PROTEINURIA IN AMBULATORY HIV-INFECTED PATIENTS MANAGED AT MUHIMBILI NATIONAL HOSPITAL

By

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A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Medicine (Internal Medicine) of the Muhimbili University of Health and Allied Sciences.

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Muhimbili University of Health and Allied Sciences a dissertation entitled: **Proteinuria in Ambulatory HIV-infected Patients Managed at Muhimbili National Hospital"**, In partial fulfilment of the requirements for the degree of Master of Medicine (Internal Medicine) of the Muhimbili University of Health and Allied Sciences.

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DEDICATION

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ABSTRACT

Background: Kidney disease has emerged as an important complication of HIV infection, highly active antiretroviral therapy (HAART) and comorbidities. Thus, there is an overwhelming implication for HIV-related kidney disease in sub-Saharan Africa (SSA) since 67.7% of the world's HIV-infected individuals do reside in SSA region. However, relatively little is known regarding the burden of HIV-associated chronic kidney disease in this region.

The HIV infection in the kidney has diverse manifestations, but most of the HIV-associated nephropathies are predominantly characterised by proteinuria. However, urine screening for proteinuria is not routinely done among HIV-infected individuals in Tanzania, and the magnitude of proteinuria and associated risk factors has not been investigated among HIV-infected population attending HIV/AIDS Care and Treatment Clinics in Tanzania.

Study Objective: To determine the magnitude of proteinuria in ambulatory HIV-infected adult patients managed at the HIV/AIDS Care and Treatment Clinic, Muhimbili National Hospital, Tanzania.

Materials and methodology: Data collection included interviews using a standard structured questionnaire, physical examination and laboratory investigations (serum biochemistry, haematology, absolute CD4+ T lymphocyte count and serology for hepatitis B and C viruses). Proteinuria was defined as a dipstick result of 1+ proteinuria or greater on the spot mid-stream urine samples.

Results: A total of 294 HIV-infected patients participated in the study; their mean age (± SD) was of 39.1 (± 6.3), range 18–69 years, and females were 68.7%. Proteinuria was detected in 11.9% (95%CI 8.5–16.3) of the study participants; with the proteinuria category being +, 2+, and ≥ 3+ in 71.4%, 22.9%, and 5.7% of the cases, respectively. Fifteen percent of participants had an estimated glomerular filtration rate (eGFR) less than 60 mL/min/1.73m^2). The presence of proteinuria was significantly associated with lower CD4+ count < 200 cells/μL ($p = 0.023$), advanced WHO HIV clinical stage ($p = 0.020$), HAART-naïve ($p < 0.0001$), lower mean haemoglobin ($p < 0.019$), lower eGFR ($p < 0.0001$), and hypoalbuminaemia ($p < 0.0001$).

In multivariate analysis, low eGFR (< 60 mL/min/1.73m^2; OR 4.07, p = 0.019), high serum creatinine (> 200 µmol/L; OR 4.94, p = 0.007), low haemoglobin (< 9g/dL; OR 2.05, p = 0.030), and hypoalbuminuria (< 30g/L; OR 3.05, p = 0.021) were independently associated with proteinuria. The eGFR < 60 mL/min/1.73m^2 had an independent association with low CD4+ count (< 200 cell/µL; OR 2.83, p = 0.033), high serum creatinine (OR 4.08, p = 0.001), low haemoglobin (< 9 g/dL; OR 1.96, p = 0.010) and WHO HIV clinical stage 3 and 4 (OR 2.65, p = 0.046).

Conclusion and recommendations: The prevalence of both proteinuria (11.9%) and impaired renal function (15%), eGFR < 60 mL/min/1.73m^2, is high in ambulatory HIV-infected patients without specific clinical symptoms and signs. These findings underscore regular proactive screening and monitoring for proteinuria and/or kidney function among HIV-infected population as an important part of the national comprehensive strategy for HIV/AIDS care and treatment in Tanzania.

Simple, versatile and cost-effective interventions such as dipstick urinalysis can easily be done at the lowest-level health facilities that provide HIV/AIDS Treatment and Care service in Tanzania. This would result in early detection and prompt treatment or referral for patients with dipstick proteinuria to higher-level health facilities for further management; and thus facilitating early diagnosis and management of HIV-associated renal disease.

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ABBREVIATIONS / ACRONYMS

ACR	:Albumin-to-creatinine ratio
AIDS	:Acquired Immune Deficiency Syndrome
ART	:Antiretroviral therapy
ARV	:Antiretroviral drug
ARVs	:Antiretroviral drugs
BMI	:Body Mass Index
BP	:Blood pressure
Hb	:Haemoglobin
CD4	:CD+ T-lymphocyte cell count
CKD	:Chronic kidney disease
CTC	:HIV/AIDS Care and Treatment clinic
ELISA	:Enzyme-linked immunosorbent assay
ESRD	:End-stage renal disease
FSGS	:Focal and segmental glomerulosclerosis
GCLP	:Good Clinical Laboratory Practice
GCP	:Good Clinical Practice
GFR	:Glomerular filtration rate
HAART	:Highly active antiretroviral therapy
HBsAg	:Hepatitis B surface antigen
HBV	:Hepatitis B virus
HCV	:Hepatitis C virus
HIV	:Human Immunodeficiency virus
HIVAN	:HIV-associated nephropathy
HRP	:Horseradish peroxidase
IDSA	:Infectious Diseases Society of America
MNH	:Muhimbili National hospital
MOHSW	:Ministry of Health and Social Welfare

MUHAS	:Muhimbili University of Health and Allied sciences
NACP	:National AIDS Control Programme
PCr	:Urine protein-to-creatinine ratio
PCR	:Polymerase chain reaction
SSA	:sub-Saharan Africa
UK	:United Kingdom
UNAIDS	:Joint United Nations Programme on HIV/AIDS
US	:United States of America
WHO	:World Health Organization

DEFINITION OF TERMS

Proteinuria: refers to urinary protein excretion that is detectable by standard dipstick test (usually > 200–300 mg/L). It also indicates increased urinary excretion of albumin or any other specific protein.

Dipstick proteinuria: a positive urine dipstick result of 1+ or greater.

Dipstick urinalysis: the qualitative assessment of urine element, such as protein, glucose, specific gravity.

Prevalence of proteinuria: the proportion of participants positive on urine dipstick analysis.

Albuminuria: refers specifically to increased urinary excretion of albumin, > 20 mg/L.

Microalbuminuria: refers to albumin excretion exceeding the normal range (> 20 mg/L), but is below the minimum level for detection by standard dipsticks (< 200–300 mg/L).

Leukocyturia: presence of leukocytes in urine.

Anaemia: defined as haemoglobin levels lower than 13.0 g/dL for men and 12.0 g/dL for women according to the World Health Organisation criteria.[1]

Mild anaemia: a haemoglobin level of ≥ 9.5 g/Dl

Moderate anaemia: a haemoglobin level of 8–9.5 g/dL

Severe anaemia: a haemoglobin level of < 8.0 g/dL

Glomerular filtration rate (GFR): is a useful index of overall renal function as it measures the amount of plasma ultra filtered across the glomerular capillaries and correlates with the ability of kidneys to filter fluids and various substances.[2,3]

Impaired kidney function: an estimated GFR less than 60 mL/min/1.73 m^2.

Body mass index (BMI): is calculated as the weight in kilograms divided by the height in metres squared (kg/m^2),[4,5] and it is the index of weight-for-height used to classify underweight, overweight and obesity in adults.

Normal serum potassium: serum potassium level of 3.5–5.0 mmol/L.[6]

Normal serum creatinine: serum creatinine level below 133 μmmol/L.[6]

Hypoalbuminaemia: serum albumin level below 30 g/L

CHAPTER ONE

1.0 BACKGROUND AND LITERATURE REVIEW

1.1 EPIDEMIOLOGY OF HIV/AIDS

HIV/AIDS still remains a major and an urgent public health problem in Tanzania[7], a developing country in sub-Saharan Africa; which is the world's most severely HIV-affected region.[8] The latest UNAIDS data (Joint United Nations Programme on HIV/AIDS, December 2009) indicate that sub-Saharan Africa accounted for 67% (22.4 million) of HIV infections globally, 68% (1.9 million) of new HIV infections among adults while 91% (390,000) of new HIV infections among children in 2008.[8] Moreover, 72% (1.4 million) of the world's AIDS-related deaths occurred in this the region.[8] An estimated 4 million HIV-infected individuals living in low- and middle-income countries were receiving antiretroviral therapy at the end of 2008.

Recently, efforts have been made to make anti-retroviral therapy available to resource-poor countries. The government of Tanzania, through the Ministry of Health and Social Welfare, and other stakeholders or partners, has taken an active role in increasing anti-retroviral therapy (ART) availability and accessibility through various initiatives. In 2007 about 2 million Tanzania persons were estimated to be living with HIV and AIDS, with approximately 600,000 (30%) in need of ART.[7] Recent data based on household surveys estimate the seroprevalence in adults aged between 15–49 years in Tanzania to be 7%, varying widely across the regions.[7] A total of 172,736 HIV-infected individuals are currently enrolled at HIV/AIDS treatment and care sites in the country, and only 22.8% (136,700/600,000) of the estimated 600,000 HIV-infected Tanzanians who qualify for ART are currently on ART.[7]

1.2 BURDEN OF KIDNEY DISEASE AND HIV

The advent of the highly active anti-retroviral therapy (HAART) has dramatically reduced morbidity and mortality rates, and improved quality of life of HIV-infected individuals.[8,9] With reduced progression to AIDS and decreased mortality, the cardiovascular, liver, and renal diseases have emerged as major causes of morbidity and mortality in individuals with HIV infection.[8]

HIV-associated renal disease is common in HIV-infected individuals, besides non-HIV kidney diseases as in general population.[10] The burden of HIV-related chronic kidney disease (CKD) has globally increased owing to improved life expectancy and unremitting growth of the population at risk in HAART era, and hence leading to incredibly increased end-stage renal disease (ESRD) in HIV-infected individuals. Studies in Western countries have demonstrated a significantly higher proportion of CKD and/or ESRD incidence in African-origin HIV-infected population than white population.[10-28] Of the HIV-associated renal diseases, HIV-associated nephropathy (HIVAN) is the largely documented and most aggressive idiosyncratic HIV-specific renal clinico-histopathology as it classically presents with nephrotic-range proteinuria and a rapid decline in renal function;[11-14,28] with predominance in African-descent populations. Morphologically, HIVAN is a characterized by foco-segmental glomerulosclerosis with collapse of the glomerular tufts; concurrently with microcystic tubular dilatation, tubulointerstitial nephritis, glomerular epithelial cell hypertrophy. Both host-specific factors, such as genetic susceptibility, and HIV-1 gene products are implicated in initiating the disease pathogenesis, though the mechanism of HIVAN susceptibility and disease progression is not clear.

Nevertheless, recent studies have reported that more than 20%–50% of the ESRD cases were attributed to other aetiology than classic HIVAN.[29,30] Other glomerular diseases that been described individuals with HIV-1 infection are immune complex glomerulonephritis, minimal change nephropathy, thrombotic microangiopathy, crescentic glomerulonephritis, and noncollapsing foco-segmental glomerulosclerosis. It is possible that many ESRD cases attributed to HIVAN are essentially reflecting a broader spectrum of HIV-associated kidney diseases as well as comorbid renal diseases[11,12] since HIV-infected patients are at increasingly high risk of developing

both acute renal failure and chronic kidney disease,[31-33] with multifactorial aetiologies including HIV infection, HAART, co-infections, comorbidities, and their treatments. While drug toxicities more frequently manifest as tubular dysfunction, the glomerular dysfunction in HIV patients may be signifying HIV-associated renal disease or other secondary diseases, for example diabetes mellitus. Glomerular involvement is indicated by the finding of proteinuria alone or combined with haematuria. Haematuria is most often caused by haemorrhage in a different place all along the genitourinary tract. The presence of haematuria alone most likely denotes an anomaly exterior to the kidney parenchyma for instance in the urethra, prostate, bladder, or ureter; and hence furthermost less specific for intrinsic renal disease.

The manifestation of HIV-associated kidney disease is wide-ranging owing to the complexity of the HIV disease process, nonetheless most of the nephropathies associated with HIV are chiefly characterised by significant proteinuria. Proteinuria is one of the most sensitive markers of renal disease owing to the fact that any urine protein occurs only subsequent to a kidney anomaly at the level of the nephron, with a few exclusions such as overflow proteinuria. As an indicator of glomerular injury, microalbuminuria may be the sole indicator of significant underlying kidney disease, with an increased risk of potential progressive renal deterioration, cardiovascular disease and mortality.[34-36] Proteinuria predicts potential cardiovascular events in the general population.[37] Although low-level proteinuria may be of little immediate clinical significance, an increased urinary albumin excretion, even microalbuminuria range (30–300mg/24hour or 30–300mg/g creatinine), is an independent risk factor cardiovascular disease and increased risk for mortality.[34-36,38-42] The proportion of patients with focal and segmental glomerulosclerosis (FSGS) increases as serum albumin increases in the nephrotic syndrome while it has been demonstrated that HIV-infected individuals with nephrotic proteinuria and a serum albumin > 35g/L consistently have FSGS or significant glomerular sclerosis on renal biopsy, with poor renal survival.[29]

Gupta et al. have revealed that HIV-related proteinuria, and particularly albuminuria, a specific indicator of glomerular function, is frequently found in both whites and blacks even in normotensive or non-diabetic individuals.[30] In comparison with the

general population, Szczech and co-workers have reported HIV-infected individuals have a greater prevalence of low-level proteinuria,[43] with 11% microalbuminuria, and the presence of HIV infection was independently associated with a fivefold risk of microalbuminuria.[43] Also, some evidence demonstrates that microalbuminuria might be an early sign of HIVAN.[44] Higher CD4+ cell count was associated with lower albumin/creatinine ratio.[43] Furthermore, the increased risk of mortality among HIV-infected patients was predicted by presence of high proteinuria ratio.[45,46]

Although the 24-hour urine collection is the gold standard for measuring protein, it is highly impractical for most patients to complete the test, unless admitted in a health facility. The random urine protein-to-creatinine ratio (PCr) is a consistent and practical method for quantifying urine protein.[47] This quantification method has shown a good correlation with 24-hour protein measurement,[47-49] and owing to the dipstick inaccuracies, any PCr value > 300 mg/g needs further timely assessment by urine microscopy, renal ultrasound and/or renal biopsy. A dipstick urinalysis for evaluation of proteinuria is recommended in the lately published guideline by the HIV Medicine Association of the Infectious Diseases Society of America (IDSA),[49] with a threshold for detection of renal disease at 1+ proteinuria. A prompt further assessment with a quantitative test for the proteinuria of > 1+ is essential so as to yield a more accurate estimation; and hence diagnosing more patients with kidney disease.[49,50]

In a study measuring proteinuria by both urine dipstick and a urine protein-to-creatinine ratio within a 48-hour period, Siedner et al[50] have reported that the dipstick did not identify 21% of 166 HIV-infected patients with proteinuria in the 300–999 mg/g creatinine range. The overall sensitivity of a > 1+ dipstick result to detect abnormal proteinuria (> 0.30 gp/gc) was 94.0% (95%CI 90.7–96.5).[50] On the other hand, the urine protein-to-creatinine ratio as a quantification method may be too expensive or inconvenient particularly in the resource-limited settings, and the dipstick urinalysis would, thus, be the most likely alternative for proteinuria screening, besides taking into account the 94% dipstick sensitivity.[50]

