University of Zambia

School of Medicine

Department of Paediatrics & Child health

PREVALENCE AND FACTORS ASSOCIATED WITH RENAL DYSFUNCTION IN HIV POSITIVE PAEDIATRIC PATIENTS ON HIGHLY ACTIVE ANTIRETROVIRAL THERAPY AT THE PAEDIATRIC CENTRE OF EXCELLENCE OF THE UNIVERSITY TEACHING HOSPITAL, IN LUSAKA, ZAMBIA

BY

DR. MABVUTO KEVIN ZIMBA

(BSc. HB, MBChB)

A Dissertation Submitted to the University Of Zambia in Partial Fulfilment of the Requirements of the Degree of Master of Medicine in Paediatrics and Child Health

(School of Medicine)

THE UNIVERSITY OF ZAMBIA

2015

DECLARATION

I declare that this dissertation is my own work. It is being submitted for the Master's degree in paediatrics and child health at the University of Zambia, Lusaka. It has not been submitted before for any degree or examination at this or any other University.

Candidate …….
Dr. Mabvuto Kevin Zimba
(BSc. HB, MBChB)

Supervisor…….
Dr. James Chipeta
(BSc. HB, MBChB, PhD)

Supervisor…….
Dr. Chipepo Kankasa
(B.Pharm, MD, MMED)

Signed.....
Head of Department

Copyright

Dr Mabvuto Kevin Zimba

2015

Approval

The University of Zambia approves this dissertation of Dr. Mabvuto Kevin Zimba as fulfilling the requirement for the award of the degree of Master of Medicine of Paediatrics and Child Health.

Head of Department

Name : . . . . . .

Signed : . . . . . .

Date : . . . . . .

Examiner- 01

Name : . . . . . .

Signed : . . . . . .

Date : . . . . . .

Examiner- 02

Name : . . . . . .

Signed : . . . . . .

Date : . . . . . .

Examiner- 03

Name : . . . . . .

Signed : . . . . . .

Date : . . . . . .

ABSTRACT

Background- Although sub-Saharan Africa has the largest number of children living with the Human Immunodeficiency Virus (HIV), little is known about the prevalence of HIV related kidney disease in these children despite the recognition of HIV infection as a strong risk factor for kidney disease [1,2]. This study investigated the prevalence and factors associated with renal dysfunction in HIV positive paediatric patients on highly active antiretroviral therapy at the Paediatric Centre of Excellence (PCOE) of the University Teaching Hospital (UTH), Lusaka, Zambia.

Methodology- The study was a cross sectional survey conducted at the PCOE of the UTH in Lusaka, Zambia. Enrolment of all eligible participants was from April to September, 2014. The Inclusion criteria were patients aged 18 months to 16 years who consented or and assented to the study and were on HAART. Renal dysfunction was defined as at least abnormal renal laboratory values in at least 1 of 3 measures of proteinuria, serum creatinine or Estimated Glomerular Filtration Rate (eGFR) 60mL/min/1.73m^2 for the age and height-adjusted value as defined by The Kidney Improving Global Outcomes (KDIGO) 2012 on two occasions. A file review and clinical evaluation was done by the study physician to determine the factors associated with renal dysfunction. Bloods were drawn for CD4 count, Haemoglobin (HB), Creatinine and Urine was taken for dipstick urinalysis.

Results- Of the 209 participants enrolled in this cross sectional study, 105(50.2%) were females. This study found a prevalence of 8.1% (CI=5.0-12.5), of renal dysfunction among paediatric HIV patients followed up at PCOE. Children aged 13 and above had on average 23 times greater odds for renal dysfunction [adjusted odds ratio (OR) = 23.76, and 95% confidence interval (CI) = (5.30 – 106.53), P-value <0.01] compared to children under 13 years old. Children receiving nephrotoxic HAART had on average 6 times greater odds for renal dysfunction [OR=5.55, CI= (1.57 – 19.65), P-value = 0.01] compared to children receiving Non-Nephrotoxic HAART.

Conclusion- The prevalence of renal dysfunction among paediatric HIV infected patients followed up at the PCOE at UTH in Lusaka Zambia is 8.1%, at 95% CI= (5.0-12.5) and associated factors include increase in age and nephrotoxic HAART.

Key words- Renal Dysfunction, Paediatric HIV patients, Proteinuria, Serum Creatinine, Estimated Glomerular Filtration Rate, Highly Active Antiretroviral Therapy (HAART).

DEDICATION

I dedicate this work to my mother and aunties for providing me with the support and parental guidance throughout my education, for without them I would not have pulled through this journey. Above all I am indebted to my ever loving Wife, Suzyo Ng'andu Zimba and our children for supporting me throughout this research and career.

ACKNOWLEDGEMENTS

I would like to thank the following for their support and contributions;

- My supervisors Dr James Chipeta and Dr Chipepo Kankasa, who corrected, supported and encouraged me while making this work interesting all the way through it.

- The staff of the Department of paediatrics and child health at the University Teaching Hospital in particular those from PCOE for the support rendered during this study.

- I also thank PCOE, UTH and Ministry of Health for the support to do this study

- Finally I would like to thank the patients that participated in the study, for without them this study would not have been possible.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

ABBREVIATIONS AND ACRONYMS/GLOSSARY

AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral Therapy
ARVs	Antiretrovirals
cART	Combination Antiretroviral Therapy
CLHIV	Children Living With Human Immunodeficiency Virus
CrCl	Creatinine Clearance
ESRD	End Stage Renal Disease
eGFR	Estimated Glomerular Filtration Rate
FI	Fusion Inhibitors
FSGS	Focal Segmental Glomerulosclerosis
GFR	Glomerular Filtration Rate
HAART	Highly Active Antiretroviral Therapy
HB	Haemoglobin
HIV	Human Immunodeficiency Virus
HIVAN	Human Immunodeficiency Virus Associated Nephropathy
HIVICK	Human Immunodeficiency Virus immune complex disease
KDIGO	Kidney Improving Global Outcomes
LPV/r	Lopinavir/Ritonovir
NRTI	Nucleoside Reverse Transcriptase Inhibitors
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitors
PCOE	Paediatric Centre of Excellence
PLHIV	People Living with HIV chronic kidney disease
PMTCT	Prevention of Mother-to-Child Transmission
PI	Protease Inhibitors
TDF	Tenofovir
UTH	University Teaching Hospital
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Although sub-Saharan Africa has the largest number of children living with the Human Immunodeficiency Virus (HIV), little is known about the prevalence of HIV related kidney disease in these children despite the recognition of HIV infection as a strong risk factor for kidney disease [1,2].This study investigated the prevalence and factors associated with renal dysfunction in HIV positive paediatric patients on highly active antiretroviral therapy (HAART) at the Paediatric Centre of Excellence (PCOE) of the University Teaching Hospital (UTH), Lusaka, Zambia.

A dramatic decline has been reported in both mortality and incidence of opportunistic and other related infections among HIV infected children, however, non-infectious complications of HIV infection, including renal disease, still occur[1]. A number of renal disorders associated with childhood HIV infection have been reported but most of these reports are based on retrospective, cross-sectional and geographically localized analyses[2-5].

Renal impairment is an important co-morbidity of HIV, whether due to direct effects of HIV on the kidney (e.g. HIV associated nephropathy-HIVAN), or due to factors related to HIV such as opportunistic infections (e.g. tuberculosis) or drug toxicity (e.g. lopinavir/ritonovir [LPV/r], tenofovir [TDF]). Renal impairment is more common in HIV patients of African origin than among HIV patients of Caucasian origin and has been shown to be an important predictor of early mortality in patients starting antiretroviral therapy (ART) in western settings as well as in Africa [2,5] .

HIV renal parenchyma disease is varied and may result from direct effect of the virus on renal epithelial cells, immune-complex mediated vasculitis, hyperviscosity of blood secondary to hyperglobulinaemia, various opportunistic infections, and also drugs such as HAART[2,5] . The commonest chronic renal parenchyma disease in HIV-positive patients is HIV-associated nephropathy (HIVAN). HIVAN progresses to end stage renal disease (ESRD) but if detected early, this progression can be slowed or even halted with the use of HAART[1,2,5] . The dearth of resources

for care of children with kidney diseases and the limited choice of antiretroviral drugs in Africa underlines a need to not only accurately assess glomerular filtration rate (GFR) in these children, but to do so early if their kidney function is to be preserved.

The incidence and occurrence of renal disease has decreased since the widespread introduction of combination antiretroviral therapy (cART) [2-7], with studies suggesting that HAART reduces the incidence of HIVAN [5], possibly by slowing the decline in renal function [2-7]. Early stages of renal dysfunction are silent and only detectable through laboratory analyses; for example, the glomerular filtration rate (GFR) which can be estimated using serum creatinine and it correlates with the severity of kidney disease and typically decreases before the onset of symptoms of kidney failure [2-7].

The prevalence rates of renal impairment in cohort studies of HIV-positive children have varied depending on the clinical status of the patients and the definition of renal impairment used[2-5]. The prevalence of renal diseases in HIV-infected children before the antiretroviral therapy era in the USA was estimated to be 40% [3-6]. Since the widespread establishment of ART in 1996, the clinical outcome of HIV-infected children in the United States has improved dramatically and HIVAN is being diagnosed at an older age than before the pre HAART era [2,7].

