KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF MOLECULAR MEDICINE

HIV Nephropathy among Children in the Ashanti Region of Ghana

By

FELIX SORVOR

JUNE, 2016

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF MOLECULAR MEDICINE

HIV Nephropathy among Children in the Ashanti Region of Ghana

Thesis submitted to the department of molecular medicine in fulfilment of requirement for the award of master of philosophy (molecular medicine).

By

FELIX SORVOR

JUNE, 2016

DECLARATION

The research work described in this thesis was carried out at the Department of Molecular Medicine-Kwame Nkrumah University of Science and Technology (KNUST), Komfo-Anokye Teaching Hospital (KATH), Suntreso Government Hospital and Agogo Presbyterian Hospital between November 2013 and March 2015. Except for references I made from published work and similar useful materials to which I have duly acknowledged, this is purely my own work, one which has never been submitted elsewhere for any other degree.

…… …..…….

FELIX SORVOR **DATE**

(Student Investigator)

…… …..…….

DR. W.K.B.A. OWIREDU **DATE**

(Supervisor)

……. ……...

PROFESSOR SAMPSON ANTWI **DATE**

(Co-Supervisor)

…… …..…….

PROFESSOR FRANCIS AGYEMANG YEBOAH **DATE**

Head of Department

(Department of Molecular Medicine)

DEDICATION

I dedicate this work to the Almighty God whose grace and mercy has brought me this far and also to my lovely wife Elizabeth Sorvor, my daughter Selase, my sons Delali and Senanu, my mother Kate Sandra Memedey and siblings Seth Sorvor and Sethina Osei Asante.

ACKNOWLEDGEMENT

Words cannot express the depth of appreciation I owe my supervisors Dr. W.K.B.A. Owiredu of the Department of Molecular Medicine, Kwame Nkrumah University of Science and Technology (KNUST) and Professor Sampson Antwi, School of Medical Sciences (SMS)/ Komfo Anokye Teaching Hospital (KATH) for their extreme patience and supportive supervision, their fatherly care and persistent calls and check-ups to ensure that this work was completed successfully. Anytime this work had challenges your words came in at the most appropriate time to give me the strength to get going. Indeed but for Dr. Owiredu this work could never have been a reality. Thank you very much and God richly bless you.

To my senior colleague and friend Dr. Richard Ephraim Dadzie of University of Cape-Coast (UCC), you were the brain behind this work and persistently you were available to make sure it came to a successful end. I am extremely grateful to you and your family for all the sleepless nights you had to tolerate me and to host me in your house and the countless number of times your wife fed me while we edited this work.

To my friend and brother Robert Adedze-Kpodo, Head of Laboratory Department, District Hospital, Sogakofe, words cannot say it all, thank you very much for your brotherly care.

My sincere gratitude also goes to Mr. Cosmos Ofori Aboagye my partner and ever hard working friend for your sacrifices and the persistent travelling you had to do weekly either on a Monday, Tuesday or Friday with me at dawn either to Agogo or Kumasi for sample collection. I am thankful for the several hours you spent with me in the various laboratories analysing the samples. I cannot ever forget the love you showed me while I was at my difficult ends on this project and I pray God grants you your very heart desire.

To Dr. Anthony Enimil and the other resident physicians, the late Madam Delaena Ami Ocloo; the lady I often referred to as my aunt and through whom I first had the courage to approach the paediatric department to seek permission to commence the sample collection process of this work, to Mrs. Eunice Osei Akoto, Mrs. Catherine Owusu Ansah and Mrs. Wilhelmina Mensah, the three very hard working state registered nurses who helped in participant recruitment and demographic data collection and all staff of the paediatric ART unit of KATH who in diverse ways helped to make this project a success I say a very big thank you.

To Dr. Agyarko Poku and Dr. Ashura Bakari of the STI clinic of Suntreso Government Hospital and their laboratory personnel, I say a big thank you for tolerating me while I worked at your facility.

Dr. Harry Boateng and Dr. Theresah Rectig at Agogo Presbyterian Hospital and the nurses at the child Health department also need special mentioning and I say a big thank you for the immense support you gave me during my sample collection sessions and also for the good record keeping.

I am grateful to Mr Laud Anthony Wihibeturo Basing for teaching me how to use the Zotero reference manager and also helping with the write up. To Miss Sylvia Karikari who works with Mr. Basing at the Parasitology department of KATH polyclinic, I say a big thank you for all the support. Miss Sylvia Karikari you are a God sent help and you came in at the right time.

To Dr. Christian Obirikorang, Dr Samuel Asamoah-Sakyi, and Dr Linda Fonjo, am grateful for the concerns and care you showed towards this work and I say thank you.

To my new friends Lawrence Quaye and Dr. Blay Nguah, I had barely known or met you but you were more than willing to analyse the data of this study. Throughout my life I have met very few people as selfless as you are and I pray you never lack in any aspect of your life and may you never cease to find help when you ever need one.

Finally, to my wife Elizabeth and our children Selase, Delali and Senanu, I cannot imagine life without you. Thank you so much for your support and prayers. I owe it all to you!

ABSTRACT

Background: HIV nephropathy (HIVAN being the commonest) remains one of the important non-infectious complications of HIV infections, affecting especially children of Sub Saharan Africa and African-Americans origin and is the third leading cause of end-stage renal disease (ESRD) among blacks. Currently the definitive diagnostic method for diagnosing HIV nephropathy is kidney biopsy, an invasive technique which requires special skills and which could pose potential risks for clients' aside the fact that the technique is not available in majority of the health – care facilities attending to and administering antiretroviral therapies (ARTs) in Ghana. The study determined the prevalence of dipstick microalbuminuria and proteinuria among the study participants and also established the prevalence of HIV nephropathy using eGFR < 60 ml $min^{-1}1.73\ m^{-2}$ in addition to microalbuminuria and or proteinuria among the HIV positive participants.

***Materials and methods*:** A total of 530 participants, including 380 HIV infected children (cases) and 150 HIV negative children (controls) attending three different ART centers namely Suntreso Government Hospital, Agogo Presbyterian Hospital and Komfo- Anokye Teaching Hospital all in the Ashanti Region were involved in the study. Five (5) mls of blood specimen was collected from each participant through a venesection into a serum gel separator tube (3mls) and an ethylenediamine tetra acetic acid (EDTA) tube (2mls). The serum samples were analysed for creatinine and cystatin-C while the EDTA samples were analysed for full blood count (FBC) and CD4 count. Additionally, urine samples were collected and urinalysis as well as microalbumin determination was done for each subject. Urine deposits were examined microscopically for urinary sediments.

Results: There was no significant difference in age between the case group (HIV infected participants) and control group (HIV negative participants) (7.4 ± 2.6 and 7.5± 2.5years, p=0.7729 respectively). The control group were significantly heavier (20.2 ± 6.6 kg) when compared with the case group (18.7 ± 6.7, p=0.0232). BMI was significantly higher in the control group (15.6 ± 2.0 kg m^{-2}) compared with the case group (14.7 ± 1.8 kg m^{-2}, p<0.0001). The proportion of participants within the case group who were on drugs was 77.1%; mean duration of infection of 3.7 ± 2.4 years with a mean duration on drugs of 3.1 ± 2.2 years. A Chi-square for trend analysis showed a significant difference in the number of the participants within the sub-categories of growth with 11.3% of the case group falling within the category of grade 3 thinness compared to 2.0% for the control group. 8.6% of the participants within the case group versus 4.0% of the participants within the control group had grade 2 thinness. 12.7% of the participants within the case group and 9.4% of those within the control group were within the grade 1 thinness category. The case group had significantly higher proteinuria 47(13.0%) compared to the control group 0(0.0%); p < 0.0001. Similarly microalbuminuria was significantly higher in the case group 95(26.2%) compared to the controls 9(6.0%); p < 0.0001. Again urinary cast and crystals were significantly higher in the case group compared to the controls 35(9.7%) and 2(1.3%); p = 0.001 and 30(8.3%) and 0(0.0%); p= 0.0003 respectively. Other urinary deposit elements such as yeast like cells were also significantly higher in the case group 16(4.4%) compared to the control group 0(0.0%); p=0.0091.The prevalence of HIV nephropathy (eGFR <60 ml min^{-1} 1.73 m^{-2} with microalbuminuria and or proteinuria) using creatinine-based (Schwartz, Counahan-Baratt and Leger equations) and Cystatin C-based (Larsson, Rule and Zapittelli equations)eGFR equations were 27.6%, 29.1% and 29.6% for Leger, Counahan-Barat and Schwartz equations and 21.1%, 28.2% and 30.6% for Rule, Larson and Zapittelli respectively.

Conclusion: Microalbumin and proteinuria are prevalent among children living with HIV/AIDS in Ghana. These in association with eGFR< 60 ml $min^{-1}1.73\ m^{-2}$ estimated using either a creatinine or cystatin-C based equation could be the easiest, cheapest, readily available and fastest way of diagnosing HIV nephropathy among such children.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

ABC:	Abacavir
ACEI:	Angiotensin converting enzyme inhibitor
ACTG:	Aids Clinical Trial Group
AIDS :	Acquired immunodeficiency syndrome
ALP:	Alkaline Phosphatase
ALT:	Alanine Transaminase
ARF:	Acute renal failure
ART:	Anti-retroviral therapy
ARV:	Antiretroviral
AST:	Aspartate Transaminase
ATN:	Acute tubular necrosis
AZT:	Azidothymidine (Zidovudine)
BMI:	Body mass index
BUN:	Blood Urea Nitrogen
CAPD:	Chronic ambulatory peritoneal dialysis
CD:	Cluster of differentiation
CIC:	Circulating immune complexes
CDC:	Center for Disease Control
CKD:	Chronic kidney disease
CKD-EPI:	Chronic Kidney Disease Epidemiology Collaboration
CDK:	Cyclic-Dependent Kinase
CrCl:	Creatinine clearance
CRF:	Chronic renal failure
d4T:	Stavudine

ddI:	Didanosine
DNA:	Deoxyribonucleic Acid
eGFR:	Estimated glomerular filtration rate
EFV:	Efavirenz
EIA:	Enzyme Immunoassay
ESKD:	End stage Kidney disease
FSGS:	Focal segmental glomerulosclerosis
FTC:	Emtricitabine
GFR:	Glomerular filtration rate
GGT:	Gamma Glutamyl Transferase
HAART:	Highly active antiretroviral therapy
HBV:	Hepatitis 'B' virus
HCV:	Hepatitis 'C' virus
HCT:	Haematocrit
HDL:	High Density Lipoprotein
HIV:	Human immunodeficiency virus
HIVAN:	Human immunodeficiency virus associated nephropathy
HTLV:	Human T-lymphotrophic Virus
HUS:	Haemolytic uraemic syndrome
IDSA:	Infectious Disease Society of America
IDV:	Indinavir
INF-α:	Interferon alpha
LAV:	Lymphadenopathy Associated Virus
LTR:	Long Terminal Repeat
KATH:	Komfo Anokye Teaching Hospital

KDOQI:	Kidney Disease Outcome Quality Initiative
KNUST:	Kwame Nkrumah University of Science and Technology
MCH:	Mean Cell Haemoglobin
MCHC:	Mean Cell Haemoglobin Concentration
MCV:	Mean Cell Volume
MDRD:	Modification of Diet in Renal Disease
MHC:	Major Histocompatibility Complex
MTC:	Mother to child
MTCT:	Mother to child transmission
NAC:	N-acetylcysteine
NFV:	Nelfinavir
NKF:	National Kidney Foundation
NNRTI:	Non-Nucleoside Reverse Transcriptase Inhibitors
NRTIs:	Nucleoside/Nucleotide reverse transcriptase inhibitors
NVP:	Nevirapine
PIs:	Protease inhibitors
3TC:	Lamivudine
TDF:	Tenofovir
TFG:	Transforming growth factor
TTP:	Thrombotic Thrombocytopaenic purpura
RBC:	Red Blood Cell
RNA:	Ribonucleic Acid
SIV:	Simian Immunodeficiency Virus
SMS:	School of Medical Sciences
STI:	Sexually Transmitted Infection

TDF:	Tenofovir
TLC:	Total Lymphocyte Count
TNF-α:	Tumour Necrosis Factor-alpha
µL:	Microlitre
µg:	Microgram
UNAIDS:	United Nations Programme on HIV/AIDS
WBC:	White Blood Cell
WHO:	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Human immunodeficiency virus (HIV) is known to cause acquired immunodeficiency syndrome, a condition in humans in which the continuous failure of the immune system allows life-threatening opportunistic infections and cancers to thrive (Alarcón *et al.,* 2012; Okolie *et al.,* 2003). Characteristically, the infection is associated with a cellular CD4 T- lymphocyte depletion (Hazenberg *et al.,* 2000). It is blood-borne and is usually transmitted via sexual intercourse, shared intravenous drug paraphernalia, and from mother-to-child (MTC); a transmission which can occur during the birth process or during breastfeeding (Foy *et al.,* 2013; Busza *et al.,* 2012). The infection is regarded an epidemic by the World Health Organisation (WHO) and from its discovery in 1981 to 2006; AIDS has killed more than 25 million people (Joint United Nations Programme on HIV/AIDS), (Mofenson *et al.,*2009; Chaudhary *et al.,*2010).The World Health Organization (WHO) approximated that in 2005 there were about 2.3 million worldwide cases of childhood HIV infection with about 90% (2.1 million) of these children living in sub-Saharan Africa (WHO,2005; Ikpeme *et al.,* 2012)

Ramifications associated with the infection appears to be relatively on a decline due to or partly as a result of the introduction and use of anti-retrovirals (ARVs) often referred to as Highly Active Anti-Retroviral Therapy (HAART) (Chopra *et al.,* 2013; Luetkemeyer *et al.,* 2014). Documented pulmonary complications include pneumocystis jirovecii pneumonia, tuberculosis and other bacterial pneumonia such as those of Streptococcus pneumonia and haemophilus influenza (Capocci and

Lipman, 2013; Chopra *et al.,* 2013). Pulmonary complications due to fungal infections are mostly those of Cryptococcus species (Estébanez-Muñoz *et al.,* 2012). Central nervous system complications associated with HIV infection include cerebral toxoplasmosis, cryptococcal meningitis, progressive multifocal leucoencephalopathy, HIV encephalopathy and peripheral neuropathy and myelopathy (Pipkin *et al.,* 2011; Bratton *et al.,* 2012; Pongmekin *et al.,* 2014). There are documented ocular complications (Chisti *et al.,* 2013; Chopra *et al.,* 2013) as well as various tumours (Kaposi's sarcosis and non-Hodgkin's lymphoma) (Pipkin *et al.*,2011).

Kidney disease is one of the important non-infectious complications of HIV infections, particularly in Sub Saharan Africa and among African-Americans (Blasi *et al.,* 2014a; Canaud *et al.,* 2014; Andiman *et al.,* 2009; Gupta *et al.,* 2005a). A variety of kidney disorders, acute or chronic may occur during the course of the infection (Anupama and Uma, 2014; Daher *et al.,* 2014; Imani *et al.,* 2013; Ando *et al.,* 2012; Atta, 2010; Kalim *et al.,* 2008). These diseases are more or less tightly linked with the virus, the expression of HIV genes in the kidney and the interaction of HIV proteins with renal cells (Blasi *et al.,* 2014; Canaud *et al.,* 2014; Ikpeme *et al.,* 2012). Approximately 50–60% of renal disease associated with HIV infection may be considered "classic" HIV-associated nephropathy (HIVAN). HIVAN is thus the most common form of chronic kidney disease resulting directly from HIV infection (Ikpeme *et al.,* 2012; Anochie *et al.,* 2008) and which often comes with proteinuria as the first sign (Chaparro *et al.,* 2008; Ramezani *et al.,* 2008). HIVAN is typically characterised by Nephrotic range proteinuria, azotaemia, low serum albumin, normal to large echogenous kidneys on ultrasound images, and focal segmental glomerulosclerosis (FSGS) on renal biopsy findings (Murray *et al.*,2014; Allan, 2011; Atta *et al.,* 2005). Globally the incidence of HIV-associated kidney disease in children

has been estimated at between 2% and 5% but this could be as high as 15% in populations of African descent (Ando *et al.,* 2012). The pathogenesis of HIVAN like other HIV nephropathies involves direct HIV infection of the kidney, with both viral and host genetic factors playing an important role (Kalim *et al.,* 2008). The use of nephrotoxic drugs such as Tenofovir as first-line antiretroviral (ARV) drugs could make people living with HIV/ AIDS (PLWHA) more susceptible to kidney diseases than the rest of the population (Bouthemy *et al.,* 2013; Ando *et al.,* 2012; Daugas *et al.,* 2005).

1.2 HYPOTHESIS

The prevalence of HIV nephropathy remains unknown among children living with HIV in Ghana due to limited access to confirmatory diagnostic tests.

1.3 PROBLEM STATEMENT

HIV nephropathy is a rapidly progressive form of chronic kidney disease; a condition highly associated with mortality (Anupama and Uma, 2014; Ikpeme *et al.,* 2012; Atta, 2010; Anochie *et al.,* 2008; Fine *et al.,* 2008). Early identification is important since appropriate therapeutic interventions could delay disease progression (Grinsztejn *et al.,* 2014). Traditionally, tests for kidney function commonly used for diagnosing renal disease involves a urine test; a simple "dipstick" followed by microscopy. Other tests include serum urea, creatinine and rate of creatinine clearance (Nagai *et al.,* 2013; Westland *et al.,* 2013; Prigent, 2008; Ledger, 2006). Predictive equations used in estimating and assessing kidney function are heavily affected by factors such as age, sex, race and body mass which affect creatinine.

Currently the definitive diagnostic method used for diagnosing HIV nephropathy is kidney biopsy, an invasive technique which requires special skills and which could

pose potential risks for clients' (Fine *et al.,* 2008) aside the fact that the technique is not available in majority of the health – care facilities attending to and administering antiretroviral therapies (ARTs) in Ghana. This case control study seeks to address this obvious diagnostic challenge.

1.4 JUSTIFICATION

HIV-related renal dysfunction is associated with high mortality (Andiman *et al.,* 2009). Recent recommendations to include the nephrotoxic drug Tenofovir in first-line ART regimens in Ghana could worsen the situation (Giacomet *et al.,* 2013; Msango *et al.,* 2011). Again the relative lack of surveillance and reporting on kidney diseases especially among children compounded by the seemingly absence of renal biopsy for histological confirmation for children living with HIV who have proteinuria, as a result of high cost, non-availability and prolonged turnaround time makes getting a surrogate for confirming HIV nephropathy a necessity in resource poor countries such as Ghana. A relatively cheaper, less invasive and easily accessible technique which does not compromise the quality of diagnosis would make it less burdensome for such clients and make it possible for more people to be diagnosed early for appropriate therapeutic intervention. Additionally the prevalence of HIV nephropathy in the Ashanti Region would be known and stimulate appropriate remedial measures to avert the progression of the condition.

1.5 AIM

The study aimed at finding a relatively easy, less invasive, timely and an affordable way of diagnosing nephropathy among children living with HIV in Ghana without compromising the quality of diagnosis.

1.5.1 Specific Objectives

The specific objectives of the study were:

1. To describe the demographic, haematological and biochemical characteristics of the study participants.

2. To determine the prevalence of dipstick proteinuria, microalbuminuria and common urinary deposits among study participants.

3. To describe the anthropometric outcomes of the study participants using varied growth reference estimations.

4. To compare the use of cystatin C and creatinine in the estimation of GFR as a diagnostic tool for muscle wasting diseases like HIV in children.

5. To estimate GFR using appropriate equations and to find the prevalence of HIVAN using eGFR< 60 ml min^{-1} 1.73 m^{-2}, with microalbuminuria and or proteinuria as definition for HIV nephropathy.

6. To explore the identification of suitable alternative biomarkers for HIV nephropathy in resource poor settings.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 HIV NEPHROPATHY

The human immunodeficiency viral infection is associated with a variety of kidney diseases, HIV-associated nephropathy (HIVAN) being the commonest and the third leading cause of end-stage renal disease (ESRD) within persons of African and or black origin aged between twenty (20) and sixty (60) years (Ikpeme *et al.,* 2012; Anochie *et al.,* 2008). As the far-flung use of competent antiretroviral therapy increases, the preponderance of persons living with HIV developing renal disease and ESRD also increases (Pipkin *et al.,* 2011; Daugas *et al.,* 2005). Renal disease seems to be comparatively the commonest ramification in patients with human immunodeficiency virus (HIV) disease (Ikpeme *et al.,* 2012; Anochie *et al.,* 2008). HIV renal disease could result from the direct infection of the kidney by HIV or from the contrary effects of antiretroviral drugs (Blasi *et al.,* 2014; Jayadev and Garden, 2009; Daugas *et al.,* 2005). Additionally, patients with HIV disease are more exposed to developing pre-renal azotaemia due to volume depletion resulting from salt wasting, poor nutrition, nausea, or vomiting (Ahuja *et al.,* 2004).

The commonest type of HIV-associated nephropathy is a collapsing focal segmental glomerulosclerosis (FSGS), though other forms of kidney disease could occur with HIV. Irrespective of the underlying health condition, renal disease in HIV-positive patients is affiliated to high risk of death (Ahuja *et al.,* 2004).

HIV nephropathy when caused by the direct infection of the renal cells by HIV-1, have impairments which result from viral gene products (Rao *et al.,* 2014; Jayadev and Garden, 2009). These impairments could also be as a result of alterations in the

release of cytokines during HIV infection. It usually occurs only in advanced disease and approximately 80% of patients with HIV nephropathy have a CD4 count of less than 200 (Coulibaly *et al.,* 2013). HIV nephropathy presents with nephrotic syndrome and progressive renal failure. Despite being a cause of chronic renal failure, kidney sizes are usually remain unchanged or enlarge (Coulibaly *et al.,* 2013; Atta *et al.,* 2005).

2.2 INCIDENCE AND RISK FACTORS

Renal impairment is an important co-morbidity of HIV, regardless of whether it is caused by the direct effects of HIV in the kidney (e.g. HIVAN) (Rao *et al.,* 2014; Jayadev and Garden, 2009), or is as a result of factors related to HIV such as opportunistic infections (e.g. tuberculosis [TB]) and adverse drug use (e.g. Tenofovir [TDF]) (Prasitsuebsai *et al.,* 2014; Rao *et al.,* 2014; Alarcón *et al.,* 2012; Lescure *et al.,*2012; Daugas *et al.,*2005). Renal impairment is more common in HIV patients of African origin compared to those of Caucasian origin and has been shown to be an important predictor of early mortality in patients starting antiretroviral therapy (ART) in western as well as Africa settings (Ikpeme *et al.,* 2012). The preponderance of renal impairment in cohort studies of HIV-positive patients have varied depending on the clinical status of the patients and the definition of renal impairment used (Alves *et al.,* 2010).

Predisposing factors for HIV nephropathy include black race, male gender, history of injection drug use, hepatitis B and C antibody, decreased CD4+ cell count, and concurrent AIDS-defining condition (Di Biagio *et al.,* 2011; Alves *et al.,* 2010). Little risk factor information exists for other renal disease histologies probably due to the relatively low incidence for each type of disease among HIV-positive persons. Despite the understanding that HIV can infect cells within the kidney and establish

renal viral reservoirs leading to HIV nephropathy (often HIVAN) and other renal diseases there appear to be limited work to confirm this fact (Alves *et al.*,2010)

Preponderance of HIVAN higher than 50% have been reported in recent times from several centres where patients have access to HAART (Ikpeme *et al.,* 2012). The reported nephropathy risk factors appear relatively different from those observed in the pre-HAART era, ranging from increased patient age to higher viral load, lower CD4 T-cell count (Antoniou *et al.,* 2005), use of Nucleotide Reverse Transcriptase Inhibitors (NRTIs) and exposure to protease inhibitor (PI) medications (Ando *et al.,* 2012). Furthermore, survival among those with HIV nephropathy has been shown to improve with increasing CD4+ cell count, decreasing serum creatinine and proteinuria levels, increasing haemoglobin level, and the receipt of corticosteroid therapy (Popova *et al.*,2013).

2.3 STAGING AND CLINICAL FEATURES

HIV nephropathy, particularly HIVAN is a World Health Organization (WHO) clinical stage IV condition, which means the need for ART regardless of their CD4 count (Ando *et al.,* 2012; Lescure *et al.,* 2012; Domingo *et al.,* 2010). ART is the main effective treatment for this condition, which otherwise leads to end-stage renal failure(Sexton *et al.,* 2014; Bansi *et al.,* 2009). Identification of HIVAN and other forms of renal impairment is important in patients in all clinical stages of HIV disease; however, it is crucial in those with clinical stage I and II, since these patients might not otherwise be identified to be in urgent need of ART (Anochie *et al.,* 2008)

Majority of people with HIVAN are of African origin with the condition portraying late in the pathogenesis of HIV-1 infection (Ikpeme *et al.,* 2012; Anochie *et al.,* 2008). Even though most of such patients are known to be HIV-1 seropositive for

several years and have CD4 counts less than 200 x 10^6 cells/l at the time they are diagnosed with HIVAN, there are reports suggesting HIVAN could occur in the setting of acute HIV-1 seroconversion (Ezeonwu *et al.,* 2012). As a lot more patients are screened early in the course of HIV-1 infection for renal disease, it is foreseeable that many more cases will be discovered (Ezeonwu *et al.*,2012; Estrella and Fine, 2010).

Diagnosis of HIV nephropathy is made when patients have severe proteinuria, commonly, but not always, in the nephrotic range (3.5g/day or greater) (Ikpeme *et al.,* 2012; Ramezani *et al.,* 2008; Atta *et al.*,2005). Majority of HIV patients with nephropathy additionally have advanced renal failure at the time of diagnosis although Burns *et al*., (2003) have reported a cohort of patients with HIVAN and mild renal insufficiency with a mean serum creatinine of 114.9µmol/l.

Notwithstanding of the presence of proteinuria that is often in the nephrotic range, more patients with HIVAN do not have noticeable peripheral oedema (Chaparro *et al.,* 2008; Atta *et al.,* 2005). Additionally, patients with HIV nephropathy are normally not hypertensive, a significant finding considering that more than 90% of black patients with renal insufficiency of other causes exhibit hypertension. These observations together with the findings that HIV-seropositive patients often have a decreased ability to conserve sodium suggest that HIV nephropathy could be a salt-wasting disease (Marik, 2014).

Laboratory investigations are not specific in HIV nephropathy except biopsy. Serologic studies are usually negative although a significant percentage of HIV-1 seropositive patients are co-infected with hepatitis C virus (HCV) (Gluhovschi *et al.,* 2014; Gupta and Singh, 2006) Because membranoproliferative glomerulonephritis

(most often related to HCV infection) is frequently diagnosed in HIV-seropositive patients with renal disease the presence of positive HCV serologies in an HIV infected patient with renal disease increases the need for renal biopsy (Gluhovschi *et al.*, 2014; Gupta and Singh, 2006). Urinalysis results often appear normal but for proteinuria and hyaline casts (Chaparro *et al.*, 2008).

In most chronic renal diseases, the kidneys become progressively smaller as renal failure progresses. In HIVAN, however, renal ultrasound commonly reveals bilateral echogenic kidneys that are often enlarged (Allan, 2011; Vourganti *et al.*, 2010)

Despite the reporting of this finding by several authors, not much has been done about the predictive value of renal ultrasound to rule in or exclude the diagnosis of HIVAN (Vourganti *et al.*, 2010). The only reliable test to confirm or rule out the presence of HIVAN is renal biopsy (Fine *et al.*,2008). In patients who are HIV-1 seropositive and undergo renal biopsy, in whom clinical suspicion of HIVAN is high, 40–55% are diagnosed with other forms of renal disease (Bani-Hani *et al.*, 2010). Since firmly establishing the diagnosis of HIVAN versus a different renal disease is important to guide treatment and provides prognostic information, clinicians would need to have a low threshold for obtaining renal biopsies in HIV-1-seropositive patients with significant proteinuria and/or renal insufficiency (Sexton *et al.*, 2014; Bani-Hani *et al.*, 2010).

2.4 COMPLICATIONS

Renal function irregularities are present in a greater proportion of patients with HIV infection (Zoja *et al.*, 2014). HIV-associated renal diseases have become a relatively common cause of end-stage renal disease (ESRD) requiring dialysis and seems to be related to the rate of progression to AIDS and death, (Bansi *et al.*, 2009). Renal failure

is a well-recognized consequence of HIV infection, even without clear signs of AIDS (Rao, 2001). The pathological scope for progressive renal failure is well-documented in literature. Collapsing focal and segmental glomerulosclerosis is often noted in patients with HIV who have a rapid diminution in kidney function, especially the African-American patient, (Valeri *et al.,* 1996). With the advent of aggressive anti-retroviral therapy, the ambit of HIV related nephropathy is changing (Betjes *et al.,* 2001). Complications of treatment are equally documented (Grinsztejn *et al.,* 2014). Complications of HIV nephropathies include; acute renal failure as well as chronic HIV nephropathies.