Of 64 HIV-positive patients with renal abnormalities, Gardner et al. reported that 71.9% (46/64) had proteinuria and 50% (32/64) had high serum creatinine.[45] HIV-infected patients have a higher prevalence of proteinuria, with microalbuminuria, when compared with the general population.[43] Compared with patients without an initial proteinuria abnormality, patients with an initial abnormality of proteinuria had a worse survival rate.[45] Also, HIV-infected patients with a creatinine level $\geq$ 1.4 mg/dL had significantly poorer survival compared with patients with a creatinine level < 1.4 mg/dL.[45]

Furthermore, HIV-positive patients with > 2+ proteinuria had a 40% increased risk of cardiovascular conditions and 50% increased risk of hospitalization.[45] In another study, Szczech et al. demonstrated that HIV-infected patients with dipstick proteinuria > 1+ prior to HAART had more than twofold hazard of death after HAART initiation.[46] Therefore, renal abnormalities in HIV-infected individuals are associated with an increased risk of mortality. Using the urine dipstick, Crowley et al.[51] showed that the prevalence of persistent asymptomatic proteinuria (>1+ proteinuria) was 14% in HIV-positive outpatient patients. Although the presence of proteinuria did not correlate with viral load, the proteinuric patients had lower average CD4 count (180 cell/μL) than non-proteinuric patients (280 cell/ μL).[51]

Szczech et al. reported that the prevalence of asymptomatic proteinuria was 32% in HIV-infected women, and the predictors of proteinuria were CD4+ count < 200/ μL, increasing HIV RNA level, black race, and presence of hepatitis C.[52] The serum creatinine levels and positive HBsAg were not significantly different between proteinuric and non-proteinuric patients.[52] Atta and colleagues[53] revealed that patients with HIVAN had significantly higher creatinine and lower CD4+ counts at the time of biopsy than those without HIVAN, while the HIVAN was not associated with presence of hepatitis C, hypertension and injection drug.[53]

In a systematic review and meta-analysis to estimate the impact of hepatitis C coinfection on the risk of kidney disease in HIV patients, Wyatt et al have recently revealed that Hepatitis C coinfection is significantly associated with increased risk of

HIV-related kidney disease.[54] The pooled prevalence of proteinuria was 28% in HIV–HCV coinfection compared with 19.3% in patients without HCV coinfection.[54] The pooled relative risk associated with hepatitis C coinfection was significantly increased for proteinuria and acute renal failure.[54]

A cross-sectional study evaluating HIV infection as an independent risk factor for microalbuminuria, among HIV-positive and control individuals, has shown that the presence of HIV infection was independently associated with a fivefold risk of microalbuminuria as compared with an age-matched cohort without HIV infection.[43] The incidence of HIVAN in black women was increasing inline with the mounting rate of HIV infection in this group[10,13] signifying that HIVAN is not sex-specific, though the male sex has also been reported as one of the risk factors.[55] Even though not for ESRD, Lucas et al. have also recently reported that female sex as a risk factor for incident kidney disease.[15,16]

In Brazil, de Mattos Cavalcante et al have reported the prevalence of persistent proteinuria was 5.6% (95%CI 3.6–8.3) in 411 HIV/AIDS patients, and 4% of patients had serum creatinine above 1.3 mg/dL.[56] Most of the participants were male (70%), and only 37.5% were black while a large proportion (44.3%) was considered dark-skinned, and the majority of the patients (92.2%) were on HAART.[56] In this study, proteinuria was significantly associated with a CD4+ lymphocyte count below 200cells/mm^3, without significant association with HIV viral load.[56] A recent retrospective study in 8 clinics in the United Kingdom reported that 96% of 54 patients with HIVAN had proteinuria level > 1.5g/24-hour at baseline,[57] and patients who developed ESRD within 3 months after the HIVAN diagnosis had a high proteinuria level at baseline, with median proteinuria of 8.2g/24-hour.[57]

A non-selected cohort study of 495 HIV-infected Norwegian patients reported the prevalence of microalbuminuria was 8.7%, and this prevalence is three-to-five times high than that of general population.[58] Compared with patients with normal albumin excretion, patients with microalbuminuria were significantly older, had higher blood pressure, longer duration of HIV infection, higher β 2-microglobulin, higher serum

creatinine and a reduced glomerular filtration rate ≤ 90mL/min.[58] Compared with patients without albuminuria, however, the HIV-infected with albuminuria had no statistically significant difference in gender, race, smoking, body mass index, cholesterol, hepatitis B and C infection, CD4+ cell count, and HIV RNA level.[58] On analysing gender separately, men with albuminuria had higher cholesterol level compared with men without albuminuria, but no statistical significant difference in β 2-microglobulin level. Only the systolic blood pressure and β 2-microglobulin were the statistically significant independent predictors of microalbuminuria.[58]

In a Swiss study, Hailemariam and co-woekers reported clinical signs of nephropathy in 36% of HIV-infected, including proteinuria (18%), abnormal urinary sediment (19.5%), and renal insufficiency (11%)[59] The participants were HAART-naïve, predominantly Caucasians (95%), and only a single African patient had classical HIVAN [70]. In a retrospective study of 27 HIV-infected Spain patients, Gutiérrez et al.[60] reported that all patients with glomerular diseases confirmed by renal biopsy had proteinuria; and 51.9% (14/27) presenting with nephrotic syndrome while 48.1% having non-nephrotic proteinuria. The study population was mainly Caucasian origin (88.9%) and male gender (85.2%).[60] Most of them presented with hepatitis C virus (77.8%) and hepatitis B virus (37%) co-infection, and only 11.1% had HIV infection without hepatitis C virus or hepatitis B virus co-infection.[60]

A cross-sectional study in India,[61] among 104 HIV-positive patients revealed that 28% had albuminuria by urine dipstick method; with albuminuria of 3+, 2+ and 1+ for 1, 9 and 19 patients, respectively. In this study, 68.2% of the patients had CD4+ < 350 cells/mm^3.[61] Albuminuria had a significant negative correlation with CD4+ count as demonstrated by a 3.5 fold increased risk of albuminuria in patients with CD4+ < 350 cells/mm^3 compared with patients with CD4+ > 350 cells/mm^3.[61] Also, albuminuria had a significant negative correlation with haemoglobin levels. Moreover, low numbers of CD4+ count were significantly associated with lower levels of haemoglobin.[61]

Afhami et al have found no electrolyte abnormality, proteinuria or renal failure among 65 HIV-positive patients in a study assessing the frequency of electrolyte imbalance, renal failure and proteinuria in HIV-infected Iranian patients.[62] All of

the study participants were Iranian nationality and non-black ethnicity. Most of these patients were asymptomatic with higher CD4+ count (mean CD4+ 367 cells/mm^3), and only 26.1% had CD4+ cell count < 200 cells/mm^3.[62] Despite the small sample size, subclinical disease, higher CD4+ count and short-term follow, the authors concluded that HIV-associated nephropathy, proteinuria and electrolyte disorders are infrequent in HIV-infected Iranian population.[62] This conclusion is contrary to the results reported by Ramezani and co-workers,[63] showing 12.3% of 171 HIV-positive Iranian patients had proteinuria using urine dipstick. Patients with proteinuria had a lower CD4+ count and creatinine clearance than those without proteinuria.[63] Hepatitis C antibody (anti-HCV) and hepatitis B surface antigen (HBsAg) were positive in 52.6% and 7.0% of patients, respectively.[63] There were statistically insignificant differences between patients with and without proteinuria regarding age, sex, risk behaviours for HIV acquisition, stage of infection, concurrent antiretroviral therapy, systolic and diastolic blood pressure, serum albumin and creatinine, glomerular filtration rate (GFR), and presence of anti-HCV or HBsAg.[63]

Despite the predominantly strong association of HIVAN and black-ethnicity than Caucasian patients, a very few studies have been done concerning the epidemiology of HIV-associated renal diseases in Africa. Emerging data suggest that the prevalence of HIV-related kidney disease in African populations is similar to that observed in African American population. Data from a retrospective study in Israel in 176 HIV-infected patients reported that both Ethiopian- and non-Ethiopian-descent patients did not meet any clinical criteria for HIVAN,[64] and therefore Behar et al. concluded that the Ethiopian-descent patients had a similar susceptibility to HIVAN as non-Ethiopian Israelis, and the genetic differences accounted for the African-Americans' predisposition to HIVAN.[64] Conversely to this conclusion, 72% of patients were Ethiopian descent,[64] and the authors did not remark on the higher overall prevalence of proteinuria in patients of Ethiopian descent (15% versus 8%), even though this difference was statistically insignificant possibly owing to small sample, and lack of renal biopsies.

In a larger study, Emem et al. have demonstrated some evidence of kidney disease in 38% of 400 HIV-infected Nigerian patients, defined by the presence of proteinuria

and/or elevated serum creatinine levels.[65] Another study also reported the median creatinine clearance of < 60 mL/min in more than 40% of ninety HIV-infected HAART-naïve Nigerian patients.[66] Han et al. revealed that on screening 615 HIV-infected HAART-naïve South African patients without overt kidney disease, 7% had overt proteinuria, and only 90 of these patients were screened for microalbuminuria.[44] The microalbuminuria was present in 36% of the 90 patients.[10] The renal biopsy done in 30 patients with microalbuminuria or overt proteinuria demonstrated a high prevalence, 83% (25/30), of biopsy-proved HIVAN.[44] Though only 30% of renal biopsies were reviewed, Gernholtz et al.[12] reported 27% HIVAN diagnosis in a series of 99 consecutive kidney biopsies in HIV-infected black South African patients. Furthermore, this study critically revealed that approximately 20% of the renal biopsies were classifies as HIV immune-complex kidney disease.[12] Despite limitation by small sample size and selection bias due to retrospective design, this study results contradict the view that HIV immune-complex kidney disease occurs more frequently in Caucasian population. Choi et al. have also recently reported similar findings in that more than 50% of the ESRD cases were attributed to aetiology than HIVAN.[11]

In a cohort of 25,249 ART-naïve HIV-infected Zambian adults, Mulenga et al. using the Cockcroft-Gault method have documented that 33.5% had renal insufficiency at baseline; of whom 73.5% with mild insufficiency, 23.4% moderate insufficiency, and 3.1% severe insufficiency.[67] When compared with individuals with CD4+ > 200cells/mm^3, individuals with CD4+ < 200cells/mm^3 were more likely to have reduced creatinine clearance, and the risk for renal insufficiency increased slightly as CD4+ cell counts decreased.[67]

In Rwanda, the prevalence of proteinuria was significantly as high as 13% in patients with a CD4+ cell count < 200 cells/mm^3 in a cohort of 740 HAART-naïve Rwandan patients.[68] In Western Kenya, a cross-sectional study of 373 medically-stable antiretroviral-naïve HIV-seropositive patients found a 6.2% prevalence of proteinuria.[69] The proteinuria was significantly associated with lower weight, lower absolute CD4+ count, lower haemoglobin, history of tuberculosis, and presence of renal insufficiency.[15]

In a cross-sectional study in Uganda, Olson et al. reported that 21% of 195 HAART-naïve HIV-infected patients had a dipstick proteinuria,[70] and 57% (20/35) of patients with any proteinuria (1+ to 3+) had a creatinine clearance of < 50 mL/min.[70] This creatinine clearance is comparable to results reported by the Nigerian study.[66]

In a study among police officers in Dar es Salaam–Tanzania,[71] Janabi et al. revealed the prevalence of albuminuria of 16.8% in 587 HIV-negative healthy participants and 28.4% of 127 HIV-1-infected participants respectively, with a significantly higher overall frequency of renal symptoms and laboratory abnormalities among latter participants.[71] In this study, even HIV-negative healthy participants had a markedly higher prevalence of albuminuria.

In a recent study in Tanzania among HIV-1 infected HAART-naïve adults, Nagu et al. have reported the prevalence of hepatitis B virus and hepatitis C virus were 17.3% and 18.1% respectively.[72] The presence of HIV-HCV co-infection significantly increases the risk for proteinuria and acute renal failure.[54]

1.3 MANAGEMENT OF PROTEINURIA AND KIDNEY DISEASE IN HIV-INFECTED INDIVIDUALS

Studies have documented that the initiation of HAART has led to a significant impact on the epidemiology of HIV-associated kidney disease[73] as the incidence of HIV-related ESRD has declined; suggesting a role of HAART in the HIVAN treatment.[74-77] HAART is recommended as first-line therapy for HIV-associated nephropathy irrespective of HIV RNA or CD4+ T cell count levels.[49] However, a study by Gupta and co-workers reported insignificant different changes in albumin-to-creatinine ratio (ACR) over the initial 64 weeks of therapy in those on protease inhibitors (PIs) and those not receiving PIs,[30] and the overall ACR change was also insignificant in the entire group.[30] These findings may be due to a small sample size of the participants.

Uncontrolled studies on adjunctive use of corticosteroids have reported some improvement in renal function and reduction of proteinuria in patients with renal insufficiency and heavy proteinuria caused by HIVAN.[78-80] Steroids are indicated as second-line therapy for patients with HIVAN, particularly those with worsening kidney function in spite of HAART use. Cyclosporine has been reported to be effective in reducing remission of proteinuria in HIVAN.[81] Basing on the evidence of benefit in other proteinuric renal diseases, both diabetic and non-diabetic, the use of angiotensin-converting enzyme inhibitors (ACEI) was associated with an increase in renal survival, with significantly lower levels of serum creatinine and proteinuria than non-ACEI treated patients.[82-84] Moreover, the transforming growth factor-β (TGF-β) has been implicated in the pathogenesis of HIVAN,[33] and the cellular synthesis of TGF-β is increased by angiotensin II.

Several recent studies have demonstrated a progressive improvement in survival among HIV-infected dialysis recipients.[85-88] A retrospective cohort study of 102 patients with biopsy-proven HIVAN in the Paris (1984–1996), Laradi and colleagues have found an overall patient survival (mean ± SD) of 73% (± 5) , 55% (± 6), and 38% (± 7) at 0.5, 1, and 3 years, respectively while the proportion of dialysis-free patients was 73% (± 5), 60% (± 7), and 18% (± 10) at 0.5, 1, and 3 years, respectively.[85] In a retrospective study to determine whether dialysis modality is a

factor in the survival of patients with HIVAN and ESRD by using the US Renal Data System database, Ahuja and co-workers[86] found that there was no difference in survival between haemodialysis and peritoneal dialysis (HR 1.01, 95%CI 0.91–1.13) among 6,053 patients with ESRD and HIVAN who started dialysis therapy in the United States (December 1995 – December 1999). Using the United States Renal Data System database (1990–1999), Ahuja and others[87] revealed a significant improvement of 1-year survival of 6,166 HIV-infected dialysis patients, from 56 to 74%; and a decrease in the annual death rates from 458 deaths to 240 deaths per 1000 patient-years.