In Sub-Saharan Africa, there are a few studies which describe the extent of renal disease in HIV-infected children. In Nigeria, Iduoriyekemwen et al. found the prevalence of renal disease in HIV-infected children on highly active antiretroviral therapy (HAART) to be 16.2% [8]. It is anticipated that with the increased availability of HAART, the knowledge in diagnosing and treating renal disease in HIV-infected children will improve [1-5].

The prevalence rates of renal impairment in cohort studies of HIV-positive subjects have varied depending on the clinical status of the patients and the definition of renal impairment used [2, 5, 22,39]. This study was aimed at investigating and finding out the prevalence and factors associated with renal dysfunction in the HIV positive paediatric patients on highly active antiretroviral therapy who present to the PCOE at UTH in Lusaka.

1.2 STATEMENT OF THE PROBLEM

Renal impairment is an important co-morbidity of HIV and a major predictor of mortality, whether due to direct effects of HIV in the kidney (e.g. HIV associated nephropathy [HIVAN]), or due to factors related to HIV such as opportunistic infections (e.g. tuberculosis) or drug toxicity (e.g. Tenofovir [TDF]/lopinavir/ritonovir) [1,2]. The survival of HIV infected children has improved due to cART hence late complications of HIV and its treatment are beginning to manifest in particular renal complications. However, the prevalence of renal dysfunction and factors associated with it in this cohort on cART have not yet been well documented among African children including Zambia. With the growing availability of ART resulting in reduced morbidity and mortality, this information has become more important for appropriate management of these children. There is no data on prevalence of renal dysfunction CLHIV on HAART in Zambia.

1.3 STUDY JUSTIFICATION

Antiretroviral therapy (ART) has reduced morbidity and mortality in HIV infected children as in adults [1, 2], but not much is known on the effect of long-term exposure to ART in children. Kidney disease is an important complication in HIV infected people, and is associated with increased risk of morbidity and mortality, related to viral infection (e.g. HIVAN), or to ART [4]. Identification of impaired kidney function in the HIV-infected population often mandates changes to antiretroviral dosing, work-up for the aetiology of kidney dysfunction, and initiation of CKD management strategies.

As ART and other supportive treatments have become widely available, the survival of Zambian children with HIV has increased as well as non communicable disease such as childhood HIV-associated renal diseases. However, the prevalence of HIV-associated renal diseases is not yet well documented among Zambian children and at the PCOE. This information is important and urgently required for appropriate management of these patients, hence the need for local studies in this field/area. The prevalence rates of renal impairment in cohort studies of HIV-positive subjects are variable depending on the clinical status of the patients and the definition of renal impairment used [2,5]. This study has provided baseline data on the prevalence and factors associated with renal dysfunction in the HIV positive paediatric patients on highly active antiretroviral therapy presenting to PCOE at UTH in Lusaka.

1.4 HYPOTHESIS

The study null hypothesis was that the prevalence of renal dysfunction in HIV positive paediatric on highly active antiretroviral therapy patients at the Paediatric Centre of Excellence in the UTH was 15 percent in comparison to other studies done elsewhere (India, Nigeria and DR-Congo) on children living with HIV (CLHIV) on HAART.

1.5 RESEARCH QUESTIONS

- What is the prevalence of renal dysfunction in HIV infected children on highly active antiretroviral therapy at the PCOE in UTH, Lusaka?
- What are the factors associated with renal dysfunction in HIV infected children on highly active antiretroviral therapy at the PCOE in UTH, Lusaka?

1.6 OBJECTIVES

1.6.1 General Objectives

To determine the prevalence and factors associated with renal dysfunction in HIV positive paediatric patients on highly active antiretroviral therapy at the Paediatric Centre of Excellence in the UTH, Lusaka, Zambia.

1.6.2 Specific Objectives

- To determine the prevalence of renal dysfunction in HIV infected children on HAART
- To evaluate the relationship between WHO clinical stage of HIV and renal dysfunction.
- To evaluate the relationship between duration of HAART and renal dysfunction.
- To evaluate relationship of other drug therapies with renal dysfunction such as herbal medication, Cotrimoxazole (CTX) and NSAIDs (brufen).

CHAPTER TWO

2.0 REVIEW OF LITERATURE

2.1 The HIV Disease Burden

Globally, the number of new HIV infections has fallen by 19%[1]. In certain sub-Saharan countries, Zambia included, incidence has fallen by more than 25%.[1] This trend has been attributed to a combination of factors such as prevention efforts (PMTCT, behavioural change etc) and the natural course of the HIV epidemic[1]. Survival of HIV-infected children continues to increase and the use of antiretrovirals (ARVs) is expanding. However, there are few data regarding the incidence and prevalence of renal dysfunction and associated risk factors among HIV-infected children and adolescents on highly active antiretroviral therapy.

In sub-Saharan Africa, approximately 2.1 million children are infected with HIV-1[1]. In the absence of antiretroviral therapy, young African children frequently died of infectious causes of AIDS-related complications before renal diseases could be manifested or diagnosed. As antiretroviral therapy has become more available, and their survival has increased, our experience in treating kidney disease in HIV-infected children needs to be improved [1].

At the end of 2013 there were 33.2-37.2 million individuals infected with human immunodeficiency virus (HIV)-1, of whom approximately 2.1 million were children younger than 15 years of age[1]. More than 85% of all HIV-infected children live in sub-Saharan Africa[1]. Considering new infections, unreported cases, and the increased survival of children receiving appropriate antiretroviral therapy (ART), this number probably has increased.

2.2 Renal Disease in HIV in the Pre- and Post HAART Era

In the absence of ART, approximately 40% of all HIV-infected children living in the United States experienced renal complications leading to poor growth, accelerated progression of acquired immune deficiency syndrome (AIDS), and/or premature death[2]. In contrast, the presence of renal disease in HIV-infected African children not treated with ART is/was overshadowed by the high prevalence of diarrheal and respiratory diseases, including *Pneumocystis jiroveci* (previously known as *Pneumocystis carinii*) pneumonia [3-5]. Without access to standard therapy, African children younger than 2 years of age frequently died from diarrhoea-induced dehydration or respiratory infections, even before renal diseases could be manifested or diagnosed [4-5]. As ART and other supportive treatments have become more available, the survival of African children has increased, however, the epidemiology and pathogenesis of HIV-associated renal diseases is not yet well documented among African children, and with the growing availability of ART, this information has become more important for appropriate management of these children.

The first cases describing an HIV-associated nephropathy (HIVAN) in African American children were reported by Strauss et al[8-9] from Miami and was confirmed almost simultaneously in children from New York [10]. These paediatric studies were published approximately 3 years after HIVAN was described in adult patients from New York and Miami [11-12]. It should be noted, however, that the focal segmental glomerulosclerosis (FSGS) lesions described in the first adult cases of HIVAN were considered similar to the glomerular lesions seen in the setting of heroin use [11-12]. What were considered unique features of HIVAN were the rapid progression of the disease and the combination of FSGS with tubular microcystic changes [11-12]. The diagnosis of FSGS in young children with HIV infection provided key evidence to support the notion that HIV-1 was capable of inducing renal disease independent of drug use [8].

During the early years of the AIDS epidemic, American children frequently died of non-renal AIDS-related complications [2,7-10]. However, autopsy reports frequently described and attributed renal tubular-interstitial lesions to acute tubular necrosis [2,7-10,13]. Subsequently, with the recognition of the renal histology of HIVAN, this renal disease was identified in perinatally

infected children at 2 to 3 years of age [2,7-10]. Although the presence of renal disease contributed to poor quality of life and premature death, these children usually died before they developed end-stage renal disease (ESRD) [2,7-10,13]. Since the widespread establishment of ART in 1996, the clinical outcome of HIV-infected children in the United States and other parts of the world has improved dramatically [7]. HIVAN is diagnosed at an older age, and the pool of HIV-infected children surviving and requiring dialysis or transplantation has increased considerably [7, 14,15]. Since ART has become available to more children in resource-limited settings, a similar epidemiologic and clinical pattern is bound to be seen in Africa.

2.3 Prevalence and Incidence of Renal Dysfunction

Studies from the United States have shown that HIVAN is a common cause of chronic kidney disease in HIV-infected adults [18-20]. Early studies in children and adults suggested that HIVAN usually develops during the late stages of HIV disease, in association with a high viral load and a low CD4+ cell count [2,8-10,19,20]. A recent study has found that reduction of the HIV viral load by ART may prevent progression of proteinuria and improve the clinical outcome of HIV-infected children [14].

A 2012 systematic review and meta-analysis by Islam FM et al, on the Relative risk of Renal disease among People Living with HIV (PLHIV) in the adult population found that PLHIV are at increased risk of renal disease with a greater risk at later stages of infection and at older ages [22]. It was also found that ART prolongs survival and decreases the risk of renal disease but however, a slight reduction in renal disease risk occurs for PLHIV on tenofovir-containing ART than for other regimens. Islam FM et al also found that, the overall relative risk of renal disease was 3.87 (95% CI: 2.85-6.85) among HIV-infected people compared to HIV-uninfected people [22]. Islam FM et al also found that, the relative risk of renal disease among people with late-stage HIV infection (AIDS) was 3.32 (95% Cl:.86-5.93) compared to other PLHIV. In the same study, the relative risk of renal disease among PLHIV who were receiving antiretroviral therapy (ART) was 0.54 (95% Cl : 0.29-0.99) compared to treatment-naïve PLHIV and that the relative risk of renal disease among PLHIV who were treated with Tenofovir was 1.56 (95% CI:0.83-2.93) compared

to PLHIV who were treated with non-Tenofovir therapy. The risk of renal disease in PLHIV was also found to significantly increase with age [22].