2.4.1 Acute renal failure

Acute renal failure (serum creatinine > 2 mg/dl) of higher incidence has been reported in a number of hospitalised HIV infected patients compared to non-HIV infected patients (Bae *et al.,* 2014). Acute renal failure (ARF) in HIV patients is often times prerenal azotaemia or acute tubular necrosis (ATN) due to diarrhoeal diseases, infection, septicaemia, bleeding, hypoalbuminaemia, or nephrotoxins (Maggi *et al.*,2012). Recent studies highlight an increased incidence of HIV – associated thrombotic microangiopathies and rhabdomyolysis, the latter from the use of statins in combination with HAART (Shah, 2012). Post-renal ARF due to tumour, lymph nodes, retroperitoneal fibrosis, or crystalluria due to Indinavir, acyclovir, or sulphadiazine is rare. Renal biopsies in thirteen (13) patients of severe ARF (where pre-renal or ATN could not be suspected) in a study showed haemolytic uraemic syndrome (HUS) in 53%, ATN in 40%, obstructive renal failure due to crystalluria in 26%, HIV associated nephropathy (HIVAN) in 23%, acute interstitial nephritis in 3%, and various other glomerulopathies in 6% (Atta, 2010)

2.4.2 Chronic HIV nephropathies

Currently, HIV infected patients make up about 1-2% of ESRD population. Three types of chronic kidney diseases are directly caused by HIV infection, namely, HIV-associated thrombotic microangiopathies; HIV-immune mediated glomerulopathies, and classic HIV-associated nephropathy (Ando *et al.,* 2012; Atta, 2010; Kalayjian, 2010; Khan *et al.,*2006).

2.4.2.1 HIV-associated thrombotic microangiopathies

Thrombotic microangiopathies involving the kidney was first described in AIDS patients in mid 1980s (Kuzmanović *et al.,* 2014; Shah, 2012). Subsequently, several cases have been reported worldwide and this appears to be the most common micro vascular injury associated with HIV infection. Thrombotic thrombocytopenic purpura (TTP) and haemolytic uraemic syndrome (HUS) are characterised by microangiopathic haemolytic anaemia with renal insufficiency along with other features such as thrombocytopenia, fever, and neurological changes. TTP traditionally has less extensive renal failure and anaemia, while thrombocytopenia is most prominent. In contrast, the microangiopathic haemolytic anaemia predominates in HUS and renal dysfunction may be extensive. The preponderance of TTP/HUS in HIV infected patients has been reported to vary widely. The clinical presentation of HIV- associated TTP/HUS is similar to the idiopathic form except for the demography. HIV associated TTP/HUS is a disease of the young (mean age of 35 years) with 80% male predominance. The prognosis is worse than the idiopathic form, with a mortality of 66% to 100%. Massive proteinuria is uncommon, which helps to differentiate it from immune mediated disease and HIVAN (Kuzmanović *et al.,* 2014; Chandran *et al.,* 2013).

Renal histology shows platelet and fibrin thrombi in glomerular capillaries, renal arterioles, and interlobular arteries. Tubuloreticular inclusions are seen in endothelial cells in blood vessels on electron microscopy. (Kuzmanović *et al.,* 2014)

The pathological process of TTP/HUS is centred on the direct endothelial damage. Renal cellular programmed cell death and the suppression of von Willebrand factor-cleaving protease may play key pathogenic roles. HIV – TTP/HUS has poor prospects with a one-third fatality rate in acute phase of the illness despite aggressive treatment regimens. HAART appears useful in refractory cases but plasmapheresis remains the anchor of therapy for HIV-associated thrombotic microangiopathies (Kuzmanović *et al.,* 2014; Shah, 2012)

2.4.2.2 HIV-associated immune - mediated glomerulopathies

The preponderance of proliferative glomerulonephritis varies between 10%and 80% in various autopsy and biopsy studies in HIV infected patients (Chandran *et al.,* 2013; Gindea *et al.,* 2010). While the HIV- associated glomerulosclerosis is relatively common in the black population, the HIV - immune mediated glomerulonephritis is mostly prevalent in Europeans and Asians (Chandran *et al.,* 2013). Membranoproliferative glomerulonephritis is the commonest of the HIV - associated immune mediated glomerular diseases (Gindea *et al.,* 2010). Membranous nephropathy, post-infectious glomerulonephritis, fibrillary glomerulonephritis and IgA nephropathy are the other lesions described. These lesions could be due to HIV infection or as a result of other associated infections; hepatitis C or B or could be a concurrent finding (Chandran *et al.,* 2013; Foy *et al.,* 2013). Deposition of circulating immune complexes (CICs) within the kidney could play an important role in the development of HIV associated glomerulonephritis. Immunoglobulins (IgG, IgM, IgA) could adhere to circulating HIV antigen (p 24, 8p G1 gp 120) resulting in

formation of CICs during all stages of HIV infection. The cellular immune factors could additionally play a role in the expression of renal disease and IgA nephropathy has been seen in several patients with HIV infection. A biopsy study done in Europe showed some level of prevalence of diffuse mesangial deposits of IgA in the patients who died of AIDS (Foy *et al.,* 2013; Cohen and Kimmel, 2008). Remarkable response has been reported with the use of highly active antiretroviral therapy (HAART), ACE-inhibitors and glucocorticoids in such situations (Adebamowo *et al.,* 2014; Foy *et al.,* 2013; Cohen and Kimmel, 2008)

2.4.2.3 HIV-associated focal glomerulosclerosis

HIV-associated focal glomerulosclerosis is characterised by monumental nephrotic proteinuria (mostly> 10 gm/day) with renal failure occurring within 1 to 2 years coupled with large, echogenic kidneys seen on ultra sound (Allan, 2011; Anochie *et al.,* 2008; Atta *et al.,* 2005). The pathologic findings are pathognomonic. Although clinical findings could be suggestive, a renal biopsy is necessary for diagnosis. The renal histology is characterised by collapsing focal and segmental glomerulosclerosis associated with microcystic tubular dilatation and interstitial inflammation (Fabian *et al.,* 2013; Wearne *et al.,* 2012). The microcystic tubular changes and interstitial oedema cause an increase in kidney size. There is visceral epithelial cell hypertrophy and 'pseudocrescent' formation in Bowman's space. Greater than 90% of renal biopsies with HIVAN contain tubuloreticular inclusions within the cytoplasm of glomerulus and other vascular endothelial cells (Fine *et al.,* 2008). Immunofluorescence frequently shows staining for IgM, C3, and less commonly C1Q in the mesangium (Lescure *et al.,* 2012). The treatment of HIVAN has not been evaluated by rigorously controlled randomised trials. Treating HIVAN with HAART or in combination with ACE inhibitors or steroids is recommended (Lescure *et al.,*

2012). HAART is renoprotective in HIVAN (Zeleniuk *et al.,* 2014). Preliminary reports indicate that HAART has beneficial effects on the prevalence and progression of HIVAN (Grinsztejn *et al.,* 2014). Suppression of viral replication is clearly a key factor in the improved outcome (Primeggia *et al.*,2013; Brady *et al.,* 2010). Less appreciated are the potential disease modulating non-viral actions of HAART, in particular anti-apoptotic effects of protease inhibitors. Two case reports of HAART in HIVAN patients have shown remission of both renal pathologic and functional abnormalities. Currently, studies on the long-term effects of HAART therapy on the patient outcomes are lacking (Daugas *et al.*,2005).

All the three renal replacement modalities, i.e., the intermittent haemodialysis, chronic ambulatory peritoneal dialysis (CAPD), and renal transplantation, are recommended in the patients of end-stage renal disease due to HIV infection. Outcomes of HIV infected patients treated with haemodialysis and peritoneal dialysis have improved. Haemodialysis is the most common renal replacement modality for HIV-infected patients. Substantial improvement in the survival of HIV - infected patients on dialysis has been noted after 1995 and this has been attributed to HAART (Trullas *et al.,* 2011). Dialyser re-use is permissible in HIV infected patients. Routine infection control precautions and routine cleaning with sodium hypochlorite solution of dialysis equipment and of surfaces that are frequently touched are sufficient in HIV - infected patients on haemodialysis. Isolation of HIV - infected patients from other dialysis patients are unnecessary (Barril*et al.*,2004; Trullas *et al.,* 2011)

The choice of renal replacement therapy often is based on patient preferences and feasibility. Continuous ambulatory peritoneal dialysis (CAPD) offers the advantage to staff of a lower risk of occupational exposure to HIV. However, peritoneal protein losses in malnourished HIV patients and severe peritonitis are the potential concerns.

Some of the immunosuppressive drugs (cyclosporine, FK506, and mycophenolate mofetil) may have anti-retroviral effects. Recent evidence suggests that addition of cyclosporine to HAART may offer other long-term beneficial effects (Frassetto *et al.*, 2005; Barril *et al.*,2004)

2.5 PATHOPHYSIOLOGY

Typical of HIV nephropathy (particularly HIVAN) is the sequential arrangement of pathologic findings involving glomerular, tubular, and interstitial compartments (Anochie *et al.,* 2008). Glomerular morbid outcomes demonstrate focal glomerulosclerosis, with eminent collapse of the glomerular tuft. Striking about the tubular disease is the development of tubular dilatation, which is followed by atrophy and flattening of tubular epithelial cells (Blasi *et al.,* 2014). Typically there are lymphocytic percolations of the interstitium. Endothelial tubuloreticular is a common pathologic finding when examined by electron microscopy in the pre-highly active antiretroviral therapy (HAART) era. Tubuloreticular inclusions, however, are found with decreasing frequency perhaps due to the efficacy of antiretroviral therapy in reducing plasma interferon levels (Ross, 2014; Heyns *et al.*, 2013; Anochie *et al.*, 2008; Atta *et al.,* 2008; Leventhal and Ross, 2008)

2.5.1 Detection of HIV-1 in renal epithelial cells

Between 1980s and 1990s investigators tried to prove the presence of HIV-1 in renal parenchymal cells using a number of techniques. While some investigators concluded that HIV-1 was present in renal epithelial cells others could not detect the virus (Blasi *et al.,* 2014).

While it was debatable whether HIV-1 infected renal epithelial cells or not in humans, work from animal experiments gave critical insight into HIVAN pathogenesis. Dickie

et al., (1991) for instance reported that HIV-1 transgenic mice contained gag/ pol deleted provirus expressed under the control of the endogenous HIV-1 long terminal repeat (LTR) promoter. These mice they observed developed excess protein in their urine, had progressive renal failure, and histologic disease that was similar to HIVAN (Kajiyama *et al.,* 2000). A mutual transplantation study by *Bruggeman et al.,* (2000) showed that the HIVAN phenotype in those mice relied on HIV-1 gene expression in the renal epithelium. Quite recently however, a rat transgenic model showing the same HIV-1 transgene have been demonstrated to cause the HIVAN phenotype (Ledger *et al,* 2006).

Work involving the use of primates by other researchers suggests that there is a role for the direct infection of renal parenchymal cells by HIV-1 in the pathogenesis of HIVAN. Research involving Macaques also showed they developed renal disease that closely looked like HIVAN following their infection with a chimeric simian-human immunodeficiency virus (SHIV). The rate at which these animals developed renal disease depended on the breed of SHIV used, evoking the hypothesis of viral determinants in such renal disease advancements. Although these researchers were able to isolate SHIV from the glomeruli of these diseased animals, it is difficult to say whether it was the renal parenchymal cells which were infected or the viruses which were isolated were from infiltrating mononuclear cells. The definitive proof of HIV infecting humans came in 2000 when Bruggeman, *et al.,* (2001) reported a series of twenty (20) HIV-seropositive patients with renal disease after they had undergone diagnostic renal biopsy. Renal tissue was collected prospectively at the time of biopsy and immediately worked on to optimize RNA preservation. Fifteen (15) of the patients were diagnosed with HIVAN and in eleven (11) of these patients; HIV-1 RNA was detected by RNA in situ hybridization. Specificity of majority of the

samples was confirmed using riboprobes for both HIV genes gag and nef and by DNA in situ PCR. Subsequently HIV-1 has been detected in epithelial cells from several segments of the nephron, including the glomerulus (visceral and parietal epithelial cells) and tubules; the proximal tubule, thick ascending limb of Henle, and the collecting duct. Additionally, the histologic pattern of the disease showed an overlapping in the distribution of epithelial infection. This notwithstanding however, the infection is yet to be conclusively identified in other renal parenchymal cells such as mesangial cells or endothelial cells. While it is still not clear the nature and mechanism by which HIV-1 enters the renal epithelial cells, the envelop receptors it uses are also not known. It is however without doubt that HIV-1 can be detected in renal epithelial cells of patients with HIVAN (Nelson *et al.*, 2002).

2.5.2 HIV-1 infection induces disease in the renal epithelium

The kidney is made up of phenotypically unique epithelial cell types along the length of each nephron. In the glomerulus, the visceral epithelial cell, or podocyte, is a highly specialized epithelial cell that forms a critical part of the glomerular filter (Blasi *et al.*, 2014b). Podocytes are known to be infected by HIV-1 in HIVAN and the phenotypic derangements associated with podocyte in renal diseases flanked by unusual increase in proteinuria are also present (Zhong *et al.*,2005). The infection inducts several irregularities including increased proliferation and reduction in the expression of markers of differentiation, including synaptopodin, WT-1, GLEPP-1, and podocalyxin. Similar abnormalities in podocyte phenotype are found in the glomeruli of HIV-1 transgenic mice. Ex vivo research involving podocytes from HIV-1 transgenic mice and that from wild-type murine show an increased level of proliferation and anchorage regardless of growth in podocytes showing HIV-1. Phenotypic changes of the tubular epithelium are similarly outstanding with increased

proliferation and apoptosis, microcystic dilatation, flattening and atrophy of epithelial cells, and loss of expression of differentiation markers, with abnormal polarization of the sodium-potassium ATPase (de Silva *et al.,* 2007; Zhong *et al.,* 2005). Abundant evidence exists to show that HIV-1 induces cell cycle progression in HIVAN. Shankland *et al.,* (2000) for instance reported that the cyclin-dependent kinase (CDK) inhibitors p27 and p57 were down regulated in podocytes in HIVAN biopsies while p21 was upregulated. Basic fibroblast growth factor is upregulated in HIVAN and has been demonstrated in other studies to increase the proliferation of renal epithelial cells ex vivo. Growth factor activity together with cell cycle regulatory proteins are thought to intercede in the increased epithelial proliferation observed in HIVAN unlike other HIV nephropathies (Bruggeman *et al.,* 2009).

HIVAN is also linked to the upregulation of transforming growth factor and to some extent, middle in the increased renal fibrosis and apoptosis that is observed during the course of the disease. Representational difference analysis has been used to examine differentially the expressed genes in podocytes from HIV-1 transgenic and normal mice. By such an approach a new small leucine-rich repeat protein, Podocan, which amass in sclerotic glomeruli of the HIV-1 transgenic mice, has been identified and is currently being studied (Zhong *et al.,* 2005). Transcriptional regulation of HIV-1 in renal epithelial cells however seems to occur in a similar fashion as occurs in lymphocytes. Transcriptions in such podocytes require binding the inducible nuclear factor-*k*B and Sp1 to the viral LTR. Inhibition of the HIV-1 transcription in such podocytes using inhibitors such as CDK-9 would result in decreased proliferation and re-expression of podocyte differentiation markers ex vivo. Administering CDK-9 inhibitors to HIV-1 transgenic mice would ameliorate the HIVAN phenotype (Nelson *et al.,* 2009; Zhong *et al.,* 2005) however the markedly low efficiency of HIV- 1

transcription in murine cells makes it difficult to predict whether CDK-9 inhibition would have similar consequences in podocytes from humans with HIVAN.

2.5.3 Contribution of HIV-1 genes to HIVAN pathogenesis as the commonest HIV nephropathy

By using animal models, the HIV-1 genes that were critical for HIVAN pathogenesis (Winston *et al.,* 2001) have been mapped out. These animals expressed a gag/pol-deleted HIV- 1 transgene under the control of endogenous viral LTR promoter but were not really required for development of the HIVAN phenotype (Kajiyama *et al.,* 2000).Hanna *et al.*, (2009) developed eighteen (18) transgenic mouse lines expressing five (5) different HIV-1 mutants' constructs under the control of the human CD4 regulatory sequences. In the said transgenic lines, the expression of HIV-1 nef was both necessary and significant to induce an AIDS-like phenotype and renal disease. The pathogenicity of HIV-1 nef in transgenic mice (with nef evinced under the control of human CD4 promoter) was abolished by mutation of the P72XXP75 SH3-binding domain of Nef (Hanna *et al.,* 2009). Hybridising these nef-mutant mice with hck-mutant ones resulted in a delay in development of the phenotype. The researchers then postulated that since Hck was known to be associated with Nef through an SH3 domain, Hck could be an important effector of Nef. However, the expression of SIV Nef, which lacked the SH3 binding domain showed the same phenotype as HIV-1 wild type Nef, suggesting that binding to Hck via the SH3- binding domain was not crucial for Nef activity (Hanna *et al.,* 2009). The multigenic vectors display the same parental backbone as that used in generating the HIV-1 transgenic model of HIVAN. Thus, Nef expressed in cultured podocytes was necessary and sufficient to induce increased proliferation and anchorage-independent growth. Nef expression has

subsequently shown to induce loss of expression of markers of differentiation in podocytes in vitro Hanna *et al.,* (2009).

2.5.4 The kidney as a reservoir for HIV-1

The infection of renal epithelial cells by HIV-1 has implications way beyond the role of the virus in promoting the development of HIVAN. Renal epithelial cells serve as a reservoir in instances where HIV-1 persist and particularly in patients who may have no detectable HIV-1 in their plasma (Ray, 2009). In the cases reported by Bruggeman *et al.*, (2000), four (4) of twenty-one (21) HIV- 1 seropositive patients with renal disease in whom diagnostic renal biopsy was performed, had an undetectable amount of HIV-1 in plasma. In each of those patients, HIV-1 RNA was detected in renal epithelial cells (Bruggeman *et al.,* 2000). After treatment with HAART, the patients had clinical and histologic resolution of the renal disease. There is also very little known about the ability of antiretroviral medications to achieve therapeutic levels in renal epithelial cells and whether the effects of these medications on the HIV-1 lifecycle in renal epithelial cells is the same as that in leukocytes (Schwartz *et al.,* 2005; Ross *et al.,* 2000). Thus, despite the dramatic response of HIVAN and other HIV nephropathies to HAART, HIV-1 RNA expression in renal epithelial cells remained unchanged (Ross *et al.,* 2000).

2.6 GENETICS

Black people have higher probability of developing of HIV nephropathy, however explanation for this remain unclear. Black people and persons of African descent normally have higher incidence of other renal diseases as well (including diabetic nephropathy, lupus); hence it is possible that black people have an underlying genetic predisposition to renal disease. Additionally the nature of the host response to the HIV

infection could determine whether or not nephropathy develops in a particular individual (Bouthemy *et al.*, 2013; Cohn and Weaver, 2006; Tang and Kaslow, 2003).

Kopp *et al.*(2008) studied the genetic variants which predisposes to idiopathic and HIV-1–associated focal segmental glomerulosclerosis (FSGS), and they concluded that genetic variation at the *MYH9* locus substantially explains why there is an increased burden of FSGS and hypertensive kidney disease among African Americans (Kuzmanović *et al.*, 2014; Cohn and Weaver, 2006). They carried out admixture-mapping linkage-disequilibrium genome scanning on one hundred and ninety (190) African American individuals with FSGS and two hundred and twenty-two (222) controls and identified a chromosome-22 region centred on *MYH9*, as a functional candidate gene expressed in kidney podocytes (Núñez *et al.*, 2010).

2.7 CLINICAL PRESENTATION

HIV nephropathy, particularly HIVAN is often a later event in the natural chronicle of the HIV disease. Persons with poorly controlled HIV infection and who have elevated viral loads and or low CD4 appears vulnerable most (Rouet *et al.*, 2010). This notwithstanding, HIVAN could be part of early manifestation of HIV infection and it is known to appear at seroconversion (Atta, 2010). Typical presentation of HIV nephropathy include proteinuria, which ranges from isolated proteinuria to nephrotic range, and significant renal dysfunction (McCulloch and Ray, 2008). Other features of nephrotic syndrome including oedema and hypoalbuminemia are barely visible. It remains unclear why such striking features are absent, but it has been postulated that it could be as a result of the salt wasting syndrome in HIV nephropathies such as HIVAN or increased oncotic pressure from increased serum immunoglobulin. Haematuria and hypertension are often absent and when present could be as a result of other causes of renal dysfunction (Atta, 2010; Atta *et al.*, 2008).

2.8 DIAGNOSTIC EVALUATION OF HIV NEPHROPATHY

Diagnosis of HIV nephropathy including HIVAN is confirmed by renal biopsy (Fine *et al.,* 2008). Histological features include both glomerular and tubular lesions involving global or focal segmental glomerulosclerosis, microcystic transformation of renal tubules and interstitial inflammation followed by fibrosis with lymphocytic infiltration.(Fabian *et al.,* 2013; Wearne *et al.,* 2012; di Belgiojoso *et al.*,2002). Glomerular collapse with podocytes hypertrophy have been reported in adults with HIV associated nephropathy, however, many children have unique presentation of mesangial hyperplasia with no development of collapsing glomerulopathy (Gu *et al.,* 2013; Zhong *et al.*, 2005). Other histological features of HIV associated nephropathy include atrophied tubular epithelium and tubuloreticular inclusions on electron microscopy (Wearne *et al.*, 2012).

Kidneys of patients with HIV nephropathy particularly HIVAN are enlarged and have hyperechogenic pattern on ultrasound (Allan, 2011; Vourganti *et al.,* 2010). Renal dysfunction is quite common with raised serum creatinine corresponding to the reduced glomerular filtration rate (Andiman *et al.*,2009). Urine examination in these patients commonly show proteinuria, other findings on urinalysis include hyaline casts and rarely haematuria (Ross, 2014; Ramezani *et al.*,2008).

In the absence of renal biopsy as confirmatory or definitive diagnosis, clinicians have depended on the presence of clinical features or presentations such as the presence of proteinuria, raised serum creatinine levels, origin and race of person as well as enlarged hyperechogenic kidneys upon ultrasound having excluded other causes of renal failure for HIV positive patients (Atta, 2010; Atta *et al.,* 2008).

2.8.1 Diagnosis of Kidney disease

Traditionally, tests for kidney function commonly used for diagnosing renal disease involves a urine test; a simple "dipstick" followed by microscopy (urinalysis). Other tests include blood urea, creatinine and rate of creatinine clearance (Nagai *et al.,* 2013). Recently microalbuminuria and sonographic images have been employed for diagnosing kidney diseases (Hadigan *et al.*, 2013; Ezeonwu *et al.,* 2012; Eke *et al.,* 2010).

2.8.1.1 Urinalysis

Urinalysis is the physical, chemical, and microscopic examination of urine (FolefackKaze *et al*., 2013). It involves a number of tests to detect and measure various elements that pass through the urine (Wongtrakul *et al*., 2014). The test is capable of revealing diseases that have gone unnoticed because they do not produce striking signs or symptoms. Examples of such diseases include diabetes mellitus, various forms of glomerulonephritis, and chronic urinary tract infections (Khatua *et al.*, 2010). While the physical examination looks at the urine colour; straw, amber, blood stained, coca cola etc. and appearance of the urine; clear, hazy or cloudy, the chemical analysis uses a ten (10) or twelve (12) parameter commercially available urine dip stick to determine the urine pH, specific gravity, glucose, micro albumin, protein, blood, ketones, leucocytes, nitrite, bilirubin and urobilinogen and microscopic examination of sediments from the urine deposit (using x 10 and x 40 objectives)(Murray *et al.*, 2014). Of particular interest in the chemical analysis using the dipstick in kidney disease is the presence of protein (macroalbumin; proteinuria), with microalbumin being a precursor (microalbuminuria) (Bertilla Uzoma *et al.,* 2012). The presence of blood using the dipstick only further indicates an aggravation of the problem once other physiological conditions have been ruled out. The presence

of cast and red blood cell in the urine deposit further establishes the possibility of a kidney disease signifying endothelial dysfunction (Murray *et al.,* 2014)

2.8.1.1.1 Proteinuria

Proteinuria is a sign of abnormal excretion of protein by the kidney but is a nonspecific term including any or all proteins excreted (Atta *et al.,* 2005). Clinically the appearance of significant amount of protein in urine is one of the earliest sign of almost all renal diseases (Ramezani *et al.,* 2008). Proteinuria is a well-known marker for renal disease. Estimation of proteinuria helps in differentiating between tubulointerstitial and glomerular diseases and also to follow the progress of renal disease and to assess the response to therapy (Chaparro *et al.,* 2008). Normally excretion in most healthy adults is between 20-150 mg of protein in urine over 24 hrs. Proteinuria more than 3.5 gm/day is taken to be diagnostic of nephrotic syndrome (Coulibaly *et al.,* 2013).

2.8.1.1.2 Microalbumin

Microalbuminuria has been reported as a predictor of subclinical renal involvement in systemic diseases including HIVAN (Mistry, 2010). It refers to albumin excretion above the normal range and has been defined as urinary albumin excretion between 30 and 300 mg/day or in concentrations 20 to 200 μg/min. It develops from progressive, subclinical, structural, and functional changes in the kidney and it is useful as an early biomarker in the detection of kidney disease (Mudi *et al.,* 2014). Studies have reported prevalence of 10–33% reported from Port Harcourt- Nigeria in Africa, India, and the United States (Mistry, 2010). Microalbuminuria; a type of glomerular proteinuria, is an early marker for the development of nephropathy and generalized endothelial dysfunction (Iseki *et al*., 2007). Urine normally contains small amounts of protein including albumin (Hadigan *et al.,* 2013). Up to 150 mg. of protein

per day could be found in normal urine of which 30 mg is albumin (Gupta *et al.*, 2005a). Microalbuminuria is the earliest indication of evolving glomerular pathology. Urinary microalbumin usually refers to urine albumin levels between 30-300mg/24 hours, (or, more commonly 30-300 mg/L, from elevated concentrations in a spot urine sample) levels of albumin which is not detectable by routine urine dipstick. Its persistent presence coupled with overt proteinuria are important markers for the subsequent development of progressive chronic kidney disease (Bertilla *et al.*, 2012). Screening for microalbuminuria and overt proteinuria, and timely referral for nephrology evaluation of these patients in the primary care setting, is critically important and offers the chance of intervention at the earliest opportunity (Masimango *et al.*, 2014).

2.8.1.1.3 Urine Protein to Creatinine Ratio (UPCR)

The urine protein to creatinine ratio (UPCR) is a test used to determine the presence of excess levels of protein in urine. The UPCR is determined by dividing the urine protein (mg/dl) by the urine creatinine (mg/dl), the numerical outcome of which is roughly equivalent to the 24-hour protein excretion in g/day per 1.73 m^2body surface area(Hadigan *et al.*, 2013). Proteinuria is recognized as an independent risk factor for renal disease and is a predictor of end organ damage. It is usually requested in cases where non-albumin proteinuria is suspected or in cases of acute kidney injuries, structural renal tract disease, recurrent renal calculi or prostatic hypertrophy (Masimango *et al.*, 2014). Its value is of essence in especially multisystem diseases where there is potential kidney involvement; systemic lupus erythematosus, and in instances where there are known family history of end-stage kidney disease or hereditary kidney disease (Johnson *et al*., 2004; Ross *et al.*, 1994). The quantification

of proteinuria is considered valuable in assessing the effectiveness of therapy and the progression of the disease (Bianchi *et al*., 1999; Redon *et al.,* 1998)

2.8.1.1.4 Urine Albumin to creatinine ratio (UACR)

This is a calculation generated from estimation of random urine albumin and creatinine expressed as a ratio and which is used to assess the risk of kidney disease development (De Zeeuw *et al.,* 2004). UACR measurement is the recommended first line test for proteinuria detection (NICE 2008). A UACR value > 30mg/g imply the presence of urine albumin and is indicative of chronic kidney disease. UACR values of 3-30mg/mmol per current KIDOQI guideline or NICE clinical guideline 182 refers to a situation of moderately increased albuminuria while values >30mg/g refers to a severely increased situation (De Zeeuw *et al.,* 2004). UACR measurement forms part of the diagnosis, staging and monitoring of chronic kidney disease (CKD) (Reynes *et al.,* 2013).