In view of results from emerging studies showing that both patient and graft survival rates in HIV-infected renal transplant recipients are comparable to the general population,[89-92] the HIV infection is not an absolute contraindication to kidney transplantation. In a retrospective cohort study of US adult deceased donor kidney transplant recipients (January 1996 – May 2001), Abbott and colleagues[90] reported that HIV-infected recipients had improved survival compared with recipients without HIV infection. In a prospective study assessing the safety and success of kidney transplantation, and the effect of immunosuppression on HIV infection, Kumar and co-workers[91] have shown that 1-year and 2-year patient survival was 85% and 82%, respectively; whereas graft survival was 75% and 71%, respectively among 40 HIV-positive dialysis patients. In this study, immunosuppression did not adversely affect HIV recipients maintained on HAART. In a prospective cohort of HIV infected kidney or liver transplant recipients followed for a median of 3.4 years in the HAART era, Roland and colleagues[92] have demonstrated that 1-year and 3-year kidney recipient survival was 94% whereas 1-year and 3-year kidney graft survival was 83%. Both the patient and graft survivals were comparable to the general population reported in the national database for transplant recipients during the same period in the United States.[92] The cumulative incidence of rejection for kidney recipients was 52% (95%CI 28–75) and 70% (95%CI 48–92) at 1-year and 3-year follow-up, respectively.[92] While episodes of acute rejection may stem, kidney transplantation is a feasible opportunity in this population even with the rational hesitancy that the immunosuppression for preventing graft rejection would hasten the HIV progression as well as high risk of severe drug interactions.

1.4 PROBLEM STATEMENT

Although the prevalence of chronic kidney disease is increasing globally, data concerning the prevalence of kidney disease in developing countries, including Tanzania, without a renal registry is generally lacking. A study among police officers in Dar es Salaam reported that albuminuria was 16.8% and 28.4% in HIV-negative and HIV-positive participants, respectively.[71] Despite matching the participants for age and sex, the study population setting[71] do limit extrapolating the study results to HIV-infected population attending the HIV/AIDS Care and Treatment clinics in Tanzania. Additionally, even HIV-negative healthy participants had a higher prevalence of albuminuria in this study.

The magnitude of proteinuria in HIV-infected individuals attending the HIV/AIDS clinics in Tanzania is primarily unknown, in spite of the fact that that the presence of proteinuria in HIV-infected individuals predicts the increased risk of morbidity and mortality.[34-36,38-43,45,46,52]

1.5 RATIONALE OF THE STUDY

Globally, HIV-associated renal disease has currently emerged as a significant public health problem. Little is known regarding the burden of HIV-associated chronic kidney disease in sub-Saharan Africa (SSA) population, despite an overwhelming implication for a potential epidemic of HIV-related renal disease in SSA given that 67.7% of the world's HIV-infected individuals do reside in this region. The HIV infection in the kidney has diverse manifestations, highlighting the complexity of the disease process, but most of the HIV-associated nephropathies are predominantly characterised by proteinuria. In addition, the presence of proteinuria in HIV-infected individuals predicts the increased risk of morbidity and mortality while glomerular dysfunction in this population is associated with a faster progressive renal decline and increased overall mortality. Proteinuria, especially microalbuminuria, is the hallmark of glomerular disease. In this view, proteinuria is, therefore, justified as a considerable screening method to identify HIV-infected individuals at risk of developing renal disease. The early identification of impaired kidney function in HIV-infected individuals is indispensable to enhanced outcomes, and intended quality of life.

Limited data is known regarding HIV-associated renal disease in Tanzania, a developing country without a renal registry. The National Guidelines for HIV/AIDS management[7] recommend monitoring of renal function through routine urine testing for the occurrence of proteinuria, and if available serum creatinine level, for patients on tenofovir-containing regimen. Despite this national guideline, neither regular urine screening for proteinuria nor serum creatinine measurement is currently done among HIV-infected individuals in Tanzania. Additionally, the magnitude and risk factors for proteinuria are principally unknown in HIV-infected population attending the HIV/AIDS Care and Treatment Clinics in Tanzania.

Therefore, this is the first study conducted in Tanzania to determine the prevalence and risk factors for proteinuria in HIV-infected adult individuals attending the HIV/AIDS Care and Treatment clinics in Tanzania, and it provides information essential for planning and implementing enhanced comprehensive care and treatment for this at-risk population.

CHAPTER TWO

2.0 OBJECTIVES OF THE STUDY

2.1 Broad Objective

To determine the magnitude of proteinuria in HIV-infected patients managed at Muhimbili National Hospital (MNH), Dar es Salaam, Tanzania

2.2 Specific Objectives

2.2.1 To determine the prevalence of proteinuria in HIV-infected patients.

2.2.2 To determine the association between proteinuria and the socio-demographic and clinical characteristics of the HIV-infected patients.

2.2.3 To determine the predictors for proteinuria in HIV-infected patients.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study design

This study was a hospital-based descriptive cross-sectional study.

3.2 Study area

This study was conducted at the HIV/AIDS Care and Treatment Centre (CTC), Muhimbili National Hospital (MNH) in Dar es Salaam. The HIV/AIDS CTC runs a daily clinic – Monday to Friday. MNH is a national referral, consultant and teaching hospital located in Dar es Salaam city. Dar es Salaam is the largest commercial city in Tanzania, situated at 6°48' South and 39°17' East on a natural harbour on the Eastern Indian Ocean coast of Africa. It encompasses two important business primes, an international airport and harbour respectively. The city had a population of 2.5 million people in 2002,[93] currently estimated to have over 4 million residents and is expected to reach 5.12 million by 2020.[94,95] With a population rate increase of 4.39% annually, Dar es Salaam has become the 3rd fastest growing city in Africa, and 9th fastest in the globally, after Bamako and Lagos, respectively.[94,95] The city is administratively divided into three districts, namely Kinondoni, Temeke and Ilala. The existing deliberate housing availability, environmental hygiene and infrastructure development as well as anticipated effectual healthcare service delivery are outgrown by this rapidly escalating population growth rate; and hence, increased the risk for disease burden.

3.3 Study Population and Participants

The study population was HIV-infected patients attending the HIV/AIDS CTC, Muhimbili National Hospital. All booked and newly registered patients attending the HIV/AIDS Care and Treatment centre were eligible for the study, unless rejected by exclusion criteria.

3.3.1 Sample size

The study sample size was calculated using the formula for cross-sectional study:

$$N = \frac{Z^2P(1-P)}{\varepsilon^2}$$

where N: expected sample size

Z: standard value, corresponding to 95% confidence; critical value 1.96

P: prevalence of proteinuria 21%[70]

ε: maximum likely error 5%

Hence, minimum sample size calculated was 255. With adjustment for 10% non-response, the adjusted sample size is N' = 281. Following systematic sampling and an informed consent, a total of 294 participants were subsequently recruited in this study.

3.3.2 Sampling method

The HIV/AIDS CTC was visited twice a week on alternating days. The number of recruited patients per day (range 6–10 patients per clinic day), depended on the number of patients attending the clinic on that day. The systematic sampling was used to identify patients following the appointment listing series at the HIV clinic, and the study participants were recruited consecutively.

The systematic sampling formula:

$$k = \frac{N}{n}$$

where k is the sampling interval; n is the number of patients recruited per interview day; and N is the number of patients attending the clinic per interview day.

Thus, every k^{th} patient in the arrival sequence was selected as study participants while patients waited for HIV/AIDS Care and Treatment service or shortly after the service. Patients who were randomly selected but could not wait for the interview, physical examination or collection of specimens on the interview day were given a next appointment within 5 days. For patients who did consent, a subsequent patient on the randomization sequence was recruited.

3.3.3 Inclusion criteria

The criteria for study participants were confirmed HIV infection, age $\geq$ 18 years, both HAART-naïve and on-HAART, and consenting to participate in the study.

3.3.4 Exclusion criteria

The exclusion criteria for this study were known comorbidities such as kidney disease, hypertension, diabetes mellitius, liver disease, malignancy, sickle cell, rheumatoid arhtiritis, dyslipidaemia, and febrile illnesss; pregnant, breastfeeding or menstruating women; leukocyturia; urinary tract infection; and abnormal genital discharge or genital ulcer.

3.4 Study Period

The study was conducted between September 2009 and January 2010.

3.5 Data Collection Technique (Procedures)

Each study participant was recruited after providing an informed consent (Appendix I & II). The participants were interviewed using a standard structured questionnaire, consisting of four parts: socio-demographic data, clinical history, physical examination, and laboratory investigations (Appendix III). The questionnaires were administered to each participant by the principal investigator or a trained research assistant under close supervision of the investigator. Clinical information was obtained by both directly asking the participants and referring to participants' clinical notes. The investigator performed the clinical physical examination[96] and HIV clinical staging according to WHO HIV staging criteria.[97,98]

The blood and urine specimens for laboratory investigation were collected after history and physical examination. The venous blood was collected in vaccutainer tubes for haematological, biochemical and immunological, hepatitis viral markers and analyses at the MNH Central Pathology and MUHAS Microbiology laboratories. The details of the laboratory methods are as shown in section 3.5.4 below.

3.5.1 History & Clinical examination

This includes medical and drug history, duration of HIV, and HAART use. The symptoms or signs in the past three months include dysuria, frequent micturition, decreased amount of urine, suprapubic pain, loin pain, facial puffiness, body swelling, and renal angle tenderness.

3.5.2 Anthropometric measurements

3.5.2.1 Weight:

The individual's body weight was measured by standardized weight-measuring scales without heavy clothing and shoes, and recorded to the nearest 0.5kg.

3.5.2.2 Height:

The study participants' height was measured with a firm measure against a vertical wall, and the height was measured to the nearest 0.5cm.

The body mass index (BMI) was calculated using the formula Kg/m^2; where Kg is body weight in kilograms and m^2 is squared height in metres. The BMI was classified according to World Health Organisation[4,5] into Underweight: BMI < 18.5; Normal weight: BMI 18.5–24.9; Overweight: BMI 25.0–29.9; and Obesity: BMI ≥ 30

3.5.3 Blood pressure:

The participants' blood pressure (BP) was measured by a nurse or doctor. After a 10-minute rest, the participants' BP was measured with a standard manual mercury sphygmomanometer while in the sitting position. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) measurements were determined by the 1st and the 5th Korotokoff's sounds respectively. The BP was measured twice, with interval of 5 minutes; the second BP measurement is considered as the patient's BP. Hypertension was defined as SBP ≥ 140mmHg and/or DBP ≥ 90 mmHg or use of hypertensive medication.[99]

3.5.4 Laboratory investigations

The laboratory investigations include venous blood and urinalysis.

3.5.4.1 Venous blood

After applying methylated spirit on cubital fossa, venopuncture was done by experienced phlebotomist to draw 6 millilitres from each subject for the laboratory investigations (random blood glucose, comprehensive serum biochemistry, full blood count, CD4+ count, hepatitis B and C seroassay). The CD4+ count was also acceptable in this study if it was done within the past three months as ascertained from the patients' clinical file.

3.5.4.2 Urine

The mid-stream urine was collected for urinalysis. All patients were given clear oral instruction on how to collect a mid-stream urine sample in a disposable clean plastic container without preservatives. The dipstick method was used for urinalysis (IND Diagnostic Inc, Canada), and the level of proteinuria was visually determined by dipstick colour change. Results of the urine test were interpreted by the principal investigator or research assistant and recorded as (–), (±), (1+), (2+), and (≥ 3+). The dipstick results recorded as (–) and (±) were defined as normal while proteinuria was defined as a dipstick record of 1+ or greater on a urinalysis.

3.5.4.3 Blood glucose

Random blood glucose was determined using the Contour TS glucometer (Bayer's CONTOUR™ TS system, Bayer Diabetes Care).

3.5.5 Laboratory Methods

3.5.5.1 Serum Creatinine, Urea, protein, lipid and Electrolytes

The serum creatinine, urea, protein, lipid and electrolyte levels were determined using the Roche COBAS MIRA Chemistry Analyzer (GMI Inc., US).[100] The classification into low, normal or high was wholly based on the calibrated laboratory reference values or as defined in the Definition of Terms above.

3.5.5.2 CD4+ T-Lymphocyte enumeration

The CD4+ T-lymphocyte cell count was determined by flow cytometry with BD FACScalibur machine (Becton and Dickinson, Immunocytometry systems, San Jose, CA, USA).[101]

3.5.5.3 Hepatitis B Surface Antigen (HBsAg) detection

The microwells were coated with HBsAg-specific monoclonal antibody as the solid phase. The patient's plasma/serum was added to the microwells together with a second mouse monoclonal antibody, conjugated with horseradish peroxidase (HRP). In the presence of HBsAg, the specific immunocomplex was formed, which was captured by the solid phase antibodies. The microwells were washed to remove unbound serum proteins and HRP conjugate.[102] This was followed by adding the chromogen/substrate to the captured HBsAg immunocomplex, and the colourless substrate was hydrolysed by the bound HRP conjugate to develop a coloured end product; with its colour intensity proportional to the amount of HBsAg present in the sample. The optical density was measured by an ELISA reader, and results interpreted as per instruction from the manufacturer. The samples below the cut-off was considered non-reactive (negative), while those above cut off considered reactive (positive). The test was repeated in cases of an indeterminate result, and the repeat reactive samples were considered positive.

3.5.5.4 Anti Hepatitis C Virus Antibody detection

The anti-Hepatitis C virus antibody (anti HCV–Antibodies) was detected using an enzyme-linked immunosorbent assay (anti-HBC ELISA) method, which was commercially available assay. The diluted sample was inoculated on microwells coated with specific recombinant Hepatitis C virus encoded antigens as the solid phase.[103] This solid phase was first treated with the diluted sample and, if the HCV antibody was present, it bound to the solid phase captured by the antigens. After washing out all other components of the sample in the second incubation bound-HCV antibodies were detected by the addition of specific polyclonal complementary anti-human IgG and M antibodies, labelled with peroxidase (HRP). The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generated an optical signal proportional to the amount of anti HCV antibodies present in the

sample. The optical density was measured by an ELISA reader, and results interpreted as per instruction from the manufacturer. The samples below the cut-off were considered non-reactive (negative), while those above cut off considered reactive (positive). The test was repeated in cases of an indeterminate result, and the repeat reactive samples were considered positive.

3.6 Data Collection Quality Control

The investigator was responsible for the overall logistics of the study. The analysis of all the laboratory specimens was performed in accordance with the manufacturer guidelines, and internal quality control directive. The observations and auditing of all recordings was done by the investigator; ensuring daily check of the data orderliness, clarity and completeness.

3.6 Estimating Glomerular Filtration Rate

The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula[104] below:

$eGFR_{CKD\text{-}EPI} = 141 \times \min(Scr/k, 1)^{\alpha} \times \max(Scr/k, 1)^{-1.209} \times 0.993^{age} \times 1.018$ (if female) x 1.159 (if black)

where Scr is serum creatinine; k is 0.7 for females and 0.9 for males

α is -0.329 for females and -0.411 for males

min is the minimum of Scr/k or 1 (i.e. minimum of standardized serum creatinine)

max is the maximum of Scr/k or 1 (i.e. maximum of standardized serum creatinine)

The eGFR was categorized using the National Kidney Foundation classification of CKD[48]

Stage 1: eGFR > 90 mL/min/1.73m^2

Stage 2: eGFR 60–89 mL/min/1.73m^2

Stage 3: eGFR 30–59 mL/min/1.73m^2

Stage 4: eGFR 15 –29 mL/min/1.73m^2

Stage 5: eGFR < 15 mL/min/1.73m^2

The CKD stages 3, 4 and 5 were combined in one stratum, and defined as impaired kidney function (i.e. eGFR < 60 mL/min/1.73m^2).