The prevalence of renal dysfunction in CLHIV is varied depending the race, location, diagnostic criteria of renal dysfunction used, the type of drugs the patient is on and the disease progression (clinical stage) among many other factors [1-5]. In the United States during the pre-HAART era, a prevalence of 7–40% renal dysfunction among CLHIV was/has been reported while studies from Lagos, Nigeria by Esezobor et al and Harare, Zimbabwe by Dondo et al have reported a prevalence of 13.3 % and 34% of renal dysfunction in ART naïve paediatric patients respectively[22-25]. These among other studies showed the wide variation in prevalence rates of renal dysfunction depending on the geographical location, race , definition of renal dysfunction use and clinical stage of the disease. The findings of a high prevalence of Renal dysfunction by Esezobor et al in the HIV-infected children is consistent with another study [24] that documented a proteinuria prevalence of 20.5% among this clinic cohort of HIV-infected children. Similarly, a large body of research [25-27] has documented high prevalence of kidney diseases in HIV-infected persons. Jones et al and Wools-Kaloustian et al reported a prevalence of renal dysfunction of 11.5% and 15.2%, respectively [27,41].

Studies on renal dysfunction in CLHIV on HAART have produced similar results with those not on HAART in terms of variation in the actual prevalence rates of renal dysfunction. Nosakhare et al and Iduoriyekemwen et al in studies done in Lagos, Nigeria reported prevalence of 16.3% and 16.2 % of renal dysfunction in CLHIV on HAART respectively [22-25]. A study in Congo by Ekulu et al on prevalence of proteinuria in CLHIV on HAART showed a prevalence of 44% renal dysfunction [15]. Differences in the criteria for defining renal disease, clinical stage of participants, race and other factors in the various studies may have accounted for the differences in prevalence.

A study 2010, in Lusaka at the UTH in the adult population by Banda et al showed high prevalence of renal dysfunction at 42% in the HIV positive patients compared to a similar cohort of HIV negative patients with a prevalence of 27%[54]. Banda et al also found that a low CD 4 count (below 200 cells) in hospitalized patients in the study was not associated with renal dysfunction which

was not in agreement with other studies done elsewhere that have found low CD 4 count to be a risk factor for renal dysfunction in PLHIV[54].

In a study by Kapakala et al, 2008, at UTH on prevalence of proteinuria in admitted children in the department of paediatrics, it was found that sixty one (49.59%) of the patients in the study were HIV positive and that being HIV positive was significantly associated with proteinuria at a prevalence of 41.8%[55]. The study showed an odds ratio of 2.44 times likely hood of proteinuria in HIV infected children than HIV negative patients[55]. However this study did not go further to analyze the relationship and factors associated with proteinuria and kidney function in the HIV infected cohort of children hence the need for further studies such as the current study that has highlighted this aspect of renal dysfunction in HIV infected children.

This study investigated and found out the prevalence as well as factors associated with renal dysfunction in the HIV positive paediatric patients on highly active antiretroviral therapy presenting to the PCOE at UTH in Lusaka.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY DESIGN

This was a Cross-sectional study and was conducted over a period of six months from April to September 2014.

3.2 TARGET POPULATION

All HIV positive children followed up at UTH, PCOE in LUSAKA between the age of 18 months and 16 years were invited for enrolment into the study.

3.3 STUDY SITE

The study site was at The UTH, PCOE in Lusaka. The PCOE is an out patient clinic in the department of paediatrics at UTH where HIV positive paediatric patients are followed up. A total of about 4000 children are followed annually and up to about 60 to 80 children are seen on a daily basis from Monday to Friday, and the patients are seen and managed as recommended by the national guide lines adopted from WHO. The majority of patients seen in the PCOE clinic are clinically stable and on HAART.

3.4 ELIGIBILITY

3.4.1 Inclusion Criteria

- All HIV positive children who followed up at UTH, PCOE in LUSAKA between the age of 18 months and 16 years old on HAART.

3.4.2 Exclusion Criteria

- HIV negative children
- Refusal to take part in the study
- Children on Tuberculosis treatment, sickle cell disease, diabetes mellitus, acute illness/fever (axillary body temperature greater than $37.5^{o}c$)
- Children known to have Hepatitis B were excluded from the study.
- Recent admission in the last 4 weeks

3.5 SAMPLE SIZE

The following prevalence formula was used to calculate sample size

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

Where

N = sample required

Z =Z statistic =1.96 (95% C I)

P= expected prevalence 0.15 (assuming 15% renal dysfunction)

E= confidence interval 0.05

Therefore $N = \frac{(1.96)^2 \times 0.15(1-0.15)}{(0.05)^2}$

= 196

Total sample considering a drop out of 5% = 1486/(1-0.05) =206

Thus the sample size was calculated to be 206

3.6 SAMPLING METHODS

Systematic sampling method was used. All children attending ART CLINIC at PCOE were invited to participate in the study. The first patient was invited to take part in the study then every alternate patient was invited to avoid selection bias. If a guardian and patient declined to be enrolled, the next possible participant was approached and only 10 participants were enrolled per day. An informed and signed consent/assent was obtained from the participants/guardians by the study physician. Details about the benefits or harm related to the study were explained to the participant/guardian. Additionally, issues of confidentiality and procedures were explained as well by the study physician. Study participants were assured of confidentiality.

A questionnaire was administered and every enrolled child was clinically evaluated by the study physician. Self-reported race and ethnicity information was provided by the parent, guardian, or subject. A maximum of 5mL of blood sample was collected from each participant by vein puncture. The blood samples were analysed in the PCOE laboratory for measurement of creatinine, haemoglobin and CD4 count while other results were obtained from the file to meet the 2 required results within 3-6 months as per study definition of renal dysfunction.

Urine samples were collected at the time of study and a urinalysis done on the spot. A second urinalysis was done on the next scheduled regular visit after 3 months by the attending physician at that time to meet the 2 required urinalysis results as per study definition of renal dysfunction.

A file review was done to collect the clinical data on; date of commencement of HAART, the HAART regimen details, other medications, baseline WHO clinic stage, baseline CD4 counts , Creatinine (see attached appendix V for details).

Enrolments were done on Mondays to Fridays in the morning as this is when the clinics at PCOE are done.

3.7 STUDY DEFINITIONS:

3.7.1 Definition of HAART

HAART was defined as a regimen that comprised at least 3 different ARVs from at least 2 drug classes (NRTI, NNRTI and PI).

ARV use was divided into mutually exclusive categories:

(1) **Nephrotoxic HAART**: Nephrotoxic HAART was defined as any HAART regimen that included tenofovir and lopinavir/ritonovir

(2) **Non-Nephrotoxic HAART**: Non-nephrotoxic HAART was defined as any HAART regimen that excluded those classified as nephrotoxic above.

The use of herbal medication and non-steroidal anti-inflammatory drugs (NSAIDs) was included as exposure to Nephrotoxic agents.

4.7.2 Definition of Renal Dysfunction

Renal dysfunction was defined as; abnormal renal laboratory values in at least 1 of 3 measures as in agreement with the kidney improving global outcomes (KDIGO) 2012 on two occasions :

(1) **Urine protein content;** Cut off for increased urine protein was ≥ +1.

(2) **Serum creatinine (Cr);** Cut offs for increased serum Creatinine was age-adjusted ;

≥ 62 umol/l. (0.7mg/dl) for children 1–12 years.

≥ 88 umol/l. (1.0 mg/dl) for adolescents ≥13 years–19 years.

(3) **Estimated Glomerular Filtration Rate (eGFR);** eGFR was computed using the Schwartz formula. The cut off for decreased eGFR was <60mL/min/1.73m^2 for the age and height-adjusted value.

3.8 DATA MANAGEMENT.

A standardized data entry questionnaire for each study participant was used for data collection and was identified by numbers. No personal details that could help identify participants appeared on the form. Double data entry was performed and data bases matched. Data was entered on an Epi Info database.

3.9 STATISTICAL ANALYSIS

The data was analysed using SPSS version 22.0. Frequency tables were used to describe the socio-demographic characteristics. Chi square tables were used to test for associations for categorical variables while t-tests were used to make associations for continuous variables. The effect size was measured using odds ratios.

Data was analysed based on gender; age group at study time; race/ethnicity; WHO clinical and immunological stage and nephrotoxic medication exposure at time of study.

In the final set of analyses, the compared risk of renal toxicities for subjects taking Nephrotoxic-HAART and other Nephrotoxic medications concurrently, was compared with non-Nephrotoxic HAART.

All analyses were restricted to participants with confirmed HIV diagnosis on HAART.

3.10 ETHICAL ISSUES

Ethical clearance was sought from the Research Ethics Committee (ERES), **Ref.no 2013-Nov-014.** Permission to carry out the study was sought from, The Department of Paediatrics and Child Health at UTH and PCOE.