2.8.1.2 Urea and Creatinine use as a kidney disease diagnostic tool

Urea is a major nitrogenous end product of protein and amino acid catabolism, produced by liver and distributed throughout intracellular and extracellular fluid. It is filtered out of the blood by glomeruli in the kidney and is partially being reabsorbed with water (Antonello *et al.,* 2015). It is useful in the differential diagnosis of acute renal failure and pre renal condition where blood urea nitrogen–creatinine ratio is increased. Increased blood urea nitrogen (BUN) is seen associated with kidney disease or failure, blockage of the urinary tract by a kidney stone, congestive heart failure, dehydration, fever, shock and bleeding in the digestive tract (Mitchell *et al.,* 2006)

Creatinine on the other hand is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body depending on muscle mass. It is a waste product that comes from the normal wear and tear on muscles of the body. The levels of creatinine in the blood can vary depending on age, race and body size (Fabiny and Ertingshausen, 1971). A creatinine level of greater than 1.2 mg/dL for women and greater than 1.4 mg/dL for men may be an early sign that the kidneys are not working properly. The level of creatinine in the blood rises, if kidney disease progresses. When the kidneys are malfunctioning, urea and creatinine are poorly excreted resulting in elevated urea and serum creatinine levels (Rule *et al.*, 2013).

2.8.1.3 Creatinine clearance

This is the volume of blood plasma that is cleared of creatinine per unit time. It could be used to approximate GFR and actually evaluates the rate and efficiency of the kidney's filtration abilities; kidney dysfunction and or decreased blood flow to the kidneys flagged by increased serum or plasma creatinine (Stevens *et al.,* 2006). Creatinine-based formulae are well-known methods used in assessing kidney function; however they are fraught with several shortcomings in the general population (Stevens *et al.,* 2006; Levey *et al.,* 1990) and particularly in HIV-infected persons (Odden *et al.,* 2007). Creatinine based estimations are imprecise, and could potentially lead to the over diagnosis of chronic kidney disease (Inker *et al.,* 2012). Notably, serum creatinine level is affected by non-renal factors, such as diet, race and muscle mass and creatinine clearance is significantly influenced by tubular secretion (Stevens *et al.,* 2006). Compounding the problem is the dependence of most laboratories on the modified kinetic Jaffe method which in itself is bedeviled with limitations (Afolabi *et al.,* 2009) for clinical decisions. Several endogenous and

exogenous interfering substances have been documented to affect the analytical specificity of creatinine when analysed by the Jaffe method. Serum protein and bilirubin in particular is documented to cause an overestimation of serum creatinine by as much as 15%–25% (Peake and Whiting, 2006). Malnutrition among children of sub-Saharan Africa origin, especially those living with HIV/AIDS (Arpadi *et al.,* 2000) further reduces the usefulness of serum creatinine-based formulae in the routine assessment of kidney function and has implications for early detection of impaired kidney function in these children (Jones *et al.,* 2008).

2.8.1.4 Use of cystatin-C (Cys-C)

Cystatin-C is a non-glycosylated low molecular weight protease inhibitor(Abiodun *et al*., 2012). It is produced by all nucleated cells at a constant rate and is freely filtrated by the glomeruli and is completely catabolized in the proximal tubules(Aaron *et al.,* 2012). The concentration of serum Cystatin-C is mainly determined by glomerular filtration, which makes Cystatin-C a better endogenous marker of glomerular filtration rate (Bevc *et al.,* 2012). In a Meta-analysis study by Dharnidharka *et al.*,(2002) it was found that Cystatin C was superior to serum creatinine as a marker of kidney function and glomerular filtration rate (Schwartz *et al.,* 2012). It has been found to be a more useful tool for detecting early renal impairment in most kidney diseases(Schwartz *et al.,* 2012).

2.8.1.5 Neutrophil gelatinase-associated lipocalin (NGAL)

Markers of renal function test whether radioactive or non-radioactive assess the normal functioning of kidneys. Their increase or decrease in valves indicates dysfunction of kidney. Neutrophil gelatinase-associated lipocalin (NGAL) also known as oncogene 24p3 or lipocalin-2is a non-radioactive biomarker involved in innate immunity by sequestrating iron that in turn limits bacterial growth (Yang *et al.,* 2002).

It has been proposed as a biomarker of acute kidney injury (Davarajan *et al.,* 2010). NGAL is secreted in high levels into the blood and urine in cases of acute kidney injuries (Benette *et al.,* 2008). Its levels are a more precise and sensitive as a marker for diagnosing AKI than serum creatinine levels and its increase in urinary excretion has been proven to be due to tubular alterations that take place before any damage can be detected by other (Blázquez-Medela *et al.,* 2014). Additionally NGAL which is a troponin-like biomarker of AKI is easy to measure, unaffected by other biological variables and is capable of both early detection and risk stratification of AKI allowing for earlier diagnosis, correct response, and reduced risk of morbidity and mortality in AKI (Haase *et al.*, 2011).

2.8.1.6 Radiography

Renal ultrasonography with arterial Doppler studies is the single most important test for evaluating all form of CKD patients with an elevated creatinine level. Key among its advantages is the fact that it is the least invasive of all the methods used in identifying obstructive uropathy, the commonest reversible form of renal failure. The Doppler part helps in the identification of patients with bilateral renal artery stenosis, whose kidney function would profit from effective angioplasty. Kidneys of patients with HIV associated nephropathy are enlarged and have hyperechogenic pattern on ultrasound (Allan, 2011; Vourganti*et al.,* 2010).

2.8.1.7 Use of predictive equations (Glomerular filtration rate; GFR and estimated GFR; eGFR)

Glomerular filtration rate is a measure of how well the kidneys remove wastes and excess fluid from the blood. It may be calculated from the serum creatinine level and to enhance the results due to challenges associated with the use of creatinine, age, weight, gender and body size are factored into various formulae during its calculation

(Stevens *et al.*, 2006; Nagai *et al.*, 2013). Other analytes have been used in estimating GFR, notably cystatin-C which is considered a better analyte because it is not affected muscle mass, age and gender (Bevc *et al.*, 2012). Normal GFR can vary according to age (as you get older it can decrease) (Glassock and Winearls, 2009). The normal value for GFR is 90 or above. A GFR below 60 is a sign that the kidneys are not working properly. A GFR below 15 indicates that a treatment for kidney failure, such as dialysis or a kidney transplant, will be needed (Tattersall *et al.*, 2011).

2.8.2 Diagnosing the commonest HIV nephropathy; HIVAN

Definitive diagnosing HIVAN involves renal biopsy, coupled with specialist microscopy (as a gold standard), a technique which is virtually non-existent in majority of health facilities attending to and administering ARTs in Ghana (Fine *et al.*, 2008)

2.9 TREATMENT OF HIV NEPHROPATHIES INCLUDING HIV ASSOCIATED NEPHROPATHY

The treatment of HIV associated nephropathy currently appears to be based on observational studies in that no randomized controlled trial results on the various interventions have been published. Anti-retroviral therapy has been reported in a number of observational studies to influence the course of HIV associated nephropathy and has been linked to reduced incidence of ESRD in HIV infected patients. (Grinsztejn *et al.*,2014; Betjes *et al.*, 2001). Peters et al reported improvement of renal functions after two years of using HAART in a study conducted among HIV infected Ugandans in the Home- Based AIDS care clinical trial (Ando *et al.*, 2012; Brady *et al.*,2010; Daugas *et al.*, 2005)

Other interventions reported to be effective in treating HIV associated nephropathy include Angiotensin-converting enzyme inhibitors (ACEIs) and steroids (Guaraldi *et al.*, 2014). Pathogenesis of HIV associated nephropathy is linked to increased cellular synthesis of transforming growth factor β (TGF-β) which is increased by angiotensin II making ACEIs logical therapy for HIV associated nephropathy (Guaraldi *et al.*, 2014; Yoshida *et al.*, 2014; Nakagawa *et al.*, 2011).

Observational studies have reported corticosteroids to be beneficial in treating HIV infected patients with nephropathy (Eustace *et al.*, 2000). Use of corticosteroids in HIV associated nephropathy is associated with reduction of serum creatinine and proteinuria (Pottel *et al.*, 2010). However the use of steroids have been reserved as second line therapy particularly for patients with deteriorating renal functions despite being on HAART (Richardson *et al.*, 2014; Mofenson *et al.*, 2009).

2.9.1 Antiretroviral use and renal dysfunction

The epoch of HAART and the administration of ARVs have been followed by a variety of harmful effects and damage to the kidneys. These effects are evidenced as acute renal failure, kidney stones, tubular necrosis or chronic renal failure (Bae *et al.*, 2014; Gupta *et al.*, 2005a). Three groups of HAART are used in most African countries including Ghana. These include nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors (PIs), (Wyatt, 2014; Brady *et al.*, 2010).

Nucleoside analogues include Lamivudine (3TC), Zidovudine (AZT or ZDV), Abacavir (ABC), Emtricitabine (FTC), Didanosine (ddl), Stavudine (d4T) and Zalcitabine (ddc). Tenofovir (TDF) is the only nucleotide analogue approved for treatment of HIV (Giacomet *et al.*, 2013; Nishijima*et al.*, 2012; Antoniou *et al.*,2005).

Nucleoside analogues have a favourable renal safety profile, with only rare reports of direct toxicity to the renal tubules (Mausumee*et al.,* 2014), Lamivudine and Abacavir have been entailed in Fanconi syndrome (Hannon *et al.*,2004), and ABC have been reported to cause immuno-allergic interstitial nephritis (Giacomet *et al.* 2013; Maggi *et al.,* 2012).

TDF is an acyclic phosphonate that has been linked with the development of renal tubular damage (Maggi *et al.,* 2012). Tenofovir toxicity result in proximal tubulopathy which may present with proteinuria, hypokalaemia, glycosuria, hypophosphatemia, phosphaturia and aminoaciduria (Jafari*et al.*, 2014; Giacomet *et al.,* 2013; Purswani *et al.,* 2013). Mild elevation in serum creatinine has been reported after starting Tenofovir with no clinically significant nephrotoxicity in retrospective study conducted in Toronto, Canada between January 2002 and December 2003 by Antoniou et al. In a multicentre prospective study which was carried on in HIV infected children in Spain, Soler-Palacin *et al*., 2008 reported abnormal urine osmolality, decreased tubular phosphate absorption and proteinuria, no significant changes in creatinine clearance was reported.

Efavirenz (EFV) and Nevirapine (NVP) are two normally used non-nucleoside analogues (NNRTIs). These two as a group have the least nephrotoxic potential. These drugs are principally metabolized by hepatic cytochrome P450 and are not actively secreted in the kidney (Gupta *et al.,* 2008).

Protease inhibitors (PIs) do not exhibit profound nephrotoxic effects as they are metabolized by hepatic cytochrome P450. Indinavir is the most commonly used PI linked with renal and urologic side effects, which manifest with reversible acute renal failure, chronic renal failure, leukocyturia, microhaematuria, mild proteinuria,

nephrolithiasis, papillary necrosis and crystalluria (Nagai *et al.*, 2013). Andiman *et al.,* (2009) reported a twofold increased risk of renal dysfunction among HIV infected children and youth, exposed to Tenofovir and or Indinavir as compared to participants exposed to other ARV drugs in a Paediatric AIDS Clinical Trial Group multi-centre study in United States.

2.10 CHRONIC KIDNEY DISEASE AND HIV INFECTION

Chronic kidney disease (CKD) is defined as presence of kidney damage or glomerular filtration rate (GFR) of less than 60mL/min/1.73 m^2 for 3 months or more, irrespective of the diagnosis (Coresh, *et al.*, 2003). Classification of CKD in children is similar to adult classification which is based on National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (NKF-K/DOQI) classification scheme (Chadha *et al.*, 2007). According to K/DOQI classification CKD is characterized by stage 1 which is mild disease through stage 5 which is end stage renal disease.

In children, glomerular filtration rate is estimated using a modified formula which was established and modified by Schwartz *et al.,* (2009) which utilizes serum creatinine and height of the child (Schwartz, 2009). Normal levels of GFR in children varies with age, gender and height, and increases with age reaching approximately adult level at the age of 2 years, therefore the GFR ranges used to define the 5 CKD stages applies only to children aged 2 years and above (Chadha *et al.*, 2007; Coresh *et al.*, 2003)

Table 2.1: National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (NKF-K/DOQI) stages of chronic kidney disease

Stage	Description	GFR(mL/min/1.73m^2)
1	Kidney damage with normal or increased GFR	>90
2	Kidney damage with mild decrease in GFR	60-89
3	Moderate decrease in GFR	30-59
4	Severe decrease in GFR	15-29
5	Kidney Failure	<15 or dialysis

Chronic kidney disease affects HIV infected persons and is one of the leading non-infectious complications (Estrella *et al.*, 2010). HIV associated nephropathy is the commonest cause of CKD in HIV infected patients (Rachakonda *et al*., 2010; Weiner *et al.*, 2003). Chronic kidney disease causes increased progression to AIDS and death in HIV infected patients even in those who are on HAART (de Silva *et al.*, 2007; Gupta *et al.*, 2005b; Szczech, 2004). Kidney disease should be detected early in HIV infected patients in order to slow its progression and to improve outcome of HIV infection.

National Kidney Foundation (NKF) defines CKD as abnormal urinalysis in the form of proteinuria and estimated GFR < 60 mL/min/1.73 m^2 of at least 3 months duration (NKF-K/DOQI, 2002). This makes screening for CKD in HIV infected patients possible by evaluating for proteinuria and renal dysfunction as recommended by

Association of the Infectious Diseases Society of America (IDSA) (Gupta *et al.,* 2005a).

Urinary protein excretion in children is <4mg/m^2/hour or <100mg/m^2/day for both sexes (Hogg *et al*., 2003). Albumin makes up 30-40% of urinary excreted protein, other components of urinary protein include Tamm-Horsfall protein (50%), immunoglobulins (5-10%) and light chains (5%) (Jefferson *et al.* 2008). Proteinuria is an excretion of protein exceeding the normal values and it is a feature of chronic kidney disease and has also been reported as a predictor of ESRD (Iseki *et al.,* 2003) Albumin excretion in urine between 30 to 300 mg/day is referred to as microalbuminuria (Vora *et al.,* 2000) and cannot be detected by tests used for testing proteinuria. Microalbuminuria is an early marker of nephropathy in both diabetic and non-diabetic patients; therefore excessive albumin excretion may signify renal glomerular disease (Hoy *et al*., 2001). Microalbuminuria has been reported as a predictor of proteinuria in HIV infected individuals and is associated with poorly controlled HIV infection with low CD4 count and high viral load (Szczech *et al.*, 2007). Eighty six percent (86%) of patients who presented with microalbuminuria had HIV associated nephropathy proven by biopsy in a study which was conducted among HIV infected adults in South Africa by Han *et al*. (2006)

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 STUDY DESIGN

The study was a case control one carried out between November 2013 and December 2015.

3.2 STUDY SITES

The study was conducted in the Kumasi Metropolis and the Asante-Akim North District all in the Ashanti region of Ghana. Per the latest HIV sentinel report released in June 2014 by the Ghana AIDS Commission, Ghana's HIV prevalence even though had declined from 1.37% in 2012 to 1.30% in 2013, that for Ashanti Region had slightly increased and now stands at 3.2%, only second to the Eastern Region which had a prevalence of 3.7%. Again the number of new HI childhood infections had increased from eight hundred and fifty-two (852) in 2012 to two thousand two hundred and forty-eight (2248) nationwide in 2013 making the selection of the study site relevant.

3.2.1 Komfo Anokye Teaching hospital (KATH)

The Komfo Anokye Teaching hospital (formerly Kumasi Central hospital), named in honour of a legendary fetish priest of the Ashanti kingdom is located on a hill overlooking the city of Kumasi in the Ashanti region, and is built on the former site of the African and European hospitals. With a bed capacity of thousand (1000), it is the second-largest hospital in Ghana, and the only tertiary health institution in the Ashanti Region. Indeed, until the recent upgrade of the Tamale Regional Hospital to Teaching Hospital, Komfo Anokye Teaching hospital (KATH) was the main referral

hospital for the Ashanti, Brong Ahafo and Northern regions of Ghana hence handling referrals from Northern, Upper East and Upper West (KATH Annual Report, 2013).

At the time of this study there were two (2) specialist doctors and a consultant paediatric nephrologist who regularly attend to children with HIV. These personnel were ably supported by five nurses (5) and a couple of rotational doctors.

3.2.2 Agogo Presbyterian Hospital

The Agogo Presbyterian Hospital is the oldest mission hospital in Ghana and is one of the Christian Health Association of Ghana (CHAG) facilities. It has a bed capacity of two hundred and fifty (250) (Agogo Presbyterian Hospital Annual Report 2014). It is located in the Asanti-Akim North Municipality were it serves as a referral site for the entire Asanti-Akim area and also for patients from all over Ghana and neighbouring countries of Togo, La Cote d'Ivoire and Burkina Faso who come for ophthalmological care. Its paediatric unit has fifty (50) beds (thirty (30) medical and twenty (20) surgical) (Agogo Presbyterian Hospital Annual Report 2014). Currently the unit has one (1) Paediatrician consultant, two (2) resident doctors in paediatrics, two (2) medical officers and two (2) house officers. Additionally there are eight (8) nurses aside one Physician assistant who attend to children with special needs including HIV.

3.2.3 Suntreso Government Hospital

The Suntreso Government Hospital is a Ghana Health Service facility located in the Suntreso North sub-metro within Kumasi. It has a total bed capacity of 116 including twenty six (26) paediatric beds (Suntreso Government Hospital Annual Report, 2014). It is one of the main referral sites for sexually transmitted infection (STI) including HIV. The STI clinic at the start of this project had four (4) doctors including a

paediatrician. There were two Physician assistants and four (4) nurses who attended to these children.

3.3 SAMPLE POPULATION

The sample population comprised both children living with HIV/AIDS (CLWHA) as well as HIV negative ones who qualified per the inclusion criteria and whose parent or care givers consented from the study sites to be part of this study.

3.4 SAMPLE SIZE AND SAMPLING

Using the formula $n = \frac{z^2 (0.50)(0.50)}{(E)^2}$

Where: Z is the standard score for the confidence level

E is the allowable error

Given that z was 1.95 and an error of 0.05 was allowed, then a total of 380 cases (HIV infected) attending the study hospitals were recruited and enrolled into the study as follows;

Agogo Presbyterian Hospital: 90

Suntreso Government Hospital: 90

Komfo Anokye Teaching Hospital: 200

Additionally, one hundred and fifty (150) HIV negative participants (control group) with similar inclusion and exclusion criteria whose parents or care givers had either signed or thumb printed the study's assent form were recruited alongside the case group in the ratio of 1:1:1 from the OPDs of the three study hospital. Except for the immunological assay (CD4 and CD3) which was not done for these HIV negative

subjects, all other parameters similar to those done for the children with HIV were done for them.

3.5 INCLUSION AND EXCLUSION CRITERIA

3.5.1 Inclusion Criteria

The case group comprised known (confirmed) HIV positive (HIV 1, HIV 2 or both) children aged between two (2) years and thirteen (13) years who attended the HIV clinics of the study sites and whose parents or care-givers had assented to be part of the study. The control group however were drawn from the out-patients department of the same study sites but from clinics other than the HIV clinics. The essences of the study was explained to their parents and care-givers and were made to understand that HIV testing as well as hepatitis B and C would be conducted on the blood samples of their children to confirm their HIV and hepatitis status before included in the study as HIV- negative control. Pre and post-test counselling was done individually for these parents and care-givers prior to sample collection. Children from the control group had similar characteristics like the case group except they were HIV-negative

3.5.2 Exclusion Criteria

HIV negative children and children living with HIV/AIDS who did not meet the age requirements of two years (2) to thirteen (13) years as well as participants who had co-infection with Hepatitis B or C viruses were excluded from the study. Additionally participants who had a recent case of pulmonary tuberculosis (TB) or were being treated for TB were excluded from the study**.**

3.6 DATA COLLECTION

3.6.1 Measurement of anthropometric variables

Anthropometric measurements included height, measured to the nearest centimeter (cm) without shoes and weight to the nearest 0.1 kilogram (kg) in light clothing. Participants were weighed on a bathroom scale (Zhongshan Camry Electronic Co. Ltd, Guangdong, China) and their height measured with a wall-mounted ruler. Percentile body mass index (BMI) was computed for each child. The CDC defines ≥95th percentile as obese, 85^{th}-94^{th} percentiles as overweight and 5^{th}-84^{th} percentile as normal and $<5^{th}$ percentile as underweight (Centers for Disease Control and Prevention, 2009; Gonzalez-Casanova *et al.*, 2013).

A total of three hundred and ninety two (392) HIV positive participants through their parents or care-givers assented to be part of the study; however twelve (12) had to be excluded because of co-infection with hepatitis B or C or both. Two (2) of the one hundred and fifty (150) HIV negative participants also had to be replaced because of Hepatitis B.

3.7 SAMPLE COLLECTION

Five (5) mls of blood sample was collected from each participant through a venesection into a serum gel separator tube (3mls; serum was analysed for urea, creatinine and cystatin-C) and an ethylenediamine tetra acetic acid (EDTA) tube (2mls; analysed for full blood count (FBC) and CD4). The gel samples were allowed to clot and later centrifuged at 500rpm for ten (10) minutes using (IEC CL 30 by Thermo Electron Corporation). The sera obtained from the gel samples were aliquoted into 3 pre-labelled cryo-tubes per subject for storage at -20^{o}C until assayed.

Additionally, fresh early morning (at least two hour bladder incubation) urine samples were collected and urinalysis as well as microalbumin determination was done for each subject. Urine deposits were examined microscopically particularly for cast, crystal, yeast-like cells, red blood cells (RBCs), and pus cells among others.

3.8 LABORATORY METHODS

3.8.1 Blood Biochemical Analysis

The sera were analysed for creatinine and urea using Elitech reagents on a fully automated biochemistry analyser (Selectra Junior; Vital Scientific NV, Netherlands). Cystatin-C (cys-C) was done using a DuoSet sandwiched enzyme linked immunosobent assay (ELISA) system. Two (2) sets of aliquotsera from each subject were brought to room temperature prior to analysis.

3.8.1.1 Serum Creatinine (CRE)

The technique which was employed in analyzing the creatinine in this study was based on the Jaffe modified kinetic method described by Fabiny *et al.,* (1971). Creatinine in each sample was reacted with picric acid in an alkaline condition and the coloured complex so obtained measured at 510 nm on the auto analyser. The rate of formation of colour was proportional to the creatinine in the sample;

$$\text{Creatinine} + \text{Sodium picrate} \xrightarrow{\text{Alkali}} \text{Creatinine picrate complex}$$

3.8.1.2 Blood Urea Nitrogen (BUN)

The method used in assaying urea in this study was based on the modification of the Urease/Glutamate dehydrogenase (GLDH) method by Talke *et al.,* (1965). Urea in each sample was hydrolysed to ammonia (NH_3) and carbon dioxide (CO_2) in the presence of water and urease. The liberated ammonia was then reacted with α-

ketoglutarate in the presence of NADH and Glutamate dehydrogenase to form L-Glutamate and NAD+. Absorbance was measured at 340 nm. The initial rate of this change was proportional to the amount of urea in the sample.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \alpha\text{-Ketoglutarate} + NADH \xrightarrow{\text{GLDH}} \text{L-Glutamate} + NAD^+$$

3.8.1.3 Cystatin C

3.8.1.3.1 Preparation

100ul of capture antibody working solution was used to coat a ninety-six (96) microplate well after which they were sealed and incubated overnight at room temperature. Subsequently, aspiration wash was done three (3) times on the wells using 400ul of wash buffer and excess buffer was blotted on a clean paper towel. Blocking was done with 300ul of the reagent diluent after which the wells were incubated for at least 1 hour at room temperature. Aspiration wash was repeated once more to make the wells ready for the samples

3.8.1.3.2 Assay Procedure

100ul of samples and or standard diluted in reagent diluent was added to the pre-coated wells and incubated at room temperature for 2 hour after sealing with an adhesive strip. Aspiration wash similar to that described above was performed prior to the addition of 100ul of detection antibody suspended in reagent diluent. The wells were sealed and incubated for 2 hour at room temperature. The addition of working solution of Streptavidin HRP (100ul) and substrate solution (100ul) before incubating wells room temperature (for 20 minutes) were preceded by aspiration wash. 50ul of

stop solution was added to each well (mixing thoroughly) before determining their optical densities at 450nm with a wave length correction at 540nm

3.8.1.3.3 Result calculation

The average of duplicate readings for the standard, control and samples were subtracted from the average zero standard optical density. A standard curve was then created by reducing the data which was obtained with computer software capable of generating a four parameter logistics (4PL) curve.

3.8.2 Haematological Analysis

The Sysmex KX-21 is a fully automated analyser capable of analysing the following parameters using three detector blocks and two separate kinds of reagents (cell pack and stromatolyser-WH):

Whole white blood cell (WBC) (Analysis principle: DC detection method)

RBC (red blood cell) (Analysis principle: DC detection method)

HGB (Haemoglobin) (Analysis principle: Non-Cyanide haemoglobin analysis method)

HCT (Haematocrit value) (Analysis principle: RBC pulse height detection method)

3.8.2.1 Principle of Operation

In the blood cell count by DC detection method, the anticoagulated blood sample is aspirated and measured to a predetermined volume, diluted to a specific ratio, and then fed into each transducer. The transducer chamber has a minute hole called the aperture besides which the electrodes generate direct current. Blood cells suspended in the diluted sample pass through the aperture and cause a direct current resistance to change between the electrodes. As direct current resistance changes, the blood cell

size is detected as an electric pulse. Blood cell count is calculated by counting the pulses, and a histogram of blood cell sizes is plotted by determining the pulse sizes. Histogram could be used to analyse the various data generated.

3.8.2.2 Non-Cyanide Haemoglobin Analysis Method

Unlike other automated analysers which use the cyanmethaemoglobin or oxyhaemoglobin technique to estimate the haemoglobin, the Sysmex KX-21 by the use of the non-cyanide analysis method combines the strength of the two techniques such that it compensates for the deficiencies of not being able to yield high results because of the presence of excess methaemoglobin as in the case of oxyhaemoglobin technique and also avoids the toxic nature of the cyanide used in the cyanmethaemoglobin technique

3.8.2.3 WBC/HGB Analysis Flow

3.8.2.3.1 Whole Blood Mode

6ul of the EDTA anticoagulated blood sample was aspirated via the sample valve and transferred into the WBC transducer chamber alongside 1.994ml of the diluent with a concurrent addition of 1.0ml of WBC/HGB lyse to give a 1:500 dilution of the sample. The mixture having been allowed to react for at least 10 seconds caused the RBC to haemolyse and the platelet to shrink while the WBC remained intact. Approximately 1ml of the diluted haemoglobin sample in the WBC transducer is transferred to the HGB flow cell. A light beam of wave length 555nm is subjected to the sample in the HGB flow cell and the concentration measured as absorbance and compared to that of the diluent alone that was measured before the addition of the sample thereby calculating the Haemoglobin value. 500ul of the sample in the WBC transducer is aspirated through the WBC aperture during which pulses generated by the cells are detected by a DC method.

3.8.2.3.2 RBC/PLT Analysis Flow in Whole Blood Mode

4ul of EDTA anticoagulated blood was aspirated via the sample probe into the sample rotar where a 1: 500 dilution of it is made by the addition of 1.996mls of the diluent and brought to the mixing chamber (first dilution). A second dilution of 1:25000 is made by adding 1.96mls of the diluent to 40ul of the first dilution and aspirated via the sample rotar valve into the RBC/Plt transducer chamber (second dilution). 250ul of the second dilution in the RBC/Plt transducer is aspirated through the aperture where RBC and platelets are counted by a DC method. Haematocrit (HCT) was measured simultaneously by the RBC pulse height detection method.

3.8.2.3.3 Calculation of RBC Constant

RBC constant (mean RBC volume, mean RBC haemoglobin, mean RBC haemoglobin concentration) is calculated from RBC, HGB, and HCT.

Mean RBC Volume (MCV)

Calculation is made from RBC and HCT by the formula below:

$$MCV\ (fl) = \frac{HCT}{RBC}$$

Mean Cell Haemoglobin (MCH)

Calculation is made from RBC and HGB by the formula below:

$$MCH\ (pg) = \frac{HGB}{RBC}$$

Mean Cell Haemoglobin Concentration (MCHC)

Calculation is made from HCT and HGB by the formula below:

$$MCHC\ (g/dl) = \frac{HGB}{HCT}$$

3.8.3 Immunological assays

Immunological assays for cell counts relating to cluster of differentiation (CD4 and CD3), was done using the FACS count flow cytometer (Becton Dickenson and Company, California, USA) on the non-haemolysed anticoagulated (EDTA) sample within two (2) hours of sample collection. The analyser also gave the ratio of CD4 and CD3 for each sample.