3.7 ETHICAL CONSIDERATION

3.7.1 Ethical Clearance

The ethical clearance to conduct this study was sought from the Research and Publication Committee of Muhimbili University of Health and Allied Sciences (MUHAS).

The consenting form was written in English and Kiswahili language. The informed written consent was obtained from all study participants. In addition to consenting, individual participants were informed on their anonymous data use. For strict confidentiality adherence, none of the participants' names appeared on the questionnaires as each questionnaire was separately coded with a study unique identification code.

All study participants were singly informed that the aim of the study was not only determining the magnitude of proteinuria, but also improved care for individuals with HIV infection.

This study was conducted according to the Helsinki Declaration,[105] and consistent with Good Clinical Practice (GCP) guidelines[106,107] and the applicable requirements by MUHAS Research and Publications Committee.

3.7.2 Patient Management

All patients with dipstick proteinuria were subjected to quantitative urinalysis, and all HIV-infected participants in this study were managed according to the national guideline and current standard of care in Tanzania.

3.9 DATA PROCESSING AND ANALYSIS

The collected data was entered into the Epi Info computer statistical programme software version 3.5.1 (Epi Info, CDC, Atlanta, US), followed by data coding, checking and cleaning. The data double entry was compulsory to ensure appropriate data consistency and quality. The data was then exported to and analyzed by SPSS statistical package software version 13.0 (SPSS Inc., Chicago, IL, US).

Descriptive statistics that characterize study participants were summarised as percentages, or dichotomized as means or medians. The baseline absolute CD4+ cell count data was $\log_{10}$-transformed to approach normality. Student's t-test was used to compare continuous variables, and the Pearson's χ^2 test or Fisher's Exact test for categorical data. Pearson's correlation coefficient was used to determine the correlation between proteinuria and other variables. For statistically significant variables ($P < 0.05$), multiple logistic regression analysis was performed to determine the independent predictors of proteinuria. The eGFR and proteinuria were also analysed as a separate renal end point in multiple logistic regression analysis to determine the effect of the predictors for proteinuria on eGFR less than 60 mL/min/1.73m^2). All statistical analyses were performed at a type I error rate of 0.05 and at confidence interval of 95% confidence interval. A two-sided P-value < 0.05 was considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Socio-demographic characteristics of the study participants.

Between September 2009 and January 2010, a total of 2302 HIV-infected patients attended the HIV/AIDS Clinic at Muhimbili National hospital, of whom 71.2% were females. During the same period, a total of 315 HIV-infected patients were eligible for enrolment in this study (figure 1). Twenty one patients (6.7%) were excluded since 8 patients did not consent and 13 patients had leukocyturia (9 patients had both leukocyturia and proteinuria).

Females accounted for 68.7% (95%CI 63.0–73.9, n = 202) of the enrolled participants. The participants' age ranged from 18 to 69 years with the mean age of 39.1 ± 6.3 years (mean ± SD), and females were significantly younger than males, with mean ages of 37.1 ± 0.50 and 42.3 ± 7.6, respectively ($p < 0.0001$, appendix IV). Majority (64.6%, 95%CI 58.8–70.0) of the participants were younger than 39 years, and most of them (47.3%, 95%CI 41.5–53.2) were in the age group 30–39 years. The socio-demographic characteristics of the study population are shown in table 1.

On marital status, the participants were 49.3%, 28.6%, 16.0% and 6.1% for married, single, widowed and divorced, respectively (table 1). On sex comparison (appendix IV), the female participants were single, widowed or divorced than male participants (31.7%, 18.3% or 6.9% versus 21.7%, 10.9% or 4.3%; $p = 0.017$), while a higher proportion of male participants were married (63.0% versus 43.1%; $p = 0.017$).

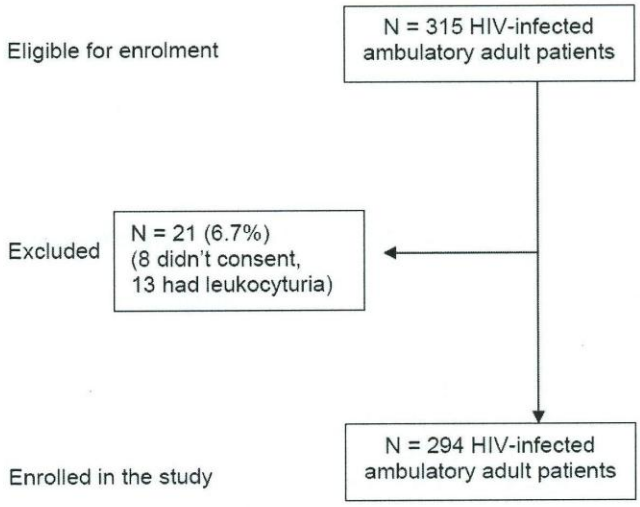


Figure 1. Flow chart for enrolment of study participants.

The majority of the participants (64.0%, 58.1–69.4, n = 188) had pre-primary or primary education (table 1). The males had significantly higher proportions in secondary and post-secondary education than female participants, respectively (46.7% versus 31.2%; $p = 0.010$, appendix IV).

The study participants had a mean body mass index (BMI) of 22.90 ± 2.08 (mean ± SD), and the majority (68.4%, 95%CI 62.7–73.6) had the BMI ≥ 18.5 (in table 1).
The study participants had mean systolic and diastolic pressures of 124.5 (± 9.3) and 78.2 (± 6.7), respectively (mean ± SD, mmHg), table 1. As compared with females (appendix IV), the male participants had significantly higher systolic pressure (129.6 ± 13.0 versus 121.4 ± 11.2; $p < 0.0001$) and diastolic blood pressure (84.3 ± 9.6 versus 73.9 ± 7.4; $p < 0.0001$), respectively. However, these differences in blood pressure are not clinically significant.

On HIV clinical stage, 20.4%, 28.2%, 40.8% and 10.5% of the participants were in WHO-HIV clinical stages 1, 2, 3, and 4, respectively. On comparing sex, the females had significantly higher proportions in WHO-HIV clinical stages 1 and 2 while the proportions of males were significantly higher in clinical stages 3 and 4 ($p = 0.008$, appendix IV). Of the study participants, 91.2% (n = 268, 95%CI 87.2–94.0) were using highly active antiretroviral therapy (HAART), and 67.7% were on HAART for

at least 18 months (table 1). The participants' median absolute CD4+ T lymphocyte count was 489.2 cells/μL, and virtually half of the patients (49.7%, 95%CI 43.8–55.5) had absolute CD4+ T lymphocyte count below 200 cells/μL (table 2).

Table 1. Sociodemographic characteristics of study population (N= 294).

Characteristics		N	(%)	95% CI
Age groups				
18 – 29		51	(17.4)	13.3 – 22.3
30 – 39		139	(47.3)	41.5 – 53.2
40 – 49		62	(21.1)	16.7 – 26.3
≥ 50		42	(14.3)	10.6 – 18.9
Mean age (± SD) years		39.1 (± 6.3)		Range 18–69
Sex Female		202	(68.7)	63.0 – 73.9
Marital Status	Single	84	(28.6)	23.6 – 34.2
	Married	145	(49.3)	43.5 – 55.2
	Widowed	47	(16.0)	12.1 – 20.8
	Divorced	18	(6.1)	3.8 – 9.7
Education	Pre- and Primary	188	(64.0)	58.1 – 69.4
	Secondary	70	(23.8)	19.1 – 29.2
	Post Secondary	36	(12.2)	8.8 – 16.7
BMI (kg/m^2)	< 18.5	93	(31.6)	26.4 – 37.3
	≥ 18.5	201	(68.4)	62.7 – 73.6
Mean BMI (±SD)		22.9 (± 2.08)		
Blood pressure		Systolic		Diastolic
Mean (±SD) mmHg		124.5 (± 9.3)		78.2 (± 6.7)
HIV–WHO stage				
1		60	(20.4)	16.0 – 25.6
2		83	(28.2)	23.2 – 33.8
3		120	(40.8)	35.2 – 46.7
4		31	(10.5)	7.4 – 14.8
On HAART		268	(91.2)	87.2 – 94.0
Duration	< 18 months	69	(23.5)	18.3 – 28.8
	≥ 18 months	199	(67.7)	62.0 – 72.9

BMI: body mass index; HAART: highly active antiretroviral therapy;
HIV: human immunodeficiency virus; SD: standard deviation; WHO: World Health Organisation

It was found that only 3.7% (95%CI 2.0–6.8, n = 11) of the study participants had urinalysis in the past six months, which was deliberately done to diagnose urinary tract infection (table 2).

The participants had mean serum creatinine 82.15 (± 5.04), and majority of patients (55.1%, 95%CI 49.2–60.9, n = 162) had serum creatinine levels < 133 μmol/L while 15.3% patients (95%CI 11.5–20.1) had serum creatinine levels above 200 μmol/L (table 2). Furthermore, thirty-five percent (n = 104, 95%CI 30.0–41.2) of study participants had an estimated glomerular filtration rate (eGFR) below 90mL/min/1.73m^2 with 15% having an eGFR less than 60 mL/min/1.73m^2.

The prevalence of anaemia in the study population was 88.8% (95%CI 84.5–92.0, n = 261), with mild anaemia in 32.3%, moderate anaemia in 55.1%, and severe anaemia in 1.4%. The haemoglobin level range was 6.0–14.3 g/dL, with the mean haemoglobin of 12.8 ± 2.9 g/dL (mean ± SD), table 2. The majority of study participants (55.1%, 95%CI 49.2–60.9, n = 162) had moderate anaemia (8.0–9.4 g/dL). Fifty two percent (95%CI 46.2–57.9, n = 153) of participants had hypoalbuminaemia (table 2).

Hepatitis C, hepatitis B, and co-infection of hepatitis B and C was found in 7.8% (95%CI 5.1–11.7, 9.9% (95%CI 6.8–14.0) and 1.7% (95%CI 0.6–4.2), respectively (table 2).

Table 2. Laboratory characteristics of study population (N= 294).

Characteristics	N	(%)	95% CI
Urinalysis in past 6 months	11	(3.7)	2.0 – 6.8
Serum creatinine (μmol/L)			
< 133	162	(55.1)	49.2 – 60.9
133 – 200	87	(29.6)	24.5 – 35.2
> 200	45	(15.3)	11.5 – 20.1
Mean (±SD)	82.15 (± 5.04)		
Potassium (mean ± SD, mmol/L)	4.16 (± 1.03)		
Hypoalbuminaemia	153 (52.0)		46.2 – 57.9
Haemoglobin (g/dL)†			
< 8.0	4	(1.4)	0.4 – 3.7
8.0 – 9.4	162	(55.1)	49.2 – 60.9
9.5 – 12 /13	95	(32.3)	27.1 – 38.0
> 12 /13	33	(11.2)	8.0 – 15.5
Mean (±SD)	12.8 (± 2.9)		Range (6.0–14.3)
Hepatitis seropositive*			
Anti-HCV	23	(7.8)	5.1 – 11.7
HBsAg / HBcAb	29	(9.9)	6.8 – 14.0
HBsAg & Anti-HCV	5	(1.7)	0.6 – 4.2
eGFR (mL/min/1.73m^2)			
≥ 90	190	(64.6)	58.8 – 70.0
60 – 89	60	(20.4)	16.0 – 25.6
< 60	44	(15.0)	11.2 – 19.7
Absolute CD4+ count (cells/μL)			
> 350	68	(23.1)	18.5 – 28.5
201 – 350	80	(27.2)	22.3 – 32.8
< 200	146	(49.7)	43.8 – 55.5
Median CD4+	489.2		Range (50–1493)
Proteinuria	35	(11.9)	8.5 – 16.3
Proteinuria category			
1+	25	(71.4)	5.7 – 12.5
2+	8	(22.9)	1.3 – 5.5
≥ 3+	2	(5.7)	0.1 – 2.7

† World Health Organisation criteria for anaemia: haemoglobin < 13.0 g/dL for men and < 12.0 g/dL for women
* The hepatitis serology results for 36 patients (12.2%) could not be traced
eGFR: estimated glomerular filtration rate; HBsAg: hepatitis B surface antigen; SD: standard deviation
Anti-HCV: anti-hepatitis C antibody; CD4+: absolute CD4+ T lymphocyte count

4.2 Proteinuria among the study participants

Proteinuria was detected in 11.9% (95%CI 8.5–16.3, n = 35) among the study participants. Of the proteinuria category, 25 (71.4%), 8 (22.9%), and 2 (5.7%) participants had dipstick proteinuria (1+), (2+), and (≥ 3+), respectively (table 2 and figure 2).

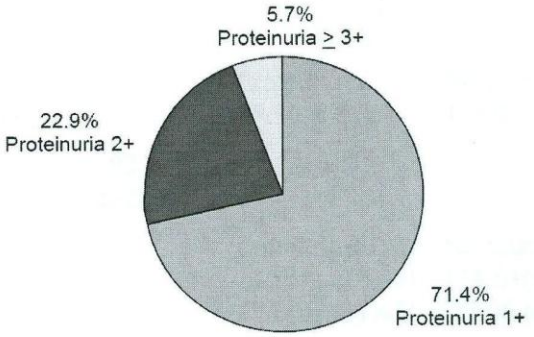


Figure 2. Pie chart showing proteinuria category.

The proportions of participants with proteinuria were comparable relative to age, sex, body mass index, blood pressure, and hepatitis infection. However, the proportion of proteinuric participants was significantly higher in the WHO-HIV clinical stage 4 than other clinical stages, and was least in clinical stage 1 (trend p = 0.020). Furthermore, proteinuria was significantly detected in participants who were not using HAART than those on HAART (73.1% versus 6.0%, p < 0.0001), and the HAART-naïve participants had an increased risk of proteinuria than those using antiretroviral therapy (RR 12.2 7, 95%CI 21–20.8; p = < 0.0001). However, the presence of proteinuria was insignificantly associated with the duration of HAART use (p = 0.267), table 3.

Table 3. Association between proteinuria and sociodemographic characteristics of study population (N= 294).