The purpose and procedures of the study was fully explained by the study physician and a written informed consent/ascent obtained from the guardian/parent and the participant where appropriate. It was emphasized that participation in the study was purely voluntary and that participants could withdraw from the study at any point. The risks (such as psychological trauma in the event that renal dysfunction was diagnosed) and benefits (such as initiation of treatment modalities in the event renal impairment was diagnosed) were fully explained to the participants as described in the consent form.

Patient results were treated as strictly confidential. All data entry forms were identified by coded numbers only. The data entry sheets were locked in a secure cabinet and all electronic entries were password protected.

Participants in need of treatment or follow up were stabilized and referred appropriately. Recommendations have been made to the relevant authorities

CHAPTER FOUR

4.0 RESULTS AND DATA ANALYSIS

4.1 SOCIAL-DEMOGRAPHIC CHARACTERISTICS OF THE PARTICIPANTS

4.1.1 Race, Sex and Age distribution

All the 209 participants enrolled in this study were black Africans and residents of Lusaka province in Zambia with a confirmed HIV diagnosis. Of the 209 recruited participants in this study, there were 105 (50.2%) female children and 104 (49.8%) male children, P-value = 0.95. The mean age at enrolment onto the study was 9.3 years (SD=3.84), while the minimum and maximum years were 2 years and 15.1 years, respectively.

4.2 CLINICAL CHARACTERISTICS OF PARTICIPANTS

4.2.1 Temperature and Weight-for-Height (Nutritional Status)

All participants had a normal body temperature as those with fever were excluded. The mean body temperature (axillary) was 35.9^{o}C (SD=0.51), while the minimum and maximum body temperature measures were 35^{o}C and 37^{o}C, respectively (table 1).

Participants enrolled into the study had a fair nutritional status with, 48 of the 209 (23%) children in enrolled into the study having a weight-for-height score < -1 SD, while the remaining 161of the 209 (77%) had median weigh-for-height standard deviation (SD).

4.2.2 Age at diagnosis and Age at of starting HAART

The mean age at diagnosis with HIV in the participants was 4.8 years (SD=3.55), while the minimum and maximum ages were 0.2 years and 13 years, respectively (table 1). The mean age for starting on HAART was 5.3 years (SD=3.80), while the minimum and maximum ages for starting on HAART were 0.4 years and 13 years, respectively(table 1).

4.2.3 Duration from diagnosis to starting HAART and Duration on HAART

The median duration from age of diagnosis of HIV to starting HAART was 0.1 years, with the minimum being immediately following diagnosis i.e. within a month of diagnosis and maximum 5 years after diagnosis. The mean duration on HAART was 4.1 years (SD=2.32), while the minimum was 0.4 years and maximum was 10 years (table 1).

Table 1; Clinical characteristic of participants

Variable	Mean in year	SD	Median	Minimum	Maximum
Body temperature	35.9ºC	0.51	36.4ºC	35ºC	37ºC
Age at enrolment (yrs)	9.3	3.84	5.00	2	15.1
Age at diagnosis (yrs)	4.82	3.54	5.00	0.2	13.0
Age at starting HAART(yrs)	5.35	3.80	5.00	0.4	13.0
Duration to starting HAART(yrs)	0.53	1.07	0.10	0.0	5.0
Duration on HAART	4.07	2.32	4.00	0.4	10.0

***NB**; all ages in years (yrs), body temperature in degrees Celsius, HAART- Highly Active Antiretroviral Therapy; SD-Standard deviation*

4.2.5 WHO clinical stage of HIV

The majority of the enrolled children (70.3%) were WHO clinical stage 1 of HIV and about 23.4 % were in WHO clinical stage 2 of HIV. 3.8% and 2.4% of the participants were in WHO clinical stage 3 and 4 respectively.

Table 2: WHO clinical stage distribution of participants.

WHO Clinical Stage	Frequency	Percent
1	147	70.3
2	49	23.4
3	8	3.8
4	5	2.4
Total	209	100.0

5.2.6 HAART and Other Drugs

About 29.7% of the children enrolled in this study were on nephrotoxic HAART as per study definition of this study, the remaining 70.3% were on non-nephrotoxic HAART regimen. About one-quarter (25.8%) of all the children enrolled into this study were on LPV/r containing regimens of HAART and only 5% were on TDF containing regimens of HAART. About 55% of the participants were on Cotrimoxasole and no patient reported having used any herbal medication and NSAIDs in the 4 (four) months prior to enrolment into this study.

TABLE 3: Type of HAART distribution among participants

HAART	Frequency	Percent
Nephrotoxic	62	29.7
Non-Nephrotoxic	147	70.3
Total	209	100.0

4.3 LABORATORY CHARACTERISTICS OF PARTICIPANTS

4.3.1 Haematological characteristics of participants

The mean haemoglobin (HB) in the study participants was 12.0 (SD=1.24), while minimum and maximum HB measures were 8.8 and 14.5, respectively.

The median baseline CD4 count prior to starting HAART was 662 and minimum and maximum CD4 counts were 35 and 2880, respectively. The median CD4 count at the time of the study was 760 and minimum and maximum CD4 counts were 18 and 3254, respectively.

Table 4: Haematological characteristics of participants of participants

variable	Participants (N)	Mean	SD	Median	Minimum	Maximum	Range
Haemoglobin (HB) g/dl	209	12.00	1.24	11.9	8.8	14.5	5.7
Study CD 4 Count (On HAART)	209	832	526	760	18	3272	3254

NB; *pre-HAART- before starting HAART, SD- standard deviation*

4.3.2 Biochemistry

4.3.2.1 Serum Creatinine adjusted for age

There were 8 (3.8%) children who had high creatinine for age on two (2) occasions over a period of 3months hence they were classified as having renal dysfunction as per study definition(s). Only 2 participants had an abnormal creatinine adjusted for age on one occasion only as shown in table 5, hence they did not the criteria for renal dysfunction.

Table 5: Participants with normal and abnormal creatinine adjusted for age

Age range	Serum creatinine	Recent creatinine (participants)	Study creatinine (participants)
1<13 yrs	**<62 *u*mol/l**	**153 (73.21%)**	**150 (71.77%)**
1<13 yrs	**>62 *u*mol/l**	**6 (2.87%)**	**9 (4.31%)**
13-16yrs	**< 88 *u*mol/l**	**48 (22.97%)**	**48 (22.97%)**
13-16yrs	**>88 *u*mol/l**	**2 (0.96%)**	**2 (0.96%)**

NB; *Recent creatinine-file review creatinine done at least three months from study creatinine. Study creatinine –done at time of study*

4.3.2.2 Estimated Glomerular Filtration Rate (eGFR)

166 (79.43%) and 152 (72.73%) participants had normal/stage 1 eGFR calculated respectively using the two creatinine results obtained above while no participants in the study had end stage renal disease/stage five. The mean recent eGFR was 104.2 (SD=23.53), and the mean study eGFR was 98.1 (SD=23.38). P-value <0.001. There were 8 (3.8%) children classified as having renal dysfunction based on eGFR results as per study definition(s) on the basis that they had eGFR on two occasions less than 60 in mL/min/1.73m^2, results shown in table 6.

Table 6: Kidney function based on estimated glomerular filtration(eGFR) rate.

Kidney function stage	eGFR range	Recent eGFR N(%)	study eGFR N (%)
STAGE 1	**>90**	**166 (79.43%)**	**152 (72.73%)**
STAGE 2	**60-89**	**35 (16.74%)**	**43 (20.57%)**
STAGE 3	**30-59**	**6 (2.87%)**	**12 (5.74%)**
STAGE 4	**15-29**	**2 (0.96%)**	**2 (0.96%)**
STAGE 5	**<15**	**0 (0%)**	**0 (0%)**

NB; eGFR- estimated glomerular filtration in mL/min/1.73m^2, *calculated using recent and study creatinine by the Schwartz formula*

4.3.2.3 Urinalysis results for participants

There were 17 (8.13%) children diagnosed with renal dysfunction based on proteinuria as per study definition. 2 participants were diagnosed with a urinary tract infection(UTI) and treated for it. 3 (three) participants had a one off proteinuria either in test one or repeat test (test Two). Haematuria was note in 3 (1.4%) participants at least on one occasion. All participant that had Haematuria were found to have had renal dysfunction as per study definition.

Table 7: Urinalysis results for participants

Urinalysis parameter	First test	Second test
Leucocytes	2 (1%)	1(0.5%)
Proteinuria ≥ 1+	20 (9.6%)	19 (9.5%)
Nitrites	2(1%)	1(0.5%)
Blood	3(1.4%)	2(1%)
Normal	182(87.1%)	186 (88.9%)

NB; *first test- urinalysis done at enrolment into study, second test- urinalysis done 3 months after first test*

4.4 PREVALENCE AND ASSOCIATION OF STUDY VARIABLES WITH RENAL DYSFUNCTION

The prevalence of renal dysfunction in HIV infected children at the PCOE in UTH, Lusaka, was found to be 8.1%, (CI- 5.0-12.5), on the basis of urinalysis proteinuria, age adjusted serum creatinine and eGFR as per study definition. All the participants with renal dysfunction had proteinuria on two occasions while only 8 out of the 17 (47%) had an abnormal creatinine and eGFR on two occasions.