3.8.3.1 Principle of Operation

When EDTA whole blood is added to the CD4/CD3 reagents, antibodies which are fluorochrome-labelled in the reagents bind specifically to antigens on the lymphocyte surface. These are then fixed in a formalin base fixative solution and analysed on the FACS flow cytometer. The cells come in contact with a laser light, which causes the fluorochrome-labelled cells to fluoresce. This fluorescent light provides the information necessary for the instrument to count the cells. In addition to containing the antibody reagent, the reagent tubes also contain a known number of fluorochrome-integrated reference beads. These beads function as a fluorescence standard for locating the lymphocytes and also as a quantitation standard for calculating the cells. Analysis is automatic. The software identifies T-lymphocyte populations and calculates the absolute counts.

3.8.4 Estimation of GFR

Estimated glomerular filtration rates (eGFRs) for the various participants were calculated using creatinine based renal function equations (Schwartz; eGFR = k (k=.055) x height (cm) / serum creatinine (mg/dl), Counahan-Barrat; eGFR = k (k= 0.43) x height (cm) / Serum creatinine (mg/dl) and Leger; eGFR= k (k= 0.542) x (weight (kg)/serum creatinine (mg/dl))+9.948 (height $(m)^2$ /serum creatinine (mg/dl)))

or cystatin based equations (Larsson; eGFR = 77.24 × ($Cys^{-1.2623}$), Rule; eGFR = 76.6 × $Cys^{-1.16}$ and Zapittelli; eGFR = 75.94 × $Cys^{-1.17}$).

3.8.5 Urinalysis

Each participant was given a 50ml urine sample container and with guidance from their parent or care-giver instructed to void directly into the container. Each participant produced at least 20mls of fresh urine (at least 2 hours of bladder incubation) which was kept in an ice chest at a temperature of about 4-8 C prior to analysis. Each urine sample was brought to room temperature prior to analysis. Urinalysis covered macroscopic (i.e. physical examination looking at colour; straw, amber, blood stained, coca cola etc and appearance of the urine; clear, hazy or cloudy), chemical analysis (using an eleven (11) parameter commercially available urine dipstick; Dirui H11-800MA from Dirui Industrial Co., Ltd, Changchun jili 130012 P.R China), and microscopic examination of sediments from the urine deposit (using x 10 and x 40 objectives).

3.8.5.1 Test Procedure

About ten (10) mls of well mixed uncentrifuged urine sample which had been brought to room temperature was poured into pre-labelled plastic centrifuge tube for each subject.

A single eleven (11) parameter Dirui urine dipstick was removed from its container (hundred (100) per container) and its reagent area completely immersed in the urine for a few seconds (between 3-5 seconds). The immersed dipstick was tapped against the rim of the urine container upon removal to take out excess urine from the strip and also possible mix up or contamination from adjacent reagent pads. Each dipstick was read by matching it against the colour chart on its container according to the

manufacturer's waiting and reporting time. Control sample were run in similar manner as described above with urine samples known to be negative or positive for the various parameters on the urine dipstick.

3.8.5.2 Test Principles and Limitation of the urine dipstick

The diagnostic strips for urinalysis are firm plastic strips to which are affixed several separate reagent areas. These tests provide information regarding the status of carbohydrate metabolism, kidney and liver function, acid-base balance, and urinary tract infection

3.8.5.2.1 Leukocytes

The test unveils the presence of granulocytes esterases. Granulocyte esterase through a hydrolytic action breaks down indoxyl esters and the resultant indoxyl interacts with diazonium salt to give a purple colour. Granulocytes esterase tests could be positive in the absence of intact microscopic cells especially when the leukocytes have been destroyed. Vaginal discharge could occasionally give false positive result. Urine glucose concentrations between 55-110mmoland high specific gravity could cause decreased test result sensitivity. Additionally the presence of cephalothin and tetracycline could cause decreased reactivity, and their high levels in urine could cause a false negative reaction.

3.8.5.2.2 Urobilinogen

This test is founded on the Ehrlich reaction in which p-dimethylaminobenzaldehyde react with urobilinogen. The test area detects urobilinogen in concentrations as low as 3µmol/L (approximately 0.2 Ehrlich's units/L) in urine with a colour range of beige through pink to dark pink. The reagent area could react with interfering substances known to react with Ehrlich's reagent. Excreted pigments and medicaments that have

intrinsic coloration in acidic medium could produce false positive results. The test is inhibited by elevated concentrations of formaldehyde. Strip reactivity increases with temperature; the optimum temperature is 22˚C-26˚C.

3.8.5.2.3 Bilirubin

The test principle is based on the van den Bergh principle in which there is the coupling of bilirubin with diazonium salt in an acid medium resulting in the formation of azobilirubin. Normally bilirubin is not detectable in urine by even the most sensitive methods. Even trace amounts of bilirubin are significantly abnormal to require additional investigation. Reagent area produces various shade of pink to violet for a positive test. Some urine components (medicines, urinary indicants) may produce a yellowish or reddish discoloration of the test paper that could interfere with interpreting the result. Ascorbic acid concentrations of 1.4mmol/L or greater could cause false negatives.

3.8.5.2.4 Blood

The test principle is based the peroxidise-like activity of haemoglobin and myoglobin. Haemoglobin and myoglobin catalyse the oxidation of the indicator by means of organic hydroperoxide contained in the test paper resulting in the formation of dimethylbenzidine. This test is highly sensitive and colour change ranges from yellow for negative through green and dark blue for positive test. Intact red blood cells give green spots on the reagent area. The sensitivity of this test may be reduced in urine with high specific gravity. The test is equally sensitive to myoglobin as to haemoglobin (Haemoglobin concentration of 150µg/L-620µg/L is approximately equivalent to 5-15 intact red blood cells per microlitre). Captopril and Lodine may also cause decreased reactivity. Blood is often found in the urine of menstruating females. Certain oxidizing contaminants, such as hypochlorite, could produce false

positive results. Microbial peroxidase associated with urinary tract infection could cause a false positive reaction. Ascorbic acid concentrations of 1.4mmol/L, or greater may cause false negatives at the trace levels.

3.8.5.2.5 Nitrite

The test is based on the Griess's test principle where dietary nitrate is converted to nitrite by the action of Gram negative bacteria in urine resulting in formation of 1, 2, 3, 4-tetrahyrobenzo (h) quinolin-3-ol-azo complex. The test is particular for nitrite. Any degree of uniform pink colour development is interpreted as a positive. A nitrite positive test indicates the presence of bacterial of about 10^5 or more organisms per ml. The colour generated on the test area does not necessarily equate to the number bacteria present and a negative result does not necessarily mean that there is no significant bacteria. Negative results could occur when urinary tract infections are caused by organisms which do not contain reductase to convert nitrate to nitrite or when urine has not been retained in the bladder long enough (4hrs- 8hrs) for reduction of nitrate to occur. Again, when dietary nitrate is absent, and organisms containing reductase are present and bladder incubation is ample specimens containing nitrite ion concentrations of 35µmol\L or less could give a negative result.

3.8.5.2.6 pH

The test is used to access the acidity or alkalinity of a urine sample. It is based on the double indicator principle and contains a mixed indicator which assures marked change in colour to cover the entire urinary pH of 5to 8.5.

3.8.5.2.7 Specific gravity (SG)

The specific gravity test assesses the diluting and concentrating ability of the kidney. The reagent area of the dipstick detects urine SG ranging from 1.000 to 1.030. Its

reagent area has a detergent which reacts with bromthymol blue indicator in the presence of ionic constituents (solute) in urine resulting in a change of colour which ranges from green to yellow. Generally SG values correlate within 0.005 with values obtained with the refractive index method. Accuracy increases by adding 0.005 for urine with pH 7 and higher and 0.010 with pH 8 and higher. Extremely alkaline urine could result in low readings when compared to other methods and presence of protein in quantities greater than 5g/L in urine could result in higher SG values.

3.8.5.2.8 Protein

The test is based on the protein error of indicator principle where tetrabromopheno-blue changes colour in the presence of protein. The reagent area of the dipstick is more sensitive to albumin could be altered in urines with elevated pH (up to 9). Residual disinfectants containing quaternary ammonium groups or chlorhexidine in the urine vessel could lead to a false positive result. Colour change ranges from yellow through various shades of green to blue.

3.8.5.2.9 Glucose

The test is based on the glucose oxidase-peroxidase reaction. Glucose oxidase catalyses the formation of gluconic acid and hydrogen peroxidise from the oxidation of glucose. The peroxidase then catalyses the reaction of hydrogen peroxide with a potassium iodide chromogen to form benzoqoinoneimine which gives a range of colours. The test is glucose sensitive; no substance voided in urine other than glucose is known to give a positive result. Ascorbic acid of more than 1.4mmol/L and high ketone concentrations (8mmol/L) could cause false negatives for specimens containing small amounts of glucose (5.5mmol/L). The responsiveness of the glucose test diminishes as SG of the urine increases. False positive reactions could be caused

by hypochlorite or peroxide (often associated with cleaning agents). Reactivity could also vary with temperature.

3.8.5.2.10 Ketone

This test is based on the legal's test principle where acetoacetic acid reacts with nitroprusside to form enamine which gives shades of colours ranging from buff-pink for negative to maroon for positive. The test is much more sensitive to acetoacetic acid than acetone. It is however not sensitive to β-hydroxybutyne acid. Some high SG and or low pH urines could give trace results. Urine specimen from healthy persons usually yields negative results for the ketone test. False positive results could occur with highly pigmented urine specimens or those containing large amounts or levodopa metabolites.

3.8.5.2.11 Urine sediment preparation

Each of the 10ml urine samples was spun at 1500rpm for 3 minutes with a centaur 2 IEC CL 30 centrifuge from Thermo Electron Corporation. Subsequently the supernatant was completely decanted and the tubes reinvented to allow traces of the urine around the inner walls of the tube to settle on the deposit. The sediments were re-suspended with a few finger taps at the base of each tube. With the help of a plastic Pasteur pipette a drop (about .01ml) of the well mixed deposit was placed on clean, grease free, dry glass slide and covered with a 22x22 mm cover slip for microscopic examination. Each prepared wet preparation was examined first with x10 objective to scan the field and also x40 for further identification of various cells. The principle of the urine sediment preparation is based on gravity, thus the relatively heavy components of the urine settled at the base for re-suspension and subsequent examination.

3.8.5.2.12 Microalbumin

Microalbuminuria refers to an albumin concentration in the urine which is greater than normal, but which is not usually detectable with routine protein dipstick assays which allow measurement of albumin at levels of 15 mg/dL or greater. Albumin is the commonest protein implicated in majority of renal diseases. Thus, monitoring lower concentrations of it in the urine is useful for early detection in patients at risk for renal disease. The test involved the use of sulfone phthalein, a highly sensitive dye for microalbumin using the protein error method (using urine dipstick from DIRUI industrial co., ltd, Changchun jili 130012 P. R China).

3.9 ETHICAL ISSUES

Ethical Clearance was obtained from the Joint Committee on Human Research, Publication and Ethics (CHRPE) of the School of Medical Science (SMS), Kwame Nkrumah University of Science and Technology (KNUST)/ Komfo Anokye Teaching Hospital (KATH). Permission was also sought from Agogo Presbyterian Hospital and Suntreso Government Hospital.

The parents and care-givers of the study participants acceded for their children to be involved in the study after they had been given enough information about the study so as to allow them make an informed voluntary decision for these children. Assent forms were given to these representatives of the study participants to sign or thumb print before the commencement of the study. Additionally, options were made for these subjects through their representatives to opt out of the study without reason at any point of the research.

The study was conducted in accordance with the Ethical Principles of the committee on human research, publication and ethics (CHRPE) - Kwame Nkrumah University of

Science and Technology/ School of Medical Sciences (KNUST/SMS) and under standard laboratory practices. All wastes were disposed in accordance with the waste management protocol (infectious materials were discarded into yellowed coloured plastic lined dust bins while the surface of the working benches were disinfected using 0.5% hypochloride solution) . The study was subjected to a high quality external quality control (EQC) checks and about 10% of samples were randomly selected and delivered to another reputable laboratory; Medilab diagnostic center-Bantama, for confirmation and validation of results. Additionally, the biochemical analysis included calibrators and controls from the reagent manufacturer (ELICAL and ELITROL; ELITEC reagents) which had to be reconstituted, analysed and passed or accepted by the Selectra junior biochemistry analyser before the study samples were analysed.

3.10 STATISTICAL ANALYSIS

Data was recorded into excel and analysed with GraphPad Prism version 5 (San Diego Carlifornia, USA, www.graphpad.com). Data was expressed as mean ± SD and proportions. Continuous data were compared using the unpaired t-test while categorical data were compared using chi square ($\chi 2$) test. The P value was set at $P < 0.05$ of confidence interval.

CHAPTER FOUR

4.0 RESULTS

Table 4.1 presents the general characteristics of the studied population stratified by infection with the case group being categorized as HIV-infected and the control group as HIV negative. There was no significant difference in age among the two classification criteria (7.4 ± 2.6 and 7.5± 2.5years, p=0.7729 for case and control groups respectively). The control group were significantly heavier (20.2 ± 6.6 kg) when compared with the case group (18.7 ± 6.7, p=0.0232). No significant difference was observed in the mean height of the two groups but the BMI was significantly increased in the control group (15.6 ± 2.0 kg m^{-2}) compared with the case group (14.7 ± 1.8 kg m^{-2}, p<0.0001). The proportion of participants within the case group who were on drugs was 77.1% mean duration of infection of 3.7 ± 2.4 years with a mean duration on drugs of 3.1 ± 2.2 years. The BMI of the study participants was categorized using BMI-for-age to estimate the proportion of participants falling within the extremes of normal weight. A Chi-square for trend analysis showed a significant difference in the number of the participants within the sub-categories with 11.3% of the case group falling within the category of grade 3 thinness compared to 2.0% for the control group. 8.6% of the participants within the case group versus 4.0% of the participants within the control group had grade 2 thinness. 12.7% of the participants within the case group and 9.4% of those within the control group were within the grade 1 thinness category. No significant differences were observed in the mean concentrations of urea (3.6 ± 2.1 vs 3.3 ± 1.4 mmol L^{-1}; p=0.0710) and creatinine (60.8 ± 25.7 vs 62.0 ± 12.3 mmol L^{-1}) as observed among the case group and the controls.

Table 4.1: Demographic and biochemical characteristics of the studied population

Variables	HIV-infected (n = 362)	HIV-negative (n = 149)	p-value
Age (years)	7.4 ± 2.6	7.5 ± 2.8	0.7729
Weight (kg)	18.7 ± 6.7	20.2 ± 6.6	0.0232
Height (cm)	111.1 ± 17.4	112.0 ± 15.2	0.5735
BMI (kg m^{-2})	14.7 ± 1.8	15.6 ± 2.0	< 0.0001
Duration of Infection (years)	3.7 ± 2.4		
Duration of drug use (years)	3.1 ± 2.2		
BMI categories			
Grade 1 thinness	46 (12.7)	14 (9.4)	
Grade 2 thinness	31 (8.6)	6 (4.0)	
Grade 3 thinness	41 (11.3)	3 (2.0)	
Normal weight	240 (66.3)	119 (79.9)	
Obese	3 (0.8)	5 (3.4)	
Overweight	1 (0.3)	2 (1.3)	< 0.0001
Sex			
Male	165 (45.6)	61 (41.9)	
Female	197 (54.4)	88 (59.1)	0.3780
Drug use			
Yes	279 (77.1)		
No	83 (22.9)		
Urea (mmol L^{-1})	3.6 ± 2.1	3.3 ± 1.4	0.0710
Creatinine (µmol L^{-1})	60.8 ± 25.7	62.0 ± 12.3	0.5712

Data are presented as means±SD and proportions. P-value defines the level of significance when HIV-infected children were compared with HIV-negative children (unpaired t-tests); BMI categories were determined for age and sex using z-anthropometry

Figure 4.1 shows the percent distribution of HIV-infected children on antiretrovirals within the three categories of nucleoside reverse transcriptase inhibitor, non-nucleoside reverse transcriptase inhibitor and a combination of both. A great majority (51.7%) of the children were on lamivudine (3TC) + zidovudine (ZDV) + efavirenz (EFV) combination followed by lamivudine + zidovudine + nevirapine (18.8%), lamivudine (3TC) + Abacavir (ABC) + efavirenz (7.9%), nevirapine + efavirenz (4.8%), lamivudine + Abacavir + nevirapine (3.8%), lamivudine + efavirenz (2.7%), Abacavir + nevirapine (2.4%), zidovudine + nevirapine (2.1%), Abacavir +

nevirapine + efavirenz (1.7%), Abacavir + efavirenz (1.7%), lamivudine + nevirapine (0.7%), lamivudine + nevirapine + efavirenz (0.3%), zidovudine + efavirenz (0.3%), nevirapine (0.3%), lamivudine (0.3%) and Abacavir (0.3%).

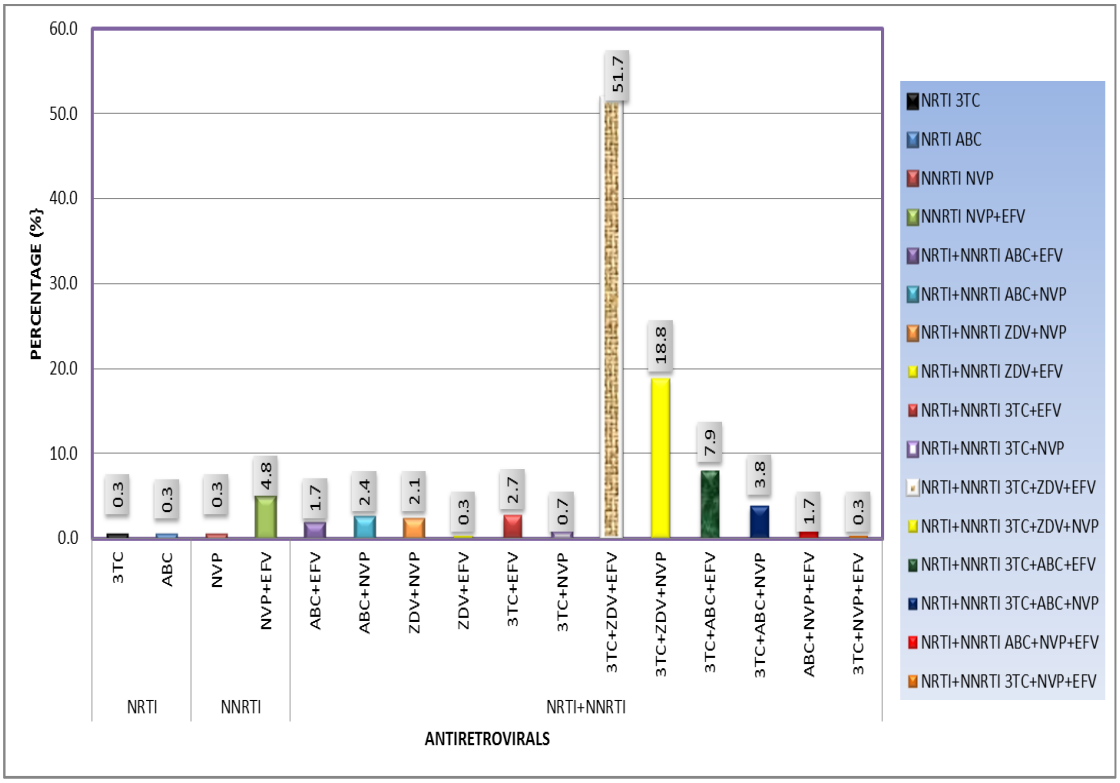

Figure 4.1: Percent distribution of HIV-infected children on antiretrovirals

The haematological characteristics of the studied population stratified by infection are as presented in Table 4.2. The mean red blood cell count (RBC) concentration estimated for the case group (4.0 ± 0.6 $x10^{12}$/L) was significantly lower when compared with that of the control group (4.2 ± 0.9$x10^{12}$/L; p=0.0074). Likewise, the mean haemoglobin concentration (10.8 ± 1.5 g dL^{-1}) of the case group was significantly lower when compared with that of the control group (11.7 ± 2.1 g dL^{-1}; p<0.0001) but a comparison of the mean packed cell volume (PCV) between the case group (33.3 ± 4.1) and the control group (32.7 ± 5.7) showed no statistically significant difference (p=0.1532). The mean white blood cell count (WBC) as

estimated in the case group (6.7 ± 3.1 $x10^9$/L) was also significantly lower when compared to that estimated in the control group (8.8 ± 3.4 $x10^9$/L; p<0.0001). The mean cell volume (MCV) estimated for the case group (83.5 ± 10.0 fL) was significantly higher when compared with that estimated in the control group (76.9 ± 10.0 fL; p<0.0001) but the mean cell haemoglobin (MCH) of (26.9 ± 3.6 pg/cell) and mean cell haemoglobin concentration (MCHC) of (32.2 ± 1.9 g L^{-1}) estimated for the case group were significantly lower compared with that of the control group (28.2 ± 3.2 pg/cell and 35.5 ± 3.1 g L^{-1} respectively). The mean platelet count (303.6 ± 114.6 $x10^9$/L) estimated in the case group was significantly higher compared with that estimated for the control group (234.3 ± 135.7 $x10^9$/L; p<0.0001). The mean CD4 count estimated in the case group was 786.3 ± 50.9 cells mm^{-3} with a mean CD3 count of 2201.9 ± 913.8 cells mm^{-3}.

Table 4.2: Haematological and immunological characteristics of the studied population

Parameters	HIV-infected (n = 362)	HIV- Negative (n = 149)	p-value
WBC ($x10^9$/L)	6.7 ± 3.1	8.8 ± 3.4	< 0.0001
RBC ($x10^{12}$/L)	4.0 ± 0.6	4.2 ± 0.9	0.0074
HB (g dL^{-1})	10.8 ± 1.5	11.7 ± 2.1	< 0.0001
PCV	33.3 ± 4.1	32.7 ± 5.7	0.1532
MCV (fL)	83.5 ± 10.0	76.9 ± 10.0	< 0.0001
MCH (pg/cell)	26.9 ± 3.6	28.2 ± 3.2	0.0002
MCHC (g L^{-1})	32.2 ± 1.9	35.5 ± 3.1	< 0.0001
PLT ($x10^9$/L)	303.6 ± 114.6	234.3 ± 135.7	< 0.0001
LYM%	46.2 ± 13.1	31.8 ± 15.0	< 0.0001
MXD%	14.6 ± 5.9	9.4 ± 6.4	< 0.0001
NEU%	39.1 ± 13.7	58.6 ± 17.2	< 0.0001
CD4 cells mm^{-1}	786.3 ± 50.9		
CD3 cells mm^{-1}	2201.9 ± 913.8		
CD4:CD3	0.4 ± 0.3		

Data are presented as means±SD. P-value defines the level of significance when HIV-infected was compared with HIV-negative

The anthropometric outcomes of the studied population based on z-score estimations of height-for-age (stunting), weight-for-age (underweight) and weight-for-height (wasting/thinness) using the United Kingdom (UK), World Health Organization (WHO) and the United States Centers for Disease Control (US) to determine the proportions under each category (Table 4.3). The anthropometric indices are categorized as adequate (z-score less than +2 and greater than or equal to -2), moderate (z-score less than -2 and greater than or equal to-3) and severe (z-score less than -3).

Height-for-age (Stunting)

A greater percentage of the participants within the case group were more likely to present with moderate and severe stunting when compared with the percentage proportion of participants within the control group. The prevalence of moderate stunting ranged from 27.6% (WHO) through 29.8% (UK) and 31.0% (US) in the HIV-infected case group compared with the 9.4% (UK) and 8.7% (US and WHO respectively) among the HIV-negative control group. The prevalence of severe stunting among participants within the HIV-infected case group ranged from 20.2% (US) through 22.9% (UK) to 24.9% (WHO) compared with the 0.7% (US, UK and WHO respectively)estimated for participants within the HIV negative group.

Weight-for-age (underweight)

A greater percentage of the participants within the HIV-infected case group were more likely to be moderately and severely underweight when compared with the HIV-negative control group using the three defining criteria. The prevalence of moderate underweight ranged from 14.6% (WHO) through 16.9% (US) and 18.0% (UK) for participants within the case group compared with the prevalence rates of 12.1% (WHO) to 13.4% (UK and US respectively) within the control group. The prevalence

of severe underweight ranged from 10.8% (WHO) through 15.5% (US) to 16.0% (UK) in participants within the case group compared with the prevalence rates of 2.0% (WHO), 6.7% (US) and 7.4% (UK) estimated for participants within the control group.

Weight-for-height (wasting/thinness)

The prevalence of moderate wasting as determined using the US and WHO criteria for the HIV-infected case group was 3.6% and 4.7% respectively but the prevalence estimated for the HIV-negative control group showed a wide variation between the US (11.0%) and WHO (4.7%) criteria. Severe wasting was around the same value for both criteria, US (5.0%) and WHO (4.7%) in the case group and 5.4% (US) and 4.0% (WHO) within the control group.

Table 4.3: Anthropometric outcomes of the studied population using varied growth reference estimations

	HIV-infected			HIV-naïve		
Anthropometric Indices	**UK N = 362 (%)**	**US N = 362 (%)**	**WHO N = 362 (%)**	**UK N =149 (%)**	**US N = 149 (%)**	**WHO N = 149 (%)**
Height-for-age (Stunting)						
Adequate (-2≤z-score<+2)	161 (44.5)	165 (45.6)	168 (46.4)	126 (84.6)	127 (85.2)	129 (86.6)
moderate (-3≤z-score<-2)	108 (29.8)	112 (31.0)	100 (27.6)	14 (9.4)	13 (8.7)	13 (8.7)
severe (z-score<-3)	83 (22.9)	73 (20.2)	90 (24.9)	1 (0.7)	1 (0.7)	1 (0.7)
Weight-for-age (Underweight)						
Adequate (-2≤z-score<+2)	229 (63.3)	233 (64.4)	225 (62.2)	118 (79.2)	119 (79.9)	120 (80.5)
moderate (-3≤z-score<-2)	65 (18.0)	61 (16.9)	53 (14.6)	20 (13.4)	20 (13.4)	18 (12.1)
severe (z-score<-3)	58 (16.0)	56 (15.5)	39 (10.8)	11 (7.4)	10 (6.7)	3 (2.0)
Weight-for-height (Wasting/Thinness)						
Adequate (-2≤z-score<+2)	-	213 (58.8)	220 (60.8)	-	77 (51.7)	88 (59.1)
moderate (-3≤z-score<-2)	-	13 (3.6)	17 (4.7)	-	17 (11.0)	7 (4.7)
severe (z-score<-3)	-	18 (5.0)	17 (4.7)	-	8 (5.4)	6 (4.0)

Data are presented as absolute values and proportions; - = parameter not determined by the UK growth reference estimation; UK-United Kingdom; US-United States; WHO-World Health Organization

Table 4.4 presents the renal characteristics of the studied population stratified by infection with the case group being categorized as HIV-infected and the control group as HIV-negative. There was no significant difference in serum creatinine and urea values of the participants (case group; 60.8 ± 25.7 and control group: 62.0 ± 12.3 p = 0.571) and (case group; 3.6 ± 2.1 and control group; 3.3 ± 1.4; p = 0.071) respectively. The case group had significantly higher proteinuria 47(13.0%) compared to none in the control group (0.0%); p < 0.0001 and even among the case group proteinuria was significantly higher among the female participants compared to their male counterparts (33; 70.2% and 14; 29.8% respectively). Similarly micro albuminuria was significantly higher in the case group 95(26.2%) compared to the controls 9(6.0%); p < 0.0001. Again urinary cast and crystals were significantly higher in the case group compared to the controls 35(9.7%) and 2(1.3%); p = 0.001 and 30(8.3%) and 0(0.0%); p= 0.0003 respectively. Other urinary deposit elements such as yeast like cells were also significantly higher in the case group 16(4.4%) compared to the control group 0(0.0%); p=0.0091

Table 4.4: **Renal biochemistry and urinalysis results of studied population**

VARIABLE	CONTROL (%)	HIV +VE (%)	P VALUE	HIV +VE		
				MALE (%)	FEMALE (%)	P VALUE
Creat	62.0 ± 12.3	60.8 ± 25.7	0.571	63.24 ± 27.7	58.66 ± 23.7	0.0909
Urea	3.3 ± 1.4	3.6 ± 2.1	0.071	3.895 ± 2.7	3.435 ± 1.5	0.0417
Proteinuria						
Yes	0(0.0)	47(13.0)	< 0.0001	14(29.8)	33(70.2)	0.0035
Microalbuminuria						
Yes	14(9.40)	95(26.24)	< 0.0001	40(42.1)	55(57.9)	0.5174
Urine Deposits						
Cast	2(1.3)	35(9.7)	0.001	15(42.9)	20(57.1)	0.5084
Crystals	0(0.0)	30(8.3)	0.0003	12(40.0)	18(60.0)	0.5217
Others						
Yeast Cells	0(0.0)	16(4.4)	0.0091	7(43.8)	9(46.2)	0.8805

Data are presented as means ± SD and proportions. Continuous data are compared using the unpaired t-test and categorical data are compared using the chi-square test.