Characteristics	Number of patients N = 294	Proteinuria n (%)		*p*-value
Number of patients	294	35 (11.9)		–
Age groups (years)				
18 – 29	51	5 (9.8)		
30 – 39	139	13 (9.4)		0.319
40 – 49	62	9 (14.5)		
≥ 50	42	8 (19.1)		
		Proteinuria	No proteinuria	
Mean age (± SD)		37.30(± 8.20)	38.60 (± 9.50)	0.441
Sex Male	92	8 (8.7)		
Female	202	27 (13.4)		0.252
Blood pressure*		Proteinuria	No proteinuria	
Systolic		112.54(±19.06)	116.01(±18.00)	0.289
Diastolic		74.01(±11.63)	73.06 (±10.97)	0.633
Body mass index kg/m^2	93	10 (10.8)		0.678
< 18.5	201	25 (12.4)		
≥ 18.5				
HIV clinical stage				
1	60	4 (6.7)		
2	83	13 (15.7)		0.020
3	120	10 (8.3)		
4	31	8 (25.8)		
On HAART use	268	16 (6.0)		< 0.0001
Not on HAART	26	19 (73.1)		
Duration on HAART				
< 18 months	69	6 (8.7)		0.267
≥ 18 months	199	10 (5.0)		

* Mean ± SD mmHg; SD: standard deviation; HAART: highly active antiretroviral therapy

The median absolute CD4+ count (range) was 396.8 (50–1279) and 506.1 (98–1493) cells/μL for proteinuric and non-proteinuric participants, respectively. The proportion of proteinuria was significantly high in participants with lower absolute CD4+ count levels compared with higher CD4+ count levels [CD4+ < 200 cells/μL (15.1%) versus CD4+ > 350 cells/μL (4.4%), $p = 0.023$], table 4.

Patients with lower haemoglobin levels had significantly higher proportions of proteinuria than non-anaemic patients ($p < 0.0001$ for haemoglobin trend). Additionally, proteinuric participants had significantly low mean haemoglobin level than non-proteinuric participants [mean ±SD, g/dL: 11.96 (±1.60) versus 12.81 (±2.05), $p = 0.019$]. The study participants with proteinuria had a significantly higher mean serum creatinine level than non-proteinuric participants (mean ±SD, μmol/L: 105.72 ±33.71 versus 89.25 ±28.53, $p = 0.002$). The participants with lower estimated glomerular filtration rates (eGFR) had higher proportions of proteinuria, which increased with a declining eGFR (6.3%, 15.0%, and 31.8% for eGFR ≥ 90, 60–89, and < 60mL/min/1.73m^2, respectively; $p < 0.0001$ for eGFR trend). Also, proteinuria was notably observed in participants with low serum albumin level (mean ±SD, g/L; 38.12 ±5.13 versus 49.05 ±6.20, $p < 0.0001$), table 4.

Of study participants, both proteinuric and non-proteinuric participants had insignificant differences for serum total protein (mean ±SD, g/L: 69.80 ±6.70 versus 71.01 ±5.44, $p = 0.231$), serum globulin (mean ±SD, g/L: 39.50 ±8.09 versus 36.30 ±9.92, $p = 0.069$), serum glucose (mean ±SD, mmol/L: 4.60 ±3.90 versus 5.00 ±2.01, $p = 0.337$), serum potassium (mean ±SD, mmol/L: 4.3 ±1.1 versus 3.9 ±1.5, $p = 0.129$), and serum total cholesterol (mean ±SD, mmol/L: 4.20 ±0.90 versus 3.92 ±0.05, $p = 0.067$), table 4.

The presence of hepatitis B or C infection or co-infection had no significant association with proteinuria, table 4.

Table 4. Association between proteinuria and laboratory characteristics of study population (N= 294).

Characteristics	Number of patients N = 294	Proteinuria n (%)			*p*-value
Serum creatinine		Proteinuria		No proteinuria	
mean ± SD (μmol/L)		105.72(±33.71)		89.25(±28.53)	0.002
Haemoglobin(g/dL) †					
< 8.0	4	4	(100)		
8.0 – 9.4	162	18	(11.1)		< 0.0001
9.5 – 12 /13	95	10	(10.5)		
> 12 /13	33	3	(9.1)		
		Proteinuria		No proteinuria	
Mean (±SD)		11.96 (±1.60)		12.81 (±2.05)	0.019
Serum albumin ‡		38.12 (±5.13)		49.05 (±6.20)	< 0.0001
Serum potassium *		4.30 ± 1.10		3.90 ± 1.50	0.129
Serum globulin ‡		39.50 ± 8.09		36.30 ± 9.92	0.069
Random blood glucose *		4.60 ± 3.90		5.00 ± 2.01	0.337
Serum total protein ‡		69.80 ± 6.70		71.01 ± 5.44	0.231
Serum total cholesterol *		4.20 ± 0.90		3.92 ± 0.05	0.067
Hepatitis seropositivity					
Anti-HCV	23	5	(21.7)		0.170
HBsAg	29	2	(6.9)		0.550
HBsAg & Anti-HCV	0		–		–
eGFR (mL/min/1.73m^2)					
≥ 90	190	12	(6.3)		
60 – 89	60	9	(15.0)		< 0.0001
< 60	44	14	(31.8)		
< 60	44	14	(31.8)		
≥ 90	190	12	(6.3)		< 0.0001
Absolute CD4+ count					
< 200	146	22	(15.1)		0.023
> 350	68	3	(4.4)		

eGFR: estimated glomerular filtration rate; HBsAg: hepatitis B surface antigen; Anti-HCV: anti-hepatitis C antibody
† World Health Organisation definition for anaemia: female (< 12g/dL) and male (< 13g/dL)
* mean ± SD (mmol/L); SD: standard deviation; ‡ mean ± SD (g/L)

4.3 Clinical symptoms and signs among study participants in the past three months.

In the past three months, the most common clinical symptoms among study participants were anorexia 48.3% (95%CI 42.5–54.2, n = 142), nausea/vomiting 36.1% (95%CI 30.6–41.9, n = 106), and body weight loss 32.0% (95%CI 26.7–37.7, n = 94). These symptoms or signs had insignificant differences among proteinuria and non-proteinuria (anorexia p = 0.693, nausea/vomiting p = 0.175, and body weight loss p = 0.063), table 5.

Dysuria was found in 8.2% (95%CI 5.7–12.5, n = 25) of the study patients, and the difference observed among proteinuric and non-proteinuric patients was not significant (p = 0.191). Foamy/smoky urine was reported in 6.8% (95%CI 4.3–10.5, n = 20) while haematuria was 5.4% (95%CI 3.3–8.9, n = 16) among the study patients, with insignificant differences for patients with proteinuria and those without proteinuria, (p = 0.073 and p = 0.418 for foamy/smoky urine and haematuria, respectively). Diarrhoea was reported in 8.2% (95%CI 5.4–12.1, n = 24) of study participants, without significant association with proteinuria (p = 1.0), table 5.

Table 5. Association between proteinuria and presence of symptoms and signs in study population (N = 294).*

Characteristics	Total population N=294 n (%)	With proteinuria N=35 n (%)	Without proteinuria N=259 n (%)	*p*-value
Anorexia	142 (48.3)	18 (51.4)	124 (47.9)	0.693
Nausea/ vomiting	106 (36.1)	9 (25.7)	97 (37.5)	0.175
Body weight loss	94 (32.0)	16 (45.7)	78 (30.1)	0.063
Dysuria	25 (8.5)	5 (14.3)	20 (7.7)	0.197
Foamy/smoky urine	20 (6.8)	5 (14.3)	15 (5.8)	0.073
Haematuria	16 (5.4)	3 (8.6)	13 (5.0)	0.418
Diarrhoea	24 (8.2)	3 (8.6)	21 (8.1)	1.0
Body swelling †	16 (5.4)	4 (11.4)	12 (4.6)	0.108
Oral candidiasis	19 (6.5)	3 (8.6)	16 (6.2)	0.482

† Body swelling (facial puffiness, lower limb/ankle swelling and/or abdominal distension)
* Symptoms and signs in the past three months

Sixteen participants (5.4%, 95%CI 3.3–8.9) had a history of body swelling (facial puffiness, abdominal distension, or lower limb/ankle swelling), and the presence of body swelling did not significantly differ for both proteinuric and non-proteinuric participants (p = 0.108). Of the study participants, nineteen patients (6.5%, 95%CI 4.0–10.1) had oral candidiasis, without significant association with proteinuria (p = 0.482), table 5.

The presence of proteinuria had significant inverse correlations with absolute CD4+ T lymphocyte count, eGFR, haemoglobin level, and serum albumin (r = -0.281, p = 0.048; r = -0.327, p = 0.027; r = -0.493, p = 0.033; and r = -0.175, $p < 0.001$); and a positive correlation with serum creatinine (r = 0.220, p = 0.016). The CD4+ count level also significantly correlated inversely with the WHO HIV clinical stage (r = -0.014, $p < 0.020$). On the other hand, there were insignificant correlations between proteinuria and BMI (r = 0.360, p = 0.114) and HAART use (r = 0.604, p = 0.150), table 6.

Table 6. Correlation between proteinuria and clinical characteristics of study population.

Variable	Pearson's correlation coefficient	*p*-value
CD4+ count versus proteinuria	- 0.281	0.048
Serum creatinine versus proteinuria	0.220	0.016
BMI versus proteinuria	0.360	0.114
eGFR versus proteinuria	- 0.327	0.027
Haemoglobin level versus proteinuria	- 0.493	0.033
HAART use versus proteinuria	0.604	0.150
HIV clinical stage versus CD4+ count	- 0.014	0.020
Serum albumin versus proteinuria	- 0.175	0.001

BMI: body mass index; CD4+: absolute CD4+ lymphocyte count; HAART: highly active antiretroviral therapy
WHO: World Health Organisation; HIV: human immunodeficiency virus; eGFR: glomerular filtration rate

Low eGFR (< 60 mL/min/1.73m^2; OR 4.07, p = 0.019), high serum creatinine level (> 200 µmol/L; OR 4.94, p = 0.007), low haemoglobin level (< 9g/dL; OR 2.05, p = 0.030), and low serum albumin (< 30g/L; OR 3.05, p = 0.021) were independently associated with proteinuria in multiple logistic regression analysis (table 7).

Table 7. Clinical characteristics of the study population as independent predictors of proteinuria.

Variable	OR †	(95% CI)	*p*-value
WHO-HIV clinical stage 3&4	2.97	(0.86 – 7.51)	0.180
Serum creatinine > 200 µmol/L	4.94	(1.64 – 17.25)	0.007
Not on HAART	6.80	(0.53 – 19.01)	0.065
Serum albumin < 30g/L	3.05	(1.48 – 9.60)	0.021
eGFR < 60 mL/min/1.73m^2	4.07	(1.99 – 8.76)	0.019
Haemoglobin level < 9 g/dL	2.05	(1.08 – 8.50)	0.030
CD4+ count < 200 cell/µL	3.68	(0.72 – 12.49)	0.092

† OR = Adjusted odds ratio; 95% CI = 95% Confidence interval; CD4+: absolute CD4+ lymphocyte count
HAART: highly active antiretroviral therapy; WHO: World Health Organisation
HIV: human immunodeficiency virus; eGFR: glomerular filtration rate

The eGFR < 60 mL/min/1.73m^2 had an independent association with high serum creatinine (OR 4.08, p = 0.001), low CD4+ count (< 200 cell/µL; OR 2.83, p = 0.033), low haemoglobin (OR 1.96, p = 0.010) and WHO HIV clinical stage 3 and 4 (OR 2.65, p = 0.046), table 8.

Table 8. Clinical characteristics of the study population as independent predictors of estimated glomerular filtration rate < 60 ml/min/1.73m^2.

Variable	OR †	(95% CI)	*p*-value
WHO-HIV clinical stage 3&4	2.65	(1.83 – 9.01)	0.046
Serum creatinine > 200 µmol/L	4.08	(1.57 – 9.13)	0.001
On HAART	0.22	(0.13 – 1.80)	0.090
Serum albumin < 30g/L	3.45	(0.63 – 4.59)	0.880
Haemoglobin level < 9 g/dL	1.96	(1.29 – 3.87)	0.010
CD4+ count < 200 cell/µL	2.83	(1.45 – 10.22)	0.033

† OR = Adjusted odds ratio; 95% CI = 95% Confidence interval
CD4+: absolute CD4+ lymphocyte count; HAART: highly active antiretroviral therapy
HIV: human immunodeficiency virus; WHO: World Health Organisation

CHAPTER FIVE

5.0 DISCUSSION

This study reveals that proteinuria (11.9%) is a significant health problem in HIV-infected individuals attending the HIV/AIDS Clinic at Muhimbili National hospital. This prevalent proteinuria may suggest jeopardy to the quality of life of individuals with HIV infection in resource-limited settings besides lack of nephrology services, like Tanzania.

This finding is consistent with results from other authors. Proteinuria was 13% and 21% in HAART-naïve patients in Rwanda and Uganda,[68,70] respectively. Dipstick proteinuria was 12.3% and 14% in HIV-positive patients Iran and New Haven USA,[51,63] respectively. Proteinuria was 18% in HIV patients in Switzerland,[59] and recently reported in 9% of 788 HIV-positive outpatients in Japan.[108] Proteinuria was lately found in 9% of 941 HIV outpatients without known CKD in Mississippi USA.[109] Microalbuminuria was 8.7% HIV-infected Norwegian patients, and was 3-to-5 times high than in the general population.[58] Nevertheless, the prevalence of proteinuria found in this study is higher than the prevalences reported in other studies. Proteinuria was 6.2% and 7% HAART-naïve patients in Kenya and South Africa,[44,69] respectively. Proteinuria was 5.6% and 6.7% HIV-positive patients in Brazil and Texas USA,[56,110] respectively.