Table 8: The prevalence of renal dysfunction

Condition	Mode of diagnosis	Number (%)
Renal dysfunction		17 (8.13%)
	Proteinuria	17 (8.13%)
	Creatinine	8 (3.83%)
	eGFR	8 (3.83%)
No Renal dysfunction		192 (91.9%)

NB ; *Renal dysfunction defined as at least an abnormal renal laboratory values in at least 1 of 3 measures of either proteinuria, serum creatinine or eGFR*

4.5 Bivariate Analysis for Factors Associated with Renal Dysfunction

At 5% significance level, age, HAART, and HB were found to be significantly associated with renal dysfunction.

Table 9: bivariate analysis results for factors associated with renal dysfunction

Variable	No Renal Dysfunction	Renal Dysfunction	P-value
Categorical variables			
Age group			
0 < 13 years	**153 (79.7%)**	**6 (35.3%)**	**<0.01[c]**
≥ 13 years	**39 (20.3%)**	**11 (64.7%)**	
Sex			
Female	**97 (50.5%)**	**8 (47.1%)**	**0.78[c]**
Male	**95 (49.5%)**	**9 (52.9%)**	
HAART			
Nephrotoxic HAART	**51 (26.6%)**	**11 (64.7%)**	**< 0.01[c]**
Lopinavir/R(LPV/R)	**43(22.4%)**	**6(35.5%)**	**<0.01[f]**
Tenofovir (TDF)	**11(5.7%)**	**0(0%)**	**0.61[f]**
Non-Nephrotoxic HAART	**141 (73.4%)**	**6 (35.3%)**	
DURATION on HAART			
< 5 Years	**122(63.5%)**	**14(82.4%)**	**0.12[c]**
> 5 Years	**70 (36.5%)**	**3(17.6%)**	
Cotrimoxazole			
On Cotrimoxazole	**103 (53.6%)**	**5 (29.4%)**	**0.18[c]**
Not Cotrimoxazole	**89 (46.4%)**	**12 (70.6%)**	
Weight-for-height			
< -1SD	**42 (21.9%)**	**6 (35.3%)**	**0.23[f]**
Median	**150 (78.1%)**	**11 (64.7%)**	
WHO Stage			
Stage I	**138 (71.9%)**	**9 (52.9%)**	**0.10[c]**
Other stages	**54 (28.1%)**	**8 (47.1%)**	

Continuous variables			
Mean study CD4 (SD)	**883.5 (526.70)**	**816.2 (538.47)**	**0.90[t]**
Mean HB (SD)	**12.1 (1.26)**	**11.2 (0.40)**	**<0.01[t]**
Mean enrolment age (SD)	**9.3 (3.63)**	**10.0 (5.81)**	**0.61[t]**
Mean age at diagnosis (SD)	**4.7 (3.37)**	**6.6 (4.88)**	**0.12[t]**
Mean age starting HAART (SD)	**5.2 (3.69)**	**6.7 (4.82)**	**0.23[t]**
Mean duration on HAART (SD)	**4.1 (2.35)**	**3.3 (1.81)**	**0.13[t]**
Mean body temperature (SD)	**35.9 (0.52)**	**35.8 (0.41)**	**0.39[t]**
[c]=Chi-square; [f]=Fisher's exact; [t]=T-test			

4.6 MULTIVARIATE LOGISTIC REGRESSION ANALYSIS PREDICTING RENAL DYSFUNCTION.

The table 10 below shows results for multivariate logistic regression analysis predicting renal dysfunction. From the bivariate analysis results, age, HAART, HB, and WHO stage were found to be associated with renal dysfunction

Table 10: Multivariate logistic regression analysis predicting renal dysfunction

Variable	Unadjusted Odds Ratio	Adjusted Odds Ratio	P-value
Age			
< 13 Years	1	1	
≥ 13 Years	7.19 (2.50 - 20.66)	23.76 (5.30 - 106.53)	<0.01
HAART			
Non-Nephrotoxic	1	1	
Nephrotoxic	5.07 (1.78 - 14.41)	5.55 (1.57 - 19.65)	0.01
WHO Stage			
Stage I	1	1	
Stage ≥ II	2.27 (0.83 - 6.19)	1.58 (0.46 - 5.50)	0.47
HB	0.55 (0.36 - 0.85)	0.28 (0.14 - 0.55)	<0.01

CHAPTER FIVE

5.1 DISCUSSION

5.11 Prevalence of renal dysfunction

The Prevalence of renal dysfunction in HIV infected children on HAART at the PCOE in UTH, Lusaka, was found to be 8.1%, at 95% confidence interval ,CI=(5.0-12.5) based on proteinuria, age adjusted serum creatinine and estimated glomerular filtration rate as per study definition, this was significantly lower than the hypothesized null of 15%, P-value = 0.008. Despite finding a significantly lower prevalence of renal dysfunction of 8.1% than hypothesized, this finding was similar and within range as has been documented in other studies of 2 % to 34% prevalence of renal dysfunction in HIV infected children in who are on HAART depending on the definition of renal dysfunction used [2, 24-27,35,36]. Differences in the criteria for defining renal disease in the various studies have accounted for the differences in prevalence hence in this study we used an internationally standardised definition of Renal dysfunction by KIDGO in order to avoid this variance in prevalence due to definitions of renal dysfunction used.

5.12 Factors associated with renal dysfunction

The male: female ratio (52.9% being male) of the children with renal disease in this study did not differ significantly statically with a p value=0.78, however this is in contrast with the majority of other studies, were there is usually a male preponderance of renal dysfunction [7,8,9] but equal male: female ratio also has been reported previously in several studies[10-14].

The median age of 10.0 years among the children infected with HIV with renal disease in this study is higher compared to the Lagos study with a median age of 5.5 years and a Jamaican study of 5.0 years[10,15]. The finding that renal dysfunction was more prevalent in the older age group children who were on HAART was not surprising as the mean age at diagnosis and starting HAART was higher compared to those who did not have renal dysfunction and may be attributed to longer periods of unsuppressed viral replication[7,8]. Renal disease in HIV is generally viewed

as a late complication, which is therefore expected at a much older age[1-5]. This finding may be because all of the study children acquired the infection by vertical transmission and living longer with the infection [12,-17]. It is possible that acquisition of HIV at a time when the kidneys are still developing may predispose to renal disease earlier than that observed in adults[8].

This study illustrated that older children infected with HIV were more likely to have renal dysfunction. Children aged 13 years and above had on average 23 times greater odds for renal dysfunction [adjusted odds ratio (OR) = 23.76, and 95% confidence interval (CI) = (5.30 – 106.53), P-value <0.01] compared to children under 13 years old. These findings are similar to Phelps et al who documented that, in children, renal disease associated with HIV progresses at a slower rate than in adults, with most children developing proteinuria within 2-5 years after HIV infection[22]. After the onset of proteinuria, end-stage renal disease can develop within 3 years. However, the rate of progression depends on the underlying cause of the disease and the presence of other AIDS-associated illnesses[5-10]. It is a well known fact that ART prolongs survival and decreases the risk and halts progression of renal disease [22].

The majority of the participant enrolled in this study were in WHO stage 1and 2 at 70.3% and 23.8% respectively. The reason for this may be effective adherence to ART and scheduled reviews by the participants and the selection criteria. This study showed that a higher WHO clinical stage was associated with renal dysfunction and this was in keeping with other studies. McCulloch et al and Kala et al both showed that a WHO clinical stage of 3 and 4 were associated with a higher prevalence of renal dysfunction (4, 5). it was noted that children with WHO stage greater than I had on average 58% increased odds for renal dysfunction compared to children with WHO stage I, but this was not statistically significant [OR=1.58, CI=(0.46 – 5.50), P-value = 0.47].

In this study, children receiving Nephrotoxic HAART as per study definition had on average 6 times greater odds for having renal dysfunction [OR=5.55, (CI=1.57 – 19.65), P-value = 0.01] compared to children receiving Non-Nephrotoxic HAART. This was in agreement with other studies such as the study by Pontrelli et al which showed a reduction in renal function in paediatric HIV patients on Nephrotoxic HAART (56). Lopinavir/Ritonovir were found to be associated with renal dysfunction with a P-value< 0.01. In literature, Lopinavir/Ritonovir is rarely associated with

renal dysfunction as it is 80-90% metabolised and excreted in the liver while only 10-20% of it is metabolised and excreted by the Kidneys[4245,56]. Lopinavir and other protease inhibitors have documented to cause renal dysfunction by causing nephrocalcinosis and renal stones [42,45,56].

Despite finding an association between the type of HAART and renal dysfunction, there was no association noted in this study between the duration the participants had been on HAART and renal dysfunction with a P-value of 0.13. The short mean duration on HAART of 3.3 years may be the reason why the duration on HAART did not show any association with renal dysfunction. However this finding may be due to the well documented fact that HAART prevents and halt the progression of renal dysfunction.[5,7]

There was a significant association between renal dysfunction and anaemia in our participants for this study. A decline in HB of 1g/dl in was associated on average with 72% decreases odds for renal dysfunction. [OR=0.28, CI=(0.46 – 5.50), P-value <0.01]. The association between renal dysfunction and a low HB (anaemia) has been well documented in literature and is attributed to a decline in the endocrine function of the kidneys and uraemia among other possible aetiologies of anaemia in renal dysfunction(54,56).