Table 4.5 describes the prevalence of renal insufficiency (GFR <60 ml min^{-1} 1.73 m^{-2}) as determined with selected creatinine-based GFR estimating equations (Schwartz, Counahan-Baratt and Leger equations) and Cystatin C-based GFR estimating equations (Le-Bricon, Larsson, Rule and Zapittelli equations). The creatinine-based equations showed varied prevalence for renal insufficiency between the HIV-infected case group and the HIV negative control group. The Schwartz equation gave a renal insufficiency prevalence rate of 34.5% and 30.2% for the case and control groups respectively. The Counahan-Baratt equation gave a prevalence rate of 30.4% and 22.8% for the case and control groups respectively. The Leger equation yielded the highest prevalence 84.0% and 73.2% for the case and control groups respectively compared with the other two creatinine-based equations. The Cystatin C-based equations showed none of the participants within the case and control group respectively had eGFR < 60 ml min^{-1} 1.73 m^{-2} using the Le-Bricon and Hoek. The Larsson, Rule and Zapittelli however showed a significant 10.8%, 5.2% and 9.9% respectively among the case group against 0% for their corresponding control groups.

Table 4.5: Prevalence of renal insufficiency among the studied participants

eGFR equations	HIV-infected N = 362 (%)	HIV-Negative N = 149 (%)	p-Value
Cr-based equation			
Schwartz			
<60	125 (34.5)	45 (30.2)	0.417
Counahan-Baratt			
<60	110 (30.4)	34 (22.8)	0.406
Leger			
<60	304 (84.0)	109 (73.2)	0.702
Cys C-based equation			
Le-Bricon			
<60	0 (0.0)	0 (0.0)	
Hoek			
<60	0 (0.0)	0 (0.0)	
Larsson			
<60	39 (10.8)	0 (0.0)	
Rule			
<60	19 (5.2)	0 (0.0)	
Zapittelli			
<60	36 (9.9)	0 (0.0)	

Data are presented as absolute numbers and proportion; Cr-creatinine; Cys C-cystatin C; eGFR-estimated glomerular filtration rate; eGFR measured in ml min^{-1} 1.73 m^{-2}

The prevalence of HIV nephropathy (GFR <60 ml min^{-1} 1.73 m^{-2}, microalbuminuria and or proteinuria) as determined with selected creatinine-based GFR estimating equations (Schwartz, Counahan-Baratt and Leger equations) and Cystatin C-based GFR estimating equations (Larsson, Rule and Zapittelli equations) is shown in table 6. The creatinine-based equations showed a HIV nephropathy prevalence of 27.6%, 29.1% and 29.6 for Leger, Counahan-Barat and Schwartz equations respectively while the cystatin C-based equations showed a prevalence of 21.1%, 28.2% and 30.6% for Rule, Larson and Zapittelli respectively.

Table 4.6: Prevalence of HIV nephropathy (using eGFR< 60 ml min^{-1} 1.73 m^{-2}, + microalbuminuria and or proteinuria)

eGFR equations	HIV-infected N = 362 (%)	HIV, Micalb + Prot N = 102 (%)	HIV nephropathy (%)
Cr-based equation			
Schwartz			
<60	125 (34.5)	37 (36.3)	29.6
Counahan-Baratt			
<60	110 (30.4)	32 (31.4)	29.1
Leger			
<60	304 (84.0)	84 (82.4)	27.6
Cys C-based equation			
Larsson			
<60	39 (10.7)	11 (10.8)	28.2
Rule			
<60	19 (5.2)	4 (3.9)	21.1
Zapittelli			
<60	36 (9.9)	11 (10.8)	30.6

Data are presented as absolute numbers and proportion; Cr-creatinine; Cys C-cystatin C; micalb; microalbumin, Prot; proteinuria eGFR-estimated glomerular filtration rate; eGFR measured in ml min^{-1} 1.73 m^{-2}

Figure 4.2 shows a comparison of the mean difference (bias) between the creatinine-based equations and their ability to estimate GFR in the HIV-negative control group. From the mean difference plot, the Schwartz vs Counahan-Baratt equations yielded the least bias of 6.9 followed by the Counaha-Baratt vs Leger (19.8) and Schwartz vs Leger (26.6) equations respectively. It is thus immediately evident that the Leger equation has the greatest probability of underestimating GFR among study participants within the control group and as such the higher prevalence estimation of participants with GFR <60 ml min^{-1} 1.73 m^{-2}. The same could be said for the Counahan-Baratt equation as against the Schwartz equation hence the ability of the

Counahan-Baratt equation to give a higher proportion of participants with eGFR <60 ml min^{-1} 1.73 m^{-2}.

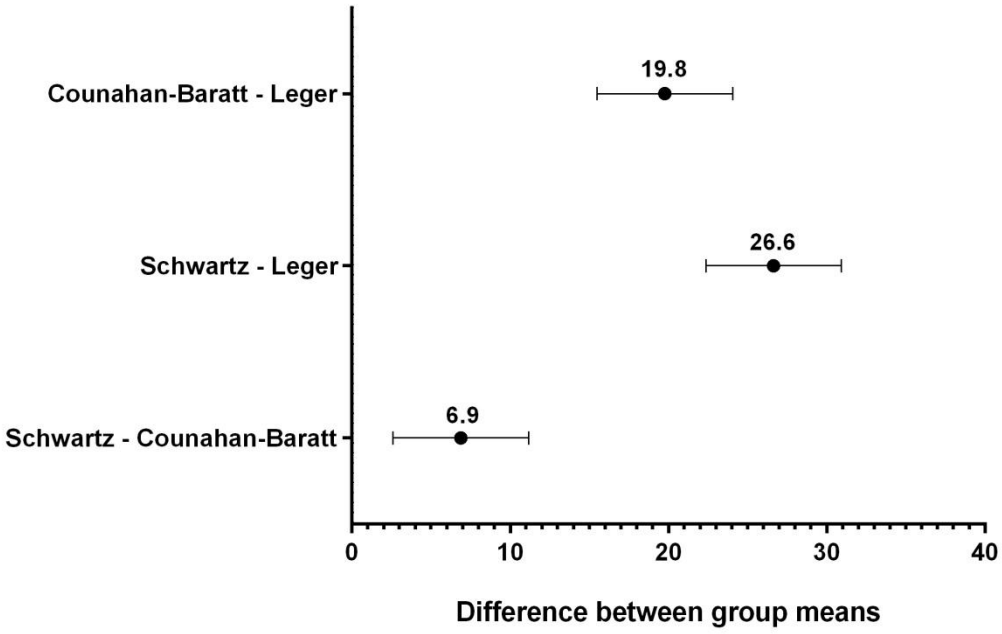

Figure 4.2: Comparison of mean difference between the Creatinine-based equations among the HIV negative group

Figure 4.3 shows a comparison of the mean difference (bias) in the values of GFR as estimated with the Cystatin C-based equations in the HIV negative control group. From the varied comparisons, the Rule vs Zapittelli equations yielded the least bias of 0.4 although none of the equations classified any of the participants within the group as having eGFR <60 ml min^{-1} 1.73 m^{-2}. It is thus obvious from the mean difference plots that the Cystatin C-based equations have the tendency to overestimate eGFR among participants within the control group hence the inability to show a participant with eGFR <60 ml min^{-1} 1.73 m^{-2}.

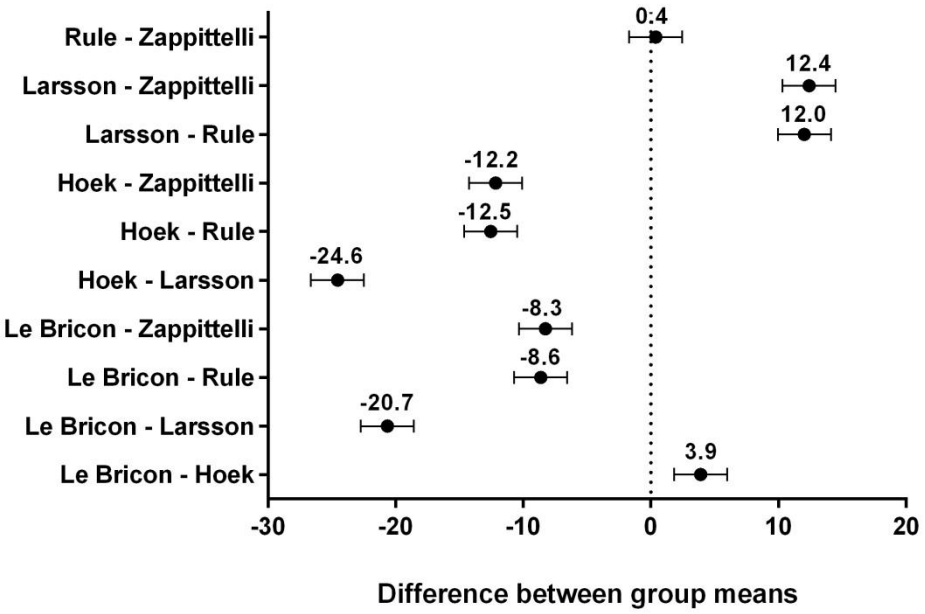

Figure 4.3: Comparison of the mean difference between the Cystatin-C based equations among the HIV negative group

A comparison of the mean differences (bias) for the Creatinine-based equations among participants within the HIV-infected case group also showed the Counahan-Baratt vs Schwartz equations yielding the least bias of -0.8 followed by the Counaha-Baratt vs Leger (29.2) and Schwartz vs Leger (37.2) equations respectively. A similar trend of underestimating GFR by the Leger equation in the control group was observed in the case group hence the ability of the Leger equation to predict a higher prevalence of eGFR <60 ml min^{-1} 1.73 m^{-2} among the study participants within the case group **(Figure 4.4)**.

For the Cystatin C-based equations, the Rule vs Zapittelli equations again yielded the least bias of 0.2 among participants within the case group. Le-Bricon and Hoek underestimated GFR and hence none of the participants within that group had eGFR <60 ml min^{-1} 1.73 m^{-2} (**Figure 4.5**)

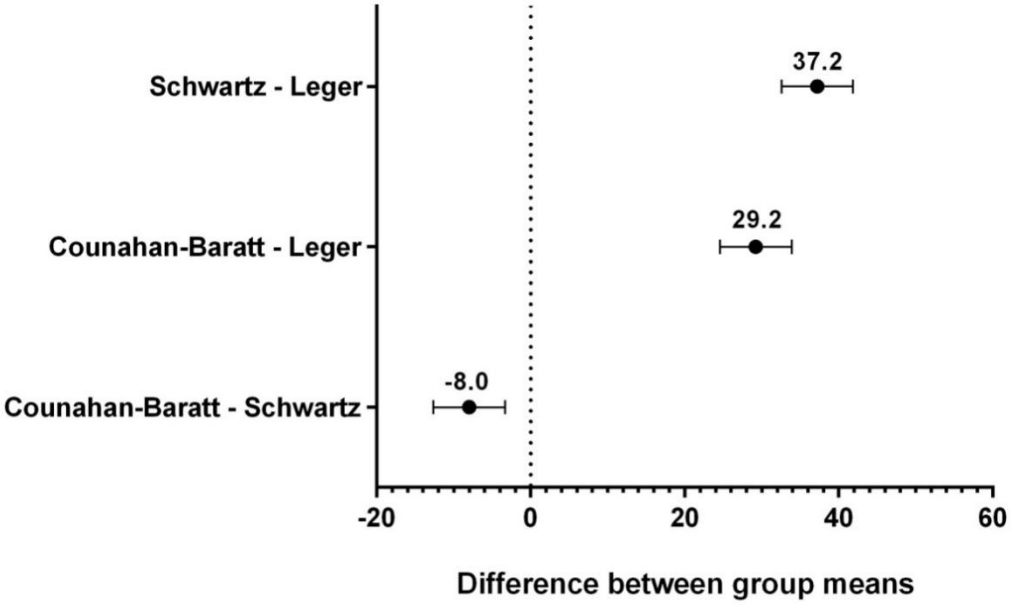

Figure 4.4: Comparison of mean difference between the creatinine-based equations among the HIV-infected group

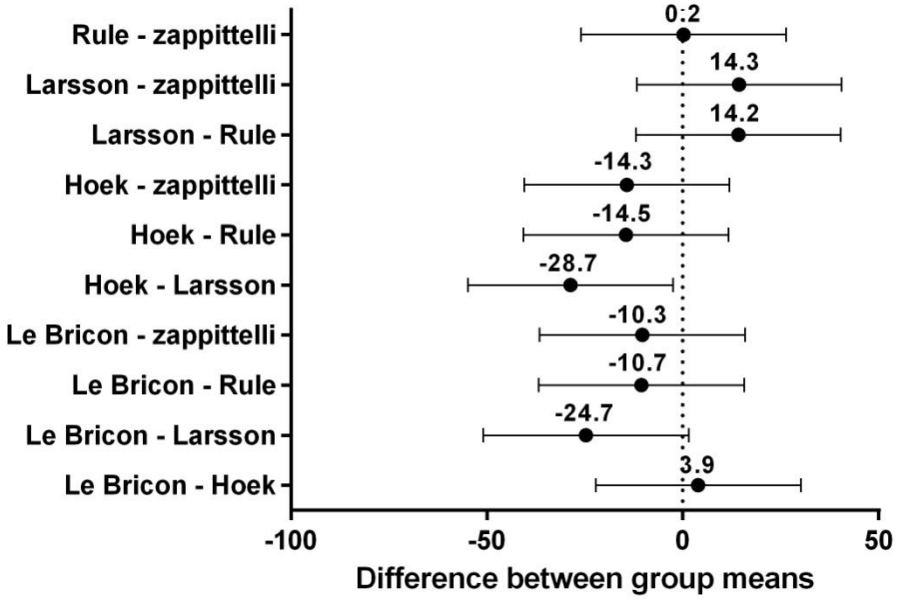

Figure 4.5: Comparison of the mean difference between the Cystatin-C based equations among the HIV-infected group

CHAPTER FIVE

DISCUSSION

HIV associated nephropathy in children has far reaching effects (Anochie *et al.,* 2008). It is a speedily progressive form of chronic kidney disease and is extremely associated with mortality (Anupama and Uma, 2014; Ikpeme *et al*., 2012). HIVAN world-wide is estimated to be between 2% and 5% but has been shown to be as high as 15% in populations of African ancestry (Ando *et al.,* 2012). Although early recognition and appropriate therapeutic interventions have been shown to retard disease advancement (Grinsztejn *et al.,* 2014), diagnosis requires kidney biopsy, an invasive technique which requires special skills (Fine *et al.*, 2008) and which is virtually non-existent in majority of the health facilities attending to and administering antiretroviral therapies (ARTs) in Ghana.

5.1 ANTHROPOMETRIC OUTCOMES OF THE STUDY PARTICIPANTS USING VARIED GROWTH REFERENCE ESTIMATIONS.

Height-for-age (Stunting)

Stunting or poor linear growth is an irreversible growth deficit characterized by deficient height or length for age. Its preponderance is relatively higher in sub-Saharan Africa and South Asia (Agedew *et al.,* 2015; Rah, *et al.,* 2010; Victoria *et al.,* 2008) and affects close to 195 million children under five years of age in the developing world (Duggan., 2010). Up to about 31% prevalence of stunting has been reported in HIV infected children of African descent (Berger *et al.,* 2008).

Findings from this study (Table 3) indicate a significantly higher prevalence of moderate and severe stunting among the case group compared to the controls. This is similar to observations made by Agedew *et al.,* (2015) who reported 18.7% in a cross

sectional studies in Southern Ethiopia. Conversely, a relatively higher prevalence of 58%, 46% and 36.6% had been reported in cross-sectional studies in South India (Padmapriyadarsini *et al*.,2009; Kapavarapu *et al.,* 2012) and Tanzania (Sunguya *et al.,* 2011) respectively. Additionally, studies in Tanzania and Mozambique have reported significantly higher prevalence of moderate to severe stunting among HIV infected children (McDonald *et al.,* 2012; Arpadi *et al.,* 2013 respectively).

Although there is general agreement that there is a high prevalence of stunting in HIV, the reasons propounded by these studies are usually varied. While Padmapriyadarsini *et al.*, (2009) attributes this to undernutrition of HIV infected children, probably due to inadequate breast feeding because of their mothers' HIV status, other studies also indicate that the virus per se has nothing to do with stunting and that in the same locality, both HIV infected and HIV negative children have similar growth patterns (Rifkin *et al.,* 2011; Lowenthal and Phelps, 2008). The relatively high prevalence of stunting reported by Padmapriyadarsini *et al.,* (2009) and Shet *et al.,* (2009) in South India could be associated with differences in characteristics of the participants. Whereas the case group (HIV infected children) in their study were HAART-naive, majority of the case group in the present study (77.1%) were on HAART. Again, while the participants in the Indian studies were from the socio-economically vulnerable grouping and were disadvantaged by poverty, poor infrastructure and limited access to basic health services, same could not be said about this study. More so, documented studies from India had reported higher prevalence for stunting in the range of 45-62% for studies conducted mostly among orphaned children (Padmapriyadarsini *et al.*,2009; Shet *et al.,* 2009) and about 46% in a mixed population of orphaned and non-orphaned children (Shet *et al.,* 2009).

Poor growth is both a manifestation of HIV/AIDS as well as an independent risk factor for death for persons living with HIV (Kawo *et al.,* 2000; Chichumba *et al.,* 2008). Stunting in such children is multi-faceted including antenatal, intrauterine, and postnatal malnutrition, more commonly due to inadequate or inappropriate nutrition and the impact of infectious disease (Agedew *et al.,* 2015).

In instances where a relatively lower prevalence of moderate and severe stunting had been reported contrary to our finding like was reported in Latin America and Caribbean countries (11%) (UNICEF, 2013), the reason has been associated with the selection of the study participants who were less than or equal to 5 years (Agedew *et al.,* 2015).

Significant increases in the prevalence of moderate and severe stunting as is observed in this study could imply a probable impairment in the response to the ART used in these children as has been reported elsewhere by other studies (Rajasekaran *et al.,* 2009). Similar result patterns have also been linked to delayed mental development, poor school performance, reduced intellectual capacity and increased mortality in HIV children (Burns *et al.,* 1999; Kawo *et al.,* 2000; Chichumba *et al.,* 2008; Mwaba *et al.,* 2015).

Weight-for-age (underweight)

Underweight (weight for age) is an important indicator of a population's nutritional health (Anigilaje *et al.,* 2015).

In agreement with earlier studies such as those by Sunguya *et al.,* (2011) who reported a 22.1% prevalence of underweight in a cross sectional study involving ART-treated HIV participants in Dar es Salaam, Tanzania, our study found a significantly higher

prevalence of moderate and severe underweight among the case group compared to the controls (Table 3).

Factors such as maternal education and maternal nutritionary condition have been linked with underweight presentations within children living with HIV infection (McDonald *et al.,* 2012). Villamor *et al.,* (2004) reported that a low level of maternal education predicted linear growth slowdown among infants under 12 months of age who were born to HIV-infected women in Tanzania. Similarly, McGrath *et al.,* (2012) found higher maternal education to decrease the risk of stunting and underweight among HIV-exposed children in Kenya. In an analysis of Demographic and Health Survey data from six countries in sub-Saharan Africa, maternal secondary education was positively associated with weight for age z-score (WAZ) in Ghana, Nigeria, and Tanzania (UNICEF, 2009). Mothers with higher levels of formal education may possess greater knowledge of proper hygiene practices and optimal child caring and feeding practices, which could be particularly important in the context of HIV (McDonald *et al.,* 2012)

Contrary to our findings however, studies from a retrospective cross-sectional study at the Federal Medical Centre, Makurdi, in Nigeria showed lower prevalence. In that study Anigilaje *et al.,* (2015) reported 12.1% of underweight in a relatively younger HIV population (ages up to 2 years).

In studies which reported a relatively higher prevalence of underweight compared with the findings of our study; like Anyabolu *et al.,* (2014) and Palumbo *et al.,* (2010) who reported a 58. 6% in a case control study in Nigeria and 50% prevalence in a South African study respectively, the participants of the study were ART-naïve contrary to that of our study where 77.1% of the participants where on ART.

The high prevalence of underweight as is seen is this study could imply that the children in the case group are relatively more vulnerable to infection. Underweight could additionally be linked to the derailed intake and absorption of vital nutrients, including amino acids, vitamins, and minerals, leading to increased risk of osteoporosis and anaemia as are documented by Gjesdal *et al.,* (2007).

Weight-for-height (wasting/thinness)

Wasting is a common clinical presentation in children with HIV infection (Agedew *et al.,* 2015). Severe malnutrition among such infected children has a higher risk of mortality than uninfected malnourished children. Factors such as low oral dietary intake, malabsorption, endocrine disorders as well as metabolic disorders have been associated with wasting in HIV infected children (Abram *et al.,* 2000).Wasting is documented to cause impairments in the functioning of the immune system and could lead to increased severity and duration of susceptibility to infectious diseases and an increased risk for death (Sunguya *et al.,* 2011).

Contrary to numerous studies on wasting in children with HIV where results have shown that higher prevalence of moderate and severe wasting was common among persons with HIV; Chiabi *et al.,* (2012) in a prospective case-control study in Cameroun reported 56. 4%, Padmapriyadarsini *et al.*,(2009) in a cross sectional study reported 63% in Southern India, Anigilaje *et al.,* (2015) in Nigeria 33.5% , and Sunguya *et al.,* (2011) who reported 13.6% in Dar es Salaam, Tanzania among HIV positive ART- treated HIV participants there was no significant difference in wasting as is observed between the HIV positive case group and the HIV-negative controls in this study (Table 3). Characteristics of the study participants and the geographical location where the studies were carried could have accounted for the difference in prevalence. For instance while majority of our case group participants were on ART,

those of the other studies were ART-naïve. Considering that about 77.1% of the case group were on ART and the initiation and continuous use of ART by such children in Ghana require that the immune and nutritional status of the children are continuously monitored, it stands to reason that the HIV infected case group had gained weights which corresponded to the height and age. Additionally study sites from Asian countries such as India where serious socio-economic challenges exist and where orphans had been used as participants for studies have reported high prevalence for wasting (Saloojee *et al.*, 2007).

ART use, coupled with good dietary supplementation as documented by Fergussion *et al.,* (2009) in their meta-analysis of HIV infected children in Sub-Saharan Africa could account for or explain the relatively low moderate and severe wasting that is observed in this study (Cobb and Bland, 2013; Sztam *et al.*, 2010; Peters *et al.*, 2008).

5.2 PREVALENCE OF PROTEINURIA, MICROALBUMINURIA AND COMMON URINARY DEPOSITS AMONG STUDY PARTICIPANTS.

Increased urinary protein, commonly detected using urine dipstick is often as a result of increased filtration across the filtration barrier (glomerular proteinuria), decreased reabsorption from the proximal tubule (tubular proteinuria) or increased secretion of protein from the tubules (secretory proteinuria).

Proteinuria (Table 4) as observed in this study was significantly higher among the case group than the control group. This observation is similar to the work of Struik *et al*., (2011) and Longo *et al.,* (2011) in cross sectional studies conducted in Democratic Republic of Congo and Congo respectively. Again Esezobor *et al.,* (2010) reported from Lagos- Nigeria, that compared with 6% of the fifty (50) controls (HIV naïve), 20.5% of the eighty-eight (88) HIV-infected children had proteinuria (p =

0.026). Additionally Ekulu *et al* (2010) showed the prevalence of proteinuria among Congolese children living in Kinshasa in Democratic Republic of Congo with HIV/AIDS was 23.8% also in agreement with the significantly increased prevalence of proteinuria among the case group in this study (Table 4). The work of Ekulu *et al.,* (2012) further showed that the HIV infected children had seven times more probability of presenting with proteinuria than the controls (OR 6.9; IC 95%: 2.3-20.8; P<0.001). The prevalence of proteinuria from this work was however relatively higher compared to the work done by Eden *et al.,* (2012) which reported 17 (7.1%) proteinuria out of 240 HIV infected participants and Steel-Duncan *et al.*,(2008) who reported a 3.3% among Jamaican children but was also relatively lower in prevalence when compared to works done by Fabian *et al*. (2011) in South Africa (44%), Mannix *et al.,* (2014) (41.3%), and Jao *et al.,* (2011) in Cameroon (39%). Whereas Mannix *et al.,* (2014) and Ekulu *et al.,* (2012) attribute the high prevalence of proteinuria to the presence of the HIV and high viral load (Mannix *et al.,* 2014; Ekulu *et al.,* 2012),Longo *et al*., (2012) attributed it to immunodeficiency, low CD4 count and nutritional status of the participants. Studies have demonstrated that the direct impact of human immunodeficiency viral components (gp120, TAT) on the endothelium, leads to the expression of adhesion which promotes and favour proteinuria (Blasi *et al.,* 2014). The study of Mannix *et al.,* (2014) like many other cohort study including that of Dimock *et al.,* (2009), reported a relatively higher prevalence of proteinuria (30-45%) in Bethesda, USA. This relative high prevalence could be attributed to difference in the characteristics of the study participants including age, physiological state, nutritional and immunological status of the participants. In instances where relatively lower prevalence was reported, participants were screened and excluded using urine dipstick for proteinuria, leucocyte and nitrite (Barisoni *et al.,* 2000).

The relatively high prevalence of proteinuria as observed in this study is suggestive of renal disorder; however this cannot be associated with drugs such as tenofovir, indinavir and atazanavir which have been documented to be nephrotoxic (Kalyesubula and Perazella, 2011) since they are not part of the administered drugs used for the children in this study.

Microalbuminuria on the other hand has been reported as a predictor of subclinical renal involvement in systemic diseases including HIV nephropathy (Mistry, 2010). It refers to albumin excretion above the normal range and has been defined as urinary albumin excretion between 30 and 300 mg/day or in concentrations 20 to 200 µg/min. It develops from progressive, subclinical, structural, and functional changes in the kidney and it is useful as an early biomarker in the detection of kidney disease (Mudi *et al.,* 2014). Microalbuminuria in this study was significantly higher in the case group 95(26.2%) compared to the controls 9(6.04%) (Table 4). This finding is similar to observations made by Mosten *et al.,* (2015) who studied the prevalence of persistent microalbuminuria in HIV infected children in a cross sectional analytical study in Tanzania. Our finding is also comparable with the prevalence of 25% and 20.4% reported in HIV infected children in South Africa and Tanzania respectively (Mistry, 2010). Han *et al.,* (2006) had a prevalence similar to those above in a cross sectional study in South Africa. The findings of this study generally compares with the reported prevalence of 10–33% reported from Port Harcourt- Nigeria in Africa, India, and the United States (Mistry, 2010). The outcome of this study, however, differed significantly from that obtained by Ezeonwu *et al.,* (2012), Mudi *et al.,* (2014), and Eke *et al.,* (2010) who obtained a prevalence of 0%, 6.7% and 12% respectively in HIV infected children in Enugu, Kano and Port Harcourt all in Nigeria. In these relatively low prevalence cases, the participant selection criteria

excluded proteinuria and other confounding factors such as hypertension, diabetes and urinary tract infections which were largely age dependent and that could have accounted for the low prevalence which was reported. Ethnic differences in most instances have also accounted for relatively low microalbuminuria preponderance(Shah, 2011). Studies that largely involved HIV positive Caucasian subjects seemed to observe a lower prevalence of microalbuminuria compared with studies among patients of African origin especially so when persons of the Sub-Saharan group have been shown to have a high prevalence of HIVAN and other HIV nephropathies (Atta *et al.*, 2005).

Such high prevalence in microalbuminuria as seen in this study could imply a decrease in immunity among particularly the case group which is surmised from low CD4 count and increased virial load (Mosten *et al.,* 2015). It further could be flagging renal complications within the case group which need attention as has been documented by Eke *et al.,* (2010) and Shah *et al.,* (2012)

Again urinary cast and crystals were significantly higher in the case group than the controls. Other urinary elements such as yeast like cells were also significantly higher in the case group 16 (4.4%) than in the controls 0 (0%).

Crystalluria refers to the precipitation of crystals in urine from super-saturated urine either in health or under pathological conditions. It leads to stone formation, which predisposes HIV patients to acute renal failure (Röling *et al.,* 2008). Several medications that are insoluble in human urine are known to precipitate within the renal tubules. Intratubular precipitation of either exogenously administered medications or endogenous crystals (induced by certain drugs) can promote crystal nephropathy (Yarlagadda *et al.,* 2008). The prevalence of 9.7% crystalluria in this

current study, though slightly lower, compares well to the 10.7% reported at the Komfo Anokye Teaching Hospital, by Ephraim *et al.,* (2014) among adults living with HIV in the Ashanti Region of Ghana. The relatively high significant prevalence of crystalluria observed in this study is an indication that the participants were at risk and probably predisposed to urolithiasis and cysteinuria as had been reported by Ephraim *et al.,* (2014) and Daudon *et al.,* (2003). Crystallization, particularly in the case group of such significant levels should be of concern because it has been reported as a risk factor for kidney disease and reduced glomerular filtration rate (Garg *et al.,* 2011) in other studies.