Higher prevalences of proteinuria have been reported particularly using albumin-specific or albumin-to-creatinine screening methods, in pre-HAART era, high-risk populations or in nephrology-referred patients. Overton et al. retrospectively found proteinuria in 30% of 845 HIV-outpatients in St. Louis,[111] and it was 26.1% in 149 HIV-infected patients starting HAART in South Africa.[112] Nephrotic range proteinuria was 21.9% in 96 patients with 24-hour urinalysis in Nigeria.[65] Proteinuria was found in all 27 HIV-infected patients with confirmed glomerular diseases in Spain;[60] with 51.9% having nephrotic syndrome. Nonetheless, such results need a vigilant interpretation in view of hepatitis C virus (77.8%) and hepatitis B virus (37%) co-infection in the participants.[60] Proteinuria was 71.9% in 64 HIV-infected patients with renal abnormalities.[45] A cross-sectional study in India

detected dipstick albuminuria in 28% of HIV-positive patients,[61] and microalbuminuria was 36% of HAART-naïve patients in South Africa.[44]
In this study proteinuria was common on all WHO HIV clinical stages, but the proportions of proteinuric participants were significantly higher in advanced HIV clinical stages. This finding may denote the impact of advanced HIV disease and kidney disease whilst it supports the amassing evidence that proteinuria predicts an increased risk of morbidity and mortality in HIV-infected individuals. Patients with an initial abnormality of proteinuria have 50% increased risk of hospitalization and a worse survival rate,[45] and high proteinuria predicts an increased risk of mortality in HIV-infected patients,[45,46] especially along with decreased renal function.[113]

Though a descriptive study, the current findings support the role of HAART in averting HIV-associated renal anomalies since HAART-naïve patients had a significantly higher proportion of proteinuria and a twelve-fold increased risk of having proteinuria than those on HAART. These results are consistent with findings from Cosgrove et al. that patients on HAART had lower levels of proteinuria than non-HAART patients[114] while contrary to other studies showing insignificant association between proteinuria and the HAART in Iran and Brazil.[56,63] The beneficial renal effects of HAART have been demonstrated in individuals with low CD4+ cell counts in a randomised Development Antiretroviral Therapy in Africa (DART) trial,[115] in a multicentre HIV Outpatient Study,[116] and in a study by Kalayjian and colleagues.[117]

The insignificant association between proteinuria and the duration of HAART found in the present study may imply a spectrum of renal syndromes in HIV-infected population that require other interventions than HAART, including treatment for comorbidities or a nephrotoxic effect of HAART or suggest HIV viral replication may not be the only mechanism leading to renal dysfunction in this population. This finding is comparable with results reported in a randomised trial by Gupta et al.[30] that no significant changes in albumin-to-creatinine ratio over the initial 64 weeks of protease inhibitors therapy. With HAART use over these 64 weeks, nevertheless, there was a resolution or substantial reduction in albumin-to-creatinine of 45–95% in subjects with elevated baseline albuminuria.[30] A recent trial showed 5% of 508

Ugandans using HAART had a significant decline in renal function despite attaining HIV viral load suppression.[118]

As participants in the current study were using different HAART regimens, more study is vindicated to determine the regimen-specific beneficial renal effects on account that specific HAART drugs, for instance tenofovir disoproxil fumarate, are associated with different changes in kidney function.[119,120] Moreover, the management of kidney disease in HIV-positive persons includes renoprotective measures such as shunning nephrotoxins, stringent blood pressure control, smoking cessation, and adjuvant therapy with renin-angiotensin system blockade and glucocorticoids.[49]

In this study, low CD4+ T lymphocyte cell count is a risk factor for proteinuria in HIV-infected population which could be attributed to kidney injury associated with the HIV-induced immunodeficiency. This finding is in keeping with authors that proteinuria is significantly associated with lower CD4+ count.[15,29,43,45,46,53,56,63,110]

The presence of hepatitis virus infection had insignificant association with proteinuria, and this could be due to small number of participants with hepatitis in this study while Wyatt and colleagues have recently demonstrated, in a systematic review and meta-analysis, that HIV–hepatitis C coinfection is significantly associated with increased risk for proteinuria and renal failure.[54]

Anaemia is common in the study population, with the mean haemoglobin of 12.8 (±2.9) g/dL; which is not in agreement with the results in Nigeria as the participants had lower mean haemoglobin (10.25 ±1.97 g/dL) and 80% having severe anaemia.[121] This difference could be explained by improved clinical status as most of participants in the current study (91.2%) were on HAART whereas the participants in Nigeria study were HAART-naïve adults. The current study also shows that anaemia is a risk factor for both proteinuria and low eGFR in HIV-infected patients. Anaemia develops during the early stages of CKD and is common in patients with end-stage renal disease (ESRD). Although the aetiologies of anaemia in CKD patients is multifactorial, the deficient erythropoietin production is the most

well-known cause of renal anaemia as anaemia causes renal hemodynamic alterations and tissue hypoxia; leading to tubulointerstitial damage and CKD progression.[122-125] The role of anaemia on the development and progression of CKD has also been demonstrated by Iseki and colleagues that the CKD incidence was 19.7% and 15.5% among subjects with anaemia and those without anaemia, respectively; with the gender-adjusted odds ratio of 1.193 (95%CI 1.020–1.395, P = 0.027).[123] On the other hand, a study conducted among African Americans revealed that proteinuria is significantly associated with lower haemoglobin level,[122] and the haemoglobin level is an independent prognostic indicator of clinical progression of HIV disease as lower haemoglobin is associated with a rapid progression to HIV disease[126-128] for HIV-infected patients.

Of the participants in the current study, 15% had impaired kidney function (i.e. eGFR < 60 ml/min/1.73m^2), and a generally a low eGFR and high serum creatinine in proteinuric participants may indicate that the prevalence of renal dysfunction is high in HIV-infected population in Tanzania. Further research on both the natural history and response to therapy of early-stage chronic kidney disease (CKD) would be helpful in preventing or slowing progression to advanced CKD this susceptible population.

Overt proteinuria was only 7% in HAART-naïve South Africans without evident kidney disease,[44] with a remarkably high prevalence (83.3%) of biopsy-confirmed HIVAN among patients with proteinuria.[44] A retrospective review of a series of 99 consecutive renal biopsies in HIV-infected black South Africans demonstrated that that 27% and approximately 20% were HIVAN and HIV immune-complex kidney disease, respectively.[12] In Zambia, a retrospective cohort study of 25,249 ART-naïve patients showed that 33.5% had renal insufficiency at baseline; with 26.5% and 25.3% having eGFR less than 60 mL/min by Cockcroft-Gault and Modification of Diet in Renal Disease (MDRD) formulas, respectively.[67] Some evidence of kidney disease was reported in 38% of HAART-naïve Nigerians, defined by the presence of proteinuria and/or elevated serum creatinine > 132 μmol/L,[65] and 52.6% had eGFR < 60 mL/min.[65] Furthermore, more than 40% of HAART-naïve Nigerians had the median creatinine clearance < 60 ml/min in another study using Cockcroft-Gault formula;[66] similar to an estimated creatinine clearance < 50 ml/min in 57% of HIV-

positive Ugandans with any dipstick proteinuria.[70] Other studies have reported a much lower prevalence ranging 2.4% to 11%.[69,111,108,109,129,130] The prevalence of impaired kidney function was 16.3% in a Kenyan study with proteinuria of 6.2% in HAART-naïve ambulatory patients, the eGFR < 60 mL/min was 11% and only 4.8% by using the Cockcroft-Gault and MDRD formulas, respectively.[69] Stöhr and colleagues also documented that baseline prevalence of stage 3 or worse CKD was only 3% when using the MDRD equation and was 7% when using the Cockcroft-Gault formula in trial of 3316 HAART-naïve HIV-infected patients in sub-Saharan Africa.[131] The observed difference for glomerular filtration estimation as well as differences in the prevalence of impaired renal function could be due to different methods for estimating glomerular filtration rate, and thus suggesting the need for validating these eGFR formulas in HIV-infected population.

Consistent with the current study, Lucas et al. recently documented that proteinuria was associated with presence of renal insufficiency in African Americans.[15] The low eGFR is also associated with higher HIV viral load level and lower CD4+ count.[46,113] Choi and co-workers have recently demonstrated that HIV-infected African Americans with eGFR < 15 mL/min have six-fold risk of mortality (HR 5.97, 95%CI 3.18–11.19).[132] A Japan population-based screening study revealed that HIV-negative participants with dipstick proteinuria and low creatinine clearance were at increasingly high risk of developing end-stage renal disease.[133] Proteinuria is an indicator of renal disease and the salient predictor of ESRD, and thus a modifiable risk factor for progression of renal disease.[133,134] Lately, an observation prospective multicenter cohort study demonstrated that HAART-mediated suppression of HIV-1 replication improves glomerular filtration rates in patients with low CD4+ counts and chronic kidney disease.[117]

Low eGFR, low CD4+ count, high serum creatinine, anaemia, and hypoalbuminaemia had significant correlations with proteinuria in the current study. This could be explained by the injury of glomerular and tubular epithelial cells due to the presence of HIV antigen-antibody complexes, HIV gene products, and viral replication along with lymphokine induction and haemodynamic alternations in the renal tissue,[135-139] including HIV-related thrombotic micro angiopathy.

In addition to antiretroviral therapy, further studies are required to determine if interventions for hypoalbuminaemia and anaemia reduce proteinuria and subsequent setback progression of kidney disease in HIV-infected individuals since low eGFR, high serum creatinine, anaemia, and hypoalbuminaemia were independently associated with proteinuria while an eGFR < 60 mL/min/1.73m^2 had an independent association with high serum creatinine, low CD4+ count, anaemia and advanced HIV disease. Though this study did not investigate the viral load (limited funding), there are conflicting results on association of viral load and proteinuria as a high HIV RNA level predicted proteinuria[52] whereas other studies documented insignificant association with proteinuria.[51,56,58]

The current study findings highlight the need to develop valid methods to estimate kidney function in HIV-positive populations at various HIV disease clinical stages and varied ethnic milieus. In view of absence of versatile tools such as urine protein-to-creatinine ratio methods in resource-limited communities, standard dipstick urinalysis exploited as a readily available screening method for proteinuria, particularly in such community settings with seldom options. Furthermore, study findings suggest that proteinuria is not only a considerable screening method to identify HIV-infected individuals at risk of developing renal disease but may also serve as a marker for HIV disease clinical progression in resource-in resource-poor settings, including Tanzania. Therefore additional research is needed on this aspect.

Of note in this study, proteinuric patients presented with various clinical symptoms and signs that did not principally differ from non-proteinuric patients. This insignificant difference could indicate that HIV-infected patients with proteinuria do not have specific clinical presentation, and therefore emphasizing the need for early and regular screening for proteinuria and kidney function in this at-risk population. Moreover, lack of specific clinical manifestations has essential implication for HIV-positive individuals who are ineligible to start HAART due to either their HIV disease clinical stage or inaccessible healthcare services in that initiating HAART even earlier could be advantageous in these patients with HIV-related kidney dysfunction by slowing the progression of earlier stages of CKD.[77,117,140]

Neither the urine screening for proteinuria nor serum creatinine measurement is currently routinely done among HIV-infected individuals in Tanzania notwithstanding the recent recommendation by the National Guidelines for HIV/AIDS management[7] to monitor renal function via testing urine for proteinuria, and if available serum creatinine level, for patients on tenofovir-containing HAART regimens. Moreover, screening for impaired renal function in all HIV-positive persons is presently not part of the national guidelines.[7] The present study shows that only 3.6% of the study participants had had quantitative urinalysis in the past six months; which was deliberately done for investigating urinary tract infections.

Therefore, there is a fundamental need to review the national guidelines to include patient-oriented screening programme for kidney disease in all HIV-infected individuals in Tanzania as well as improving knowledge of patients, and the general population, regarding the risk factors for kidney disease. All healthcare workforce involved in the management of HIV-infected individuals should be aware of the risk factors for proteinuria and impaired kidney function, and thus facilitate early vigilant diagnosis and timely intervention of HIV-associated kidney disease.

5.1 STUDY LIMITATIONS

Renal ultrasonography and biopsy were not performed due to limited funding of the present study. In the absence of renal biopsy capacity in resources-limited settings and the aggressive nature of HIV-associated nepropathy (HIVAN), predominately affecting African-descent individuals, the suppression of viral replication should be the principal treatment goal for HIV-related renal disease given the tremendous effectiveness of HAART for HIVAN in African Americans. Although the role played by race on the risk for developing HIVAN and its progression to ESRD is not well established in individuals with suppressed viral load, progression to ESRD is almost inevitable in patients with HIVAN and uncontrolled HIV-1 viraemia.[26] Additionally, kidney biopsy in individuals infected with HIV is essential for aggressive, progressive or deteriorating renal function to therapy for the definitive diagnosis of HIVAN; including renoprotective measures as the same approach in HIV-negative patients explained earlier.

The principal investigator acknowledges the single measurement for proteinuria and serum creatinine could have resulted in underestimation, and this needs to be addressed by the prospective cohort studies. The investigator also acknowledges the use of a standard dipstick urinalysis instead of quantitative measures for proteinuria may have reduced the ability to find other significant correlates of proteinuria. In view of the absence of the urine protein quantification methods, the standard dipstick urinalysis is a simple, versatile and cost-effective method which can easily be done at the lowest-level health facilities that provide HIV/AIDS Treatment and Care service in Tanzania; and thereby facilitating early detection and prompt treatment or referral for patients with dipstick proteinuria to higher-level facilities for further management. Furthermore, the standard urine dipstick tests have the sensitivity of 80.7% for detecting low levels of protein,[141] with increasing sensitivity (96%) and specificity (87%) in the presence of significant proteinuria ($\geq$ 3 g/L).[142] However, further cohort research is undoubtedly needed using urine protein quantification methods to investigate precisely the associations or predictors of proteinuria at various proteinuria levels, including developing a risk stratification model that may guide the treatment of HIV-associated kidney disease.

The United Kingdom guidelines from the National Institute for Health and Clinical Excellence (NICE) recommend the urine dipsticks should only be used to identify proteinuria if microalbuminuria can be measured[143] while both the United States National Kidney Foundation (NKF)[48] and the HIV Medicine Association of the Infectious Diseases Society of America (IDSA)[49] recommend urine dipsticks for proteinuria screening, and a prompt further quantitative assessment for dipstick > 1+ proteinuria.[49] Nevertheless, the NKF advocates screening for proteinuria using urine albumin-based methods for instance albumin-specific dipstick and spot urine albumin-to-creatinine ratios (particularly first morning urine), and subsequently monitoring for persons with proteinuria or albuminuria using spot urine protein-to-creatinine or spot urine albumin-to-creatinine ratios.[143] These urine protein quantification methods, which are clinically practical methods, correlate well with 24-hour urinary protein excretion,[47,144-147] since the 24-hour urine collection is greatly unfeasible for most patients unless admitted in a health facility. Notwithstanding, the remarkable advantage of being cheap, easy to perform and interpret with an immediate result; the use of these urine protein quantification methods is entirely restricted by largely unavailability if such strips in the resources-limited settings like Tanzania.

5.2 STUDY STRENGTHS

The unique attribute of this study is offsetting these limitations since using systematic sampling technique reduced selection bias and the characteristics of study participants are uniformly distributed over the study population. Therefore, the study findings may be generalized to the entire HIV-infected population in Tanzania. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula, a new GFR estimating equation, is presently the most accurate method for estimating GFR; with fewer false-positive diagnoses of chronic kidney disease, and greater precision in predicting risk for adverse outcomes for diverse populations compared with Modification of Diet in Renal Disease (MDRD) equation.[104,148-150] Further clinical research is required to validate the CKD-EPI equation in HIV-infected individuals as it has not been validated in Tanzania population or in HIV-infected population globally.

This is the first study in Tanzania to determine the magnitude of proteinuria in HIV-infected adult patients attending the HIV/AIDS Care and Treatment clinics; and it reveals essential information for planning enhanced comprehensive treatment and care for individuals with HIV infection in Tanzania.

The study also provides a new baseline evidence for, and will hopefully help to guide, future research to further understanding pathogenesis and determine optimal treatment for proteinuria and kidney disease in HIV-infected patients in Tanzania.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

The evidence of both proteinuria and impaired kidney function is relatively high in ambulatory HIV-infected individuals in Tanzania without specific clinical manifestations.

As an essential part of comprehensive HIV/AIDS management policy, the National Guidelines for the Management of HIV and AIDS should take into account the screening for proteinuria and impaired kidney function at baseline work-up and regularly during follow up of HIV-infected individuals. This would result not only in early detection of HIV-associated kidney disease, but also avert rapid progression of HIV disease.

In view of lack of resources for urine protein quantification methods and serum creatinine measurement; simple, versatile and cost-effective interventions such as dipstick urinalysis can easily be done at the lowest-level health facilities that provide HIV/AIDS Treatment and Care service in Tanzania. This would result in early detection and prompt treatment or referral for patients with dipstick proteinuria to higher-level health facilities for further management.

REFERENCES

1. Blanc B, Finch CA, Hallberg L, et al. Nutritional anaemias. Report of a World Health Organisation Scientific Group. *WHO Tech Rep Ser.* 1968;405:1-40.