There was no association noted between renal dysfunction and other drug therapies. No participants in the study reported having used Herbal medication or NSAIDs in the 4 months preceding enrolment in to the study. There was no significant association between renal dysfunction and Cotrimoxazole (Septrin) use in our participants in this study P=0.18. This finding has been documented is several studies and is attributed to low dosage of Cotrimoxazole used as prophylaxis is for PCP.[9,10,11,56]

5.2 LIMITATIONS

The results obtained from this study cannot be generalized to all HIV positive paediatric patients in Lusaka due to the tertiary nature of the study site at PCOE which may have had a selection bias. In addition, the results from this study were from a small sample size that was calculated using a prevalence formula for disease burdens affecting less than 10 000 participants as the cohort at PCOE is only 4000 and due to limitation of finances to carry out a multicentre study.

Renal ultrasounds and biopsies among participants with renal dysfunction were not done for radiological and histological confirmation and further classification of types of nephropathy in Paediatric patients with HIV.

As participants in the study were stable out-patients, and that the acutely ill patient were not included in the study such as those on TB treatment and with known hepatitis B infection, this may have resulted in under estimating the prevalence of renal dysfunction in paediatric HIV patients.

CHAPTER SIX

6.0 CONCLUSION

In conclusion, the Prevalence of renal dysfunction in HIV infected children on HAART at the PCOE in UTH, Lusaka, was found to be 8.1%, at 95% confidence interval ,CI=(5.0-12.5) in keeping with other previous studies world wide. Increase in age, WHO stage greater than I (one), and nephrotoxic HAART, were associated with renal dysfunction among HIV paediatric patients on HAART in this study. This study did not find any association between duration on HAART and renal dysfunction nor was there an association with other drug therapies such as Cotrimoxazole.

6.1 RECOMMENDATIONS

1. This study provides evidence to strongly recommend regular kidney function screen in older children and those with WHO clinical stage greater than one (1).
2. This study recommends that urinalysis be done on HIV positive patients regularly.
3. A longitudinal and multicentre study should be done to look at the outcome of these paediatric HIV infected patients with renal dysfunction in a resource limited country like Zambia.

CHAPTER SEVEN

7.0 REFERENCES

1. UNAIDS. AIDS epidemic update. 2013. Available from:www.unaids.org/en/KnowledgeCentre/HIVData/EpiUpdate/EpiUpdArchive/2013. Accessed on 2nd November 2014
2. Ray PE, Rakusan TM, Loechelt BJ, Selby DM, Liu X-H, Chandra RS. Human immunodeficiency virus (HIV)-associated nephropathy in the children from the Washington D.C. area; 12 Years' experience. Semin Nephrol 1998;18:396–05. [PubMed: 9692352]
3. Benson C, Kaplan J, Masur H. Treating opportunistic infections among HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association/Infectious Diseases Society of America. Clin Infect Dis 2005;40 (Suppl 1):S1–84. [PubMed: 15655768]
4. McCulloch MI. Introduction to paediatric HIV disease [abstract]. Pediatr Nephrol 2007;22:1438, 298.
5. Kala U, Petersen K, Faller G, Goetsch S. Spectrum of severe renal disease in children with HIV/Aids at Chris Hani Baragwanath Hospital, Johannesburg [abstract]. Pediatr Nephrol 2007;22:1439, 301.
6. Fauci SA. HIV-1 and AIDS. 20 years of science. Nat Med 2003;9:839–43. [PubMed: 12835701]
7. Ray PE, Xu L, Rakusan T, Liu XH. A 20 year history of childhood HIV-associated nephropathy. Pediatr Nephrol 2004;19:1075–92. [PubMed: 15300477]
8. Pardo VL, Meneses R, Ossa L, Jaffe DJ, Strauss J, Roth D, et al. AIDS-related glomerulopathy: occurrence in specific risk groups. Kidney Int 1987;31:1167–73. [PubMed: 3599656]
9. Strauss J, Abitbol CL, Zilleruelo G, Scott G, Paredes A, Malaga S, et al. Renal disease in children with the acquired immunodeficiency syndrome. N Engl J Med 1989;321:625–30. [PubMed: 2770791]

10. Connor E, Gupta S, Joshi V, DiCarlo F, Offenberger J, Minnefor A, et al. Acquired immunodeficiency syndrome associated renal disease in children. J Pediatr 1988;113:39–44. [PubMed: 3385527].
11. McConnell MS, Byers RH, Frederick T, et al. Trends in Antiretroviral Therapy Use and Survival Rates for a Large Cohort of HIVInfected Children and Adolescents in the United States, 1989–2001. J Acquir Immune Defic Syndr 2005;38:488-94.
12. Pardo V, Aldana M, Colton RM, Fischl MA, Jaffe D, Moskowitz L, et al. Glomerular lesions in the acquired immunodeficiency syndrome. Ann Intern Med 1984;101:429–34. [PubMed: 6476632].
13. Ray PE, Liu XH, Xu L, Rakusan T. Basic fibroblast growth factor in HIV-associated hemolytic uremic syndrome. Pediatr Nephrol 1999;13:586–93. [PubMed: 10460507]
14. Chaparro AI, Mitchell CD, Abitbol CL, Wilkinson JD, Baldarragi G, Lopez E, et al. Proteinuria in children infected with the human immunodeficiency virus. J Pediatr 2008;152:844–9. [PubMed:18492529].
15. Ekulu PM, Nseka NM, Aloni MN, Gini JL, Makulo JR, Lepira FB, Sumali EK,Mafuta EM, Nsibu CN, Shiku JD: Prevalence of proteinuria and itsassociation with HIV/AIDS in Congolese children living in Kinshasa,Democratic Republic of Congo. Nephrol Ther 2012, 8(3):163–7.
16. Haas M, Kaul S, Eustace JA. HIV-associated immune complex glomerulonephritis with "lupus-like"features: a clinicopathologic study of 14 cases. Kidney Int 2005;67:1381–90. [PubMed: 15780090].
17. Fabian J, Katz I, Gerntholtz T, Goetsch S, Naicker S. Chronic kidney disease in human immunodeficiency virus infection. Panminerva 2007;49:51–66.
18. Wyatt CM, Klotman PE. HIV-1 and HIV-associated nephropathy 25 years later. Clin J Am Soc Nephrol 2007;2 (Suppl):S20–4. [PubMed: 17699507]
19. Winston JA, Klotman ME, Klotman PE. HIV-associated nephropathy is a late, not early, manifestation of HIV-1 infection. Kidney Int 1999;55:1036–40. [PubMed: 10027941]
20. Emen CP, Arogundade F, Sanusi A, Adelusola K, Wokoma F, Akinsola A. Renal disease in HIV seropositive patients in Nigeria: an assessment of prevalence, clinical features and risk factors. Nephrol Dial Transplant 2008;23:741–6. [PubMed: 18065807].

21. Gerntholtz TE, Goetsch SJ, Katz I. HIV-related nephropathy: a South African perspective. Kidney Int 2006;69:1885–91. [PubMed: 16625149]

22. Islam et al. BMC Public Health 2012, 12:234

23. Esezobor CI, Iroha E, Oladipo O, et al. Kidney function of HIV-infected children in Lagos, Nigeria: using Filler's serum cystatin C- based forumular. J Int AIDS Soc 2010;13:17.

24. Nourse P, Bates W, Gajjar P, Sinclair P, Sinclair-Smith, McCulloch M. Paediatric HIV renal disease in Cape Town, South Africa [abstract]. Pediatr Nephrol 2007;22:1597, 750.

25. Gonzalez C, Arietta G, Langmann CB, Zibaoui P, Escalona L, Dominguez LF, et al. Hypercalciuria is the main renal abnormality finding in human immunodeficiency virus-infected children in Venezuela. Eur J Pediatr 2008;16:509–15. [PubMed: 17593389]

26. Friedman AL, Ray PE. Maintenance fluid therapy: what it is and what it is not. Pediatr Nephrol 2008;23:677–80. [PubMed: 17955266].

27. Hussain S, Khayat A, Tolaymat A, Rathmore MH. Nephrotoxicity in a child with perinatal HIV on tenofovir, didanosine and lopinavir/ritonavir. Pediatr Nephrol 2006;21:1034–6. [PubMed:16773419]

28. Ray PE, Liu XH, Henry D, Dye L, Xu L, Orestein JM, et al. Infection of human primary renal epithelia cells with HIV-1 from children with HIV-associated nephropathy. Kidney Int 1998;53:1217–29.[PubMed: 9573536].

29. Wyatt CM, Klotman PE. HIV-associated nephropathy in the era of antiretroviral therapy. Am J Med2007;120:488–92. [PubMed: 17524746].

30. Kimmel PL, Barisoni L, Kopp JB. Pathogenesis and treatment of HIV-associated renal diseases:lessons from clinical and animal studies, molecular pathologic correlations, and genetic investigations. Ann Intern Med 2003;139:214–26. [PubMed: 12899589]

31. Gupta SK, Eustace JA, Winston JA, Boydstun II, Ahuja TS, Rodriguez RA, et al. Guidelines for the management of chronic kidney disease in HIV-infected patients: recommendations of the HIV Medicine Association of the Infectious Diseases Society of America. Clin Infect Dis 2005;40:1559–85.[PubMed: 15889353].

32. Szczech LA, Gupta SK, Habash R, Guasch A, Kalayjian R, Appel R, et al. The clinical epidemiology and course of the spectrum of renal diseases associated with HIV infection. Kidney Int 2004;66:1145–52. [PubMed: 15327410].