Urinary cast are cylindrical structures formed from Tamm-Horsfall mucoprotein, cells and debris within the renal tubules. Casts, whether cellular or acellular could be seen in a routine urine examination especially after strenuous exercise and also in aggravated renal disease condition and are favoured by acidic urine conditions (Chawla *et al.,* 2008). Presence of cellular casts in urinary deposits could be interpreted to mean; red cell casts are seen in glomerular disease, white cells casts are classically associated with glomerulonephritis, renal tubular epithelial cell casts are always indicative of tubular damage, and both coarse granular casts and waxy casts are formed from the breakdown of cellular casts and therefore are indicative of renal pathology, possibly chronic or end-stage renal disease, although might not be always so (Israni *et al.,* 2007). The 8.3% prevalence of cast observed in this study could be attributed to aside other reasons the elaboration of cytokines and other protein elucidated as a result of presence of the HIV.

5.3 HAEMATOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF THE STUDY PARTICIPANTS

Haematological manifestations have been documented to be the second most common cause of morbidity and mortality in HIV patients (Munyazesa *et al*., 2012). Typically there is decreased cellularity and myelodysplasia (abnormal change of the cellular structure, affecting cells of the erythroid lineage as well as those of the megakaryocytes) as well as dysplastic changes of the granulocyte-macrophage lineage with arrest of maturation in the marrow of HIV infected persons (Owiredu *et al.,* 2011). Several studies including those of Renner *et al.*, (2013), Makubi *et al.,* (2012) and Calis *et al.,* (2008) have shown result patterns similar to those observed in this present study. The mean red blood cell count (RBC) was lower in the cases group compared to the controls group, similarly the mean haemoglobin concentration of the case group (10.8 ± 1.5 g dL^{-1}) was significantly lower when compared with that of the control group (11.7 ± 2.1 g dL^{-1}; $p<0.0001$). Anaemia of varying prevalence have be reported by Ruhinda *et al*., (2012) who reported 57.6% anaemia among HIV infected children aged 3 months and 18 years in Uganda and also 66%, 70%, 74.6%, 79.9% reported by Shet *et al.,* (2009), Shah and Katira (2011), Okechukwu (2010) and Adetifa *et al.,* (2006) respectively. Other studies reported relatively low prevalence for anaemia in HIV infected children such as the 3% reported by Ezeonwu et al. (2014). The varying prevalence have been associated with figures used in classifying anaemia such as the < 10.0g/dl used by Okechukwu (2010) and Adetifa *et al.,* 2006 (WHO standard) and the < 11.0g/dl used in other studies such as Shet *et al.,* (2009), Shah and Katira (2011). The haematologic abnormalities observed in this study may occur as a result of the HIV infection itself, as sequelae of HIV related infections or malignancies or as a consequence of therapies used for HIV infections

and associated conditions (De Santis *et al.,* 2011; Mathews *et al.,* 2013; Enawgaw *et al.,* 2014). Whereas age, drug use (particularly bone marrow suppressing ones like ZDV), stunting and advanced stages of the HIV infections have been shown as predictors of anaemia in children from Asian origin (Renner *et al.,* 2013; Shet *et al.,* 2009), a high incidence of anemia which is not drug- related have been demonstrated in black persons, although the black race has not been associated with drug-related anemia (Redig and Berliner, 2013). This association has been documented to be as a result of a number of black persons having sickle cell anemia and glucose-6-phosphate dehydrogenase (G-6-PD) deficiency. The prevalence of glucose-6-phosphate dehydrogenase (G-6-PD) deficiency ranges between up to 13% for black men and 3% for black women. Sickle cell anemia and G-6-PD are not routinely diagnosed in children prior to ARV administration in most African countries including Ghana where ZDV remains a first line drug (Renner *et al.*, 2013; Ruhinda *et al.,* 2012). Again, malaria which is one of the leading causes of anaemia in children and which is a confounder for HIV morbidity in Sub-Saharan African continuous to be a challenge in Ghana (Arinaitwe *et al.,* 2012). Malnutrition has been shown to contribute significantly to anaemia due to the deficiency of iron, folate and B12 as well as increased infection. Dietary supplements have been shown to correct anaemia and weight loss in HIV-infected children (Simpore *et al.,* 2005). Low CD4 counts (< 25%) and high HIV-1 RNA levels in plasma have also been associated independently with increased risk of anaemia (Volberding *et al.,* 2004) in HIV. The mean white blood cell count (WBC) as estimated in the case group (6.7 ± 3.1 x10^9/L) was also significantly lower when compared to that estimated in the control group (8.8 ± 3.4 x10^9/L; $p<0.0001$). Statistically significant low white cell counts as seen in this study (case group; 6.7 ± 3.1 x10^9/L) compared to that estimated in the control group ($8.8 \pm$

3.4 x10^9/L; p<0.0001) have been reported as a characteristic haematological feature in HIV infection. Regardless of whether or not ART had been initiated, leucopaenia typically in the neutrophil population (Neutropenia; though prevalence could indicate a relative decline in children on HAART) or in the lymphocyte population (lymphopaenia; though prevalence could indicate a relative decline in children on HAART) have been observed. Enawgaw *et al.,* (2014) in a comparative cross-sectional study among adults reported 35.9% leucopaenia in HAART treated participants and 16.7% in HAART-naïve participants in Gondar-Northwest Ethiopia, Patwardhan *et al.,* (2002) reported 15.5% the in adult population in India while Matthews *et al.,* (2013) reported 5.9% in New Delhi. The mean cell volume (MCV) estimated for the case group was significantly higher (83.5 ± 10.0 fL) when compared with that estimated in the control group (76.9 ± 10.0 fL; p<0.0001) but the mean cell haemoglobin (MCH) of (26.9 ± 3.6 pg/cell) and mean cell haemoglobin concentration (MCHC) of (32.2 ± 1.9 g L^{-1}) estimated for the case group were significantly lower compared with that of the control group (28.2 ± 3.2 pg/cell and 35.5 ± 3.1 g L^{-1} respectively). The results pattern obtained in this study which is typical of normocytic hypochromic anaemia have been reported widely among children with HIV compared to HIV naïve children. Owiredu *et al.,* (2011), Moyle *et al.,* (2002), Beach *et al.,* (1998) and Burkes *et al.,* (1987) reported similar results pattern in their work. Moyle *et al.,* (2002) like others mentioned above associated the observed normocytic anaemia with vitamin B12 or folate deficiency and in the setting of HIV treatment reflects the use of zidovudine.

The mean platelet count (303.6 ± 114.6 x10^9/L) estimated in the case group even though normal was significantly higher compared with that estimated for the control group (234.3 ± 135.7 x10^9/L; p<0.0001), a situation which was unusual particularly

between children with HIV and those who did not have the infection. Several reasons however could be assigned to why HIV naive children could have a relatively low platelet count even in the absence of infection. Decreased production; usually as a result of viral infection other than HIV and other conditions that affect the bone marrow, increased platelet destruction as in idiopathic thrombocytopenic purpura (ITP) in which case the body targets its own platelets and considers them as foreign cells and produces antibodies against them in a highly uncommon situation or hemolytic-uremic syndrome (HUS) which occurs from a particular strain of E.coli that causes food poisoning and diarrhoea in children and increased platelet sequestration in which case the spleen raptures as a result of liver cirrhosis caused by Hepatitis infection in children (Munyazesa *et al.,* 2012). The relatively normal mean platelet cell count seen in the case group compared to the control have been considered under ITP

5.4 USE OFCREATININE AND CYSTATIN-C IN THE ESTIMATION OF GFR IN HIV/AIDS CHILDREN

Creatinine-based formulae are established methods for assessing kidney function; however they are known to be fraught with several shortcomings in the general population (Stevens *et al.,* 2006; Levey *et al.,* 1990) and particularly in HIV-infected persons (Odden *et al.,* 2007). Creatinine based estimations appear to be imprecise, and could potentially lead to the over diagnosis of chronic kidney disease (Inker *et al.,* 2012).

Results from this study (Table 5) shows an increased prevalence of renal insufficiency (eGFR <60 ml min^{-1} 1.73 m^{-2}) in both case group and control group regardless of the type of creatinine based equation used and the number further increases when the Leger equation is used.

Notably, serum creatinine level is affected by non-renal factors, such as diet, race, medication and muscle mass. The corresponding increase in renal insufficiency (characterized by increased creatinine values) as was seen among the control group could be as a result of dehydration (due to vomiting and diarrhoea) and starvation (low dietary intake due to loss of appetite) resulting in malnutrition which could be seen in sick children in Ghana. Creatinine clearance is additionally significantly influenced by tubular secretion (Stevens *et al.,* 2006). Compounding the problem is the dependence of most laboratories on the modified kinetic Jaffe method which in itself is bedeviled with limitations (Afolabi *et al.,* 2009) for clinical decisions in Ghana. Several endogenous and exogenous interfering substances have been documented to affect the analytical specificity of creatinine when analysed by the Jaffe method. The huge number of participants within the control group (Table 5); a relatively healthy population, who were graded as having renal insufficiency (eGFR< 60 ml min^{-1} 1.73 m^{-2}) using the creatinine based equations surmise the fact that creatinine based equations are not very specific and tend to underestimate GFR when they are not standardised (Inker *et al.,* 2012). Serum protein and bilirubin in particular is documented to cause an overestimation of serum creatinine by as much as 15%–25% (Peake and Whiting, 2006).The escalating prevalence seen when the Leger equation was used could further buttress the fact that the Leger equation is not suitable for use in children. Malnutrition among children of sub-Saharan Africa origin, especially those living with HIV/AIDS (Arpadi *et al.,* 2000) further reduces the usefulness of serum creatinine-based formulae in the routine assessment of kidney function and has implications for early detection of impaired kidney function in these children (Jones *et at.,* 2008). Results from this study (Table 5) indicate a high prevalence of renal insufficiency among the case compared to the controls. This was

however not significantly different from that observed among the control group and is similar to the results documented from a cross sectional study in an adult population by Odden *et al.,* (2007). Wools-Kaloustain *et al.,* (2007) however reported a relatively lower prevalence of 11.5% using the Cockcroft–Gault creatinine based equation in a HAART naïve Kenyan population compared to our study. Compared to creatinine however, cystatin C is less affected by non-glomerular factors, such as muscle mass, medication, diet and tubular secretion (Laterza *et al.,* 2002; Bokenkam *et al.,* 1998). Studies have shown that, cystatin C-based formulae for estimation of GFR closely mirror gold-standard measures of GFR and reflect changes in GFR earlier than creatinine-based formulae (Filler *et al.,* 2003; Jones *et al.,* 2008).

Results from this study (Table 5) is similar to that reported by Esezobor *et al.,* (2010) in a case -control study in Nigeria where they reported using a cystatin-C based formula a prevalence of 13.3% renal insufficiency in children infected with HIV compared with none (0%) among relatively healthy uninfected children. Our finding of high prevalence of renal insufficiency (eGFR less than 60 ml/min/1.73 m^2) in the HIV-infected children is consistent with findings in an earlier study (Esezobor *et al.,* 2009) which documented a proteinuria prevalence of 20.5% among a cohort of HIV-infected children. Other studies have documented high prevalence of kidney diseases in HIV-infected persons (Eke *et al.,* 2007; Wools-Kaloustain *et al.,* 2007; Gupta *et al.,* 2005). Jones *et al.,* (2008) in a cross sectional study in Boston, Massachusetts and Providence, Rhode Island, Jaroszewicz *et al.,* (2006) in Poland, Odden *et al.,*(2007) in USA and Wools-Kaloustian and colleagues reported prevalence of renal insufficiency between 11.5% and 15.2%, similar to the findings of this study.

The significantly high prevalence of renal insufficiency shown by GFR less than 60 ml/min/1.73 m^2 in this study supports the association between HIV infection and

kidney disease (Eke *et al.,* 2007; Kimmel *et al.,* 2003), and implies that HIV-related kidney disease may be as common in Ghanaian children infected and living with HIV as there are in other parts of the world (Eke *et al.,* 2007; Gupta *et al.,* 2005).

Again the fact these participants with reduced eGFR were selected from an outpatient population probably indicates a chronic, rather than a rapidly evolving, reduction in GFR. The high prevalence of glomerular dysfunction observed in the HIV-infected children in this study warrants early detection of kidney involvement in HIV infection and the institutionalization of measures that may halt progression to end-stage kidney disease as has been reported by Esezobor *et al.,* (2010).

5.5 PREVALENCE OF HIV NEPHROPATHY USING EGFR< 60 ML MIN^{-1} 1.73 M^{-2}, WITH MICROALBUMINURIA AND OR PROTEINURIA AS

HIVAN is the commonest form of chronic kidney disease ensuing directly from HIV infection and occurs almost entirely among Africans (Herman and Klotman, 2003). Its prevalence has been determined in diverse ways including the use eGFR ml min^{-1} 1.73 m^{-2} and the presence of proteinuria and or use of sonographic description. The prevalence range of 21.2% -30.6% obtained in this study compares favourably with the 31.6%, 24.2% and 29.8% reported by some workers in Nigeria, South Africa and Washington DC respectively (Estrella and Fine, 2010; Ikpeme *et al.,* 2012). Other workers have reported prevalence of 19.4% (Szczech *et al.,* 2010), and 20.6% (Ikpeme *et al.,* 2012). The relatively higher prevalence from this study may be attributable to the fact that all the patients in the study population were Africans (Choi *et al.,* 2009). Early detection of HIV nephropathy may be beneficial in evaluating early treatment and thereby preventing further disease progression to end stage renal disease, needing renal replacement therapy among the study participants.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

Stunting and underweight was prevalent among the HIV positive case group compared to the HIV negative control group.

The study observed typical haematological derangements such as lower means for RBC count, WBC count, MCH and MCHC within the case group compared to the control group. The mean MCV and mean platelet counts were however higher among the case group compared to the control group.

Cystatin-C better discriminated renal dysfunction among the study participants.

The Counahan-Baratt vs Schwartz creatine equations and the Rule vs Zapittelli cystatin-C equations yielded better results in identifying renal diseases among the study participants.

Yeast cells, cast and crystals were significant and relatively common among children with HIV infection.

Finally the study showed that microalbumin and proteinuria were prevalent among children living with HIV/AIDS. These together with an estimated GFR< 60 ml min^{-1} 1.73 m^{-2} either by a creatinine or cystatin-C based equation could be the easiest, cheapest, and readily available way of diagnosing HIV nephropathy among such children in Ghana.

6.2 RECOMMENDATIONS

We recommend strongly that microalbuminuria or proteinuria screening whether by use of a urine dipstick or through estimations should be considered a routine test for people living with HIV.

Creatinine should not be used as a standalone test or renal function marker in children unless in addition to microalbuminuria and or proteinuria or in estimation equations such as eGFR which accounts for factors such as age, sex and body mass which are known to affect creatinine estimations,

Cystatin-C is much sensitive and a more specific renal marker and should be preferred to creatinine as a renal marker

Additionally children living with HIV with eGFR< 60 ml min^{-1} $1.73m^{-2}$ and who have microalbuminuria and or proteinuria should have their treatment regimen revised in that their result pattern points to HIV nephropathy.

6.3 LIMITATIONS

The study did not include kidney biopsy which is the known gold standard for the definitive diagnosis of HIVAN; hence the prevalence or proportion of HIV nephropathy children who had HIVAN could not be establish.

REFERENCES

Aaron, K.J., Kempf, M.-C., Christenson, R.H., Wilson, C.M., Muntner, P., and Shrestha, S. (2012). Prevalence of proteinuria and elevated serum cystatin C among HIV-Infected Adolescents in the Reaching for Excellence in Adolescent Care and Health (REACH) study. J. Acquir. Immune Defic. Syndr. 1999 *61*, 499–506.

Abiodun, M.T., Iduoriyekemwen, N.J., and Abiodun, P.O. (2012). Cystatin C-Based Evaluation of Kidney Function of HIV-Infected Children in Benin City, Southern Nigeria. Int. J. Nephrol. *2012*.

Abrams DI. (2000) Potential interventions for HIV/AIDS wasting: an overview. J Acquir Immune Defic Syndr 25(Suppl 1):74-80.

Adebamowo, C.A., Casper, C., Bhatia, K., Mbulaiteye, S.M., Sasco, A.J., Phipps, W., Vermund, S.H., and Krown, S.E. (2014). Challenges in the Detection, Prevention, and Treatment of HIV-Associated Malignancies in Low- and Middle-Income Countries in Africa. J. Acquir. Immune Defic. Syndr. 1999 *67 Suppl 1*, S17–S26.

Adetifa IM, Temiye EO, Akinsulie AO, Ezeaka VC, Iroha EO (2006). Haematological abnormalities associated with paediatric HIV/AIDS in Lagos. Ann. Trop. Paediatr. 2:121-125.

Afolabi MO, Abioye-Kuteyi AE, Arogundade FA, Bello IS. (2009) Prevalence of chronic kidney disease in a Nigerian family practice population. S Afr Fam Pract. 51:132–137

Agedew E. and Tefera C. (2015) Prevalence of Stunting among Children Aged 6-23 Months in Kemba Woreda, Southern Ethiopia: A Community Based Cross-Sectional Study. J Nutr Food Sci 2015, 5:4

Ahuja, T.S., Abbott, K.C., Pack, L., and Kuo, Y.-F. (2004). HIV-associated nephropathy and end-stage renal disease in children in the United States. Pediatr. Nephrol. Berl. Ger. *19*, 808–811.

Allan, P.L. (2011). CHAPTER 24 - Infectious diseases of the kidney. In Clinical Ultrasound (Third Edition), P.L. Allan, G.M. Baxter, and M.J. Weston, eds. (Edinburgh: Churchill Livingstone), pp. 460–466.

Alarcón JO, Freimanis-Hance L, Krauss M, Reyes MF, Cardoso CA, Mussi-Pinhata MM, Cardoso E, Hazra R (2012). NISDI Pediatric Study Group 2011. Opportunistic and other infections in HIV-infected children in Latin America compared to a similar cohort in the United States. AIDS Res Hum Retroviruses. 28(3):282-8.

Alves, T.P., Hulgan, T., Wu, P., Sterling, T.R., Stinnette, S.E., Rebeiro, P.F., Vincz, A.J., Bruce, M., and Ikizler, T.A. (2010). Race, Kidney Disease Progression, and Mortality Risk in HIV-Infected Persons. Clin. J. Am. Soc. Nephrol. CJASN *5*, 2269–2275.

Andiman, W.A., Chernoff, M.C., Mitchell, C., Purswani, M., Oleske, J., Williams, P.L., Spiegel, H., Gona, P., and Seage, G.R. (2009). Incidence of Persistent Renal

Dysfunction in Human Immunodeficiency Virus-Infected Children. Pediatr. Infect. Dis. J. *28*, 619–625.

Ando, M., Tsuchiya, K., and Nitta, K. (2012). How to manage HIV-infected patients with chronic kidney disease in the HAART era. Clin. Exp. Nephrol. *16*, 363–372.

Anígilájé EA, Dabit OJ, Ageda B, Hwande S, Bitto TT. (2013) The prevalence and predictors of HIV infection among children of mothers who missed prevention of mother to child transmission of HIV interventions in Makurdi, Nigeria. J AIDS Clin Res. 4:249

Anochie, I.C., Eke, F.U., and Okpere, A.N. (2008). Human immunodeficiency virus-associated nephropathy (HIVAN) in Nigerian children. Pediatr. Nephrol. Berl. Ger. *23*, 117–122.

Antonello, V.S., Poli-De-Figueiredo, C.E., Antonello, I.C.F., and Tovo, C.V. (2015). Urinary protein-to-creatinine ratio versus 24-h proteinuria in the screening for nephropathy in HIV patients. Int. J. STD AIDS *26*, 479–482.

Antoniou, T., Raboud, J., Chirhin, S., Yoong, D., Govan, V., Gough, K., Rachlis, A., and Loutfy, M. (2005). Incidence of and risk factors for tenofovir-induced nephrotoxicity: a retrospective cohort study. HIV Med. *6*, 284–290.

Anupama, Y.J., and Uma, G. (2014). Prevalence of chronic kidney disease among adults in a rural community in South India: Results from the kidney disease screening (KIDS) project. Indian J. Nephrol. *24*, 214–221.

Anyabolu, H.C., Adejuyigbe, E.A., and Adeodu, O.O. (2014). Under nutrition and anaemia among HAART-naïve HIV infected children in Ile-Ife, Nigeria: a case-controlled, hospital based study. Pan Afr. Med. J. *18*.

Arinaitwe E, Gasasira A, Verret W, Homsy J, Wanzira H, Kakuru A, Sandison TG, Young S, Tappero JW, Kamya MR, Dorsey G (2012). The association between malnutrition and the incidence of malaria among young HIV-infected and -uninfected Ugandan children: a prospective study. Malar J. 11: 90-10.1186/1475-2875-11-90

Arpadi, S., Shiau, S., Strehlau, R., Martens, L., Patel, F., Coovadia, A., Abrams, E., and Kuhn, L. (2013). Metabolic Abnormalities and Body Composition of HIV-infected South African Children on Ritonavir-Boosted Lopinavir-based or Nevirapine-based Antiretroviral Therapy: A Comparative Study. Arch. Dis. Child. *98*, 258–264.

Atta, M.G. (2010). Diagnosis and Natural History of HIV-Associated Nephropathy. Adv. Chronic Kidney Dis. *17*, 52–58.

Atta, M.G., Choi, M.J., Longenecker, J.C., Haymart, M., Wu, J., Nagajothi, N., Racusen, L.C., Scheel Jr, P.J., Brancati, F.L., and Fine, D.M. (2005). Nephrotic range proteinuria and CD4 count as noninvasive indicators of HIV-associated nephropathy. Am. J. Med. *118*, 1288.e21–e1288.e26.

Atta, M.G., Lucas, G.M., and Fine, D.M. (2008). HIV-associated nephropathy: epidemiology, pathogenesis, diagnosis and management. Expert Rev. Anti-Infect. Ther. *6*, 365–371.

Bae, E.J., Hwang, K., Jang, H.N., Kim, M.J., Jeon, D.-H., Kim, H.-J., Cho, H.S., Chang, S.-H., and Park, D.J. (2014). A retrospective study of short- and long-term effects on renal function after acute renal infarction. Ren. Fail. 1–5.

Bani-Hani, S., Patel, V., Larsen, C.P., Walker, P.D., Cooke, C.R., and Showkat, A. (2010). Renal disease in AIDS: it is not always HIVAN. Clin. Exp. Nephrol. *14*, 263–267.

Bansi, L., Hughes, A., Bhagani, S., Mackie, N.E., Leen, C., Levy, J., Edwards, S., Connolly, J., Holt, S.G., Hendry, B.M., et al. (2009). Clinical epidemiology of HIV-associated end-stage renal failure in the UK. AIDS Lond. Engl. *23*, 2517–2521.

Barisoni, L., Bruggeman, L.A., Mundel, P., D'Agati, V.D., and Klotman, P.E. (2000). HIV-1 induces renal epithelial dedifferentiation in a transgenic model of HIV-associated nephropathy. Kidney Int. *58*, 173–181.

Barril, G., González Parra, E., Alcázar, R., Arenas, D., Campistol, J.M., Caramelo, C., Carrasco, M., Carreño, V., Espinosa, M., García Valdecasas, J., et al. (2004). [Guidelines on hemodialysis-associated viral infections]. Nefrol. Publ. Of. Soc. Esp. Nefrol. *24 Suppl 2*, 43–66.

Beach RS, Mantero-Atienza E, Eisdorfer C, Fordyce-Baum MK. Altered (1988) folate metabolism in early HIV infection. JAMA.259 (4):519

Bennett DE, Bertagnolio S, Sutherland D, Gilks CF. (2008). The World Health Organization's global strategy for prevention and assessment of HIV drug resistance. Antivir Ther. 13 Suppl 2:1-13

Berger MR, Fields-Gardner C, Wagle A, Hollenbeck CB. (2008) Prevalence of malnutrition in human immunodeficiency virus/acquired immunodeficiency syndrome orphans in the Nyanza province of Kenya: a comparison of conventional indexes with a composite index of anthropometric failure. J Am Diet Assoc. 108:1014–1017.

Bertilla Uzoma, E., Henrietta Uchenna, O., Anthony Nnaemeka, I., and Tagbo, O. (2012). Screening for Microalbuminuria in HIV-Positive Children in Enugu. Int. J. Nephrol. *2012*, 805834.

Betjes, M.G.H., Weening, J., and Krediet, R.T. (2001). Diagnosis and treatment of HIV-associated nephropathy. Neth. J. Med. *59*, 111–117.

Bevc, S., Hojs, R., Ekart, R., Zavr&#x161, Nik, M., Gorenjak, M., and Puklavec, L. (2012). Simple Cystatin C Formula for Estimation of Glomerular Filtration Rate in Overweight Patients with Diabetes Mellitus Type 2 and Chronic Kidney Disease. J. Diabetes Res. *2012*, e179849.

Bianchi S, Bigazzi R, Campese VM. (1999). Microalbuminuria in essential hypertension: significance, pathophysiology, and therapeutic implications. Am J Kidney Dis. 34(6):973-95

Blasi, M., Balakumaran, B., Chen, P., Negri, D.R.M., Cara, A., Chen, B.K., and Klotman, M.E. (2014). Renal epithelial cells produce and spread HIV-1 via T-cell contact. AIDS Lond. Engl.

Blázquez-Medela, A.M., García-Sánchez, O., Blanco-Gozalo, V., Quiros, Y., Montero, M.J., Martínez-Salgado, C., López-Novoa, J.M., and López-Hernández, F.J. (2014). Hypertension and hyperglycemia synergize to cause incipient renal tubular alterations resulting in increased NGAL urinary excretion in rats. PloS One *9*, e105988.

Bökenkamp A, Domanetzki M, Zinck R, Schumann G, Byrd D, Brodehl J. (1998) Cystatin C--a new marker of glomerular filtration rate in children independent of age and height. Pediatrics. 101(5):875-81.

Bouthemy, C., Nel, I., Oudot Mellakh, T., and Theodorou, I. (2013). [Host's genetics in HIV disease]. Pathol. Biol. (Paris) *61*, 17–20.

Brady, M.T., Oleske, J.M., Williams, P.L., Elgie, C., Mofenson, L.M., Dankner, W.M., and Van Dyke, R.B. (2010). Declines in Mortality Rates and Changes in Causes of Death in HIV-1-Infected Children during the HAART Era. J. Acquir. Immune Defic. Syndr. 1999 *53*, 86–94.

Bratton, E.W., El Husseini, N., Chastain, C.A., Lee, M.S., Poole, C., Stürmer, T., Juliano, J.J., Weber, D.J., and Perfect, J.R. (2012). Comparison and temporal trends of three groups with cryptococcosis: HIV-infected, solid organ transplant, and HIV-negative/non-transplant. PloS One *7*, e43582.

Bruggeman, L.A., Adler, S.H., and Klotman, P.E. (2001). Nuclear factor-kappa B binding to the HIV-1 LTR in kidney: implications for HIV-associated nephropathy. Kidney Int. *59*, 2174–2181.

Bruggeman L.A, Bark C, and Kalayjian R.C. (2009) HIV and the Kidney. Curr Infect Dis Rep 11(6): 479–48

Bruggeman, L.A., Ross, M.D., Tanji, N., Cara, A., Dikman, S., Gordon, R.E., Burns, G.C., D'agati, V.D., Winston, J.A., Klotman, M.E., et al. (2000). Renal Epithelium Is a Previously Unrecognized Site of HIV-1 Infection. J. Am. Soc. Nephrol. *11*, 2079–2087.

Burkes RL, Cohen H, Krailo M, Sinow RM, Carmel R. (1987) Low serum cobalamin levels occur frequently in the acquired immune deficiency syndrome and related disorders. Eur J Haematol.; 38(2):141–147

Burns JM, Baghurst PA, Sawyer MG, McMichael AJ, Tong SL. (1999) Lifetime low-level exposure to environmental lead and children's emotional and behavioral development at ages 11–13 years. The Port Pirie Cohort Study. Am J Epidemiol. 149:740–749

Burns P, Gough S, Bradbury AW. Management of peripheral arterial disease in primary care. BMJ. 2003; 326:584–8

Busza, J., Walker, D., Hairston, A., Gable, A., Pitter, C., Lee, S., Katirayi, L., Simiyu, R., and Mpofu, D. (2012). Community-based approaches for prevention of mother to child transmission in resource-poor settings: a social ecological review. J. Int. AIDS Soc. *15 Suppl 2*, 17373.