2. Tierney LM, Papadakis MA, McPhee SJ, editors. Current Medical Diagnosis and Treatment 2008. 47 ed. New York: McGraw-Hill, Medical Publishing Division; 2008. p. 786,787.

3. Kasper DL, Braunwald E, Hauser SL, et al, editors. Harrison's Principles of Internal Medicine. 16 ed. New York: McGraw-Hill, Medical Publishing Division; 2004. p. 246,247.

4. World Health Organisation. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. *WHO Technical Report Series* 854. 1995. Geneva: World Health Organization.

5. World Health Organisation expert consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet.* 2004;157-63.

6. Kasper DL, Braunwald E, Hauser SL, et al, editors. Harrison's Principles of Internal Medicine. 16 ed. New York: McGraw-Hill, Medical Publishing Division; 2004. p. A4, A9.

7. The United Republic of Tanzania, 2009. National Guidelines for the Management of HIV and AIDS. 3rd Ed. (revised February 2009), NACP. Ministry of Health: Dare es Salaam.

8. AIDS epidemic update 2009. Joint United Nations Programme on HIV/AIDS (UNAIDS) and World Health Organization (WHO) 2009. AIDS epidemic update: November 2009. *http://www.unaids.org/en/HIV_data/2009EpiUpdate/default.asp*. Accessed May 10th, 2010.

9. Palella FJ Jr, Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med.* 1998;338(13):853-60.

10. Eggers PW, Kimmel PL. Is There an Epidemic of HIV Infection in the US ESRD Program? *J AM Soc Nephrol.* 2004;15:2477-85.

11. Choi AI, Rodriguez RA, Bacchetti P, et al. Racial differences in end-stage renal disease rates in HIV infection versus diabetes. *J Am Soc Nephrol.* 2007;18:2968-74.

12. Gerntholtz TE, Goetsch SJW, Katz I. HIV-related nephropathy: a South African perspective. *Kidney Int* 2006;69:1885-91.

13. Monahan M, Tanji N, Klotman PE. HIV-associated nephropathy: an urban epidemic. *Semin Nephrol* 2001;21:394-402.
14. Rao TK, Friedman EA, Nicastri AD. The types of renal disease in the acquired immunodeficiency syndrome. *N Engl J Med* 1987;316:1062-8.

15. Lucas GM, Mehta SH, Atta MG, et al. End-stage renal disease and chronic kidney disease in a cohort of African-American HIV-infected and at-risk HIV-seronegative participants followed between 1988 and 2004. *AIDS* 2007;21:2435-43.

16. Lucas GM, Lau B, Atta MG, et al. Chronic kidney disease incidence, and progression to end-stage renal disease, in HIV-infected individuals: a tale of two races. *J Infect Dis.* 2008;197:1548-57.

17. Lu TC, Ross M. HIV-associated nephropathy: a brief review. *Mt Sinai J Med.* 2005; 72(3):193-9.

18. Abbott KC, Hypolite I, Welch PG, et al. Human immunodeficiency virus/ acquired immunodeficiency syndrome-associated nephropathy at end-stage renal disease in the United States: patient characteristics and survival in the pre highly active antiretroviral therapy era. *J Nephrol.* 2001;14(5):377-83.

19. Lucas GM, Eustace JA, Sozio S, et al. Highly active antiretroviral therapy and the incidence of HIV-1-associated nephropathy: a 12-year cohort study. *AIDS* 2004;18(3):541-6.

20. Williams DI et al. Presentation, pathology, and outcome of HIV associated renal disease in a specialist centre of HIV/AIDS. *Sex Transm Infect.* 1998;74(3):179-84.

21. Cohen AH, Cohen GM. Distinguished Scientists Lecture Series. HIV-associated nephropathy. *Nephron.* 1999; 83(2);111-6.

22. Kimmel PL. HIV-associated nephropathy: virologic issues related to renal sclerosis. *Nephrol Dial Transplant.* 2003;18(S6):vi59-63.

23. Mocroft A, Kirk O, Gatell J, et al. Chronic renal failure among HIV-1-infected patients. *AIDS.* 2007;21(9):1119-27.

24. Choi AI, Rodriguez RA, Bacchetti P, et al. The impact of HIV on chronic kidney disease outcomes. *Kidney Int* 2007;72:1380-7.

25. Wyatt CM, Winston JA, Malvestutto C, et al. Chronic kidney disease in HIV infection: an urban epidemic. *AIDS.* 2007;21(15):2101-3.

26. Szczech LA, Gupta SK, Habash R, et al. The clinical epidemiology and course of the spectrum of renal diseases associated with HIV infection. *Kidney Int* 2004:66:1145-52.

27. Wyatt C.M. Editorial Commentary. HIV and the Kidney: A Spotlight on Racial Disparities. *J Infect Dis.* 2008;197:1490-2.

28. Rao TK , Filippone EJ, Nicastri AD, et al. Associated focal and segmental glomerulosclerosis in the acquired immunodeficiency syndrome. *N Engl J Med* 1984; 310(11):669-73.

29. Gupta K, Iskandar SS, Daeihagh P, et al. Distribution of pathologic findings in individuals with nephrotic proteinuria according to serum albumin. *Nephrol Dial Transplant* 2008;23:1595-9.

30. Gupta SK, Parker RA, Robbins GK, et al. The effects of highly active antiretroviral therapy on albuminuria in HIV-infected persons: results from a randomized trial. *Nephrol Dial Transplant.* 2005;20(10):2237-42.

31. Kimmel PL, Barisoni L, Kopp JB. Pathogenesis and treatment of HIV-associated renal diseases: lessons from clinical and anima studies, molecular pathologic correlations, and generic investigations. *Ann Intern Med.* 2003;139(3):214-26.

32. Brook MG, Miller RF. HIV-associated nephropathy: a treatable condition. *Sex Transm Infect.* 2001;77(2);97-100.

33. Weiner NJ, Goodman JW, Kimmel PL. The HIV-associated renal diseases: Current insight into pathogenesis and treatment. *Kidney Int.* 2003;63:1618-31.

34. Stehouwer CD, Nauta JJ, Zeldenrust GC et al. Urinary albumin excretion, cardiovascular disease, and endothelial dysfunction in non-insulin-dependent diabetes mellitus. *Lancet* 1992;340:319-23.

35. Russo LM, Comper WD, Osicka TM. Mechanism of albuminuria associated with cardiovascular disease and kidney disease. *Kidney Int Suppl* 2004: S67-S68.

36. Weir MR. Microalbuminuria and cardiovascular disease. *Clin J Am Soc Nephrol* 2007; 2:581-90.

37. Kannel WB, Stampfer MJ, Castelli WP, et al. The prognostic significance of proteinuria: the Framingham study. *Am Heart J* 1984;5:1347-52.

38. Hillege HL, Fidler V, Diercks GF, et al. Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population. *Circulation* 2002;106(14):1777-82.

39. Stuveling EM, Hillege HL, Bakker SJL et al. C-reactive protein and microalbuminuria differ in their associations with various domains of vascular disease. *Atherosclerosis* 2004;172:107-14.

40. Klausen K, Borch-Johnsen K, Feldt-Rasmussen B et al. Very low levels of microalbuminuria are associated with increased risk of coronary heart disease and death independently of renal function, hypertension, and diabetes. *Circulation* 2004; 110:32-35.

41. Romundstad S, Holmen J, Kvenild K et al. Microalbuminuria and all-cause mortality in 2,089 apparently healthy individuals: a 4.4-year follow-up study. The Nord-Trondelag Health study (HUNT), Norway. *Am J Kidney Dis* 2003;42:466-73.

42. Arnlov J, Evans JC, Meigs JB, et al. Low-grade albuminuria and incidence of cardiovascular disease events in nonhypertensive and nondiabetic individuals: the Framingham Heart Study. *Circulation* 2005;112(7):969-75.

43. Szczech LA, Grunfeld C, Scherzer R, et al. Microalbuminuria in HIV infection. *AIDS* 2007;21:1003-9.

44. Han TM, Naicker S, Ramdial PK et al. A cross-sectional study of HIV-seropositive patients with varying degrees of proteinuria in South Africa. *Kidney Int* 2006;69:2243-50.

45. Gardner LI, Klein RS, Szczech LA, et al. Rates and risk factors for condition-specific hospitalizations in HIV-infected and uninfected women. *J Acquir Immune Defic Syndr.* 2003;34(3):320-30.

46. Szczech LA, Hoover DR, Feldman JG, et al. Association between renal disease and outcomes among HIV-infected women receiving or not receiving antiretroviral therapy. *Clin Infect Dis* 2004;39(8):1199-206.

47. Ginsberg JM, Chang BS, Matarese RA, et al. Use of single voided urine samples to estimate quantitative proteinuria. *N Engl J Med* 1983;309(25):1543-6.

48. National Kidney Foundation. KDOQI Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification, and Stratification. *http://www.kidney.org/professionals/kdoqi/guidelines_ckd/p4_class_g1.htm.* Accessed May 31st 2010.

49. Gupta SK, Eustace JA, Winston JA, et al. Guidelines for the management of chronic kidney disease in HIV-infected patients: recommendations of the HIV Medicine Association of the Infectious Diseases Society of America. *Clin Infect Dis.* 2005; 40(11):1559-85.

50. Siedner MJ, Atta MG, Lucas GM, et al. Poor Validity of urine dipstick as a screening tool for proteinuria in HIV-positivepatients. *J Acquir Immune Defic Syndr.* 2008; 47(2):261-63.

51. Crowley ST, Cantwell B, Abu-Alfa A, et al. Prevalence of persistent asymptomatic proteinuria in HIV-infected outpatients and lack of correlation with viral load. *Clin Nephrol* 2001;55:1-6.

52. Szczech LA, Gange SJ, Van der Horst C, et al. Predictors of proteinuria and renal failure among women with HIV infection. *Kidney Int* 2002;61:195-202.

53. Atta MG, Choi MJ, Longenecker JC, et al. Nephrotic range proteinuria and CD4 count as non-invasive indicators of HIV-associated nephropathy. *Am J Med* 2005;118(11): 1288.

54. Wyatt CM, Malvestutto C, Coca SG, et al. The impact of hepatitis C virus coinfection on HIV-related kidney disease: a systematic review and meta-analysis. *AIDS* 2008; 22:1799-807.

55. Rao TK, Friedman EA, Nicastri AD. The types of renal disease in the acquired immunodeficiency syndrome. *N Engl J Med* 1987;316:1062-8.

56. de Mattos Cavalcante MAG, Coelho SN, Lacerda HR. Prevalence of Persistent Proteinuria in Stable HIV/AIDS Patients and Its Association with HIV Nephropathy. *Bra J Infect Dis.* 2007;11(5):456-61.

57. Post FA, Campbell LJ, Hamzah L, et al. Predictors of Renal Outcome in HIV-Associated Nephropathy. *Clin Infect Dis* 2008;46:1282-9.

58. Baekken M, Os I, Sandvik L and Oektedalen O. Microalbuminuria Associated with Indicators of Inflammatory Activity in an HIV-Positive Population. *Nephrol Dial Transplant* 2008;23:3130-7.

59. Hailemariam S, Walder M, Burger HR, et al. Renal pathology and premortem clinical presentation of Caucasian patients with AIDS: an autopsy study from the era prior to antiretroviral therapy. *Swiss Med Wkly* 2001;131:412-7.

60. Gutiérrez E, Morales E, Gutiérrez Martínez E, et al. HIV Infection-Associated Glomerulopathies: A Spanish Perspective. *Nefrología* 2007;27(4):439-47.

61. Janakiraman H, Abraham G, Matthew M, et al. Correlation of CD4 Counts with Renal Disease in HIV Positive Patients. *Saudi J kidney Dis Transplant* 2008;19(4):603-07.

62. Afhami S, Rasoulinejad M, Razeghi E, et al. Renal Disorders in HIV-Infected Patients. *Arch Iranian Med* 2007;10(3):335-8.

63. Ramezani A, Mohraz M, Banifazl M et al. Frequency and associated factors of proteinuria in Iranian HIV-positive patients. *Int J Infect Dis.* 2008;12:490-4.

64. Behar DM, Shlush LI, Maor C, et al. Absence of HIV-associated nephropathy in Ethiopians. *Am J Kidney Dis.* 2006;47(1):88-94.

65. Emem CP, Arogundade F, Sanusi A, et al. Renal Disease in HIV-Seropositive Patients in Nigeria: an Assessment of Prevalence, Clinical Features and Risk Factors. *Nephrol Dial Transplant.* 2008;23:741-6.

66. Agbaji O, Gwanzhi N, Idoko J. Factors affecting the GFR of ARV-naïve HIV-1-infected patients at the Jos Teaching Hospital, Nigeria [Abstract]. *XVI International AIDS Conference*, Toronto, Canada. 2006 Aug 13-18.
67. Mulenga LB, Kruse G, Lakhi S, et al. Baseline renal insufficiency and risk of death among HIV-infected adults on antiretroviral therapy in Lusaka, Zambia. *AIDS.* 2008; 22:1821-7.

68. Mugabo JS, Lu J, Binagwaho A, et al. Proteinuria, hematuria, and creatinine clearance in Rwandan women with and without HIV infection [Abstract]. XVI *International AIDS Conference*, Toronto, Canada. 2006 Aug 13-18.

69. Wools-Kaloustian, Gupta SK, Muloma E, et al. Renal disease in an antiretroviral-naïve HIV-infected outpatient population in Western Kenya. *Nephrol Dial Transplant* 2007;22(8):2208-12.

70. Olson D, Thomas E, Allheimen M, et al. Impaired renal function of patients initiating HAART in two developing countries [Abstract]. *13th Conference on Retroviruses and Opportunistic Infections,* Denver, Colorado. 2006 Febr 5-8.

71. Janabi MY, Chale S, Magao P, et al. Renal abnormalities associated with human immunodeficiency virus infection among police officers in Dar-es-Salaam, Tanzania. *Int Conf AIDS.* 2002 Jul 7-12; 14: abstract no. ThPeB7197.

72. Nagu TJ, Bakari M, Matee M. Hepatitis A,B, and C viral co-infections among HIV-infected adults presenting for care and treatment at Muhimbili National Hospital in Dar es Salaam, Tanzania. *BMC Public Health.* 2008, 8:416.

73. Schwartz EJ, Szczech LA, Ross MJ, et al. HAART and the epidemic of HIV+ end stage renal disease. *J Am Soc* Nephrol 2 005;16:2412-20.

74. Szczech LA, Edwards LJ, Sanders LL, et al. Protease inhibitors are associated with a slowed progression of HIV-related renal diseases. *Clin Nephrol* 2002;57:336-41.

75. Wali RK, Drachenberg CI, Papadimitriou JC, et al. HIV-1-associated nephropathy and response to highly-active antiretroviral therapy. *Lancet* 1998;352:783-4.

76. Winston JA, Bruggeman LA, Ross MD, et al. Nephropathy and establishment of a renal reservoir of HIV type 1 during primary infection. *N Engl J Med* 2001;344:1979-84.

77. Atta MG, Gallant JE, Rahman MH, et al. Antiretroviral therapy in the treatment of HIV-associated nephropathy. *Nephrol Dial Transplant* 2006;2:2809-13.

