact me in case of any issue as pertains to this study and procedure therein via P.O BOX 14023-00400 or 0721501144 or my supervisor Dr Mecha on 0722842741

CONSENT

I-------------------------------------having read the above consent information form and understood the purpose of the study, my rights and obligations as outlined above, and also being aware that participation is voluntary; I can withdraw from the study at any time without any compromise to my continued care do hereby give my informed consent to participate.

Signed---------------------------------------------Date---------------------

Witness……………………………………Date……………………..

I Dr kairu Brian Njogu hereby confirm that I have adequately explained to the study participant the above information and he/she has understood all the information

Signed---------------------------------------------Date ----------------------------
APPENDIX IV: KNH/UON-ERC LETTER OF APPROVAL

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and approved your above proposal. The approval periods are 8th May 2014 to 7th May 2015.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.

b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.

c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.

d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.

e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (Attach a comprehensive progress report to support the renewal).

f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.

g) Submission of an executive summary report within 90 days upon completion of the study.

This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website www.uonbi.ac.ke/activities/KNH/UoN.
Yours sincerely,

PROF. M. L. CHINDIA
SECRETARY, KNH/UoN-ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director CS, KNH
The Chairperson, KNH/UoN-ERC
The Assistant Director, Health Information, KNH
The Dean, School of Medicine, UoN
The Chairman, Dept of Clinical Medicine and Therapeutics, UoN
Supervisors: Prof. Joshua Kayima, Dr. Jared Mecha, Dr. Enoch Omonge

Protect to Discover