Calis, J.C.,Phiri., K.S., Faragher,E. B.,Brabin , B.J.,Bates,I.& Cuevas L.E.(2008) Severe anemia in Malawian children. New Eng l and Journal of Medicine 358,888-99

Canaud, G., Dejucq-Rainsford, N., Avettand-Fenoël, V., Viard, J.-P., Anglicheau, D., Bienaimé, F., Muorah, M., Galmiche, L., Gribouval, O., Noël, L.-H. (2014). The kidney as a reservoir for HIV-1 after renal transplantation. J. Am. Soc. Nephrol. JASN *25*, 407–419.

Capocci, S., and Lipman, M. (2013). Respiratory infections in HIV-infected adults: epidemiology, clinical features, diagnosis and treatment. Curr. Opin. Pulm. Med. *19*, 238–243.

Centers for Disease Control and Prevention (2009). BMI for children and teens

Chadha V, Warady BA. (2005) Epidemiology of pediatric chronic kidney disease. Adv Chronic Kidney Dis 12: 343–352

Chandran, S., Jen, K.-Y., and Laszik, Z.G. (2013). Recurrent HIV-Associated Immune Complex Glomerulonephritis with Lupus-like Features after Kidney Transplantation. Am. J. Kidney Dis. *62*, 335–338.

Chaparro, A.I., Mitchell, C.D., Abitbol, C.L., Wilkinson, J.D., Baldarrago, G., Lopez, E., and Zilleruelo, G. (2008). Proteinuria in Children Infected with the Human Immunodeficiency Virus. J. Pediatr. *152*, 844–849.

Chaudhary, M., Gupta, S., Khare, S., and Lal, S. (2010). Diagnosis of tuberculosis in an era of HIV pandemic: a review of current status and future prospects. Indian J. Med. Microbiol. *28*, 281–289.

Chawla, L.S., Dommu, A., Berger, A., Shih, S., and Patel, S.S. (2008). Urinary Sediment Cast Scoring Index for Acute Kidney Injury: A Pilot Study. Nephron Clin. Pract. *110*, c145–c150.

Chiabi A, Lebela J, Kobela M, Mbuagbaw L, Obama MT, Ekoe T. (2012)The frequency and magnitude of growth failure in a group of HIV-infected children in Cameroon. Pan Afr Med J. 11:15.

Chisti, M.J., Ahmed, T., Pietroni, M.A.C., Faruque, A.S.G., Ashraf, H., Bardhan, P.K., Hossain, I., Das, S.K., and Salam, M.A. (2013). Pulmonary tuberculosis in severely-malnourished or HIV-infected children with pneumonia: a review. J. Health Popul. Nutr. *31*, 308–313.

Choi, A.I., Shlipak, M.G., Hunt, P.W., Martin, J.N., and Deeks, S.G. (2009). HIV-infected persons continue to lose kidney function despite successful antiretroviral therapy. AIDS Lond. Engl. *23*, 2143–2149.

Chopra, V., Garg, N., and Mrigpuri, P. (2013). Spontaneous pneumopericardium an unusual complication in a patient of HIV and pulmonary tuberculosis. Lung India Off. Organ Indian Chest Soc. *30*, 148–150.

Cobb, G., and Bland, R.M. (2013). Nutritional supplementation: the additional costs of managing children infected with HIV in resource-constrained settings. Trop. Med. Int. Health *18*, 45–52.

Cohen, S.D., and Kimmel, P.L. (2008). Immune Complex Renal Disease and Human Immunodeficiency Virus Infection. Semin. Nephrol. *28*, 535–544.

Cohn, S.K., and Weaver, L.T. (2006). The Black Death and AIDS: CCR5-Delta32 in genetics and history. QJM Mon. J. Assoc. Physicians *99*, 497–503.

Coresh J, Astor BC, Greene T, Eknoyan G, Levey AS. (2003) Prevalence of chronic kidney disease and decreased kidney function in the adult US population: Third National Health and Nutrition Examination Survey. Am J Kidney Dis.41 (1):1-12.

Coulibaly, G., Kouéta, F., Ouédraogo, O., Dao, L., Lengani, A., and Yé, D. (2013). [Prevalence of proteinuria in children followed-up for HIV infection at Pediatric University Hospital Charles-de-Gaulle (CHUP-CDG) of Ouagadougou]. Bull. Société Pathol. Exot. 1990 *106*, 13–17.

Counahan R, Chantler C, Ghazali S, Kirkwood B, Rose F, Barratt TM. Estimation of glomerular filtration rate from plasma creatinine concentration in children. Arch Dis Child. 1976; 51:875-878

Daher, E.D.F., Junior Silva, G.B. da, Vieira, A.P.F., Souza, J.B. de, Falcão, F.D.S., Costa, C.R. da, Fernandes, A.A.C. da S., and Lima, R.S.A. (2014). Acute kidney injury in a tropical country: a cohort study of 253 patients in an infectious diseases intensive care unit. Rev. Soc. Bras. Med. Trop. *47*, 86–89.

Daudon M, Cohen-Solal F, Barbey F, et al. Cystine crystal volume determination: a useful tool in the management of cystinuric patients. Urol Res 2003; 31:207.

Daudon M, Jungers P, Lacour B. Clinical value of crystalluria study. Ann Biol Clin (Paris) 2004; 62:379–93.

Daugas, E., Rougier, J.-P., and Hill, G. (2005). HAART-related nephropathies in HIV-infected patients. Kidney Int. *67*, 393–403.

Devarajan P. (2010) Neutrophil gelatinase-associated lipocalin (NGAL): A troponin-like biomarker for human acute kidney injury. Nephrology (Carlton) 15:419–428

De Santis, G.C., Brunetta, D.M., Vilar, F.C., Brandão, R.A., de Albernaz Muniz, R.Z., de Lima, G.M.N., Amorelli-Chacel, M.E., Covas, D.T., and Machado, A.A. (2011). Hematological abnormalities in HIV-infected patients. Int. J. Infect. Dis. *15*, e808–e811.

De Silva, T.I., Post, F.A., Griffin, M.D., and Dockrell, D.H. (2007). HIV-1 Infection and the Kidney: An Evolving Challenge in HIV Medicine. Mayo Clin. Proc. *82*, 1103–1116.

De Zeeuw D. (2004) Albuminuria, not only a cardiovascular/renal risk marker, but also a target for treatment? Kidney international l66 (Suppl 92):2–6.

Dharnidharka VR, Kwon C, Stevens G (2002). Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. *Am J Kidney Dis 40*: *221–* 226

Di Belgiojoso, G.B., Ferrario, F., and Landriani, N. (2002). Virus-related glomerular diseases: histological and clinical aspects. J. Nephrol. *15*, 469–479.

Di Biagio, A., Rosso, R., Vitale, F., Cardinale, F., Sormani, M.P., Secondo, G., Di Stefano, L., and Viscoli, C. (2011). Risk factors for chronic kidney disease among human immunodeficiency virus-infected patients: A European case control study. Clin. Nephrol. *75*, 518–523.

Dimock D, Vakkalanka S, Kopp J, Purdy J, Hazra R, Hadigan C. (2009). Microalbuminuria in a longitudinal cohort of adolescents and young adults with HIV infection acquired in infancy or childhood. Antiviral Therapy. 14:A47

Dimock D, Thomas V, Cushing A, Purdy JB, Worrell C, Kopp JB, Hazra R, Hadigan C. (2011). Longitudinal assessment of metabolic abnormalities in adolescents and young adults with HIV-infection acquired perinatally or in early childhood. Metabolism. 60(6):874–880.

Domingo, P., Knobel, H., Gutiérrez, F., Barril, G., and Fulladosa, X. (2010). [Assessment and management of kidney disease in the HIV-1-infected patient. A practical review]. Enfermedades Infecc. Microbiol. Clínica *28*, 185–198.

Duggan, M.B. (2010). Anthropometry as a tool for measuring malnutrition: impact of the new WHO growth standards and reference. Ann. Trop. Paediatr. *30*, 1–17.

Eke, F.U., Anochie, I.C., Okpere, A.N., Eneh, A.U., Ugwu, R.O., Ugwu, R.N., Ejilemele, A.A., and Ugboma, H.U. (2010). Microalbuminuria in children with human immunodeficiency virus (HIV) infection in Port Harcourt, Nigeria. Niger. J. Med. J. Natl. Assoc. Resid. Dr. Niger. *19*, 298–301.

Ekulu PM, Nseka NM, Aloni MN, Gini JL, Makulo JR, Lepira FB, Sumaili EK, Mafuta EM, Nsibu CN, Shiku JD. (2012) Prevalence of proteinuria and its association with HIV/AIDS in Congolese children living in Kinshasa, Democratic Republic of Congo Nephrol Ther. 8(3):163-7

Enawgaw, B., Alem, M., Addis, Z., and Melku, M. (2014). Determination of hematological and immunological parameters among HIV positive patients taking highly active antiretroviral treatment and treatment naïve in the antiretroviral therapy clinic of Gondar University Hospital, Gondar, Northwest Ethiopia: a comparative cross-sectional study. BMC Hematol. *14*, 8.

Ephraim R. K. D., Brenyah R. C., Osei R., Bossipe B. D., Adoba P., Osakunor D. N. M., Agbodzakey H., (2014) Crystalluria in HIV/AIDS patients on highly active anti-retroviral therapy in the Kumasi metropolis; a cross sectional study. Niger Med J. 55(6): 504–507

Esezobor CI, Iroha E, Onifade E, Akinsulie AO, Temiye EO, Ezeaka C.(2009) Prevalence of proteinuria among HIV-infected children attending a tertiary hospital in Lagos, Nigeria. J Trop Pediatr. 56(3):187-90

Esezobor CI, Iroha E, Oladipo O, Onifade E, Soriyan OO, Akinsulie AO, Temiye EO, Ezeaka C. (2010) Kidney function of HIV-infected children in Lagos, Nigeria: using Filler's serum cystatin C-based formula. J Int AIDS Soc doi: 10.1186/1758-2652-13-17

Estébanez-Muñoz, M., Soto-Abánades, C.I., Ríos-Blanco, J.J., and Arribas, J.R. (2012). Updating our understanding of pulmonary disease associated with HIV infection. Arch. Bronconeumol. *48*, 126–132.

Estrella, M.M., and Fine, D.M. (2010). Screening for chronic kidney disease in HIV-infected patients. Adv. Chronic Kidney Dis. *17*, 26–35.

Eustace, J.A., Nuermberger, E., Choi, M., Scheel, P.J., Moore, R., and Briggs, W.A. (2000). Cohort study of the treatment of severe HIV-associated nephropathy with corticosteroids. Kidney Int. *58*, 1253–1260.

Ezeonwu BU, Ikefuna AN, Oguonu T, Okafor HU (2014). Prevalence of hematological abnormalities and malnutrition in HIV infected under five children in Enugu. Niger. J. Clin. Pract. 17:303-8

Ezeonwu BU, Okafor HU, Ikefuna AN, Oguonu T. (2012) Screening for microalbuminuria in HIV-positive children in Enugu. *International Journal of Nephrology*. 2012(5): 805-834

Fabian, J., Naicker, S., Goetsch, S., and Venter, W.D.F. (2013). The clinical and histological response of HIV-associated kidney disease to antiretroviral therapy in South Africans. Nephrol. Dial. Transplant. Off. Publ. Eur. Dial. Transpl. Assoc. - Eur. Ren. Assoc. *28*, 1543–1554.

Fabiny, D.L., and Ertingshausen, G. (1971). Automated Reaction-Rate Method for Determination of Serum Creatinine with the CentrifiChem. Clin. Chem. *17*, 696–700.

Fergusson P, Chinkhumba J, Grijalva-Eternod C, Banda T, Mkangama C, Tomkins A. (2009) Nutritional recovery in HIV-infected and HIV-uninfected children with severe acute malnutrition. Archives of Disease in Childhood. 94:512–516.

Filler G, Lepage N. (2003) Should the Schwartz formula for estimation of GFR be replaced by cystatin C formula? Pediatr Nephrol. 18(10):981-5

Fine, D.M., Perazella, M.A., Lucas, G.M., and Atta, M.G. (2008). Kidney Biopsy in HIV: Beyond HIV-Associated Nephropathy. Am. J. Kidney Dis. *51*, 504–514.

FolefackKaze, F., Kengne, A.-P., PefuraYone, E., NdamFemben, N., and Ashuntantang, G. (2013). Renal function, urinalysis abnormalities and correlates among HIV-infected cameroonians naive to antiretroviral therapy. Saudi J. Kidney Dis. Transplant. *24*, 1291.

Fowler, M.G., Lampe, M.A., Jamieson, D.J., Kourtis, A.P., and Rogers, M.F. (2007). Reducing the risk of mother-to-child human immunodeficiency virus transmission: past successes, current progress and challenges, and future directions. Am. J. Obstet. Gynecol. *197*, S3–S9.

Foy, M.C., Estrella, M.M., Lucas, G.M., Tahir, F., Fine, D.M., Moore, R.D., and Atta, M.G. (2013). Comparison of risk factors and outcomes in HIV immune complex kidney disease and HIV-associated nephropathy. Clin. J. Am. Soc. Nephrol. CJASN *8*, 1524–1532.

Frassetto, L., Baluom, M., Jacobsen, W., Christians, U., Roland, M.E., Stock, P.G., Carlson, L., and Benet, L.Z. (2005). Cyclosporine pharmacokinetics and dosing modifications in human immunodeficiency virus-infected liver and kidney transplant recipients. Transplantation *80*, 13–17.

Garg P, Rabelink T. (2011) Glomerular proteinuria: a complex interplay between unique players. Adv Chronic Kidney Dis. 18(4):233-42

Giacomet, V., Cattaneo, D., Viganò, A., Nannini, P., Manfredini, V., Ramponi, G., Clementi, E., and Zuccotti, G.V. (2013). Tenofovir-induced renal tubular dysfunction in vertically HIV-infected patients associated with polymorphisms in ABCC2, ABCC4 and ABCC10 genes. Pediatr. Infect. Dis. J. *32*, e403–e405.

Gindea, S., Schwartzman, J., Herlitz, L.C., Rosenberg, M., Abadi, J., and Putterman, C. (2010). Proliferative Glomerulonephritis in Lupus Patients with Human Immunodeficiency Virus Infection: A Difficult Clinical Challenge. Semin. Arthritis Rheum. *40*, 201–209.

Gjesdal CG, Vollset SE, Ueland PM, Refsum H, Drevon CA, Gjessing HK, Tell GS. . (2006) Plasma total homocysteine level and bone mineral density: the Hordaland Homocysteine Study. Arch Intern Med 166:88–94.

Gjesdal C.G., Vollset S.E., Ueland P.M., Refsum H., Meyer H.E., Tell G.S. (2007) Plasma homocysteine, folate, and vitamin B 12 and the risk of hip fracture: The hordaland homocysteine study. J. Bone Miner. Res. 22:747–756

Glassock, R.J., and Winearls, C. (2009). Ageing and the Glomerular Filtration Rate: Truths and Consequences. Trans. Am. Clin. Climatol. Assoc. *120*, 419–428.

Gluhovschi, C., Sporea, I., Gădălean, F., Kaycsa, A., Curescu, M., Velciov, S., Petrica, L., Bălgrădean, C., Vernic, C., and Gluhovschi, A. (2014). Are other factors besides albuminuria important for the progression of HCV chronic hepatitis towards CKD? A survey from a hepatology department in western Romania. Romanian J. Intern. Med. Rev. Roum. Médecine Interne *52*, 13–17.

Gonzalez-Casanova I, Sarmiento OL, Gazmararian JA, Cunningham SA, Martorell R, Pratt M, Stein AD. (2013). Comparing three body mass index classification systems to assess overweight and obesity in children and adolescents. Rev Panam Salud Publica. 33(5):349-55

Grinsztejn, B., Hosseinipour, M.C., Ribaudo, H.J., Swindells, S., Eron, J., Chen, Y.Q., Wang, L., Ou, S.-S., Anderson, M., McCauley, M., et al. (2014). Effects of early

versus delayed initiation of antiretroviral treatment on clinical outcomes of HIV-1 infection: results from the phase 3 HPTN 052 randomised controlled trial. Lancet Infect. Dis. *14*, 281–290.

Gu, L., Dai, Y., Xu, J., Mallipattu, S., Kaufman, L., Klotman, P.E., He, J.C., and Chuang, P.Y. (2013). Deletion of podocyte STAT3 mitigates the entire spectrum of HIV-1-associated nephropathy. AIDS Lond. Engl. *27*, 1091–1098.

Guaraldi, G., Dolci, G., Bellasi, A., and Di Iorio, B. (2014). [Inhibition of the renin-angiotensin system in HIV nephropathy]. G. Ital. Nefrol. Organo Uff. Della Soc. Ital. Nefrol. *31*.

Gupta, S., and Singh, S. (2006). Hepatitis B and C virus co-infections in human immunodeficiency virus positive North Indian patients. World J. Gastroenterol. WJG *12*, 6879–6883.

Gupta, S.K., Parker, R.A., Robbins, G.K., and Dubé, M.P. (2005a). The effects of highly active antiretroviral therapy on albuminuria in HIV-infected persons: results from a randomized trial. Nephrol. Dial. Transplant. Off. Publ. Eur. Dial. Transpl. Assoc. - Eur. Ren. Assoc. *20*, 2237–2242.

Gupta, S.K., Eustace, J.A., Winston, J.A., Boydstun, I.I., Ahuja, T.S., Rodriguez, R.A., Tashima, K.T., Roland, M., Franceschini, N., Palella, F.J., et al. (2005b). 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. *40*, 1559–1585.

Gupta, S.K., Rosenkranz, S.L., Cramer, Y.S., Koletar, S.L., Szczech, L.A., Amorosa, V., and Hall, S.D. (2008). The pharmacokinetics and pharmacogenomics of efavirenz and lopinavir/ritonavir in HIV-infected persons requiring hemodialysis. AIDS Lond. Engl. *22*, 1919–1927.

Haase, M., Devarajan, P., Haase-Fielitz, A., Bellomo, R., Cruz, D.N., Wagener, G., Krawczeski, C.D., Koyner, J.L., Murray, P., Zappitelli, M., et al. (2011). The outcome of neutrophil gelatinase-associated lipocalin-positive subclinical acute kidney injury: a multicenter pooled analysis of prospective studies. J. Am. Coll. Cardiol. *57*, 1752–1761.

Hadigan, C., Edwards, E., Rosenberg, A., Purdy, J.B., Fleischman, E., Howard, L., Mican, J.M., Sampath, K., Oyalowo, A., Johnson, A., et al. (2013). Microalbuminuria in HIV disease. Am. J. Nephrol. *37*, 443–451.

Hannon, H., Bagnis, C.I., Benhamou, Y., Beaufils, H., Sullivan, M., Brosgart, C., Izzedine, H., Poynard, T., and Deray, G. (2004). The renal tolerance of low-dose adefovir dipivoxil by lamivudine-resistant individuals co-infected with hepatitis B and HIV. Nephrol. Dial. Transplant. Off. Publ. Eur. Dial. Transpl. Assoc. - Eur. Ren. Assoc. *19*, 386–390.

Han TM, Naicker S, Ramdial PK, Assounga AG. A cross-sectional study of HIV-seropositive patients with varying degrees of proteinuria in South Africa. Kidney International 2006; 69: 2243-50

Hanna Z, Priceputu E, Chrobak P, Hu C Dugas V , Goupil M , Marquis M , de Repentigny L , Jolicoeur P (2009). Selective expression of human immunodeficiency virus *Nef* in specific immune cell populations of transgenic mice is associated with distinct AIDS-like phenotypes. J Virol. 83(19):9743–9758.

Hazenberg, M.D., Hamann, D., Schuitemaker, H., and Miedema, F. (2000). T cell depletion in HIV-1 infection: how CD4+ T cells go out of stock. Nat. Immunol. *1*, 285–289.

Herman, E.S., and Klotman, P.E. (2003). HIV-associated nephropathy: Epidemiology, pathogenesis, and treatment. Semin. Nephrol. *23*, 200–208.

Heyns, C.F., Smit, S.G., van der Merwe, A., and Zarrabi, A.D. (2013). Urological aspects of HIV and AIDS. Nat. Rev. Urol. *10*, 713–722.

Hogg RJ, Furth S, Lemley KV, Portman R, Schwartz GJ, Coresh J, Balk L, Lau J, Levin A, Kausaz AT, EknoyanG, Levey AS (2003). National Kidney Foundation's Kidney Disease Outcomes Quality Initiative Clinical Practice Guidelines for Chronic Kidney Disease in Children and Adolescents: Evaluation, Classification, and Stratification. Pediatrics. 111(6):1416-20

Hogg RJ, Portman RJ, Milliner D, Lemley KV, Eddy A, Ingelfinger J. (2000) Evaluation and management of proteinuria and nephrotic syndrome in children: recommendations from a pediatric nephrology panel established at the National Kidney Foundation Conference on Proteinuria, Albuminuria, Risk, Assessment, Detection, and Elimination (PARADE). Pediatrics 105:1242– 1249

Hoy WE, Wang Z, VanBuynder P, Baker PR, McDonald SM, Mathews JD. (2001). The natural history of renal disease in Australian Aborigines. Part 2: Albuminuria predicts natural death and renal failure. Kidney Int 60: 249–256

Ikpeme, E.E., Ekrikpo, U.E., Akpan, M.U., and Ekaidem, S.I. (2012). Determining the prevalence of human immunodeficiency virus-associated nephropathy (HIVAN) using proteinuria and ultrasound findings in a Nigerian paediatric HIV population. Pan Afr. Med. J. *11*, 13.

Imani, P.D., Odiit, A., Hingorani, S.R., Weiss, N.S., and Eddy, A.A. (2013). Acute kidney injury and its association with in-hospital mortality among children with acute infections. Pediatr. Nephrol. Berl. Ger. *28*, 2199–2206.

Inker L.A, Shaffi K, Levey S.A (2012) Estimating GFR Using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Creatinine Equation: Better Risk Predictions. Circ Heart Fail. 5(3): 303–306.

Iseki K, Ikemiya Y, Iseki C, Takishita S. (2003). Proteinuria and the risk of developing end-stage renal disease. Kidney Int 63: 1468–1474

Israni A, Kasiske B. (2007) Laboratory assessment of kidney disease: clearance, urinalysis, and kidney biopsy. In: *Brenner and Rector's the Kidney (8th Edition).* Brenner (Ed.). Saunders, NY, USA, 724–756

Jafari, A., Khalili, H., and Dashti-Khavidaki, S. (2014). Tenofovir-induced nephrotoxicity: incidence, mechanism, risk factors, prognosis and proposed agents for prevention. Eur. J. Clin. Pharmacol. 70(9):1029-40

Jao J, Palmer D, Leus I, Tih P, Baweja M, Klotman M, Sperling R, and Wyatt C. (2011) Prevalence and Predictors of Proteinuria in HIV-infected and Uninfected Pregnant Women in Cameroon. Nephrology Dialysis Transplantation. 26(9):3051-3.

Jaroszewicz J, Wiercinska-Drapalo A, Lapinski TW, Prokopowicz D, Rogalska M, Parfieniuk A. (2006). Does HAART improve renal function? An association between serum cystatin C concentration, HIV viral load and HAART duration. Antivir Ther. 11(5):641–5

Jayadev, S., and Garden, G.A. (2009). Host and viral factors influencing the pathogenesis of HIV-associated neurocognitive disorders. J. Neuroimmune Pharmacol. Off. J. Soc. NeuroImmune Pharmacol. *4*, 175–189.

Jefferson JA, Shankland SJ, Pichler RH. 2008. Proteinuria in diabetic kidney disease: A mechanistic viewpoint. Kidney Int 74(*1*):22–36.

Johnson DW 2004. Evidence-based guide to slowing the progression of early renal insufficiency. Internal Medicine Journal 34:50–7

Jones CY, Jones CA, Wilson IB, Knox TA, Levey AS, Spiegelman D, Gorbach SL, Van Lente F, Stevens LA. (2008). Cystatin C and creatinine in an HIV cohort: the nutrition for healthy living study. Am J Kidney Dis. 51:914–924.

Kajiyama, W., Kopp, J.B., Marinos, N.J., Klotman, P.E., and Dickie, P. (2000). Glomerulosclerosis and viral gene expression in HIV-transgenic mice: role of nef. Kidney Int. *58*, 1148–1159.

Kalayjian, R.C. (2010). The Treatment of HIV-Associated Nephropathy. Adv. Chronic Kidney Dis. *17*, 59–71.

Kalim, S., Szczech, L.A., and Wyatt, C.M. (2008). Acute kidney injury in HIV-infected patients. Semin. Nephrol. *28*, 556–562.

Kalyesubula, R., and Perazella, M.A. (2011). Nephrotoxicity of HAART. AIDS Res. Treat. *2011*, 1–11.

Kapavarapu, P.K., Bari, O., Perumpil, M., Duggan, C., Dinakar, C., Krishnamurthy, S., Arumugam, K., and Shet, A. (2012). Growth patterns and anaemia status of HIV-infected children living in an institutional facility in India. Trop. Med. Int. Health TM IH *17*, 962–971.

Kawo G, Karlsson K, Lyamuya E, Kalokola F, Fataki M, Kazimoto T, Kitundu J, Msaky H, Munubhi E, Ostborn A, Bredberg-Rådén U, Swai A, Mbise R, Msengi A, Mhalu F, Biberfeld G. (2000) Prevalence of HIV type 1 infection, associated clinical features and mortality among hospitalized children in Dar es Salaam, Tanzania. Scand J Infect Dis. 32(4):357-63

Khan, S., Haragsim, L., and Laszik, Z.G. (2006). HIV-Associated Nephropathy. Adv. Chronic Kidney Dis. *13*, 307–313.

Khatua, A.K., Taylor, H.E., Hildreth, J.E.K., and Popik, W. (2010). Non-productive HIV-1 infection of human glomerular and urinary podocytes. Virology *408*, 119–127.

Kimmel PL, Barisoni L, Kopp JB. (2003) Pathogenesis and treatment of HIV-associated renal diseases: lessons from clinical and animal studies, molecular pathologic correlations, and genetic investigations. Ann Int Med 139*:*214-27

Kopp JB, Smith MW, Nelson GW, Johnson RC, Freedman BI, Bowden DW, Oleksyk T, McKenzie LM, Kajiyama H, Ahuja TS, Berns JS, Briggs W, Cho ME, Dart RA, Kimmel PL, Korbet SM, Michel DM, Mokrzycki MH, Schelling JR, Simon E, Trachtman H, Vlahov D, Winkler CA (*2008*)*:* MYH9 is a major-effect risk gene for focal segmental glomerulosclerosis. Nat Genet 40: 1175–1184

Kuzmanović, M., Kunishima, S., Putnik, J., Stajić, N., Paripović, A., and Bogdanović, R. (2014). Congenital thrombocytopenia with nephritis - The first case of MYH9 related disorder in Serbia. Vojnosanit. Pregl. Mil.-Med. Pharm. Rev. *71*, 395–398.

Laterza OF, Price CP, Scott MG. (2002) Cystatin C: an improved estimator of glomerular filtration rate? Clin Chem. 48(5):699-707.

Ledger, S. (2006). Laboratory assessment of kidney function. CANNT J. J. ACITN *16*, 34–37; quiz 38–39.

Leger F, Bouissou F, Coulais Y, Tafani M, Chatelut E. Estimation of glomerular filtration rate in children. Pediatr Nephrol. 2002; 17:903–907.

Lescure, F.-X., Flateau, C., Pacanowski, J., Brocheriou, I., Rondeau, E., Girard, P.-M., Ronco, P., Pialoux, G., and Plaisier, E. (2012). HIV-associated kidney glomerular diseases: changes with time and HAART. Nephrol. Dial. Transplant. Off. Publ. Eur. Dial. Transpl. Assoc. - Eur. Ren. Assoc. *27*, 2349–2355.

Leventhal, J.S., and Ross, M.J. (2008). Pathogenesis of HIV-associated nephropathy. Semin. Nephrol. *28*, 523–534.

Levey AS. Measurement of renal function in chronic renal disease. Kidney Int 1990; 38:167.

Longo AL, Lepira FB, Sumaili EK, Makulo JR, Mukumbi H, Bukabau JB, Mokoli VM, Kayembe PK, Nseka NM. (2011) Prevalence of low estimated glomerular filtration rate, proteinuria, and associated risk factors among HIV-infected black patients using Cockroft-Gault and modification of diet in renal disease study equations. J Acquir Immune Defic Syndr. 59(1):59–64

Luetkemeyer, A.F., Havlir, D.V., and Currier, J.S. (2014). CROI 2014: viral hepatitis and complications of HIV disease and antiretroviral therapy. Top. Antivir. Med. *22*, 602–615.

Maggi, P., Montinaro, V., Bellacosa, C., Pietanza, S., Volpe, A., Graziano, G., Strippoli, G.F.M., and Angarano, G. (2012). Early markers of tubular dysfunction in

antiretroviral-experienced HIV-infected patients treated with tenofovir versus abacavir. AIDS Patient Care STDs *26*, 5–11.