78. Eustace JA, Nuermberger E, Choi M, et al. Cohort study of the treatment of severe HIV-associated nephropathy with corticosteroids. *Kidney Int* 2000;58:1253-60.

79. Smith MC, Austen JL, Carey JT, et al. Prednisone improves renal function and proteinuria in human immunodeficiency virus-associated nephropathy. *Am J Med* 1996;101:41-8.

80. Watterson MK, Detwiler RK, Bolin P. Clinical response to prolonged corticosteroids in a patient with human immunodeficiency virus-associated nephropathy. *Am J Kidney Dis.* 1997;29:624-6.

81. Ingulii E, Tejani A, Fikrig S, et al. Nephrotic syndrome associated with acquired immunodeficiency syndrome in children. *J Paediatr.* 1991;119(5):710-6.

82. Kimmel PL, Mishkin GJ, Umana WO. Captopril and renal survival in patients with human immunodeficiency virus nephropathy. *Am J Kidney Dis*1996;28:202-8.

83. Burns GC, Paul SK, Toth IR, et al. Effect of angiotensin converting enzyme inhibition in HIV-associated nephropathy. *J Am Soc Nephrol.* 1997;8:1140-6.

84. Wei A, Burns GC, Williams BA, et al. Long-term renal survival in HIV-associated nephropathy with angiotensin-converting enzyme inhibition. *Kidney Int.* 2003;64:1462-71.

85. Laradi A, Mallet A, Beaufils H, et al. HIV-associated nephropathy: outcome and prognosis factors. Groupe d'Etudes Nephrologiques d'Ile de France. *J Am Soc Nephrol.* 1998;9(12):2327-35.

86. Ahuja TS, Collinge N, Grady J, Khan S. Is dialysis modality a factor in survival of patients with ESRD and HIV-associated nephropathy? *Am J Kidney Dis.* 2003; 41(5): 1060-4.

87. Ahuja TS, Grady J, Khan S. Changing trends in the survival of dialysis patients with human immunodeficiency virus in the United States. *J Am Soc Nephrol.* 2002; 13(7):1889-93.

88. Vigneau C, Guiard-Schmid JB, Tourret J, et al. The clinical characteristics of HIV infected patients receiving dialysis in France between 1997 and 2002. *Kidney Int.* 2005; 67(4):1509-14.

89. Stock PG, Roland ME, Carlson L, et al. Kidney and liver transplantation in human immunodeficiency virus-infected patients: a pilot safety and efficacy study. *Transplantation* 2003;76(2):370-5.

90. Abbott KC, Swanson SJ, Agodoa LY, Kimmel PL. Human immunodeficiency virus infection and kidney transplantation in the era of highly active antiretroviral therapy and modern immunosuppression. *J Am Soc Nephrol* 2004;15(6):1633-9.

91. Kumar MS, Sierka DR, Damask AM, et al. Safety and success of kidney transplantation and concomitant immunosuppression in HIV-positive patients. *Kidney Int.* 2005;67(4):1622-9.

92. Roland ME, Barin B, Carlson L, et al. HIV-Infected Liver and Kidney Transplant Recipients: 1- and 3-Year Outcomes. *Am J Transplant.* 2008;8(2):355-65.

93. United Republic of Tanzania. Bureau of Statistics. Population and Housing Report, 2002.

94. City Mayors Statistics.The World's Fastest Growing Cities and Urban Areas from 2006 to 2020. *http://www.citymayors.com/statistics/urban_growth1.html.* Accessed May 20th, 2010.

95. *The World Encyclopaedia:* Dar es Salaam. *http://www.newworldencyclopedia.org/ entry/Dar_es_Salaam#cite_note-0.* Accessed June 20th, 2010.

96. Swash M, Glynn M, editors. Hutchison's Clinical Methods: An Integrated Approach to Clinical Practice, 22 ed. Edinburgh: New York: Saunders Elsevier, 2007.

97. World Health Organisation, 2006. Antiretroviral Therapy for HIV Infection in Adults and Adolescents: Recommendations for a Public Health Approach. - *2006 rev.*

98. World Health Organization: Treat 3 million by 2005. Interim WHO clinical staging of HIV/AIDS and HIV/AIDS case definitions for surveillance. African region.WHO/HIV/2005.02. *http://www.who.int/hiv/pub/guidelines/clinicalstaging.pdf.* Accessed June 30th 2010.

99. World Health Organization. International Society of Hypertension guidelines for the management of hypertension. *J Hypertens* 1999;17:151-83.

100. Roche COBAS MIRA Chemistry Analyzer (GMI Inc, US). *http://www.gmi-inc.com/ Products/rochemira.htm* . Accessed March 16th 2010.

101. Becton Dickson Facs Calibur Machine. *http://www.bdbiosciences.com.* Accessed April 10th, 2010.

102. Adaltis – EIAgen HBsAg kit. Adaltis Advanced Laboratory Diagnostic Systems. *http://www.adaltis.com/upload/documents/package/1323.pdf.* Accessed March 16th 2010.

103. Adaltis – EIAgen Anti HCV Ab kit. Adaltis Advanced Laboratory Diagnostic Systems. *http://www.adaltis.com/upload/documents/package/1869.pdf.* Accessed March 16th 2010.

104. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Meg.* 2009;150(9):604-12.

105. The World Medical Association, WMA, 2008. The Declaration of Helsinki, version 2008. *http://www.wma.net.* Accessed April 10th 2010.

106. International Conference on Harmonisation (ICH). Guideline for Good Clinical Practice (GCP). *http://www.ich.org/LOB/media/MEDIA482.pdf.* Accessed January 10th 2009.

107. Ezzelle J, Rodriguez-Chavez IR, Darden JM, et al. Guidelines on Good Clinical Laboratory Practice. Bridging Operations between Research and Clinical Research Laboratories. *J Pharm Biomed Anal.* 2008;46(1):18-29.

108. Yanagisawa N, Ando M, Suganuma A, et al. Prevalence of kidney disease in HIV-infected patients in Japan. A single center study. *Kansenshogaku Zasshi.* 2010; 84(1):28-32.

109. Fulop T, Olivier J, Meador RS, et al. Screening for chronic kidney disease in the ambulatory HIV population. *Clin Nephrol.* 2010 Mar; 73(3):190-6.

110. Ahuja TS, Boruckia M, Funtanilla M, et al. Is the prevalence of HIV-Associated Nephropathy Decreasing? *Am J Nephrol.* 1999; 19:655-9.

111. Overton ET, Nurutdinova D, Freeman J, et al. Factors associated with renal dysfunction within an urban HIV-infected cohort in the era of highly active antiretroviral therapy. *HIV Med.* 2009;10(6):343-50.

112. Franey C, Knott D, Barnighausen T, et al. Renal impairment in a rural African antiretroviral programme. *BMC Infect Dis* 2009, 9:143.

113. Gardner LI, Holmberg SD, Williamson JM, et al. Development of proteinuria or elevated serum creatinine and mortality in HIV-infected women. *J Acquir Immune Defic Syndr.* 2003;32:203-9.

114. Cosgrove CJ, Abu-Alfa AK, Perazella MA. Observations on HIV-associated Renal Disease in the Era of HAART. *Am J Med Sci.* 2002;323(2):102-6.

115. Reid A, Stöhr W, Walker AS, et al. Glomerular dysfunction and associated risk factors following initiation of ART in adults with HIV infection in Africa. XVI International Conference on AIDS. Toronto, July 13 –18 2006 [THAB0105].

116. Krawczyk CS, Holmberg SD, Moorman AC, et al. Factors associated with chronic renal failure in HIV-infected ambulatory patients. *AIDS* 2004;18:2171-78.

117. Kalayjian RC, Franceschini N, Gupta SK, et al. Suppression of HIV-1 replication by antiretroviral therapy improves renal function in persons with low CD4 cell counts and chronic kidney disease. *AIDS.* 2008;22(4):481-7.

118. Peters PJ, Moore DM, Mermin J, et al. Antiretroviral therapy improves renal function among HIV-infected Ugandans. *Kidney Int.* 2008;74:925-29.

119. Gallant JE, Parish MA, Keruly JC, et al. Changes in renal function associated with tenofovir disoproxil fumarate treatment, compared with nucleoside reverse-transcriptase inhibitor treatment. *Clin Infect Dis* 2005;40:1194-98.

120. Reilly RF, Tray K, Perazella MA. Indinavir nephropathy revisited: a pattern of insidious renal failure with identifiable risk factors. *Am J Kidney Dis* 2001; 38:E23.

121. Erhabor O, Ejele OA, Nwauche CA, et al. Some haematological parameters in human immunodeficiency virus (HIV) infected Africans: the Nigerian perspective. *Niger J Med.* 2005;14(1):33-8.

122. Nangaku M. Chronic hypoxia and tubulointerstitial injury: a common pathway to end-stage renal failure. *J Am Soc Nephrol* 2006; 17: 17-25.

123. Iseki K, Kohagura K. Anemia as a risk factor for chronic kidney disease. *Kidney Int Suppl.* 2007;(107):S4-9.

124. National Kidney Foundation. Kidney Early Evaluation Program. Anaemia and chronic kidney disease. *Am J Kidney Dis* 2005;45(Suppl 2): S71–S80.

125. Rossert J, Froissart M. Role of anaemia in progression of chronic kidney disease. *Semin Nephrol* 2006;26: 283-89.

126. Russell EC, Charalambous S, Pemba L, et al. Low haemoglobin predicts early mortality among adults starting antiretroviral therapy in an HIV care programme in South Africa: a cohort study. *BMC Public Health* 2010, 10:433.

127. Obirikorang C, Yeboah FA. Blood haemoglobin measurement as a predictive indicator for the progression of HIV/AIDS in resource-limited setting. *J Biomed Sci.* 2009;16:102.

128. Kowalska JD, Mocroft A, Blaxhult A, et al.; for the EuroSIDA Study Group. Current hemoglobin levels are more predictive of disease progression than haemoglobin measured at baseline in patients receiving antiretroviral treatment for HIV type 1 infection. *AIDS Res Hum Retroviruses.* 2007;23(10):1183-8.

129. Jones CY, Jones CA, Wilson IB, et al. Cystatin C and creatinine in an HIV cohort: the nutrition for healthy living study. *Am J Kidney Dis.* 2008;51(6):914-24.

130. Ferdinando SK, Finkelstein FO, Moore BA, et al. Prevalence of chronic kidney disease in an urban HIV infected population. *Am J Med Sci.* 2008;335(2):89-94.

131. Stöhr W, Walker AS, Munderi P, et al. Estimating glomerular filtration rate in HIV-infected adults in Africa: Comparison of Cockcroft-Gault and Modification of Diet in Renal Disease formulae. *Antivir Ther* 2008;13:761-70.

132. Choi AI, Rodriguez RA, Bacchetti P, et al. Lower rates of antiretroviral therapy among HIV-infected patients with chronic kidney disease. *Clin Infect Dis.* 2007; 45:1633-9.

133. Iseki K, Kinjo K, Iseki C, et al. Relationship between predicted creatinine clearance and proteinuria and the risk of developing ESRD in Okinawa, Japan. *Am J Kidney Dis.* 2004;44(5):806-14.

134. Iseki K, Ikemiya Y, Iseki C, et al. Proteinuria and the risk of developing end-stage renal disease. *Kidney Int.* 2003;63:1468-74.

135. He JC, Husain M, Sunamoto M, et al: Nef stimulates proliferation of glomerular podocytes through activation of Src dependent Stat3 and MAPK1,2 pathways. *J Clin Invest.* 2004;114(5):643-51.

136. Brugggeman LA, Dikman S, Meng C, et al. Nephropahty in human immunodeficiency virus-1 transgenic mice is due to renal transgene expression. *J Clin Invest.* 1997;100:84-92.

137. Ray PE, Liu XH, Robinson LR, et al. A novel HIV-1 transgenic rat model of childhood HIV-1-associated nephropathy. *Kidney Int.* 2003; 63(6):2242-53.

138. Kajiyama W, Kopp JB, Marinos NJ, et al. Glomerulosclerosis and viral gene expression in HIV-transgenic mice: role of nef. *Kidney Int.* 2000;58(3):1148-59.

139. Barisoni L, Bruggeman LA, Mundel P, et al. HIV-1 induces renal epithelial dedifferentiation in a transgenic model of HIV-associated nephropathy. *Kidney Int.* 2000;58(1):173-81.

140. El-Sadr WM, Grund B, Neuhaus J, et al. Risk for opportunistic disease and death after reinitiating continuous antiretroviral therapy in patients with HIV previously receiving episodic therapy: a randomized trial. *Ann Intern Med* 2008;149:289-99.

141. Siedner MJ, Gelber AC, Rovin BH, et al. Diagnostic accuracy study of urine dipstick in relation to 24-hour measurement as a screening tool for proteinuria in lupus nephritis. *J Rheumatol* 2008;35:84-90.

142. Simerville JA, Maxted WC, Pahira JJ. Urinalysis: a comprehensive review. *Am Fam Physician* 2005;71:1153-62.

143. National Institute for Health and Clinical Excellence. Chronic kidney disease. London: NICE, 2008. *www.nice.org.uk/Guidance/CG73*. Accessed August 15th 2010.

144. Schwab SJ, Christensen RL, Dougherty K, et al. Quantitation of proteinuria by the use of protein-to-creatinine ratios in single urine samples. *Arch Intern Med.* 1987;147(5):943-4.

45. Price CP, Newall RG, Boyd JC. Use of Protein:Creatinine Ratio Measurements on Random Urine Samples for Prediction of Significant Proteinuria: A Systematic Review. *Clinical Chemistry* 2005;51:9 1577-86.

146. Guy M, Newall R, Borzomato J, et al. Use of a first-line urine protein-to-creatinine ratio strip test on random urines to rule out proteinuria in patients with chronic kidney disease. *Nephrol Dial Transplant* 2009;24:1189-93.

147. Guy M, Borzomato JK, Newall RG, et al. Protein and albumin-to-creatinine ratios in random urines accurately predict 24 h protein and albumin loss in patients with kidney disease. *Ann Clin Biochem*. 2009;46(6):468-76.

148. Levey AS and Stevens LA. Estimating GFR Using the CKD Epidemiology Collaboration (CKD-EPI) Creatinine Equation: More Accurate GFR Estimates, Lower CKD Prevalence Estimates, and Better Risk Predictions. *Am J Kidney Dis.* 2010; 55(4):622-7.

149. White SL, Polkinghorne KR, Atkins RC, et al. Comparison of the Prevalence and Mortality Risk of CKD in Australia Using the CKD Epidemiology Collaboration (CKDEPI) and Modification of Diet in Renal Disease (MDRD) Study GFR Estimating Equations: The AusDiab (Australian Diabetes, Obesity and Lifestyle) Study. *Am J Kidney Dis.* 2010;55(4):660-70.

150. Matsushita K, Selvin E, Bash LD, et al. Risk implications of the new Epidemiology Collaboration (CKD-EPI) equation compared with the MDRD Study equation for estimated GFR: the Atherosclerosis Risk in Communities (ARIC) study. *Am J Kidney Dis.* 2010;55(4):648-59.