33. Abitbol CL, Friedman LB, Zilleruelo G. Renal manifestations of sexually transmitted diseases and the kidney. Adolesc Med Clin 2005;16:45–65. [PubMed: 15844383]
34. Tzakis AG, Cooper MH, Dummer JS, Ragni M, Ward JW, Starzl TE. Transplantation in HIV+ patients. Transplantation 1990;49:354–8. [PubMed: 2305465]
35. Dondo et al.: Renal abnormalities among HIVinfected, antiretroviral naive children, Harare, Zimbabwe: a crosssectional study. BMC Pediatrics 2013 13:75.
36. Shah I, Gupta S, Shah DM, Dhabe H, Lala M: Renal manifestations of HIV-infected highly active antiretroviral therapy naïve children in India. World J Paediatr 2012, 8(3):252–5.
37. McCulloch, MI. Infections in paediatric transplantation in developing countries. Paper presented at: 4th Congress of the International Pediatric Transplant Association (IPTA) on Pediatric Transplantation; 2007 March 20; Cancun, Mexico.
38. McCulloch, MI. Resource allocation and outcome of dialysis and transplantation in a developing country [abstract]. Pediatr Nephrol; Paper presented at: 14th Congress of the International Paediatric Nephrology Association; 2007. p. 1432p. 25437.
39. Bruzzone P, Berloco PB. Ethical aspects of renal transplantation from living donors. Transplant Proc 2007;39:1785–6. [PubMed: 17692612]
40. Fine DM, Atta MG. Kidney disease in the HIV-infected patient. AIDS Patient Care STDS 2007;21:813–24. [PubMed: 18240891].
41. 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 Int 2006;69:2243–50. [PubMed:16672914].
42. Naicker S, Han TM, Fabian J. HIV/AIDS—dominant player in chronic kidney disease. Ethn Dis2006;16 (Suppl 2):S56–60.McCulloch and Ray Page 10 *Semin*.
43. Jones CY, Jones CA, Wilson IB, Knox TA, Levey AS, Spiegelman D,Gorbach SL, Van Lente F, Stevens LA: Cystatin C and creatinine in an HIV cohort: the nutrition for healthy living study. Am J Kidney Dis 2008, 51:914-924.
44. Laterza OF, Price CP, Scott MG: Cystatin C: An improved estimator of glomerular filtration rate? Clin Chem 2002, 48:699-707.
45. Jaroszewicz J, Wiercinska-Drapalo A, Lapinski TW, Prokopowicz D,Rogalska M, Parfieniuk A: Does HAART improve renal function? An association between serum

cystatin C concentration, HIV viral load and HAART duration. Antivir Ther 2006, 11:641-645.

46. Grubb AO: Cystatin C - properties and use as diagnostic marker. Adv Clin Chem 2000, 35:63-99
47. Bokenkamp A, Domanetzki M, Zinck R, Schumann G, Byrd D, Brodehl J: Cystatin C- A new marker of glomerular filtration rate in children independent of age and height. Pediatrics 1998, 101:875-881
48. White C, Akbari A, Hussain N, Dinh L, Filler G, Lepage N, Knoll GA: Estimating glomerular filtration rate in kidney transplantation: a comparison between serum creatinine and cystatin C-based methods. J Am Soc Nephrol 2005, 16:3763-3770.
49. Baxmann AC, Ahmed MS, Marques NC, Menon VB, Pereira AB, Kirsztajn GM,Heilberg IP: Influence of muscle mass and physical activity on serum and urinary creatinine and serum Cystatin C. Clin J Am Soc Nephrol 2008,3:348-354.
50. Finney H, Newman DJ, Thakkar H, Fell JME, Price CP: Reference ranges for plasma cystatin C and creatinine measurements in premature infants, neonates and older children. Arch Dis Child 2000, 82:71-75.
51. Sharma AP, Kathiravelu A, Nadarajah R, Yasin A, Filler G: Body mass does not have a clinically relevant effect on cystatin C eGFR in children. Nephrol Dial Transplant 2009, 24(2):470-474.
52. Wools-Kaloustian K, Gupta SK, Muloma E, Owino-Ong'or W, Sidle J,Aubrey RW, Shen J, Kipruto K, Zwickl BE, Goldman M: Renal disease in an antiretroviral-naïve HIV-infected outpatient population in western Kenya. Nephrol Dial Transplant 2007, 22:2208-2212.
53. Eke FU, Anochie IC, Okpere AN, Eneh AU, Ugwu RN, Ejilemele AA, et al. Microalbuminuria in children with human immunodeficiency virus (HIV) infection in Port Harcourt, Nigeria. Niger J Med 2010;19:298-301.
54. J. Banda et al 2010, prevalence and factors associated with renal dysfunction in HIV positive and negative adults at the UTH in Lusaka. Medical journal of Zambia. Vol37,number 3, 2010.
55. Kapakala et al 2008, prevalence of proteinuria in children admitted to the UTH Lusaka.(a dissertation study).

56. Pontrelli et al.: Renal function in HIV-infected children and adolescents treated with tenofovir disoproxil fumarate and protease inhibitors. BMC Infectious Diseases 2012 12:18.

Appendix I: INFORMATION SHEET

(A STUDY ON PREVALENCE AND FACTORS ASSOCIATED WITH RENAL DYSFUNCTION IN HIV POSITIVE PAEDIATRIC PATIENTS AT THE PAEDIATRIC CENTRE OF EXCELLENCE OF THE UNVERSITY TEACHING HOSPITAL, IN LUSAKA, ZAMBIA.)

Why are we giving you this form?

We are giving you this form, so as to give you information about the named study and also to give you a chance to ask questions about this study. Then you can decide if you would like to take part in this study that is trying to find out how often Kidney disease(s) occur in HIV infected children at the Paediatric Centre of Excellence (PCOE) of the University Teaching Hospital (UTH) .

2. Who is carrying out this study?

Dr Mabvuto Kevin Zimba is doing the study as part of specialist training at the University Of Zambia School Of Medicine.

3. Background Information

You are being asked to take part in the above mentioned study, were we would like to find out how often Kidney disease(s) occur in children infected with HIV at the PCOE of the UTH. By participating in this study we will be able to get the information that may help in order to make relevant policies and interventions for this problem of kidney disease(s) in children infected with HIV. We believe this is very vital information to all of us and you would help by participating in this study.

4. What Happens In This Research Study?

You will be interviewed now and then your child will be examined, and some blood and urine taken for tests. **A total of 4 mL only of blood will be collected and sent to the laboratory to assess the kidney function and immunity level (CD 4). 10 mL of urine will be collected and tested to assess the kidney function**. The information collected will be kept confidential.

5. Possible Problems

We believe that the processes being used will not be harmful to you and the **child** participating in this study although needle prick will cause pain to your child while collecting blood samples. However if we notice anything peculiar to you or your child during or after information is collected, we will let you know and facilitate your (you and your child) seeking appropriate medical help **at the UTH paediatrics emergency room.**

6. Benefits

It is hoped that the study will help produce information on how often kidney disease(s) occur in children infected with HIV and will result appropriate measures being taken to control and treat the disease.

7. Confidentiality

Your name will never be made public by the investigators. The medical record will be treated the same as all medical records at the health centres. A code number that makes it very difficult for anyone to identify you will identify the research information gathered during this study from you. All information will be stored in a secure place. Information from this study maybe used for research purposes and may be published; however, your name will not be made public by the investigators**.** It is possible that, after the study is over, we may want to look again at the laboratory and interview record data collected during this study to help us answer another question. If this happens, still your name will not be made public by the investigators. The Laboratory and interview data will be stored for five (5) years and there after the data will be shredded and burnt.

8. Research Related Injury

In the event that a problem results from a study-related procedure, **Dr Mabvuto Kevin Zimba** in LUSAKA should be notified (0n **+260 977 821128**) or contact the **ERES CONVERGE IRB** (see contact details section), and you or your child will be stabilized and facilitated to seek and receive appropriate medical care at the health facility.

9. Contact Details

Should you want further information about this study or your rights as a participant please use the details provided below.

Dr. Mabvuto Kevin Zimba **Principle Investigator.** **University Teaching Hospital,** **Department of Paediatrics and Child Health.** **Lamya: +260-977 821128** **Email: drkevinmzimba@gmail.com**	**The Secretary,** **ERES CONVERGE IRB,** **33 Joseph Mwilwa Road,** **Rhodes Park,** **LUSAKA**. **Lamya: +260 966765 503** **+260 955 t55 634** **Email: eresconverge@yohoo.co.uk**

Appendix II: CONSENT FORM

(A STUDY ON PREVALENCE AND FACTORS ASSOCIATED WITH RENAL DYSFUNCTION IN HIV POSITIVE PAEDIATRIC PATIENTS AT THE PAEDIATRIC CENTRE OF EXCELLENCE OF THE UNVERSITY TEACHING HOSPITAL, IN LUSAKA, ZAMBIA.)

Participant

I_____ (participant's parent or guardian's name, signature or thumb-print) have been informed about the study .I volunteer to have my child and I participate in the study. A copy of this form signed by me and one of the study investigators is being given to me.

Signature/Thumb_____

Date (D/M/Y) _____

Interviewer

I have explained this research study to the Participant. I am available to answer any questions now or in the future regarding the study and the Participant's rights.