Makubi NB, Mugusi F, Magesa MP, Roberts D, Quaresh A. (2012) Risk factors for anaemia among HIV infected children attending care and treatment clinic at Muhimbili national hospital in Dar es Salaam, Tanzania. Tanzan J Health Re. 14(1):1–9

Mannix IM, Ernest K, Sumaili Michel J, Pierre W, Dieudonné K, Mubagwa Rissassy JR, François B L, and Nazaire MN. (2014). Prevalence of microalbuminuria and diagnostic value of dipstick proteinuria in outpatients from HIV clinics in Bukavu, the Democratic Republic of Congo BMC Nephrol 15:146.

Marik, P.E. (2014). Iatrogenic salt water drowning and the hazards of a high central venous pressure. Ann. Intensive Care *4*, 21.

Masimango, M.I., Sumaili, E.K., Jadoul, M., Wallemacq, P., Mubagwa, D.K., Makulo, R.J.-R., Lepira, F.B., and Nseka, N.M. (2014). Prevalence of microalbuminuria and diagnostic value of dipstick proteinuria in outpatients from HIV clinics in Bukavu, the Democratic Republic of Congo. BMC Nephrol. *15*, 146.

Mathews, S.E., Srivastava, D., BalaYadav, R., and Sharma, A. (2013). Association of Hematological Profile of Human Immunodeficiency Virus-Positive Patients with Clinicoimmunologic Stages of the Disease. J. Lab. Physicians *5*, 34–37.

Mausumee, G., Frank, S., Shawn, C., Dara, H., Zhao, Y., Soleil, P.M., Sanderson, T.P., Michael, G., and Marc, D. (2014). Nonclinical Safety Profile of BMS-986001, a Nucleoside Transcriptase Inhibitor for Combination Retroviral Therapy. Int. J. Toxicol. *33*, 204–218.

McGrath CJ, Nduati R, Richardson BA, Kristal AR, Mbori-Ngacha D, Farquhar C, John-Stewart GC. (2012). The prevalence of stunting is high in HIV-1-exposed uninfected infants in Kenya. J Nutr 142(4):757-63.

McCulloch, M.I., and Ray, P.E. (2008). Kidney Disease in HIV-Positive Children. Semin. Nephrol. *28*, 585–594.

McDonald, C.M., Kupka, R., Manji, K.P., Okuma, J., Bosch, R.J., Aboud, S., Kisenge, R., Spiegelman, D., Fawzi, W.W., and Duggan, C.P. (2012). Predictors of stunting, wasting, and underweight among Tanzanian children born to HIV-infected women. Eur. J. Clin. Nutr. *66*, 1265–1276.

Mistry, B.J. (2010). Relevance of microalbuminuria in screening for HIV-Associated Nephropathy. Faculty of Health Sciences, University of Witwatersrand, Johannesburg [Electronic theses and Dissertations (ETD)],WIReDSpace, http://hdl.handle.net/10539/7650.

Mitchell HR and Kline W. (2006) Core curriculum in nephrology, Renal Function Testing. Am J Kidney Dis.47:174–183.

Mofenson, L.M., Brady, M.T., Danner, S.P., Dominguez, K.L., Hazra, R., Handelsman, E., Havens, P., Nesheim, S., Read, J.S., Serchuck, L., et al. (2009).

Guidelines for the Prevention and Treatment of Opportunistic Infections Among HIV-Exposed and HIV-Infected Children: Recommendations from CDC, the National Institutes of Health, the HIV Medicine Association of the Infectious Diseases Society of America, the Pediatric Infectious Diseases Society, and the American Academy of Pediatrics. MMWR Recomm. Rep. Morb. Mortal. Wkly. Rep. Recomm. Rep. Cent. Dis. Control *58*, 1.

Mosten, I.K., Hamel, B.C., and Kinabo, G.D. (2015). Prevalence of persistent microalbuminuria and associated factors among HIV infected children attending a Tertiary Hospital in Northern Tanzania: a cross sectional, analytical study. Pan Afr. Med. J. *20*.

Moyle G (2002) Anaemia in persons with HIV infection: prognostic marker of contribution to morbidity. AIDS Rev 4(1): 13–20.

Msango, L., Downs, J.A., Kalluvya, S.E., Kidenya, B.R., Kabangila, R., Johnson, W.D., Fitzgerald, D.W., and Peck, R.N. (2011). Renal dysfunction among HIV-infected patients starting antiretroviral therapy. AIDS Lond. Engl. *25*, 1421–1425.

Mudi, A., Alhaj, B.U., Hassan-Hanga, F., Yahaya, I.A., Mudi, A., Alhaj, B.U., Hassan-Hanga, F., and Yahaya, I.A. (2014). Persistent Microalbuminuria in Human Immunodeficiency Virus Infected Children in Kano, Nigeria, Persistent Microalbuminuria in Human Immunodeficiency Virus Infected Children in Kano, Nigeria. Int. J. Nephrol. Int. J. Nephrol. *2014*, *2014*, e567838.

Munyazesa, E., Emile, I., Mutimura, E., Hoover, D.R., Shi, Q., McGinn, A.P., Musiime, S., Muhairwe, F., Rutagengwa, A., Dusingize, J.C., et al. (2012). Assessment of haematological parameters in HIV-infected and uninfected Rwandan women: a cross-sectional study. BMJ Open *2*, e001600.

Murray, C.J.L., Ortblad, K.F., Guinovart, C., Lim, S.S., Wolock, T.M., Roberts, D.A., Dansereau, E.A., Graetz, N., Barber, R.M., Brown, J.C., et al. (2014). Global, regional, and national incidence and mortality for HIV, tuberculosis, and malaria during 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet.

Nagai, T., Uemura, O., Ishikura, K., Ito, S., Hataya, H., Gotoh, Y., Fujita, N., Akioka, Y., Kaneko, T., and Honda, M. (2013). Creatinine-based equations to estimate glomerular filtration rate in Japanese children aged between 2 and 11 years old with chronic kidney disease. Clin. Exp. Nephrol. *17*, 877–881.

Nakagawa, N., Fujino, T., Kabara, M., Matsuki, M., Chinda, J., Kikuchi, K., Hasebe, N., and NICE-Combi Study Group (2011). Angiotensin II receptor blocker and long-acting calcium channel blocker combination therapy decreases urinary albumin excretion while maintaining glomerular filtration rate. Hypertens. Res. Off. J. Jpn. Soc. Hypertens. *34*, 1121–1126.

Nelson M, Azwa A, Sokwala A, Harania RS, Stebbing J. (2008). Fanconi syndrome and lactic acidosis associated with stavudine and lamivudine therapy. AIDS. 22(11):1374–6.

Nelson TJ, Balza R, Xiao Q, Misra RP. (2005) SRF-dependent gene expression in isolated cardiomyocytes: regulation of genes involved in cardiac hypertrophy. J Mol Cell Cardiol. 39:479–489

Nelson PJ, Gelman IH, Klotman PE. (2002). Suppression of HIV-1 expression by inhibitors of cyclin-dependent kinases promotes differentiation of infected podocytes. J Am Soc Nephrol 12*:* 2827–2831

Nelson PJ, Sunamoto M, Husain M, Gelman IH. (2002) HIV-1 expression induces cyclin D1 expression and pRb phosphorylation in infected podocytes: cell-cycle mechanisms contributing to the proliferative phenotype in HIV-associated nephropathy. BMC.Microbiol. 2:e26.

NICE (2008) Clinical Guideline 73: Chronic kidney disease http://www.nice.org.uk/ CG73.

NICE clinical guideline 73(2008). Chronic kidney disease; early identification and management of chronic kidney disease in adults in primary and secondary care. London: National Institute for Health and Clinical Excellence.

Nishijima, T., Komatsu, H., Higasa, K., Takano, M., Tsuchiya, K., Hayashida, T., Oka, S., and Gatanaga, H. (2012). Single nucleotide polymorphisms in ABCC2 associate with tenofovir-induced kidney tubular dysfunction in Japanese patients with HIV-1 infection: a pharmacogenetic study. Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. *55*, 1558–1567.

Núñez, M., Saran, A.M., and Freedman, B.I. (2010). Gene-Gene and Gene-Environment Interactions in HIV-Associated Nephropathy: A Focus on the MYH9 Nephropathy Susceptibility Gene. Adv. Chronic Kidney Dis. *17*, 44–51.

Odden MC, Scherzer R, Bacchetti P, Szczech LA, Sidney S, Grunfeld C, Shlipak MG. Cystatin C level as a marker of kidney function in human immunodeficiency virus infection: the FRAM study. (2007) Arch Intern Med. 167(20):2213-9.

Okechukwu AA, Gambo D, Okechuk wu IO (2010). Prevalence of Anaemia in HIV-Infected Children at the University of Abuja Teaching Hospital, Gwagwalada. Niger. J. Med. 19:50-57.

Okolie, M.N., Eghafona, N.O., and Omoregie, R. (2003). Anti-Human Immunodeficiency Virus (HIV) Agents. J. Med. Lab. Sci. *12*, 1–14.

Owiredu, W., Quaye, L., Amidu, N., and Addai-Mensah, O. (2011). Prevalence of anaemia and immunological markers among Ghanaian HAART-naïve HIV-patients and those on HAART. Afr. Health Sci. *11*, 2–15.

Padmapriyadarsini C, Pooranagangadevi N, Chandrasekaran K, Subramanyan S, Thiruvalluvan C, Bhavani P. K, and Swaminathan S (2009). Prevalence of Underweight, Stunting, and Wasting among Children Infected with Human Immunodeficiency Virus in South India. Int J Pediatr.837627

Palumbo, P., Lindsey, J.C., Hughes, M.D., Cotton, M.F., Bobat, R., Meyers, T., Bwakura-Dangarembizi, M., Chi, B.H., Musoke, P., Kamthunzi, P., et al. (2010).

Antiretroviral Treatment for Children with Peripartum Nevirapine Exposure. N. Engl. J. Med. *363*, 1510–1520.

Patwardhan MS, Golwilkar AS, Abhyanakar JR, Atre MC. (2002) Hematological profile of HIV positive patients. Indian J Pathol Microbiol. 45:147–50

Peake, *Michael, and Whiting, M. (2006). Measurement of Serum Creatinine – Current Status and Future Goals. Clin. Biochem. Rev. *27*, 173–184.

Perazella MA. (2010) Tenofovir-induced kidney disease: an acquired renal tubular mitochondriopathy. *Kidney International*. 78(11):1060–1063.

M. A. Perazella (2011). "Tenofovir-induced kidney disease: an acquired renal tubular mitochondriopathy," Kidney International, vol. 78, no. 11, pp. 1060–1063,

Peters, P.J., Moore, D.M., Mermin, J., Brooks, J.T., Downing, R., Were, W., Kigozi, A., Buchacz, K., and Weidle, P.J. (2008). Antiretroviral therapy improves renal function among HIV-infected Ugandans. Kidney Int. *74*, 925–929.

Pipkin, S., Scheer, S., Okeigwe, I., Schwarcz, S., Harris, D.H., and Hessol, N.A. (2011). The effect of HAART and calendar period on Kaposi's sarcoma and non-Hodgkin lymphoma: results of a match between an AIDS and cancer registry. AIDS Lond. Engl. *25*, 463–471.

Pongmekin, P., Chongtrakool, P., Santanirand, P., and Kiertiburanakul, S. (2014). Clinical characteristics and mortality risk factors of cryptococcal infection among HIV-negative patients. J. Med. Assoc. Thail. Chotmaihet Thangphaet *97*, 36–43.

Popova, A.A., Kravchenko, A.V., Kozhevnikova, G.M., and Zimina, V.N. (2013). [The value of changes in CD8+CD28+ and CD4+CD28+ cells in patients with HIV infection concurrent with tuberculosis during treatment]. Ter. Arkhiv *85*, 54–57.

Pottel, H., Mottaghy, F.M., Zaman, Z., and Martens, F. (2010). On the relationship between glomerular filtration rate and serum creatinine in children. Pediatr. Nephrol. Berl. Ger. *25*, 927–934.

Prasitsuebsai, W., Kariminia, A., Puthanakit, T., Lumbiganon, P., Hansudewechakul, R., Moy, F.S., Law, M., Kumarasamy, N., Razali, K., Sirisanthana, V., et al. (2014). Impact of Antiretroviral Therapy on Opportunistic Infections of HIV-Infected Children in the TREAT Asia Pediatric HIV Observational Database. Pediatr. Infect. Dis. J. *33*, 747–752.

Prigent, A. (2008). Monitoring renal function and limitations of renal function tests. Semin. Nucl. Med. *38*, 32–46.

Primeggia, J., Timpone Jr., J.G., and Kumar, P.N. (2013). Pharmacologic Issues of Antiretroviral Agents and Immunosuppressive Regimens in HIV-infected Solid Organ Transplant Recipients. Infect. Dis. Clin. North Am. *27*, 473–486.

Purswani, M., Patel, K., Kopp, J.B., Seage, G.R., Chernoff, M.C., Hazra, R., Siberry, G.K., Mofenson, L.M., Scott, G.B., and Van Dyke, R.B. (2013). Tenofovir Treatment

Duration Predicts Proteinuria in a Multi-Ethnic United States Cohort of Children and Adolescents with Perinatal HIV-1 Infection. Pediatr. Infect. Dis. J. *32*, 495–500.

Rachakonda A, Kimmel P. (2010) CKD in HIV-infected patients other than HIV-associated nephropathy. Adv Chronic Kidney Dis. 17:83-93

Rah J.H., Akhter N., Semba R.D., de Pee S., Bloem M.W., Campbell A.A., Moench-Pfanner R., Sun K., Badham J., Kraemer K. (2010) Low dietary diversity is a predictor of child stunting in rural Bangladesh. Eur. J. Clin. Nutr. 64:1393–1398

Rajasekaran S, Jeyaseelan L, Ravichandran N, Gomathi C, Thara F, Chandrasekar C. (2009) Efficacy of antiretroviral therapy program in children in India: prognostic factors and survival analysis. J Trop Pediatr. 55(4):225–232

Ramezani, A., Mohraz, M., Banifazl, M., Jam, S., Gachkar, L., Yaghmaie, F., Eslamifar, A., Zadsar, M., Kalantar, N., Nemati, K., et al. (2008). Frequency and associated factors of proteinuria in Iranian HIV-positive patients. Int. J. Infect. Dis. *12*, 490–494.

Rao, T.K. (2001). Human immunodeficiency virus infection and renal failure. Infect. Dis. Clin. North Am. *15*, 833–850.

Rao, V.R., Ruiz, A.P., and Prasad, V.R. (2014). Viral and cellular factors underlying neuropathogenesis in HIV associated neurocognitive disorders (HAND). AIDS Res. Ther. *11*, 13.

Ray, P.E. (2009). Taking a hard look at the pathogenesis of childhood HIV-associated nephropathy. Pediatr. Nephrol. Berl. Ger. *24*, 2109–2119.

Redig, A.J., and Berliner, N. (2013). Pathogenesis and clinical implications of HIV-related anemia in 2013. ASH Educ. Program Book *2013*, 377–381.

Renner, L.A., Dicko, F., Kouéta, F., Malateste, K., Gueye, R.D., Aka, E., Eboua, T.K., Azondékon, A., Okomo, U., Touré, P. (2013). Anaemia and zidovudine-containing antiretroviral therapy in paediatric antiretroviral programmes in the IeDEA Paediatric West African Database to evaluate AIDS. J. Int. AIDS Soc. *16*.

Redon J. (1998) Renal protection by antihypertensive drugs: insights from microalbuminuria studies. J Hypertens.16 (12 Pt 2):2091-100.

Reynes, J., Cournil, A., Peyriere, H., Psomas, C., Guiller, E., Chatron, M., Cristol, J.-P., and Badiou, S. (2013). Tubular and glomerular proteinuria in HIV-infected adults with estimated glomerular filtration rate ≥ 60 ml/min per 1.73 m2. AIDS Lond. Engl. *27*, 1295–1302.

Richardson, E.T., Grant, P.M., and Zolopa, A.R. (2014). Evolution of HIV treatment guidelines in high- and low-income countries: Converging recommendations. Antiviral Res. *103*, 88–93.

Röling J, Schmid H, Fischereder M, Draenert R, Goebel FD. (2006) HIV-associated renal diseases and highly active antiretroviral therapy-induced nephropathy. Clin Infect Dis. 42:1488–95.

Ross MJ, Klotman PE, Winston JA. (2000) HIV-associated nephropathy: case study and review of the literature. AIDS Patient Care STDS 14*:* 637–645.

Ross RP, McCrea JB, Besarab A. (1994) Erythropoietin response to blood loss in hemodialysis patients in blunted but preserved. ASAIO J. 40(3):M880-5.

Ross, M.J. (2014). Advances in the pathogenesis of HIV-associated kidney diseases. Kidney Int. *86*, 266–274.

Rouet, F., Foulongne, V., Viljoen, J., Steegen, K., Becquart, P., Valéa, D., Danaviah, S., Segondy, M., Verhofstede, C., Van de Perre, P. (2010). Comparison of the Generic HIV Viral Load assay with the Amplicor HIV-1 monitor v1.5 and Nuclisens HIV-1 EasyQ v1.2 techniques for plasma HIV-1 RNA quantitation of non-B subtypes: the Kesho Bora preparatory study. J. Virol. Methods *163*, 253–257.

Ruhinda, E.N., Bajunirwe, F., and Kiwanuka, J. (2012). Anaemia in HIV-infected children: severity, types and effect on response to HAART. BMC Pediatr. *12*, 170.

Rule, A.D., Bailey, K.R., Lieske, J.C., Peyser, P.A., and Turner, S.T. (2013). Estimating the glomerular filtration rate from serum creatinine is better than from cystatin C for evaluating risk factors associated with chronic kidney disease. Kidney Int. *83*, 1169–1176.

Saloojee H, De Maayer T, Garenne M, Kahn K. (2007) What's new? Investigating risk factors for severe childhood malnutrition in a high HIV prevalence South African setting. Scand J Public Health Suppl 69:96–106

Schwartz EJ, Szczech LA, Ross MJ, Klotman ME, Winston JA, Klotman PE.(2005) Highly active antiretroviral therapy and the epidemic of HIV+ end-stage renal disease. J Am Soc Nephrol.16 (8):2412-20

Schwartz GJ, Muñoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, Furth SL. (2009) New equations to estimate GFR in children with CKD. J Am Soc Nephrol. 20(3):629-37.

Schwartz, G.J., Schneider, M.F., Maier, P.S., Moxey-Mims, M., Dharnidharka, V.R., Warady, B., Furth, S.L., and Muñoz, A. (2012). Improved equations estimating GFR in children with chronic kidney disease using an immunonephelometric determination of cystatin C. Kidney Int. *82*, 445–453.

Sexton, D.J., Reule, S., Solid, C., Collins, A.J., and Foley, R.N. (2014). End-stage renal disease from human immunodeficiency virus–associated nephropathy in the United States, 2001 through 2010. JAMA Intern. Med. *174*, 809–811.

Shah, I. (2011). Nephrotic proteinuria and renal involvement in HIV-infected children. Indian J. Sex. Transm. Dis. *32*, 111–113.

Shah, I. (2012). Recurrent thrombosis in an HIV-1 infected child. Indian J. Sex. Transm. Dis. *33*, 121–123.

Shah I, Katira B (2011). Hematological manifestation in HAART naive HIV-1 infected children in India in a resource limited setting. Pediatr. Oncol 8:5.

Shankland SJ, Eitner F, Hudkins KL, Goodpaster T, D'Agati V, Alpers CE. (2000) Differential expression of cyclin-dependent kinase inhibitors in human glomerular disease: role in podocyte proliferation and maturation. Kidney Int. 58:674–683

Shet A, Mehta S, Rajagopalan N, DinakarI C, Ramesh E, Samuel NM, Indumathi CK, Fawzi WW, Kurpad AV (2009). Anemia and growth failure among HIV-infected children in India: a retrospective analysis. BMC Pediatrics 9:37.

Simpore, J., Zongo, F., Kabore, F., Dansou, D., Bere, A., Nikiema, J. B., & Musumeci, S (2005). Nutrition rehabilitation of HIV-infected and HIV-negative undernourished children utilizing spirulina. *Annals of nutrition and metabolism*, *49*(6), 373-380.

Soler-Palacin P, Melendo S, Noguera-Julian A, Fortuny C, Navarro ML, Mellado MJ, Garcia L, Uriona S, Martín-Nalda A, Figueras C. (2011). Prospective study of renal function in HIV-infected pediatric patients receiving tenofovir-containing HAART regimens. AIDS. 25:171–176

Steel-Duncan J, Miller M, Pierre RB, Dunkley-Thompson J, Palmer P, Evans-Gilbert T, Rodriquez B, Christie CD. (2008) Kingston Paediatric and Perinatal HIV/AIDS Study Group. Renal manifestations in HIV-infected Jamaican children. West Indian Med J. 57(3):246-52.

Stevens, L.A., Coresh, J., Greene, T., and Levey, A.S. (2006). Assessing kidney function--measured and estimated glomerular filtration rate. N. Engl. J. Med. *354*, 2473–2483.

Struik GM, den Exter RA, Munthali C, Chipeta D, Van Oosterhout JJ, Nouwen JL, Allain TJ. (2011). The prevalence of renal impairment among adults with early HIV disease in Blantyre, Malawi. Int J STD AIDS. 22(8):457–462

Sunguya, B.F., Poudel, K.C., Otsuka, K., Yasuoka, J., Mlunde, L.B., Urassa, D.P., Mkopi, N.P., and Jimba, M. (2011). Undernutrition among HIV-positive children in Dar es Salaam, Tanzania: antiretroviral therapy alone is not enough. BMC Public Health *11*, 869.

Szczech LA, Grunfeld C,Schezer R, Canchola JA, van der Horst C, Sidney S, Wohl D, Shlipak MG. (2007). Microalbuminuria in HIV infection. AIDS. 21(8):1003-9

Szczech, L.A., Menezes, P., Quinlivan, E.B., van der Horst, C., Bartlett, J.A., and Svetkey, L.P. (2010). Microalbuminuria predicts overt proteinuria among patients with HIV-infection. HIV Med. *11*, 419–426.

Sztam, K.A., Fawzi, W.W., and Duggan, C. (2010). Macronutrient Supplementation and Food Prices in HIV Treatment. J. Nutr. *140*, 213S – 223S.

Talke H, Schubert G.E. (1965). Enzymatic urea determination in the blood and serum in the warburg optical test. Klin Wochenschr. 43:174-5.

Tang, J., and Kaslow, R.A. (2003). The impact of host genetics on HIV infection and disease progression in the era of highly active antiretroviral therapy. AIDS Lond. Engl. *17 Suppl 4*, S51–S60.

Tattersall, J., Dekker, F., Heimbürger, O., Jager, K.J., Lameire, N., Lindley, E., Biesen, W.V., Vanholder, R., and Zoccali, C. (2011). When to start dialysis: updated guidance following publication of the Initiating Dialysis Early and Late (IDEAL) study. Nephrol. Dial. Transplant. gfr168.

Trullas, J.C., Cofan, F., Tuset, M., Ricart, M.J., Brunet, M., Cervera, C., Manzardo, C., López-Dieguez, M., Oppenheimer, F., Moreno, A., (2011). Renal transplantation in HIV-infected patients: 2010 update. Kidney Int. *79*, 825–842.

Valeri, A., Barisoni, L., Appel, G.B., Seigle, R., and D'Agati, V. (1996). Idiopathic collapsing focal segmental glomerulosclerosis: a clinicopathologic study. Kidney Int. *50*, 1734–1746.

Victora C.G., Adair L., Fall C., Hallal P.C., Martorell R., Richter L., Sachdev H.S. (2008) Maternal and Child Undernutrition Study Group. Maternal and child undernutrition: Consequences for adult health and human capital. Lancet. 371:340–357.

Villamor E, Fataki MR, Bosch RJ, Mbise RL, Fawzi WW. (2004) Human immunodeficiency virus infection, diarrheal disease and sociodemographic predictors of child growth. Acta Paediatr. 93(3):372–9.

Villamor E, Msamanga G, Urassa W, Petraro P, Spiegelman D, Hunter DJ, Fawzi W W: (2006) Trends in obesity, underweight, and wasting among women attending prenatal clinics in urban Tanzania 1995-2004. Am J Clin Nutr. 83: 1387-1394

Volberding PA, Levine AM, Dieterich D, Mildvan D, Mitsuyasu R, Saag M; (2004) Anemia in HIV infection: clinical impact and evidence-based management strategies. Clin Infect Dis. 38(10):1454-63

Vora JP, Ibrahim HAA, Bakris GL (2000). Responding to the challenge of diabetic nephropathy, the historic evolution of detection, prevention, and management. JHum Hypertens. 14:667

Vourganti, S., Agarwal, P.K., Bodner, D.R., and Dogra, V.S. (2010). Ultrasonographic Evaluation of Renal Infections. Ultrasound Clin. *5*, 355–366.

Wearne, N., Swanepoel, C.R., Boulle, A., Duffield, M.S., and Rayner, B.L. (2012). The spectrum of renal histologies seen in HIV with outcomes, prognostic indicators and clinical correlations. Nephrol. Dial. Transplant. Off. Publ. Eur. Dial. Transpl. Assoc. - Eur. Ren. Assoc. *27*, 4109–4118.

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

Weiner DE, Tighiouart H, Amin MG, (2004) l. Chronic kidney disease as a risk factor for cardiovascular disease and all-cause mortality: a pooled analysis of community-based studies. J Am Soc Nephrol 15:1307.

Weiner DE, Tighiouart H, Griffith JL, (2007). Kidney disease, Framingham risk scores, and cardiac and mortality outcomes. Am J Med 120:552.

Westland, R., Abraham, Y., Bökenkamp, A., Stoffel-Wagner, B., Schreuder, M.F., and van Wijk, J.A.E. (2013). Precision of estimating equations for GFR in children with a solitary functioning kidney: the KIMONO study. Clin. J. Am. Soc. Nephrol. CJASN *8*, 764–772.

Winston JA, Bruggeman LA, Ross MD, Jacobson J, Ross L, D'Agati VD, Klotman PE, Klotman ME. (2001) Nephropathy and establishment of a renal reservoir of HIV type 1 during primary infection. N Engl J Med 344:1979-84.

Wongtrakul, J., Thongtan, T., Roytrakul, S., Kumrapich, B., Janphen, K., Praparattanapan, J., Supparatpinyo, K., and Smith, D.R. (2014). Proteomic analysis of serum and urine of HIV-monoinfected and HIV/HCV-coinfected patients undergoing long term treatment with nevirapine. Dis. Markers, 315824.

Wools-Kaloustian K, Gupta SK, Muloma E, Owino-Ong'or W, Sidle J, Aubrey RW, Shen J, Kipruto K, Zwickl BE, Goldman M. (2007) Renal disease in an antiretroviral-naïve HIV-infected outpatient population in Western Kenya Nephrol Dial Transplant.;22(8):2208-12

World Health Organization (WHO). Nutrition and HIV/AIDS. Geneva*:* WHO*;* 2005. [Accessed October 11, 2015].

Wyatt, C.M. (2014). Antiretroviral therapy and the kidney. Top. Antivir. Med. *22*, 655–658.

Yang, J., Goetz, D., Li, J.Y., Wang, W., Mori, K., Setlik, D., Du, T., Erdjument-Bromage, H., Tempst, P., Strong, R., Barasch, J. (2002). An iron delivery pathway mediated by a lipocalin. Mol. Cell *10*, 1045–1056.

Yarlagadda SG, Perazella MA. (2008) Drug-induced crystal nephropathy: An update. Expert Opin Drug Saf. 7:147–58

Yoshida K, Hanass-Hancock J, Nixon S, Bond V. (2014) Using intersectionality to explore experiences of disability and HIV among women and men in Zambia. Disabil Rehabil. 36(25):2161-8.

Yoshida S., Nagase M., Shibata S., Fujita T. (2008) Podocyte injury induced by albumin overload in vivo and in vitro: involvement of TGF-beta and p38 MAPK. Nephron Experimental Nephrology.108 (3):e57–e68

Zeleniuk, V.H., Zamors'kyĭ, I.I., and Horoshko, O.M. (2014). [Renoprotective efficacy of different doses of statins in experimental acute renal failure]. Fiziolohichnyĭ Zhurnal Kiev Ukr. 1994 *60*, 75–81.

Zhong, J., Zuo, Y., Ma, J., Fogo, A.B., Jolicoeur, P., Ichikawa, I., and Matsusaka, T. (2005). Expression of HIV-1 genes in podocytes alone can lead to the full spectrum of HIV-1-associated nephropathy. Kidney Int. *68*, 1048–1060.

Zoja C, Abbate M, Remuzzi G. (2015) Progression of renal injury toward interstitial inflammation and glomerular sclerosis is dependent on abnormal protein filtration. Nephrol Dial Transplant. 30: 706–712