Signature of Investigators & Printed Names Date of signature

Signature_____

Date (D/M/Y) _____

APPENDIX III: ASSENT INFORMATION SHEET

(A STUDY ON PREVALENCE AND FACTORS ASSOCIATED WITH RENAL DYSFUNCTION IN HIV POSITIVE PAEDIATRIC PATIENTS AT THE PAEDIATRIC CENTRE OF EXCELLENCE OF THE UNVERSITY TEACHING HOSPITAL, IN LUSAKA, ZAMBIA.)

1. Why are we giving you this form?

We are giving you this form, so as to give you information about the named study and also to give you a chance to ask questions about this study. Then you can decide if you would like to take part in this study that is trying to find out how often Kidney disease(s) occur in children at the Paediatric Centre of Excellence (PCOE) of the University Teaching Hospital (UTH) .

2. Who is carrying out this study?

Dr. Dr Mabvuto Kevin Zimba who is training to become a children's doctor

3. Background Information

Kidney disease(s) are becoming common in children your age with your condition (some Children may not have their status disclosed to). This is a study were we are trying to see how often Kidney disease(s) occur in children like you at the (PCOE) of the University Teaching Hospital.

The doctor will talk to you and your guardian then he will examine you. The doctor will collect 4 ml of blood. This will be a little painful.

The importance of you taking part in the study is that you will assist the doctor to try and come up with information that will be useful in helping to treating children with kidney disease(s) who are HIV positive.

APPENDIX IV: ASSENT FORM

(A STUDY ON PREVALENCE AND FACTORS ASSOCIATED WITH RENAL DYSFUNCTION IN HIV POSITIVE PAEDIATRIC PATIENTS AT THE PAEDIATRIC CENTRE OF EXCELLENCE OF THE UNVERSITY TEACHING HOSPITAL, IN LUSAKA, ZAMBIA.)

Participant

I_____ **(**participant's name, signature or thumb-print) have been informed about the study .I volunteer participate in the study. A copy of this form signed by me and one of the study investigators is being given to me.

Signature/Thumb_____

Date (D/M/Y) _____

Interviewer

I have explained this research study to the Participant. I am available to answer any questions now or in the future regarding the study and the Participant's rights.

Signature of Investigators & Printed Names Date of signature

Signature_____

Date (D/M/Y) _____

APPENDIX V: DATA COLLECTION SHEET

A STUDY ON PREVALENCE AND FACTORS ASSOCIATED WITH RENAL DYSFUNCTION IN HIV POSITIVE PAEDIATRIC PATIENTS AT THE PAEDIATRIC CENTRE OF EXCELLENCE OF THE UNVERSITY TEACHING HOSPITAL, IN LUSAKA, ZAMBIA.

Identification code :

Initials of participant :

Participant study number:

Part I: Demographics

a) Age: …….. Years ……. Months

b) Sex: 1) Male 2) Female

c) Race: 1) Asian 2) Arabs 3) blacks 4) Whites

Part II: Presenting Complaints:

III: Review of systems:

a) Cardio-Respiratory system:

1) Normal 2) abnormal ,specify …….

b) Gastrointestinal system:

1) Normal 2) abnormal ,specify …….

c) Genital-urinary system:

1) Normal 2) abnormal ,specify …….

d) Neurology system:

1) normal 2) abnormal ,specify …….

e) other systems

1) normal 2) abnormal ,specify ……. system:

Part IV: Past medical history

1) Confirmation of HIV:

a) DNA-PCR date ……. Age at diagnosis …….

b) Antibody test/Elisa date ……. Age at diagnosis ……

2) a) any recent admissions (< 4 weeks): 1) Yes 2) No

b) 1). Renal disease 2) SCD 3) DM 4)TB 5) others …….

Part V: Drug history

A) Date of starting HAART: dd/mm/yy

B) Combination of HAART:

NRTIs	NNRTIs	PIs	Other drugs
zidovudine (AZT)	**Nevirapine (NVP)**	**Lopinavir/Ritonovir (LPV/r)**	**Septrin**
Stavudine (D4T)	**Efavirenz (EFV)**	**Indinavir (IDV)**	
Lamivudine (3TC)		**Nelfinavir (NFV)**	
Abacavir (ABC)			
Tenofovir (TDF)			
Didanosine(DDI)			
Emtricitabine (FTC)			

C) Date of change of HAART:

D) Combination changed to:

NRTIs	NNRTIs	PIs	Other drugs
Zidovudine (AZT)	**Nevirapine (NVP)**	**Lopinavir/Ritonovir (LPV/r)**	**Septrin**
Stavudine (D4T)	**Efavirenz (EFV)**	**Indinavir (IDV)**	
Lamivudine (3TC)		**Nelfinavir (NFV)**	
Abacavir (ABC)			
Tenofovir (TDF)			
Didanosine(DDI)			
Emtricitabine (FTC)			

e) Other drugs:

Drugs in last 4 wks	**Yes**	**No**
Gentamicin		
Sulfadiazine		
Sulfamethoxazole		
ibuprofen		
Acyclovir		
Amphotericin B		
herbal medication		
Others		

Part VI: PHYSICAL EXAMINATION

a) General appearance: 1) Well 2) Ill

b) Vitals

Pulse:

Respiratory rate:

Temp:

c) Anthropometry

Weight:

Height:

Weight for height standard deviation:

BP:

d) General examination

Pallor: 1) mild 2) moderate 3) severe

Oedema: 1) yes 2) no

e) systems/organs

System/organ	Normal	abnormal	Specify findings
skin			
Eyes			
Ears, Nose			
Oral			
Lymph nodes			
Heart			
Lungs			
Abdomen			
Urogenital			
Musculoskeletal			
Neurological			

Part VII: WHO Clinical stage: (tick highest)

Stage 1	**Stage 2**	**Stage 3**	**Stage 4**

Part VIII: base line data and LABORATORY DATA SHEET

A) CD 4 count

	Date sample collected	CD 4 count	CD %	WHO stage based on CD 4
Base line CD4 count				
Recent CD 4 count (with in 6 months)				
Study CD 4 COUNT				

B) Blood biochemistry and proteinuria

	Date of collection	creatinine	urea	sodium	potassium	chloride	eGFR	proteinuria
Base line								
Recent (within 6 months)								
Study result								

Name of doctor : ……. Signature:……. Date ……

Data entry date

Data entry number

Appendix vi: EHICAL APPROVAL

33 Joseph Mwilwa Road
Rhodes Park, Lusaka
Tel: +260 955 155 633
+260 955 155 634
Cell: +260 966 765 503
Email: eresconverge@yahoo.co.uk

I.R.B. No. 00005948
FWA. No. 00011697

14th February, 2014

Ref. No. 2013-Nov-014

The Principal Investigator
Dr. Kevin Mabvuto Zimba
The University Teaching Hospital
Dept. of Paediatrics and Child Health
P/Bag 1X RW,
LUSAKA.

Dear Dr. Zimba,

RE: Prevalence and factors associated with renal dysfunction in HIV positive paediatric patients at the Paediatric Centre of Excellence of the University Teaching Hospital in Lusaka, Zambia.

Reference is made to your corrections dated 14th February, 2014. The IRB resolved to approve this study and your participation as principal investigator for a period of one year.

Review Type	Ordinary	Approval No. **2013-Nov-014**
Approval and Expiry Date	Approval Date: 14th February, 2014	Expiry Date: 13th February, 2015
Protocol Version and Date	February 2014/?	13th February, 2015
Information Sheet, Consent Forms and Dates	• English.	13th February, 2015
Consent form ID and Date	Version-Nil	13th February, 2015
Recruitment Materials	Nil	13th February, 2015
Other Study Documents	Data Collection Sheet.	13th February, 2015
Number of participants approved for study	206	13th February, 2015

Specific conditions will apply to this approval. As Principal Investigator it is your responsibility to ensure that the contents of this letter are adhered to. If these are not adhered to, the approval may be suspended. Should the study be suspended, study sponsors and other regulatory authorities will be informed.

Conditions of Approval

- No participant may be involved in any study procedure prior to the study approval or after the expiration date.
- All unanticipated or Serious Adverse Events (SAEs) must be reported to the IRB within 5 days.
- All protocol modifications must be IRB approved prior to implementation unless they are intended to reduce risk (but must still be reported for approval). Modifications will include any change of investigator/s or site address.
- All protocol deviations must be reported to the IRB within 5 working days.
- All recruitment materials must be approved by the IRB prior to being used.
- Principal investigators are responsible for initiating Continuing Review proceedings. Documents must be received by the IRB at least 30 days before the expiry date. This is for the purpose of facilitating the review process. Any documents received less than 30 days before expiry will be labelled "late submissions" and will incur a penalty.
- Every 6 (six) months a progress report form supplied by ERES IRB must be filled in and submitted to us.
- ERES Converge IRB does not "stamp" approval letters, consent forms or study documents unless requested for in writing. This is because the approval letter clearly indicates the documents approved by the IRB as well as other elements and conditions of approval.

Should you have any questions regarding anything indicated in this letter, please do not hesitate to get in touch with us at the above indicated address.

On behalf of ERES Converge IRB, we would like to wish you all the success as you carry out your study.

Yours faithfully,
ERES CONVERGE IRB

Dr. E. Munalula-Nkandu
BSc (Hons), MSc, MA Bioethics, PgD R/Ethics, PhD
CHAIRPERSON